Harinder Gill
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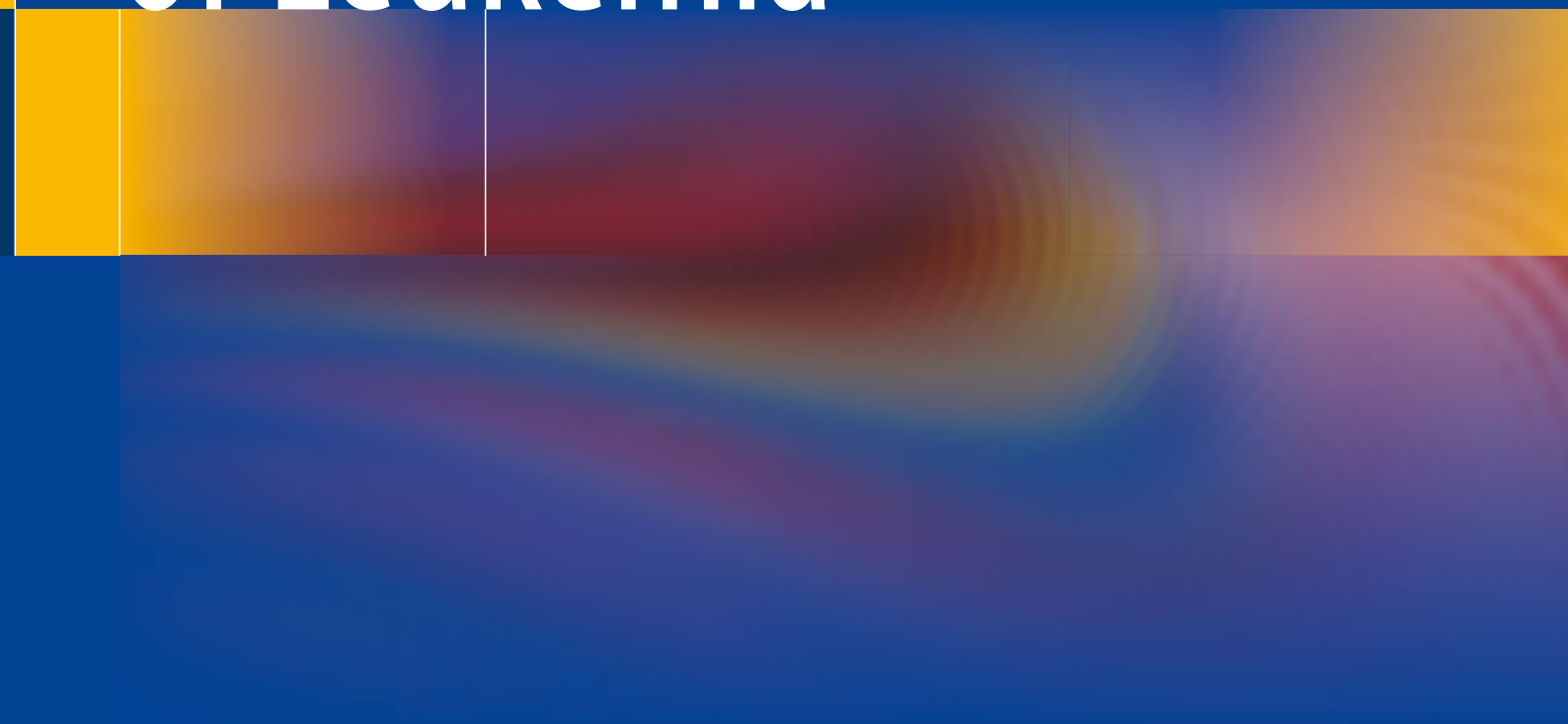
Pathogenesis and Treatment of Leukemia



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Contents

Basic Hematopoiesis and Leukemia Stem Cells

1

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Abstract

There have been significant advances in the knowledge and understanding of hematopoiesis over the last century. Detailed functional, phenotypic, and genetic studies on hematopoietic stem and progenitor cells as well as cellular subsets have facilitated efforts in the diagnosis and prognostication of various diseases of the bone marrow. Identification of myriad cellular pathways has also facilitated the development of new drugs for the treatment of these diseases, especially for the blood cancers. Current development of novel techniques for the expansion and genetic modification of hematopoietic stem cells, mesenchymal stromal cells, and immune cells will further expand the toolbox for treating patients with otherwise fatal cancers and bone marrow diseases.

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1.1 Introduction

Hematopoiesis is essential for life in humans and most animals. Hematopoietic stem and progenitor cells (HSPCs) give rise to erythroid precursors which produce red blood cells (RBCs) that carry oxygen to tissues; myeloid precursors which produce cells that contribute mostly to the innate immune system; lymphoid precursors which produce cells that contribute to the adaptive immune system; and megakaryocyte precursors which produce platelets that help arrest bleeding.

Due to its vital functions, HSPCs must begin function and production of progeny shortly after conception until death of the animal. In early human embryonic life, hematopoiesis begins briefly in the yolk sac, followed by its first definitive site in the aorto-gonado mesonephros from about 3 to 8 weeks of embryogenesis. Hematopoiesis then continues in the fetal liver (6 weeks to birth) and fetal spleen (10–28 weeks) before transiting to the fetal bone marrow (18 weeks to adult life) [1]. At birth, some HSPCs may be harvested from the umbilical cord blood (UCB) and, subsequently, HSPCs may be harvested from the bone marrow (BM) or from the peripheral blood after mobilization (mPB).

The discovery of hematopoietic cells and understanding of their function have progressed tremendously after the invention of the first compound microscope (inventor unknown) in 1620 [2]. Thereafter, the first description of RBCs was made by Jan Swammerdam in 1658 and later described in detail by Anthony van Leeuwenhoek in 1695. White blood cells (WBCs) were later discovered by Gabriel Andral and William Addison in 1843, while platelets and their function were discovered by Alfred Donne in 1842 [3], who also observed a maturation arrest of WBCs in some

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patients. This was rapidly followed by publications by a series of authors who described "leucocythemia" in 1845, a reversed WBC and RBC balance called "leukämie" in 1847, and the first diagnosis of leukemia in a living patient using microscopy in 1946 [4].

Since then, treatments involving hematopoietic cells have advanced from primitive bloodletting practices to current safe and rational blood transfusions after the discovery of human blood groups by Karl Landstenier in 1900 [5]. The threat of nuclear warfare or radiation-induced BM failure led to a series of animal experiments including one, which showed that mice that received an infusion of BM cells from a syngeneic mouse could recover fully from total body irradiation [6]. This was followed by another study where mice which were given total body radiation to eradicate their leukemia followed by infusion of syngeneic marrow were able to recover hematopoiesis without leukemia relapse [7]. This was followed, shortly after, by the first HSC transplants (HSCTs) carried out using intensive radiation or chemotherapy to cure BM disease at the expense of normal hematopoiesis, followed by infusion of fresh BM cells to reconstitute the hematopoietic system [8].

While the first HSCTs were less successful, subsequent ones had resounding success with the use of immunosuppressive drugs and histocompatibility matching for donors. To date, over 1.5 million HSCTs have been performed for a variety of BM disorders and cancers with improving outcomes and extension of HSPC sources to include peripheral blood stem cells (PBSCs/mPB) and UCB [9, 10]. The scientific discoveries and cell processing techniques; as well as clinical infrastructure, expertise and workflow developed due to HSCTs have laid the foundation for modern day cell-based immunotherapy (for various cancers) and cell-based regenerative medicine (for aging-related diseases).

New tools have emerged in the last few decades, which have rapidly enhanced our understanding of normal and abnormal hematopoiesis. These include methods from the last few decades which continue to be refined today, including multi-parametric flow cytometry for cell surface markers and polymerase chain reaction (PCR) techniques for nucleic acid studies. The last decade has seen further improvements in advanced techniques including whole genome sequencing, single cell gene analysis, genetic barcoding, cytometry by time-of-flight (CyTOF), and multi-omic immune profiling. This book outlines many of the recent discoveries that have been made in the field of malignant hematopoiesis due to these technologies as well as the new therapies that have been made using small molecules, proteins, antibodies, and cells for the treatment of these diseases.

1.2 Hematopoietic Stem and Progenitor Cells

BM is the major site for hematopoiesis. The BM niche and its stromal cell components provide a specialized microenvironment for the maintenance of HSPCs, differentiation of lineage-restricted progenitor cells, and serve as a reservoir of mature leukocytes. It is well established that HSPCs can circulate between peripheral tissues after release from the BM [11, 12]. While the functional relevance of this phenomenon is not fully understood, it has been proposed that these tissue resident HSPCs are essential for extramedullary hematopoiesis. The classical model of hematopoiesis is defined by the hierarchical differentiation of HSPCs into common myeloid progenitors (CMP) and common lymphoid progenitors (CLP), which subsequently give rise to mature myeloid and lymphoid cells, respectively. While this hierarchical model has been instrumental for understanding the process involved in hematopoiesis, data generated from the single cell RNA sequencing (scRNA) technology have challenged this hematopoietic hierarchy. Specifically, these studies have provided evidence to show heterogeneity in the HSPCs population, forming the basis for a "continuum" differentiation model instead of a "concrete" step-wise differentiation model.

1.2.1 Hematopoietic Stem and Progenitor Cell Heterogeneity

HSPCs are defined by their ability to repopulate the entire blood system after transplantation into lethally irradiated recipient/s. However, single cell and serial dilution transplantation studies have revealed significant difference in the engraftment activities and lineage-biased cell output. Moreover, single cell RNA (scRNA) analyses of HSPCs have also further confirmed the heterogeneity [13]. Of note, Wilson et al. have shown that there are two functionally distinct HSPC populations, i.e., repopulating HSPC and non-repopulating HSPC in the transplantation model [14]. Additionally, HSPC subsets that are myeloid, lymphoid, and platelet-biased have also been reported [15]. Collectively, these data illustrate that HSPCs are heterogenous in terms of their molecular signature, as well as their function. It is important to point out that most of these studies focus on HSPC function in the context of transplantation. Thus, these results may not fully represent HSPC function during normal hematopoiesis. Indeed, in situ clonal tracking of HSPCs provided evidence to show that HSPCs have a minimal contribution to the mature leukocyte output, and that lineage-restricted progenitors are the cells driving steady state hematopoiesis [16, 17].

1.2.2 Lineage Commitment from the Hematopoietic Stem and Progenitor Cell

One major feature of hematopoietic stem cells (HSCs) is their ability to self-renewal. In contrast, hematopoietic progenitor cells (HPCs) lack the capability of extended self-renewal, and they are lineage-restricted—for example CLP and CMP. Lineage commitment decisions involve a series of transcriptional events leading to lineage specification and commitment. The classical model suggests that first lineage commitment step is triggered by a strict bifurcation of HSC into CMP and CLP. However, there is growing evidence to support the view that there is still plasticity in the lineage restriction of multipotential progenitors [18–20], indicating that hematopoiesis does not follow a strict myeloid-lymphoid segregation as previously thought. Transcription factors play a major role in lineage priming and commitment of multipotent cells. This is best exemplified by the cross-antagonism of transcription factors like GFI1 and IRF8, whereby the balance between these transcription factors is the major determinant for neutrophil or macrophage fate choice [21].

1.3 Hematopoietic Stem and Progenitor Cell Assays

The characterization and enumeration of HSPCs are essential in guiding both research and clinical workflows. The key tools used in assessing quality and quantity of HSPCs will be described in this section using phenotypic and functional assays.

1.3.1 Phenotypic Characterization

Multi-parametric flow cytometry is a common tool that is used in research and clinical laboratories to perform immunotyping of HSPCs and various other lineages [22]. We are able to monitor the highly orchestrated process for hematopoiesis in blood and bone marrow samples using differentiation and self-renewal stage-specific markers that are expressed on HSPCs and its progenies. Almost all HSPCs express the pan-leucocyte marker, CD45, as well as the stem cell-specific glycoprotein, CD34, and exhibit low forward and side scatter (small cells) [23]. To date, expression of antigens such as CD90 [24], CD49f [25], CD133 [26], CD117 [27], and CD166 [28] have been shown to be present in long-term HSCs that concurrently lack expression of maturation markers such as CD45RA, CD38, and Lin [29]. With maturation of the HSCs to multipotent progenitors (MPPs), the expression of CD90, CD133, and CD49f is downregulated [23, 25]. Based on various studies lineage-specific markers such as CD38, CD135, CD45RA, CD110, and CD123 become highly expressed in the multiple subsets of CMP [19, 30]; while CD10 and CD7, along with CD34, are primarily used to identify the CLP that gives rise to mature T, B, and NK cells [31]. Viability being a key factor in determining the quality of HSPCs is measured using dyes such as 7-Aminoactinomycin D (7-AAD) [32] or 4′,6-diamidino-2-phenylindole (DAPI) [33] in almost all multi-parametric flow cytometry panels. Cell cycle of HSPCs is yet another key parameter to monitor, especially for those that are put in ex vivo cultures using propidium iodide (PI) staining, while cell doubling is monitored through the use of carboxyfluorescein diacetate succinimidyl ester (CFSE) [34] or bromodeoxyuridine (BrdU) [35] coupled with flow cytometer-based analysis. In clinical workflow, CD34 or CD133 antibodies conjugated with magnetic nanoparticles are widely used for isolation and purification of HSPCs for therapeutic and diagnostic purposes [36]. Conventional morphology analysis using hematoxylin and eosin (H&E) and May Grunwald-Giemsa (MGG) stain is unable to allow specific recognition of HSPCs as their appearance closely resembles small, mononuclear lymphocytes [37].

1.3.2 Colony Forming Unit (CFU) and Long-Term Culture-Initiating Cell (LT-CIC) Assays

The discovery and implementation of the CFU assay for HSPCs have been pivotal in assessing the quality of the myeloid progenitor cells prior to therapeutic usage and to measure experimental outcomes [38]. In the CFU assay, a fixed number of input cells are cultured using a semisolid media supplemented with various hematopoietic-specific growth factors, which leads to the formation of myeloid progenitor-type-specific colonies [39]. The number and morphology of the colonies are used to determine the differentiation and proliferation capacity of the cultured progenitor cells [40]. In many instances, cells from the colonies can be harvested for further identification using flow cytometry and morphological analysis. CFU assays are appropriate for identification of progenitors that can give rise to granulocytic, erythroid, monocytic, and megakaryocytic lineages [41]. A major limitation of the CFU assay is its inability to assess the in vitro functional capacity of lymphoid progenitors.

The LT-CIC assay is directed towards evaluating primitive hematopoietic progenitors, especially myeloid clonogenic progenitors [42], and has now been extended to quantification of lymphoid-lineage populations as well [43, 44]. In this assay, a feeder layer (e.g., M2-10B4) is generated following which HSPCs are added using serial dilution and those cultures are maintained greater than 5 weeks with

appropriate media changes [42]. At end of the culture, CFU is plated using the harvested cells and generated colonies are scored. The data generated from LT-CIC assays enable to detect and quantitate primitive HSPCs that share phenotype and functionality with in vivo repopulating HSCs [45].

1.3.3 Xenotransplantation Studies

The long-term ability of human HSPCs to survive and give rise to multi-lineage, mature immune cells can only be assessed using xenotransplantation studies involving immunodeficient mice models. The most widely used mice strain is the nonobese diabetic (NOD) severe combined immunodeficiency (*scid*) gamma (NSG™) that are extremely immunodeficient and prevent host rejection of nonself-tissues [46, 47]. The NOD inbred background ensures impaired innate function; the *Prkdc*^{scid} mutation prevents maturation of mouse T and B cells; and *l2rg*^{tm1Wjl} mutation disrupts cytokine signalling and maturation of mouse NK cells [46, 47]. Furthermore, the unique allele of Sirpa along with myeloablative, sublethal total body irradiation (of up to 400 cGy) further enhances engraftment of human HSPCs in the NSG model [46, 47]. To date, there are over 40 variants of NSG™ mice, such as (i) NOD *Rag* gamma (NRG) mice that have similar capacity to engraft human HSPCs without the need to perform sublethal gamma irradiation [48]; (ii) NOD *scid* gamma Il3-GM-SF (NSG-SGM3) mice that have secretion of cytokines—IL-3, GM-CSF, and SCF in the BM niche which expedites myeloid lineage and regulatory T cell engraftment [49]; and (iii) NSG-IL15 mice that better support development of human NK cells [50]. A typical xenotransplantation study involves the following main steps: (i) pretransplantation preparatory activities that include antimicrobial drug prophylaxis (to prevent opportunistic infections); and myeloablation (to create space in the mouse BM niche); (ii) transplantation of the human HSPCs (purified CD34+ cells with or without mature immune cells) primarily via the intravenous route (tail vein injections); and (iii) posttransplantation follow-up that includes quantification of the human blood cells in the mouse peripheral blood (survival procedure); and BM and spleen (end of life procedures) primarily using flow cytometry [51]. In some cases, immunosuppressive drugs such as ciclosporin are administered to transplanted immunodeficient mice to alleviate symptoms of xeno-graft-versus-host-disease [52]. The NSG™ mice, once transplanted with CD34 expressing human HSPCs, start to show presence of human cells within 4–8 weeks posttransplantation and could remain alive for up to 12 months. In some instances, the human cells harvested from the BM of primary mice recipients are further transplanted to secondary immunodeficient mice to monitor the long-term self-renewal capacity of human HSPCs [25]. Prior to starting any clinical studies for new types of cellular therapy grafts comprising of engineered or expanded human HSPCs, such animal studies are essential in establishing preclinical safety, efficacy, and lay the foundation for clinical protocols.

1.4 Hematopoietic Stem and Progenitor Cell Expansion

HSCT has been effective in the cure of many hematological malignancies. In this clinical procedure, primary disease is treated by myeloablation of the BM followed by restoration of normal hematopoiesis through the infusion of healthy HSPCs from autologous or allogeneic sources. However, this 6-decade old procedure is associated with a significant period of post-conditioning pancytopenia as the infused HSPCs need to undergo engraftment, proliferation, and differentiation that lead to repopulation of the recipients' BM and reestablishment of normal hematopoiesis [53]. Based on various studies, it has been established that the time to recovery is dependent on the quality and quantity of the HSPCs infused to the patients as well as the graft source (BM, mPB, or UCB) [54].

HSPC expansion that involves culturing cells in cGMP-grade cell therapy manufacturing facilities could help increase the infused cell dosage and improve the outcomes of both autologous and allogeneic HSCT by accelerating hematopoietic recovery [55]. However, while conventional hematopoietic cytokines such as stem cell factor (SCF), thrombopoietin (TPO), FMS-like tyrosine kinase 3 ligand (Flt-3L), and various interleukins (ILs) can promote the growth of late or committed progenitor cells (CMP and CLP) from HSCs, these cells lose long-term proliferative potential and become unusable as a HSCT graft that can impart life-long hematopoiesis [56]. Various efforts have been employed to expand hematopoietic progenitors while maintaining or enhancing long-lived HSCs. These include the use of novel cytokines or small molecules and mesenchymal stromal cell (MSC) coculture to mimic the innate microenvironment of the HSC niche in the BM [54]. Many of these studies have either shown no improvement in times to engraftment or failure of the expanded graft to contribute to long-term hematopoiesis. However, these studies have yielded important insights into the mechanisms of HSPC expansion, including the importance of maintenance of cell viability during ex vivo cultures [32, 57] and the presence of intercellular cytosolic and mitochondrial transfer during MSC coculture [58].

Recent studies involving the use of SR1 [59], UM171 [60], and nicotinamide [61] in ex vivo HSPC cultures have shown significant improvements in neutrophil and platelet engraftment times, while also maintaining long-term engraftment of the expanded cells. In a randomized phase 3 study of

Omidubicel in cord blood transplantation using the nicotinamide platform, median time to neutrophil engraftment shortened from 22 to 12 days ($p < 0.001$) with a lower incidence of infection and an increase in time spent out of hospital during the first 100 days posttransplant. Platelet recovery was also accelerated [62]. Similarly, SR-1 [59] or UM171 [60] expanded grafts have shown the ability to reduce time to recovery in early phase trials.

While HSPC expansion is mostly used in the setting of umbilical cord blood transplantation (UCBT), it could also be useful for enhancement of bone marrow (BMT) and peripheral blood stem cell transplants (PBSCT). Manufacturing of mega doses of HSPCs from BM or PB could further accelerate engraftment that will eventually enable transplants to be carried out as outpatient procedures [56]. Expansion of gene-edited HSPCs for gene therapy of inherited BM diseases like thalassemia major and sickle cell disease could also be a potential life-saving procedure for many patients [63, 64]. In addition, expansion of mature progenitors followed by infusion to patients could also help to accelerate recovery from intensive chemotherapy regimens used in diseases like acute myeloid leukemia (AML). Further studies into the mechanisms of HSPC expansion as well as the directed expansion of selected immune cells like T cells and NK cells could further expand this field of cellular therapy.

1.5 Aging Hematopoiesis, Including Telomeres

1.5.1 Influence of Aging on Hematopoiesis

The hematopoietic system produces 10^{11}–10^{12} mature blood cells every day, compensating for the daily loss of a similar number of blood cells. This enormous proliferative capacity of the hematopoietic system is important for maintaining a healthy body status. The production of blood cells is tightly regulated by self-renewal and differentiation of HSPCs, which maintain a normal blood cell count throughout life. Despite this precise regulatory mechanism, HSPCs still age in humans [65] and mice [66].

There are several characteristics of aged HSPCs. One of the key features of aged HSPCs is their reduced ability to self-renew. However, the number of HSPCs in the BM is known to increase with age in humans [65] and mice [66], which is thought to compensate for the age-related decline in the self-renewal capacity of these cells. Aged HSPCs have been shown to have a smaller number of daughter clones than young counterparts after serial transplantation [67]. Aged HSPCs are also known to have a higher association with quiescence [68] and a lower proliferation rate [69]. As HSPCs age, the expression profile of cell cycle genes is significantly altered [70, 71]. Another change that occurs in aging HSPCs is a bias in differential potentials. Aged HSPCs have a reduced ability to differentiate into lymphoid lineage cells and tend to differentiate into myeloid lineage cells [65, 72, 73]. Subsequently decreased common lymphoid progenitor cells, increased megakaryocyte/erythrocyte progenitors [74], and increased platelets [75] have also been reported. During HSCTs, homing refers to the ability of HSPCs to migrate to the BM, and engulfment refers to the ability of transplanted HSPCs to contribute to stable blood production. It has been shown that these two important properties are impaired in aged HSPCs [76, 77]. Other phenotypic changes in aged HSPCs include clonal expansion, increased polarity, and high mobility. Traditionally, HSPCs were thought to be homogeneous. However, recent experiments have shown that aged HSPCs are heterogeneous and that their clonal distribution increases with age [78]. Polarity is tightly linked to the symmetrical or asymmetrical mode of division of HSPCs; however, aged ones divide more symmetrically than younger counterparts [79]. HSPCs reside in the BM niche to maintain their homeostasis, but aged HSPCs become more mobile and migrate from the BM to the PB in response to granulocyte colony-stimulating factor (G-CSF) [80]. Importantly, aged HSPCs have a higher risk of malignant transformation [81]. Elucidating underlying mechanisms of various phenotypic changes in aged HSPCs will help in the development of new therapeutic approaches for blood cancers and age-related blood diseases.

Many potential mechanisms of HSPCs aging have been proposed. A well-known cause of HSPCs aging is telomere shortening, which potentially limits the self-renewal of these cells. The details of this mechanism are discussed in the later section. Another important cause of HSPCs aging is the accumulation of DNA damage. This was shown experimentally by *XPD*-null mice lacking the nucleotide excision repair pathway or *Ku80*-null mice lacking the nonhomologous end-joining pathway. The HSPCs of these mice showed not only increased DNA damage, but also reduced reconstitution and proliferation, decreased self-renewal ability, increased apoptosis, and functional exhaustion [82]. Accumulation of DNA damage is also observed in the HSPCs of wild-type aged mice [83]. Reactive oxygen species (ROS), which induce DNA damage, are also known to be associate with HSPCs aging. As described earlier, HSPCs reside in the BM niche that maintains a hypoxic microenvironment and protects these cells from oxidative stress. The Forkhead O (FOXO) family of transcription factors such as Foxo1, Foxo3a, and Foxo4 plays critical roles in reducing oxidative stress, maintaining quiescence, and improving survival, and these functions are required for the long-term regenerative potential of HSPCs [84, 85]. Besides DNA damage and ROS, many studies have shown that epigenetic changes are also associated with HSPCs aging. For example, the genes involved in HSPC

differentiation tend to be hypermethylated in their promoter, while genes associated with HSPC maintenance tend to be hypomethylated [86]. Indeed, the studies in mice have shown that the ectopic expression of different epigenetic writers affects the balance between self-renewal and differentiation of HSPCs [87]. The changes in polarity may also contribute to HSPCs aging. Increased activity of Cdc42, small RhoGTPase, in aged HSPCs is associated with enhanced polarity toward symmetrical division, and inhibitors of Cdc42 restore the altered polarity of aged HSPCs and improve the function of HSPCs after transplantation [88]. Reduced autophagy in aged HSPCs has also been proposed as a contributing factor to aging. Reduced autophagy in HSPCs leads to the accumulation of impaired mitochondria, resulting in their differentiation into myeloid and a decreased self-renewal and regenerative potential of HSPCs [89]. Reducing mitochondrial stress in aged HSPCs can restore their regenerative capacity [90]. However, another report showed that mutations in mitochondrial DNA itself are not a major driver of HSPCs aging [91]. Other factors that may contribute to the functional decline of aged HSPCs include replication stress [92], switching from canonical to noncanonical Wnt signaling [93], and changes in protein homeostasis [94].

In addition to the potential intrinsic causes of HSPC aging described above, recent evidence suggests that the alteration of the BM microenvironment may contribute to HSPC aging. These changes include the location of HSPCs within niches, imbalanced BM-MSC differentiation, remodeling of the BM endothelial vasculature, increased pro-inflammatory cytokines, and increased senescent cell populations [95–97]. Although there is a strong association between HSPC aging and microenvironment alterations, further studies are needed to determine whether these changes are the cause or consequence of HSPC's aging process.

Recent advances in understanding mechanisms of HSPC aging have led to the search for ways to rejuvenate the blood forming cells. Cellular senescence is a state of irreversible growth arrest, and an increase in the number of senescence cells in the body is associated with aging [98]. Senescent cells secrete many pro-inflammatory cytokines called senescence-associated secretory phenotype (SASP), which are associated with tissue dysfunction and an increased risk of developing age-associated diseases including cancer [98]. The CDK inhibitor p16 is one of the well-known markers of senescence and contributes to the onset and maintenance of senescent cells. The depletion of p16-positive senescent cells in mice suppressed age-related deterioration of several organs [99]. Although it has been shown that aged HSPCs do not express p16 [100], pharmacological removal of senescent cells by ABT263 rejuvenates aged HSPCs [101], suggesting that senescent cells in the BM niche may contribute to aging of these cells. In addition, several other approaches have recently been shown for HSPCs rejuvenation. These include the treatment with CASIN, CDC42 inhibitor, or rapamycin, mTOR inhibitor, inhibition of RANTES, the inflammatory cytokine, transplantation of young endothelial cells, activation of Notch signaling in old endothelial cells in the bone marrow, Sirt3 or Sirt7 overexpression, and restoration of the sympathetic nervous system in the bone marrow by supplementation of adrenoreceptor β3 signals [97]. Developing ways to rejuvenate HSPCs will not only help gene therapy, such as BMT used to treat leukemia, other cancers, and blood disorders, but will also prevent diseases caused by HSPCs aging and improve the health of the aging population.

1.5.2 Telomeres in Hematopoiesis

Telomeres are unique nucleoprotein structures with tandem TTAGGG DNA repeats that are essential to protect the ends of human linear chromosomes. Synthesized by the reverse transcriptase enzyme called telomerase, the core components comprise the protein catalytic subunit, hTert, and the RNA subunit, *hTERC/hTR/hTER* [102, 103]. Other telomerase-associated components, including dyskerin, pontin, and reptin, are also required for telomerase holoenzyme assembly and function [104], which were identified through characterization of genetic mutations in a spectrum of human telomere diseases including dyskeratosis congenita, aplastic anemia, and pulmonary fibrosis [105–107].

The physiological expression of *hTERC/hTR/hTER* is ubiquitous, whereas *hTERT* is detectable only in germ cells, undifferentiated stem, and progenitor cells of various tissue types including hematopoietic lineage [108, 109]. Differentiation and maturation into somatic cells result in the repression and eventual silencing of *hTERT* expression [110]. Cells lacking telomerase activity inevitably experience telomere shortening by 50–200 bp with every cell division due to the incomplete replication by DNA polymerase and end processing of the newly replicated DNA strand [111]. Consequently, when telomere length reaches critical limit, exposure of chromosomal ends triggers DNA damage response which induces replicative senescence, apoptosis, or genomic instability [102, 103]. Therefore, the expression of *hTERT* is the rate-limiting step for telomerase activity in vivo.

In the hematopoietic system, telomerase expression is detectable in the HSPCs [111]. Owing to the limited lifespan of mature hematopoietic cells, HSPCs divide constantly to provide replacements. As such, telomeres of HSPCs are serially shortened and their self-renewal capability is progressively diminished, which is a hallmark of aging [112, 113]. This age-related shortening was estimated to be 485 bp (early life), 74 bp (childhood), and 28 bp (adult) per year in

humans [114]. However, when telomeres are aberrantly eroded by genotoxic exposures or by inherited mutations of telomerase genes, HSPCs can undergo premature DNA damage signaling. This, in turn, leads to accelerated aging or genome destabilization [113, 115]. Such telomere dysfunctions significantly deplete the HSPC population in the BM and increase the predisposition of hematological neoplasms including myelodysplastic syndrome (MDS) and leukemia [113, 115]. MDS is a heterogeneous group of hematopoietic diseases that are distinguished by inefficient hematopoiesis, dysplasia, and persistent DNA damage in the stem and progenitor cells [113, 116]. Patients diagnosed with MDS have an increased risk of developing leukemia [113, 115, 116]. Such genetic predisposition is recapitulated in telomerase knockout mouse models, with late generation telomerase knockout mice exhibiting impaired HSPCs proliferation and differentiation, thereby causing skewed myeloid differentiation, anemia, and lymphocyte deficiency [113, 117]. Serial transplant potential of HSPCs from these mice was severely reduced as they lack the ability to repopulate the blood of irradiated mice [118, 119]. Additionally, it was shown that the aged G4/G5 telomerase-knockout mice exhibited persistent DNA damage signals, a distinct increase of BM myeloid blasts, and a small percentage of the mice developing acute myeloid leukemia (AML) [113]. These studies, therefore, establish a strong clinical link between the loss of telomere functions and hematopoietic disorders.

Telomerase reactivation was documented in more than 85–90% of all human cancers [110, 120]. However, leukemia exhibits diverse hematological origins and complex etiology. Telomerase activation is a frequent phenotype observed in the progression of leukemia via a spectrum of mechanisms, including epigenetic modulations, gene amplification, and microRNAs [121]. Epigenetic status on the *hTERT* gene promoter is a determining factor for telomerase activity. In cancer cells, DNA demethylation is required at the core promoter of *hTERT*, while *hTERT* promoter of normal cells is either hypomethylated or unmethylated [122, 123]. For B-cell CLL, telomerase was reported to be expressed at high levels while exhibiting low levels of methylation at the *hTERT* promoter [124]. Additionally, ALL was found to possess hypomethylation at the *hTERT* promoter [125, 126]. Therefore, treatments targeting the methylation status of *hTERT* promoter in these cancers could prove feasible. Indeed, DNA methyltransferase inhibitors (DNMTI), including azacitidine and decitabine, were shown to reduce hTERT gene expression and telomerase activity [127–129]. Thus, DNMTIs have been approved for the treatments of MDS and AML patients [130]. Histone deacetylation and methylation were also documented to reflect the repressive status of the *hTERT* promoter [131]. Histone methylation is involved in *hTERT* regulation through histone H3K4. Trimethylation of histone H3K4 by a histone methyltransferase called SMYD3 was shown capable of inducing *hTERT* transcription and telomerase activity in normal human fibroblasts and cancer cell lines [132]. Additionally, methylation of H3K4 may play an essential role for the trans-activation of *hTERT* gene by regulating the c-Myc transcription [133]. Woo et al. demonstrated that inhibition of histone deacetylase (HDAC) suppressed *hTERT* expression and telomerase activity, thereby inducing antiproliferation and cell death in leukemic cells [134]. Thus, HDAC inhibitors are increasingly explored as anticancer treatments. Examples of clinical trials include chronic lymphoid leukemia, CLL (NCT01016990); acute myeloid leukemia, AML (NCT00006240, NCT00305773, NCT01802333); and acute lymphoblastic leukemia, ALL (NCT00462605). Amplification of the *hTERT* gene frequently occurs in human tumors and hematological neoplasms, and amplified regions encompassed most of chromosome 5p region or via chromosomal translation [135, 136]. In ALL and CLL, rare recurring somatic chromosomal translocation was found for the telomerase reverse transcriptase-cleft lip and palate transmembrane protein 1-like protein locus that carries the *hTERT* gene to immunoglobulin (IG) heavy and non-IG loci [137, 138]. Such chromosomal rearrangement could potentially relieve the repressive epigenetic modifications on the *hTERT* promoter leading to its transcription.

MicroRNAs (miRNAs) were implicated in telomerase activation through crosstalk with other pathways that drive malignancy [139]. For leukemia, Bhatia et al. showed that the loss of miR-196b function in B-cell ALL significantly upregulated c-myc, which elevated telomerase activity via hTERT expression, and this could be reverted by restoring miR-196b expression [140]. Yan, Ooi et al. have shown that the *hTERT* expression in human embryonic stem cells (hESC) is negatively regulated by miR-615-3p and HoxC5 via targeting hTERT 3'UTR and the 20 kb upstream enhancer region of hTERT, respectively [141]. Additionally, both miR-615-3p and HoxC5 are found on the same locus, and overexpression experiments in cancer cells showed tumor growth suppression in vivo indicating tumor suppressive function. Furthermore, the miR-615-3p and HoxC5 expressions were progressively upregulated and *hTERT* expression subsequently suppressed upon the transition of hESCs to neuronal fate, suggesting a mechanistic role in *hTERT* suppression during cell differentiation [141]. Notably, Bijl et al. observed that matured and differentiated lymphoid cells exhibited strong HoxC5 expression. In contrast, HoxC5 expression in leukemic cells with activated *hTERT* expression was undetectable [142]. These observations in combination with Yan, Ooi et al. findings indicate that leukemic cells with activated *hTERT* expression could originate from an undifferentiated hematopoietic stage.

Current telomerase targeting therapeutics that are undergoing clinical trials include immunotherapy, small molecule

inhibitors, and antisense oligonucleotide [143]. The immunotherapy GRNVAC1 is a dendritic cell (DC)-based vaccine that utilizes patient-derived immature DCs to produce telomerase fragments for antigen presentation, thereby enhancing immune response. GRNVAC1 vaccine is being assessed in an AML randomized phase II clinical trial. For small molecule inhibitors, BIBR1532 selectively inhibits the telomerase active site in a dose-dependent manner and transcriptionally suppresses survivin-mediated c-Myc to induce apoptosis in patient-derived AML or CLL [144, 145]. Imetelstat or GRN163L is an antisense oligonucleotide that is complementary to the hTERC sequence in design, thereby blocking telomerase from telomere elongation. Imetelstat is evaluated in stage I and II clinical trials of patients with refractory and relapsed MM [146]. Moreover, Imetelstat has shown favorable overall survivability in high-risk myelofibrosis patients exhibiting Janus Kinase inhibitors resistance [147]. While these results are promising, significant dose-dependent hematological side effects, liver function abnormalities, and other adverse events remain a potential hurdle for using telomerase inhibitors in clinical therapy [148, 149]. This is likely due to the simultaneous telomerase inhibition in both cancer cells and tissue-specific stem/progenitor cells (such as hematopoietic stem/progenitor cells) at the high dose of telomerase inhibitor, leading to toxicity and failure of therapy.

Overall, telomerase reactivation is a hallmark in the oncogenesis and progression of leukemia and numerous other neoplasms. Hence, designing anticancer strategies to effectively inhibit telomerase activity is critical and essential.

1.6 Leukemic Stem Cells

Cancer stem cells (CSCs) have an almost infinite capacity for self-renewal and production of tumorigenic progeny. It is believed that only a small proportion of the cells that are considered part of the malignant clone in a patient with cancer are CSCs. Leukemia stem cells (LSCs) have also been shown to exist, and therapies aimed at targeting slowly dividing LSCs could be more effective in eradication of leukemia in the long-term than cytotoxic chemotherapies that target mainly the more rapidly dividing mature progenitors. This is not to be confused with the cell of origin of leukemia (COL), which is defined as the normal cell that is able to transform into a leukemia cell [150].

For example, CML, a hematopoietic stem cell malignancy, despite effective BCR-ABL1 tyrosine kinase inhibitors (TKIs), remains incurable. LSCs in CML have been shown to persist in a quiescent state, while TKI-sensitive progenitors and differentiated myeloid cells are eradicated [151]. LSCs have been demonstrated in the BM of CML patients even in the presence of successful inhibition of the BCR-ABL fusion oncoprotein [152], and even when the *BCR-ABL* transcript is completely undetectable by clinically available laboratory tests with high sensitivity [153]. It has been shown that TKI-treated LSCs are capable of self-renewal in vitro LT-CIC and long-term engraftment in in vivo NSG mice [154]. Reservoir of quiescent yet fully leukemogenic LSCs is hence thought to survive chemotherapeutic agents through various mechanisms.

Heterogeneous genetic mutations, and complex interactions of transcriptomic and epigenomic alterations in leukemia, affect multiple downstream pathways and provide survival advantage for LSCs over normal HSCs [155]. Somatic loss-of-functions mutations in *TET2* gene can create myeloid-biased differentiation of HSCs and increased self-renewal of stem and progenitor compartment, leading to leukemic transformation [156]. Signaling pathways like the Hedgehog pathway are involved in the cell cycle of normal HSCs and LSCs [157]. In vivo CML stem cell quiescence involves the cooperation of stemness factors FOXO and β-catenin [158]. Genomic instability induced by oxidative DNA damage and repair mechanisms may result in further genetic aberrations and clonal evolution of LSCs, rendering chemotherapy resistance [159].

In addition, intracellular nuclear-cytoplasmic transport may also inactivate tumor suppressors, favoring survival of LSCs. For example, CRM1 (chromosome region maintenance 1/exportin 1) has been shown to be upregulated in LSCs in myeloid and lymphoid malignancies, compared to normal HSCs [160, 161]. A recent meta-analysis of published CML microarray datasets has revealed that differentially upregulated genes in primitive LSCs are enriched for cell cycle and DNA replication genes with nuclear export signals regulated by CRM1 [154].

Another crucial factor for persistence of leukaemia is thought to be mediated through intricate cross-interaction between LSCs and BM stroma and altered immune modulation in the microenvironment [162]. In the BM tumor microenvironment, extracellular matrix proteins also contribute by altering the physiology of LSCs, thus influencing leukemia progression [163]. Mobilizing LSCs from the BM stem cell niche could overcome resistance to therapeutic measures by triggering a release from dormancy and deprivation from survival factors in the marrow microenvironment [164]. Modulation of stromal cells in the marrow by epigenetic modulation has also been shown to promote normal hematopoiesis while suppressing leukemic and myelodysplastic progenitors [165].

Altered immunomodulation in the leukemic BM microenvironment may also facilitate the immune escape of LSCs. Ineffective immune surveillance and response by cytotoxic T lymphocytes (CTLs) against CML has been mediated by the interaction of the programmed death (PD-1) receptor

expressed on CTLs with its inhibitory ligand PD-L1 expressed on LSCs [166]. Targeting the PD-1/PD-L1 interactions has been shown to eradicate LSCs and prevent disease development in CML mouse models [167].

Upregulation of inflammatory pathways in LSCs and mesenchymal stroma is another common finding in leukemia. Transcriptomic analysis by RNA sequencing of purified leukemic cells from chronic myelomonocytic leukemia (CMML) demonstrates highly pro-inflammatory signature, with increased expression of pathways including tumor necrosis factor and interleukin (IL)-6 signaling, compared to age-matched healthy controls [168]. Similarly, an upregulated p53-S100A8/9-TLR inflammatory signaling in purified mesenchymal cells of preleukemia or myelodysplastic syndrome (MDS) has been associated with genotoxic stress and leukemic evolution [169].

Studies are underway to target LSCs by intracellular signaling pathways and modulation of the tumor microenvironment as well as checkpoint molecules and unique combinations of cell surface molecules [170]. These are probably more important in the myeloid leukemias where there is more phenotypic heterogeneity than with the lymphoid neoplasms. The genetic and hierarchical complexity of these myeloid neoplasms contributes to the development of tumor resistance in subclones and underscores the need for development of therapies that target LSCs [171, 172].

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2 Modern Classification of Acute and Chronic Leukemias: Integrating Biology, Clinicopathologic Features, and Genomics

Harinder Gill

Abstract

The modern classification of acute leukemias and myeloid neoplasms has much refinement incorporating clinicopathologic and genomic features with the objective of defining disease entities with information on the clinical behaviour, pathobiology, and prognosis. In this chapter, we discuss the current classification of acute leukemias and myeloid neoplasms, highlighting the fifth edition of the World Health Organization (WHO) Classification published in 2022, and the International Consensus Classification (ICC) of Myeloid Neoplasms and Acute Leukemia. Details on the classification of acute lymphoblastic leukemia and myeloid neoplasms with germline predisposition are discussed elsewhere.

Keywords

Acute leukemia · Myeloid neoplasms · Classification

2.1 Introduction

The major categories of myeloid neoplasms and acute leukemias are listed in Table 2.1 [1, 2]. The genetic characteristics and etiology are emphasized.

Table 2.1 Major categories of myeloid neoplasms and acute leukemias according to the International Consensus Classification (ICC) [1, 2]

Myeloproliferative neoplasms
Myeloid/lymphoid neoplasms with eosinophilia and tyrosine gene fusions
Mastocytosis
Myelodysplastic/myeloproliferative neoplasms
Pre-malignant clonal cytopenias and myelodysplastic syndromes
Pediatric and/or germline mutation-associated disorders
Acute myeloid leukemia
Myeloid proliferation associated with down syndrome
Blastic plasmacytoid dendritic cell neoplasm
Acute leukemia of ambiguous lineage
B-lymphoblastic leukemia/lymphoma
T-lymphoblastic leukemia/lymphoma

2.2 Myeloproliferative Neoplasms (MPN)

Integration of molecular markers has improved the current diagnostic criteria of MPN [2, 3]. Diagnostic assessment of MPN involved correlation between clinical features, bone marrow morphologic feature, and genomic information. Chronic myeloid leukemia with *BCR*::*ABL1* was classically a tri-phasic disease in the pre-tyrosine kinase inhibitor (TKI) era. With the advent of TKI and molecular monitoring, progression is uncommon and the 10-year overall survival is in excess of 80% [4, 5]. While the International Consensus Classification (ICC) retains the classification and definitions of the tri-phasic disease (Table 2.2) [2], the WHO 2022 classification has removed the designation for accelerated phase as this has become less relevant in the TKI era [3].

For the classical *BCR::ABL1*-negative MPNs, the diagnostic criteria for PV, ET, PMF, post-PV MF (PPV MF), and post-ET MF (PET MF) rely on the constellation of clinical features, peripheral blood features, bone marrow morphology, and genomics (Tables 2.3 and 2.4) [2, 3, 6]. Chronic neutrophilic leukemia (CNL) is a rare *BCR::ABL1* negative MPN that is diagnosed based on the sustained peripheral

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blood neutrophilia with white blood cell (WBC) count ≥25 × 10^9/L (with ≥80% segmented neutrophils and bands), hypercellular bone marrow due to neutrophilic proliferation, hepatosplenomegaly, and the presence of *CSF3R* mutations (usually present in more than 60% of cases and is diagnostic of CNL) [7–10]. Mutations in SETBP1, ASXL1, SRSF2, and other signalling genes frequently co-occur in patients with CNL [10, 11]. In additional to the diagnostic implication, the presence of *CSF3R* mutations (*CSF3R* T618I in particular) has important therapeutic implications due to its sensitivity of *CSF3R*-positive CNL to the JAK inhibitor ruxolitinib [12]. Chronic eosinophilic leukemia (CEL) is characterized by sustained eosinophilia for ≥4 weeks and clonal proliferation of morphologically abnormal eosinophils and eosinophilic precursors in the bone marrow [3]. The abnormal bone marrow morphology may also include erythroid and megakaryocytic dysplasia. Proof of clonality and abnormal bone marrow morphology are essential criteria in differentiating CEL from idiopathic hypereosinophilic syndrome [13, 14]. In the latest edition of the WHO classification, juvenile myelomonocytic leukemia (JMML) is categorized under MPN and is recognized as an MPN of early childhood [3]. Diagnostic molecular studies are essential in establishing the diagnosis of JMML. Demonstration of mutations in genes involving the RAS pathway is emphasized [15, 16]. The most aggressive form of JMML comprises somatic mutations of *PTPN11* and germline mutations associated with type 1 neurofibromatosis [16]. Less aggressive forms of JMML may harbour germline *CBL* mutations and may rarely undergo spontaneous remissions [16]. *KMT2A* rearrangements must be excluded before establishing the diagnosis of JMML [3, 16]. The presence of monosomy 7 is no longer required in establishing the diagnosis of JMML [3].

Table 2.2 Diagnosis of accelerated phase and blast phase of chronic myeloid leukemia (CML) based on the International Consensus Classification (ICC) and the World Health Organization (WHO) 2022 Classification [2, 3]

Accelerated phase	Blast phase
ICC [2]	
BM or PB blasts 10–19%	BM or PB blasts ≥20%
PB basophils ≥20%	Extramedullary myeloid sarcoma or blast proliferation
Presence of additional clonal cytogenetic abnormality in Ph + cells (ACA): Second Ph, trisomy 9, isochromosome 17q, trisomy 19, complex karyotype, or abnormalities of 3q26.2	Presence of lymphoblasts (confirmed by immunophenotype) ≥ 5% suggests lymphoblastic crisis
WHO 2022 [3]	
No designation for accelerated phase	BM or PB myeloid blasts ≥20%
	Extramedullary blast proliferation
	The presence of increased lymphoblasts in peripheral blood or bone marrow (optimal cut-off and significance of low level B-lymphoblasts unclear)

ICC International Consensus Classification, *WHO* World Health Organization, *BM* bone marrow, *PB* peripheral blood

Table 2.3 Diagnostic criteria for classical *BCR::ABL* negative MPN [2, 3]

Criteria	PV[a]	ET[b]	prePMF[c]	Overt PMF[c]
Major				
1.	Elevated Hb concentration or Hct (men: Hb > 16.5 g/dL, Hct > 49%; women: Hb > 16 g/dL; Hct > 48%)	Platelet ≥450 × 10^9/L	BM biopsy showing megakaryocytic proliferation with atypia[d]; BM reticulin fibrosis grade 0–1; increased age-adjusted BM hypercellularity, granulocytic proliferation, and decreased erythropoiesis	BM biopsy showing megakaryocytic proliferation with atypia[d]; reticulin, and/or collagen fibrosis grade 2–3
	Increased RBC mass (>25% above mean normal predicted value)			
2.	Presence of *JAK2* V617F or *JAK2* exon 12 mutation[e]	Presence of *JAK2*, *CALR* or *MPL* mutation[f]	Presence of JAK2, CALR, or MPL mutation[f]; or	Presence of JAK2, CALR, or MPL mutation[f]; or
			Presence of another clonal marker[g]; or	Presence of another clonal marker[g]; or
			Absence of reactive BM fibrosis[h]	Absence of reactive BM fibrosis[h]

Table 2.3 (continued)

Criteria	PV[a]	ET[b]	prePMF[c]	Overt PMF[c]
3.	Age-adjusted BM hypercellularity with trilineage proliferation (panmyelosis) with pleomorphic mature megakaryocytes without atypia	BM biopsy showing mainly megakaryocytic proliferation with increased number of large, mature megakaryocyte with hyperlobulated staghorn-like nuclei; infrequent dense clusters[i]; no significant increase or left-shift in granulopoiesis or increase in erythropoiesis; no significant marrow fibrosis[j]	Diagnosed criteria for *BCR::ABL1*-positive CML, PV, ET, MDS, or other myeloid neoplasms are not met	Diagnosed criteria for *BCR::ABL1*-positive CML, PV, ET, MDS or other myeloid neoplasms are not met
		Diagnosed criteria for *BCR::ABL1*-positive CML, PV, PMF, MDS, or other myeloid neoplasms are not met		
Minor				
1.	Subnormal EPO level	Presence of a clonal marker[k] or no evidence of reactive thrombocytosis[l]	Anemia not attributed to a comorbidities	Anemia not attributed to a comorbidities
2.			Leukocytosis $\geq 11 \times 10^9$/L	Leukocytosis $\geq 11 \times 10^9$/L
3.			Palpable splenomegaly	Palpable splenomegaly
4.			Elevated LDH	Elevated LDH
5.				LE blood picture

[a]The diagnosis of PV requires either all three major criteria or the first two major criteria plus the minor criterion. A BM biopsy may not be required in patients with sustained absolute erythrocytosis (Men: Hb > 18.5 g/dL and Hct > 55.5%; Women: Hb > 16.5 g/dL and Hct > 49.5%) and the presence of *JAK2*V617F or *JAK2* exon 12 mutation

[b]The diagnosis of ET requires either all major criteria or the first three major criteria plus the minor criterion

[c]The diagnosis of pre-PMF or overt PMF requires all three major criteria and at least one minor criterion confirmed in two consecutive assessments

[d]Megakaryocytic atypia is a distinctive feature of pre-PMF and overt PMF. Features include variation in size from small to giant megakaryocytes and severe maturation defects (cloud-like, hypolobulated, and hyperchromatic nuclei) and the presence of abnormally large and dense clusters (>6 megakaryocytes lying strictly adjacent)

[e]A highly sensitive assay for JAK2V617F (sensitivity <1%). In *JAK2*V617F negative cases, non-canonical or atypical *JAK2* mutations in exons 12–15 should be sought

[f]The recommended sensitivity levels of molecular assays: <1% for *JAK2* V617F; 1–3% for *CALR* and *MPL*

[g]Clonal markers are assessed by cytogenetics or next-generation sequencing (NGS); the presence of mutations in genes associated with myeloid neoplasm (e.g. *ASXL1*, *EZH2*, *IDH1*, *IDH2*, *SF3B1*, *SRSF2*, and *TET2* mutations) supports the clonal nature of the disease

[h]Reticulin fibrosis may occur secondary to infection, autoimmune/chronic inflammatory conditions, hairy cell leukemia or other lymphoid neoplasms, metastatic malignancies, or toxic myelopathies

[i]A cluster is defined as ≥3 megakaryocytes lying adjacent to each other without other cells in between; the presence of huge clusters (>6 cells) accompanied by granulocytic proliferation suggests the diagnosis of prePMF instead

[j]Rarely, grade 1 reticulin fibrosis may be observed

[k]Clonal marker(s) detected by cytogenetics or NGS

[l]Reactive causes include iron deficiency, subacute/chronic infections, chronic inflammatory disorders, non-hemic malignancies, and history of splenectomy

Table 2.4 Diagnostic criteria for post-polycythemia vera and post-essential thrombocythemia myelofibrosis [2]

Criteria	Post-PV MF	Post-ET MF
Required		
1.	Prior established diagnosis of PV	Prior established diagnosis of ET
2.	BM fibrosis of grade 2–3	BM fibrosis of grade 2–3
Additional		
1.	Anemia or sustained loss of requirement for phlebotomy or cytoreduction	Anemia and >2 g/dL reduction in Hb from baseline
2.	LE blood picture	LE blood picture
3.	>5 cm increase in palpable splenomegaly from baseline or new development of palpable splenomegaly	>5 cm increase in palpable splenomegaly from baseline or new development of palpable splenomegaly
4.	Development of 2 or more of the following symptoms: >10% weight loss in 6 months; night sweats; unexplained fever (>37.5 °C)	Elevated LDH
5.		Development of 2 or more of the following symptoms: >10% weight loss in 6 months; night sweats; unexplained fever (>37.5 °C)

The diagnosis of post-PV MF or post-ET MF is established with the presence of all required criteria and at least two additional criteria

2.3 Myeloid/Lymphoid Neoplasms with Eosinophilia and Tyrosine Kinase Gene Fusions (MLN-TK)

MLN-TK are currently defined as myeloid or lymphoid neoplasms driven by specific gene fusions that activate receptor tyrosine kinases [2, 3]. They are commonly associated with eosinophilia. Common associated gene fusions, clinical associations, and their therapeutic implications are shown in Table 2.5 [2, 3]. Of note, MLN-TK must be excluded before the diagnosis of CEL, mastocytosis, or HES is made.

Table 2.5 Genomic and clinical characteristics of myeloid/lymphoid neoplasms with eosinophilia and tyrosine kinase gene fusions [2, 3, 17–21]

Gene	Commonest gene fusions	Other fusion partners	Clinical features	Treatment implications
PDGFRA	*FIP1L1::PDGFRA*/cryptic 4q12 deletion	*CDK5RAP2, STRN, KIF5B, TNKS2 ETV6, BCR*	Eosinophilia +/− end-organ damage	Responsive to imatinib
PDGFRB	*ETV6::PDGFRB*/t(5;12) (q32;p13)	>30 other partners	Eosinophilia +/− end-organ damage, and monocytosis	Responsive to imatinib
FGFR1	*ZMYM2::FGFR1*/t(8;13) (p11.2;q12.1)	15 other partners	T-ALL/LL with eosinophilia or BM showing MPN in blast phase	Responsive to FGFR inhibitor
JAK2	*PCM1::JAK2*/t(8;9) (p22;p24.1)	*ETV6, BCR*	MPN or MDS/MPN with eosinophilia	Variable responses to ruxolitinib
FLT3	*ETV6::FLT3*/t(12;13) (p13.2;q12.2)	*ZMYM2, TRIP11, SPTBN1, GOLGB1, CCDC88C, MYO18A, BCR*	T-ALL/LL or myeloid sarcoma with eosinophilia or BM MDS/MPN features	Variable response to FLT3 inhibitors
ABL1	*ETV6::ABL1*/t(9;12) (q34.1;p13.2)	–	MPN with eosinophilia	Variable responses to dasatinib/nilotinib

T-ALL/LL T-acute lymphoblastic leukemia/lymphoblastic lymphoma, *BM* bone marrow, *MDS* myelodysplastic syndrome, *MPN* myeloproliferative neoplasm; +/− with or without, *FGFR* fibroblast growth factor receptor, *IFN-α* interferon-alfa

2.4 Mastocytosis

Mastocytosis is a group of rare heterogeneous hematologic neoplasms characterized by the accumulation of morphologically abnormal mast cells in the bone marrow, organs, or tissues. The 2022 WHO classification recognizes three major subtypes of mastocytosis: cutaneous mastocytosis, systemic mastocytosis, and mast cell sarcoma [3]. Subtypes of systemic mastocytosis comprise bone marrow mastocytosis, indolent systemic mastocytosis, smoldering systemic mastocytosis, aggressive systemic mastocytosis, and systemic mastocytosis with an associated hematologic neoplasm (SM-AHN) [3]. Somatic *KIT* mutations at codon 816 are present in more than 90% of patients with systemic mastocytosis, while rare mutations in the extracellular or juxtamembrane are present in less than 1% [3]. Co-occurring mutations in *TET2, SRSF2, ASXL1, RUNX1,* and *JAK2* are frequently present in SM-AHM [3, 22]. Patients with somatic *KIT* mutations may respond to treatment with the tyrosine kinase inhibitor midostaurin [23, 24].

2.5 Myelodysplastic/Myeloproliferative Neoplasms

The major subtypes of myelodysplastic/myeloproliferative neoplasm (MDS/MPN) under the WHO 2022 classification comprise chronic myelomonocytic leukemia (CMML), MDS/MPN with neutrophilia (formerly atypical chronic myeloid leukemia), MDS/MPN with *SF3B1* mutation and thrombocytosis (formerly MDS/MPN with ring sideroblasts and thrombocytosis), and MDS/MPN not otherwise specified [3]. The diagnostic criteria for CMML have been refined (Table 2.6) most notably with the lower threshold for absolute monocytosis to 0.5×10^9/L and the elimination of the category CMML-0 [3]. The designation of CMML-0 lacks prognostic significance and was thus removed. Somatic mutations are detected in more than 90% of patients with CMML, most frequently in *SRSF2*, *TET2,* and *ASXL1* [25, 26]. Other somatic mutations that are associated with CMML involve the genes *SETBP1*, *NRAS/KRAS*, *RUNX1*, *CBL,* and *EZH2* [3, 25, 26]. MDS/MPN with neutrophilia or atypical chronic myeloid leukemia (aCML) is characterized by WBC $\geq 13 \times 10^9$/L (with ≥10% neutrophils and their precursors), cytopenia, blasts <20%, dysgranulopoiesis, no/minimal monocytosis or no eosinophilia, and a hypercellular marrow with granulocytic proliferation and dysplasia [2, 3]. Dyserythropoiesis or dysmegakaryopoiesis may or may not be present. *BCR::ABL1* or other known driver genes or gene fusions associated with MPN or MLN-TK must be absent. The presence of *SETBP1* in association with *ASXL1* supports the diagnosis of MDS/MPN with neutrophilia [2, 3, 26].

Table 2.6 Updated WHO 2022 diagnostic criteria and classification for chronic myelomonocytic leukemia [3]

Prerequisite criteria
1. Persistent absolute (≥ 0.5 x 10^9/L) and relative (≥ 10%) PB monocytosis
2. Blasts/blasts equivalents <20% in the PB and BM
3. Not meeting the diagnostic criteria for CML and other forms of MPN
4. Not meeting the diagnostic criteria for MLN-TK
Supporting criteria
1. Dysplasia in 1 or more myeloid lineage(s)
2. Acquired clonal cytogenetic or molecular abnormality
3. Abnormal partitioning of PB monocyte subsets: Increased classical monocytes (>94%) in the absence of active autoimmune or inflammatory conditions
Subgroups
CMML-1: <5% blasts/blast equivalents in PB and <10% blasts/blast equivalents in BM
CMML-2: 5–19% blasts/blast equivalents in PB, 10–19% blasts/blast equivalents in BM or the presence of Auer rods
Variants
Myelodysplastic CMML: WBC < 13×10^9/L
Myeloproliferative CMML: WBC ≥ 13×10^9/L

The diagnosis of CMML requires the presence of all prerequisite criteria and: 1 or more supporting criteria (if absolute PB monocytosis ≥1×10^9/L); or supporting criteria 1 and 2 (if absolute PB monocytosis is from 0.5 to <1×10^9/L)

PB peripheral blood, *BM* bone marrow, *CML* chronic myeloid leukemia, *MPN* myeloproliferative neoplasm, *MLN-TK* myeloid/lymphoid neoplasm with eosinophilia and tyrosine kinase gene fusion, *CMML* chronic myelomonocytic leukemia

2.6 Myelodysplastic Syndromes or Myelodysplastic Neoplasms (MDS)

In the latest edition of the WHO classification, the term myelodysplastic neoplasm replaces myelodysplastic syndrome with the aim of emphasizing the neoplastic nature of MDS and to harmonize terminology with MPN. MDS is defined as a clonal hematological neoplasm associated with ineffective hematopoiesis, cytopenia, morphologic dysplasia, and a propensity for clonal progression to AML [2, 3]. Cytopenia is typically present for more than 4 months and is not explained by nutritional deficiencies (e.g. vitamin B12/folate, pyridoxine, and copper deficiencies), drugs, toxins, or chronic medical diseases. The threshold for bone marrow dysplasia is 10% for all lineages. Megakaryocytic dysplasia, specifically the presence of micromegakaryocytes, is most indicative of MDS [3]. Somatic mutations are present in more than 90% of patients with MDS. The most significant update to the classification of MDS is the genomic categorization (Table 2.7) [2, 3].

Table 2.7 Classification of myelodysplastic neoplasms

Category	Blast percentage	Cytogenetics	Mutations
MDS with low blasts and isolated 5q deletion (MDS-5q)	<5% BM and <2% PB	5q deletion alone or with 1 other abnormality other than monosomy 7 or 7q deletion	
MDS with low blasts and *SF3B1* mutation (MDS-*SF3B1*)	<5% BM and <2% PB	Absence of 5q deletion, monosomy 7, or complex karyotype	*SF3B1* (usually with VAF ≥ 10% and without multi-hit *TP53* or *RUNX1* mutations)
MDS with biallelic *TP53* inactivation (MDS-bi*TP53*)	<20% BM and PB	Usually complex	≥2 *TP53* mutations or 1 mutation with *TP53* copy number loss or copy neutral loss of heterozygosity
MDS with low blasts (MDS-LB)	<5% BM and <2% PB		
Hypoplastic MDS (MDS-h)	<5% BM and <2% PB		
MDS with increased blasts (MDS-IB)			
MDS-IB1	5–9% BM or 2–4% PB		
MDS-IB2	10–19% BM or 5–19% PB or Auer rods		
MDS with fibrosis (MDS-f)	5–19% BM; 2–19% PB		

BM bone marrow, *PB* peripheral blood, *VAF* variant allele frequency

2.7 Acute Myeloid Leukemia

The genomic classification of AML is emphasized and the distinction between MDS with blasts ≥10% and AML is softened (Fig. 2.1; Table 2.8) [2, 3]. It has to be emphasized that in patients with blasts 10–19%, AML can be diagnosed with AML defining genetic abnormalities that have important prognostic and therapeutic implications (Tables 2.8 and 2.9) [27]. In situations without AML-defining genetic abnormalities, the distinction between AML and MDS should be maintained to avoid overtreatment of the latter.

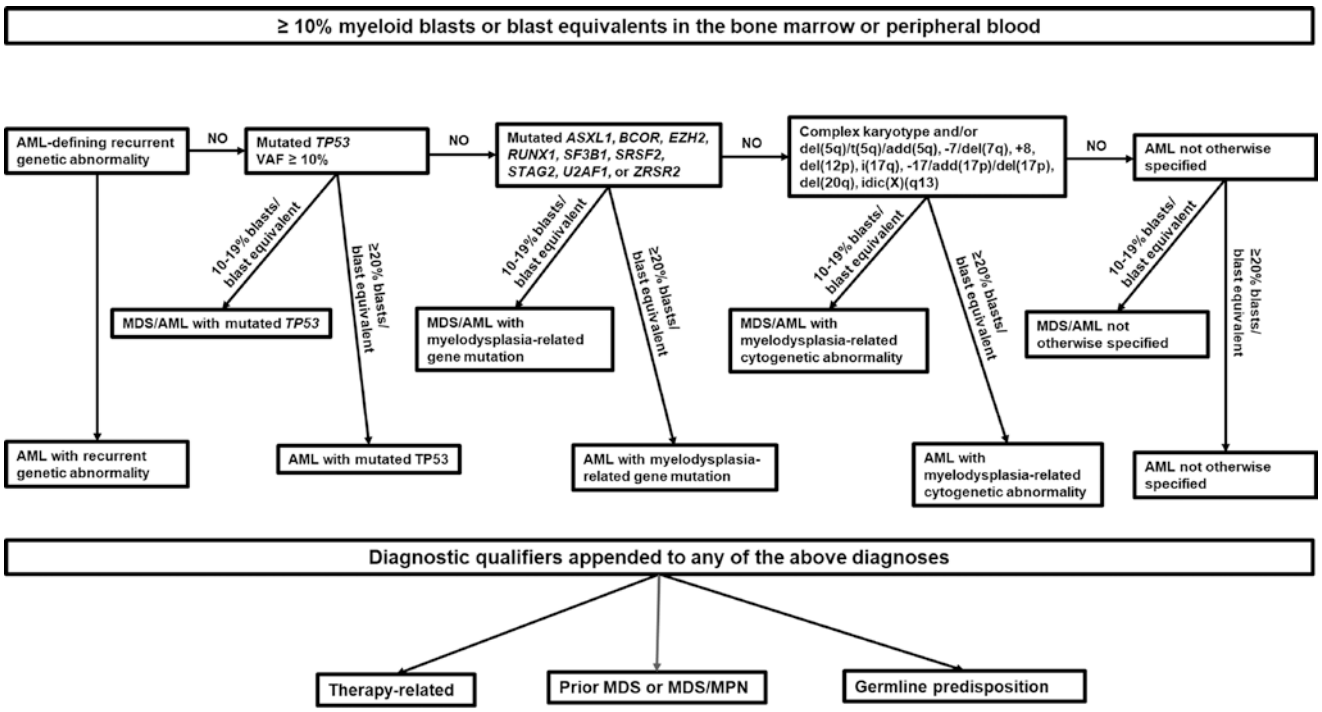

Fig. 2.1 A hierarchal classification of acute myeloid leukemia (AML) based on the international consensus classification. *MDS* myelodysplastic syndrome, *MPN* myeloproliferative neoplasm, *VAF* variant allele frequency

Table 2.8 The 2022 EuropeanLeukemiaNet (ELN) classification of acute myeloid leukemia [27]

AML with recurrent genetic abnormalities (may not require ≥20% blasts in PB/BM)
• APL with t(15;17)(q24.1;q21.2)/*PML::RARA*
• AML with t(8;21)(q22;q22.1)/*RUNX1::RUNX1T1*
• AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/*CBFB::MYH11*
• AML with t(9;11)(p21.3;q23.3)/*MLLT3::KMT2A*
• AML with t(6;9)(p22.3; q34.1)/*DEK::NUP214*
• AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/*GATA, MECOM*
• AML with mutated *NPM1*
• AML with in-frame bZIP mutated *CEBPA*
• AML with t(9;22)(q34.1;q11.2)/*BCR::ABL1*
Specific subtypes designated AML (if ≥20% blasts in PB/BM) or MDS/AML (if 10–19% blasts in PB/BM)
• AML or MDS/AML with mutated *TP53*
• AML or MDS/AML with myelodysplasia-related gene mutations (*ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, or ZRSR2*)
• AML or MDS/AML with myelodysplasia-related cytogenetic abnormalities
• AML or MDS/AML not otherwise specified
Myeloid sarcoma
Myeloid proliferations related to down syndrome
Blastic plasmacytoid dendritic cell neoplasm

Table 2.9 Classical acute myeloid leukemia-defining genetic abnormalities and the 2022 EuropeanLeukemiaNet (ELN) Risk Categorization [27]

Risk classification[a, b]	Genetic abnormality
Favourable	• t(8;21)(q22;q22); *RUNX1::RUNX1T1*
	• Inv(16)(p13.1q22) or translocation t(16;16) (p13.1;q22); *CBFB::MYH11*
	• Mutated *NPM1* without *FLT3*-ITD
	• Basic leucine zipper (bZIP) in-frame mutated *CEBPA*[c]
Intermediate	• Mutated *NPM1* with *FLT3*-ITD
	• Wild-type *NPM1* with *FLT3*-ITD
	• t(9;11)(p21.2;q23.2); *MLLT3::KMT2A*
	• Cytogenetic abnormalities not classified as favourable or adverse
Unfavourable/adverse/poor	• t(6;9)(p23;q34.1); *DEK::NUP214*
	• t(v;11q23.3); *KMT2A*-rearranged
	• t(9;22)(q34.1;q11.2); *BCR::ABL1*
	• t(8;16)(p11;p13)/*KAT6A::CREBBP*
	• Inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2,MECOM(EVI1)*
	• −5 or del(5q), −7; −17/abn(17p)
	• Complex (≥3 clonal chromosomal abnormalities) karyotype, Monosomal karyotype
	• Mutated *ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1 or ZRSR2*
	• Mutated *TP53*

[a]The ELN AML risk categorization was developed based on data from intensively treated patient and may need modifications for patients receiving less intensive therapies

[b]Initial risk assignment may change based on treatment

[c]Only in-frame mutations affecting basic leucine zipper (bZip) region of *CEBPA*, irrespective they occur as monoallelic or biallelic mutations have been associated with favourable outcome

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3 Molecular Techniques in the Diagnosis and Monitoring of Acute and Chronic Leukaemias

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Abstract

This chapter discusses the use of current molecular techniques in the clinical laboratories for investigating acute and chronic leukaemias, including short-read massively parallel sequencing, measurable residual disease monitoring by real-time quantitative PCR or digital PCR, and gene expression profiling. Practical implementation of these molecular techniques will be discussed, with emphases on the special considerations related to acute and chronic leukaemias, including discussion on the bioinformatic analyses of NGS data. Newer genomic techniques, including long-read sequencing, single-cell sequencing, optical genome mapping, and circulating tumour DNA testing, will be briefly covered.

Keywords

Molecular techniques · PCR · Quantitative PCR · Digital PCR · Massively parallel sequencing · Bioinformatics

3.1 Introduction

Genetic testing is increasingly important in the investigations of patients with acute and chronic leukaemias [1]. The advent of massively parallel sequencing or next-generation sequencing (NGS) has enabled major advancement in our understandings of how genetic features contribute to the development of leukaemias. The prevailing model suggests that leukaemias begin as clonal haematopoiesis with genetic variants that likely confer survival advantages, leading to clonal expansion of certain haematopoietic cells [2, 3]. The acquisition of certain genetic variants would result in preleukaemic states. Depending on the profile of preleukaemic genetic variants, variable risks of progression to acute myeloid leukaemias (AML) have been observed presumably due to the survival advantage conferred by these genetic variants on the preleukaemic clone [4, 5]. Apart from pathogenetic mechanisms, genomic studies have also enabled more refined diagnostic classifications in leukaemias [6, 7] and facilitated the characterization of prognostic significance of various genetic variants. These have facilitated the practice of precision medicine [8], such that the best treatment can be prescribed to individual patients according to the characteristics of their disease conditions. With the increasing availability of tailor-made therapeutic strategies for leukaemia patients, one can only expect a steep increase in demand for faster and more comprehensive genetic testing in leukaemia patients in the immediate future [9].

This chapter will focus on the current and upcoming molecular techniques used in the diagnosis, prognostication, and monitoring of leukaemias, with emphases on AML, acute lymphoblastic leukaemias (ALL), chronic myeloid leukaemia (CML), and myeloproliferative neoplasms (MPN). Over the past decade, NGS has emerged as a standard technology for genetic testing [1], with the field witnessing significant improvements in wet-bench procedures, bioinformatic strategies for variant detection, standardization of methods, and reporting. This chapter will first review these issues in NGS, including different NGS assay designs targeting different types of genetic variants, with some practical considerations in acute and chronic leukaemias. This will be followed by a review of real-time quantitative PCR and digital PCR in the detection of measurable residual disease (MRD) in acute and chronic leukaemias. Techniques for gene expression profiling with specific applications in leukaemias will then be discussed. The chapter will end by discussing newer genomic techniques, including long-read sequencing and single-cell sequencing, that have emerging clinical applications recently. Conventional molecular diagnostic techniques, including assays with various end-point PCR-based detection methods and Sanger sequencing, will not be covered in the interest of

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space. Readers are referred to excellent reviews elsewhere for these topics [10, 11]. This chapter will use some representative genetic variants from various leukaemias for illustrative purpose, but for a comprehensive review of genetic features in association with specific types of haematological malignancy, readers are directed to subsequent chapters covering the corresponding disease categories in this book.

3.2 Short-Read NGS

Since its inception in the mid-2000s, NGS has emerged as a highly versatile and paradigm-shifting technique in clinical diagnostic laboratories. In contrast to conventional molecular techniques that can only interrogate single to a handful of genetic loci, NGS can test millions to billions of genetic loci in a single sequence run. NGS also has the advantage of being agnostic to the input genetic loci to be sequenced, such that it can sequence any compatible genetic material without the need of prior knowledge of the genetic sequence. This feature facilitates the detection of novel fusion transcripts in human cancers and discovery of novel genetic sequences from previously uncharacterized pathogens. Building on the agnostic nature of NGS to the genetic material to be sequenced, scientific researchers have devised numerous NGS techniques that capture the targets of biological interests for input into the sequencer, for example enriching a subset of genomic DNA for sequencing targeted genes implicated in diseases [12]; converting RNA to complementary DNA (cDNA) for measurement of gene expression [13]; capturing protein-bound DNA by immunoprecipitation for determination of chromatin status [14]; determining open chromatin regions in the genome [15]; determining chromosomal conformation by measurement of crosslinked DNA regions [16]; etc. These techniques have contributed enormously to our current understandings of biological and pathogenetic mechanisms in cells and diseases.

Despite the promising roles of NGS in genomic testing, the roll-out of this technique in clinical diagnostics was hindered to some extent by the challenging bioinformatic analysis of voluminous raw data generated from the sequencers. In recent years, such hindrance has been gradually resolved due to the wider availability of bioinformatic expertise, efforts in method standardization, and development of commercial plug-and-play solutions. This section will focus on DNA sequencing and RNA sequencing techniques to build an in-depth understanding of these NGS techniques in clinical application.

3.3 General Principles of NGS

For detection of somatic mutations in leukaemias, a paired tumour-normal sequencing strategy is commonly employed. Tumour specimens are usually in the form of marrow aspirate or peripheral blood. Matched normal specimen is usually in the form of buccal swab, hair follicle, or skin. Tumour-only sequencing is also performed by many clinical laboratories when matched normal specimens are not readily available for testing. In a typical NGS library preparation procedure, genomic DNA is first fragmented randomly into sizes of less than 550 to 600 bp (including adapters) by means of ultrasonication or enzymatic digestion, with end-repair performed. Adapters with universal priming sites are then ligated to the fragment ends, with subsequent PCR amplification by library amplification primers. The adapter-ligated fragments then undergo clonal amplification, so that signals generated are strong enough for sequence detection systems to detect, with the advantage of high accuracy for base calling. The techniques of clonal amplification vary in different platforms, for example, bridge amplification for random flow cell in Illumina, exclusion amplification for patterned flow cell in Illumina, emulsion PCR in Ion Torrent, and in-solution nanoball generation in BGI. Such use of clonal template amplification methods defines the category of "second-generation" sequencing that all short-read sequencing techniques belong to. Sequencing can then be performed by sequencing-by-synthesis technique that most of the sequencers employ nowadays. For a more detailed review of principles in short-read NGS, with comparisons between different platforms of sequencers, readers are referred to reviews elsewhere [17, 18].

Sequencing-by-synthesis technique can be further classified into cyclic reversible termination (CRT), as used by Illumina and BGI sequencers, and single-nucleotide addition (SNA), as used by Ion Torrent sequencers. For CRT [17], sequencing primers bind complementarily to adapters of clonal amplified libraries on the flow cell and incorporate DNA polymerase that performs chain elongation of one nucleotide at a time using the four types of fluorochrome-labelled and 3′-blocked deoxynucleotides (dNTPs). After one dNTP has been incorporated complementarily to the template, the 3′-blocking group prevents further incorporation of dNTPs. An image is then captured, so that the colour of the fluorochrome will denote the identity of the incorporated dNTP. The fluorochrome is then cleaved, with removal of the 3′-blocking group. The cycle begins again, with incorporation of the next dNTP. This process is performed simultaneously on millions of DNA fragments, thus the sequencing is performed in a massively parallel manner. Paired-end sequencing can be performed to sequence both ends of a fragment for higher accuracies in downstream read alignment and variant calling (especially for indels and structural variants). For SNA [18], the incorporation of dNTP is detected by the change in pH due to the release of hydrogen ions at dNTP incorporation. The release of the hydrogen ions is detected by semiconductor. As such change in pH cannot distinguish between the types of dNTP that have been incorporated, the four types of dNTP therefore need to be added one after another to pinpoint the incorporation of certain dNTP. Since SNA does not require image capture at every cycle, the

sequencing speed is higher than CRT. SNA can also yield longer reads in a single round of sequencing.

3.4 DNA Sequencing

3.4.1 Principles of NGS Assay Design

3.4.1.1 Panel Selection

The most common application of NGS in clinical laboratories is sequencing the genomic DNA to look for variants that have diagnostic, prognostic, or therapeutic significance in diseases. Despite the capability of NGS to sequence whole human genomes in a short period of time, it is often not practical to perform genome sequencing (GS) for every patient in clinical laboratories, given the high sequencing costs, voluminous data for computation and storage, and relative scarcity of established clinically actionable genetic features in the human genome. In view of this, target enrichment strategies are available for selection of a subset of the genome for sequencing in smaller scales. The selection is usually in the form of all protein-coding genes (i.e. exome sequencing, ES) or a panel of genes (i.e. panel sequencing) that have clinical importance in some specified diseases. The major considerations are summarized in Table 3.1.

Due to its lower sequencing costs and capabilities of detecting somatic variants with lower variant allele frequencies (VAF), panel sequencing is currently the most common form of NGS in clinical laboratories for investigations of acute and chronic leukaemias. In view of its wide availability, the remaining discussions in this section will focus on panel sequencing.

3.4.1.2 Gene Selection in Panel Sequencing

Many commercial vendors provide highly flexible means of target enrichment nowadays. The two major options of target enrichment are amplicon sequencing and hybridization capture. Users can select off-the-shelf commercial panels or customized panels with user-selected genes. The selection of genes to be included in a clinical NGS panel should take into account the most updated list of genes that are of diagnostic, prognostic, and/or therapeutic significance in the diseases to be investigated. Different commercial designs may cover varying regions in a gene, with some designs covering only mutation hotspots for certain genes. After the initial design, the proposed genomic regions for each gene in the panel should be carefully verified against the original intended regions to make sure the regions with reported clinically actionable variants have been covered. The exonic regions of each gene are available in the updated version of reference sequence databases, e.g. Ensembl [19] and RefSeq [20]. The verification step can be performed using the "intersect" function of Bedtools [21], or using visualization platforms, such as Integrative Genomics Viewer (IGV) [22] or UCSC Genome Browser [23].

Table 3.1 Major considerations between the selection of genome sequencing, exome sequencing, and panel sequencing

	Genome sequencing	Exome sequencing	Panel sequencing
Cost per sample	Highest	Medium to high	Usually the lowest
Data volume	About 60–150 Gb	About 8–15 Gb	Varies depending on panel size
Scale of sequencer	High	Medium to high	Low to medium depending on panel size
Sequencing depth	Typically 10–30×	Typically 50–100×	Typically >500×
Coverage	Major parts of genome Except repeat regions	Protein-coding genes	Selected target genes
Noncoding genome	Included	Not included	Usually not included. Can customize according to need
Detection of variant			
SNV and short indels	Yes. Cannot reliably detect subclonal variants	Yes. Need higher sequencing depth to detect subclonal variants	Yes, can reliably detect subclonal variants
Copy number variant	Weak, due to low sequencing depth, especially for subclonal variants	Weak, due to low sequencing depth, especially for subclonal variants	Potentially strong in the targeted regions
Structural variant	Yes, with appropriate bioinformatic analysis	Not included	Only focused detection possible if breakpoints of structural variants have been targeted by panel

3.4.1.3 Considerations of Variant Types During Panel Design

Common genetic variants can be categorized into single nucleotide variants (SNV), short indels (≤21 bp), long indels (>21 bp), copy number variants (CNV), and structural variants (SV). Conventional testing to detect the whole range of genetic variants in leukaemias requires methods that span cytogenetics for the detection of large-scale structural variants to a compendium of molecular methods for highly focused detection of smaller variants (Fig. 3.1). In the context of NGS, as panel sequencing usually covers only the protein-coding regions of genes, their strengths lie in the detection of SNV and short indels within these regions. The detection of long indels depends on whether the regions of the long indels are enriched

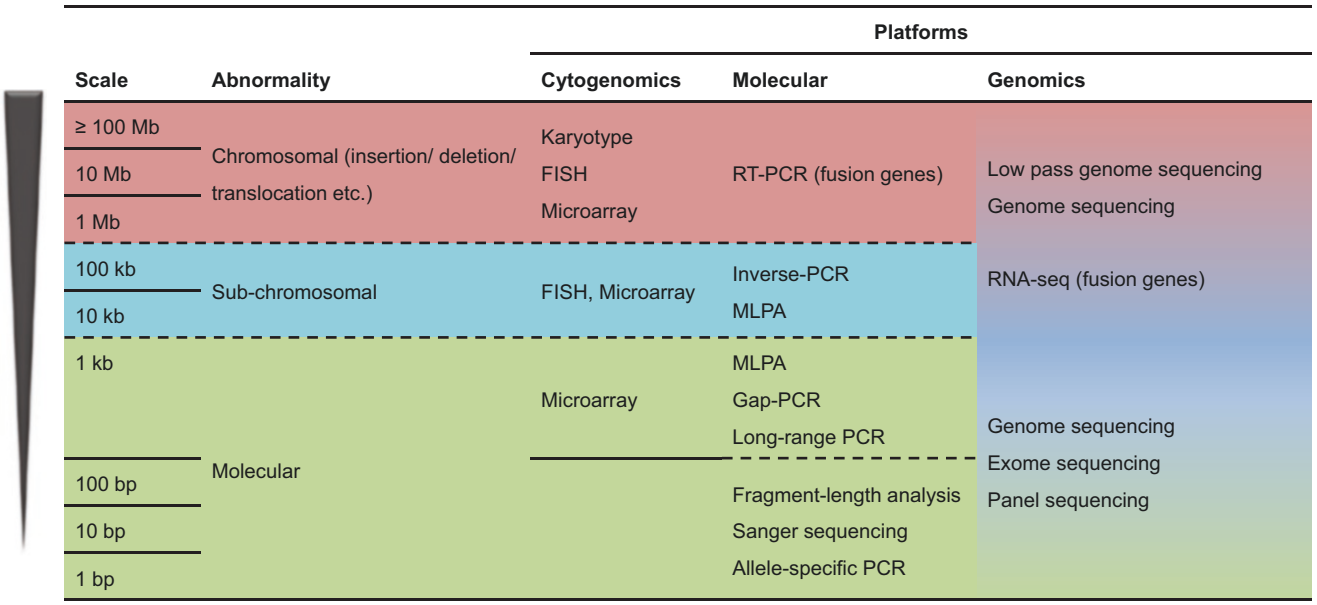

Fig. 3.1 Detection of genetic variants using cytogenomic and molecular methods vs. using NGS. Diagnostic laboratories conventionally use cytogenomic methods to cover chromosomal abnormalities (red region), supplemented with focused FISH assays for interrogation of specific copy number or structural variants and microarray for subchromosomal copy number variants (blue region). The detection of small abnormalities requires the use of a variety of high-resolution molecular techniques that cover small genomic areas with highly specific scopes (green region). Depending on the assay design and data analysis strategy, the advent of NGS has enabled one testing platform to traverse multiple testing "regions" (spectrum of colours). For example, genome sequencing can potentially detect molecular abnormalities at base-pair resolution and also structural variants at the megabase level. Abbreviations: *FISH* fluorescence in-situ hybridization, *MLPA* multiplex ligation-dependent probe amplification, *RT-PCR* reverse transcription-PCR

in the panel design and the bioinformatic strategies used to detect them. CNVs for the captured genes can be determined by counting the number of sequence reads, with subsequent normalization with specimens in the same batch or with a panel of normal samples tested using the same panel. SVs remain the most difficult type of variant to be detected by panel sequencing, as many known SVs have chromosomal breakpoints in the intronic regions of genes or intergenic regions, and these regions are usually not enriched in panel sequencing. Many commercial vendors provide highly flexible ways of customizing a panel; users may utilize such service to design panels that cover specific intronic or intergenic regions to facilitate the detection of certain SVs. An example of customization strategy in haematology is Karyogene [24], which utilized hybridization capture to enrich 49 genes implicated in myeloid neoplasms and known intronic breakpoints of *PML::RARA*, *CBFB::MYH11*, *RUNX1::RUNX1T1,* and *KMT2A* genes for detection of these translocations. Karyogene also captured a backbone of single nucleotide polymorphisms (SNPs) spaced every 300 kb to facilitate the detection of CNVs and copy neutral loss of heterozygosity in the whole genome [24]. Such strategy would result in a larger panel size, due to the relatively large intronic regions that need to be captured, in order to ensure high sensitivity to detect the specified gene rearrangements. An alternative approach to detect gene rearrangements is to perform RNA sequencing, which will be covered in a later section in this chapter. An example of variant detection strategy for common clinically actionable variants in acute myeloid leukaemia using NGS is summarized in Table 3.2.

3.4.2 Bioinformatic Analysis for Variant Detection

In contrast to other molecular platforms, the strategies of bioinformatic analysis for NGS data have a critical role in the analytical sensitivity and specificity for the detection of various clinically actionable variants. This section therefore includes the discussion of bioinformatic strategies, regarding bioinformatics as an integrated component of NGS platform [25] (Fig. 3.2).

3.4.2.1 Pre-processing Procedures

NGS sequencers output raw sequence FASTQ files. The quality of sequencing reads in the FASTQ files can be investigated by FastQC. The FASTQ data are first processed to remove potential adaptors and low-quality bases using trimming software, such as Trim-Galore or Trimmomatic [26]. The adaptor- and quality-trimmed FASTQ data are then aligned to a human reference genome using aligners, such as BWA-MEM [27] or Bowtie2 [28]. The resulting BAM file is then sorted by coordinate and marked for PCR duplicates using tools from GATK [29]. Base quality score recalibration is then performed by GATK. The resulting analysis-ready BAM file can be used for variant calling. The bioinformatic steps to process FASTQ files to aligned BAM files are reasonably standardized for short-read NGS. Readers can refer to the current best practices [29, 30] for stepwise procedures, with the section on somatic short variant discov-

Table 3.2 Detection of clinically actionable variants in acute myeloid leukaemia: Conventional approach vs. NGS approach

Variants	Cytogenetics	Molecular	NGS
t(8;21)	Karyotype	RT-PCR	Fusion detection by RNA-seq Targeted DNA-seq
inv(16) / t(16;16)	Karyotype, FISH	RT-PCR	Fusion detection by RNA-seq Targeted DNA-seq
KMT2A rearrangement	Karyotype, FISH	RT-PCR	Fusion detection by RNA-seq Targeted DNA-seq
t(6;9)	Karyotype, FISH	RT-PCR	Fusion detection by RNA-seq Targeted DNA-seq
inv(3) / t(3;3)	Karyotype, FISH		Gene expression by RNA-seq Targeted DNA-seq
Complex/ monosomal karyotype	Karyotype		Copy number detection in DNA-seq
NPM1		Fragment length Sanger	Targeted DNA-seq
FLT3-ITD		Fragment length Sanger	Targeted DNA-seq
CEBPA (biallelic)		Sanger	Targeted DNA-seq
RUNX1		Sanger	Targeted DNA-seq
ASXL1		Sanger	Targeted DNA-seq
TP53		Sanger	Targeted DNA-seq
IDH1		Sanger, HRM	Targeted DNA-seq
IDH2 (R140)		Sanger, HRM	Targeted DNA-seq
IDH2 (R172)		Sanger, HRM	Targeted DNA-seq
DNMT3A		Sanger	Targeted DNA-seq
TET2		Sanger	Targeted DNA-seq
KMT2A-PTD		RT-PCR	Copy number detection by DNA-seq
NRAS		Sanger	Targeted DNA-seq
SRSF2		Sanger, ARMS	Targeted DNA-seq
SF3B1		Sanger	Targeted DNA-seq
ZRSR2		Sanger	Targeted DNA-seq
U2AF1		Sanger, ARMS	Targeted DNA-seq
STAG2		Sanger	Targeted DNA-seq
RAD21		Sanger	Targeted DNA-seq
SMC1A		Sanger	Targeted DNA-seq
SMC3		Sanger	Targeted DNA-seq
EZH2		Sanger	Targeted DNA-seq

NGS next-generation sequencing, *ARMS* amplification refractory mutation system, *FISH* fluorescence in situ hybridization, *HRM* high resolution melting, *RT-PCR* reverse transcription PCR

ery in GATK being the most relevant for the variant detection in acute and chronic leukaemias.

Before calling variants, the analysis-ready BAM file should be subjected to routine quality control procedures. Clinical laboratories should establish acceptance criteria for selected core quality metrics [31], for example, mapping quality, duplicate rate, and depth of coverage. The importance of interpreting these quality metrics before analysis of results cannot be overemphasized, as failure to meet certain quality standards may result in inaccurate results. For instance, insufficient depth of coverage may adversely affect the sensitivity of variant detection, especially for variants with low variant allele frequencies. Also, genomic regions with no or poor coverage may result in false negativity during variant calling, as these regions may not be properly sampled and examined. These quality metrics are also applicable to the validation of newly acquired NGS panels. As an example, in the context of leukaemias, *CEBPA* is consistently reported to have suboptimal coverage in some off-the-shelf panels [32, 33]. Depth of coverage should also be closely monitored for genes that have been reported to have high sequence homology with other genes, if applicable [34].

3.4.2.2 Variant Calling for SNVs and Short Indels

For variant calling, there are numerous variant callers available for the detection of SNVs and short indels in short-read NGS. These variant callers typically take analysis-ready BAM file as input and generate Variant Call Format (VCF) file recording the called variants. Multiple studies have been reported to compare the performance of these variant callers [35–39]. It has been consistently observed that all variant call-

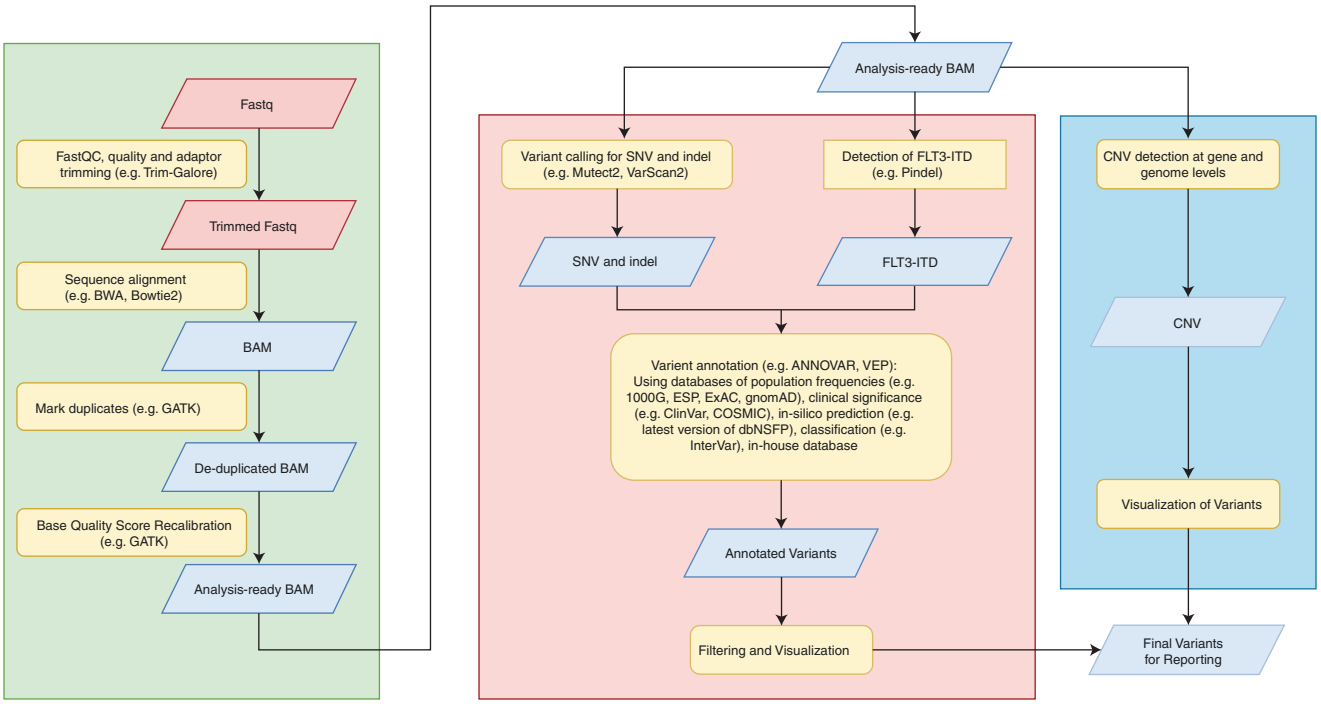

Fig. 3.2 Bioinformatic analysis for NGS data using acute myeloid leukaemia as an example. The input to the workflow are Fastq files of tumour specimen with or without matched germline specimen. The Fastq files undergo standard pre-processing workflow (green background) to generate analysis-ready BAM file(s). The analysis-ready BAM file(s) can be used for various downstream analysis workflow, including variant calling (pink background) and copy number variant detection (blue background). Abbreviations: *CNV* copy number variant, *COSMIC* Catalogue of Somatic Mutations in Cancer, *ESP* Exome Sequencing Project, *ExAC* Exome Aggregation Consortium, *gnomAD* Genome Aggregation Database, *ITD* internal tandem duplication, *SNV* single nucleotide variant, *VEP* Ensembl Variant Effect Predictor

ers have their strengths and weaknesses, such that no single variant caller is sensitive enough for the detection of all clinically relevant variants. To address this, an ensemble approach that incorporates the results of multiple variant callers is recommended [30]. In the context of clinical interpretation, each variant caller can be regarded as an independent diagnostic test, with each caller carrying its own sensitivity and specificity for detection of different types of variants. Clinical laboratories should ascertain the performance of commonly used variant callers and their collective performance for detection of different variant types in representative genes during method validation. Tools that manipulate and combine VCF files, for example BCFtools, can be used for merging the variant lists into a single VCF file to facilitate unified downstream analysis.

3.4.2.3 Variant Annotation

The called variants can then be processed by variant annotation. Variants in VCF file typically contain information regarding their genomic location, their nucleotide change and basic variant information, e.g. genotype call, sequencing depth, and read counts for reference allele and alternate allele. To facilitate downstream interpretation of the clinical relevance of given variants, information from various genomic databases needs to be matched with and supplemented for each variant. Typical information would include the location of the variants in relation to nearby gene(s), the impact on exonic function for variants in protein-coding genes, predicted amino acid changes, HGVS nomenclature in relation to selected or canonical transcripts, frequency of the variants in the population, database matches in dbSNP, ClinVar and COSMIC, and *in-silico* prediction scores for variant effect. Common tools for variant annotation include Variant Effect Predictor (VEP) by Ensembl [40], ANNOVAR [41], and SnpEff [42]. Readers can refer to a published stepwise protocol for an example of typical annotation workflow [43]. The annotated variants can be interpreted for their clinical relevance. Various classification schemes of variant interpretation are available for guidance [44, 45]. It is advisable for clinical laboratories to record the variant classes assigned to all the interpreted variants for future reference and annotation, in order to permit consistent result reporting and future review of historical variants reported by the laboratories. During the interpretation of clinical significance of variants, it is recommended to visualize the sequence reads containing the variants for manual review, e.g. using IGV [46]. The visualization step may detect sequencing artefacts or errors in the adjacent regions for the sequence reads and facilitate the appreciation of horizontal or vertically complex variants [47] (Figs. 3.3 and 3.4). The additional information may positively or negatively affect the clinical interpretation of such variants.

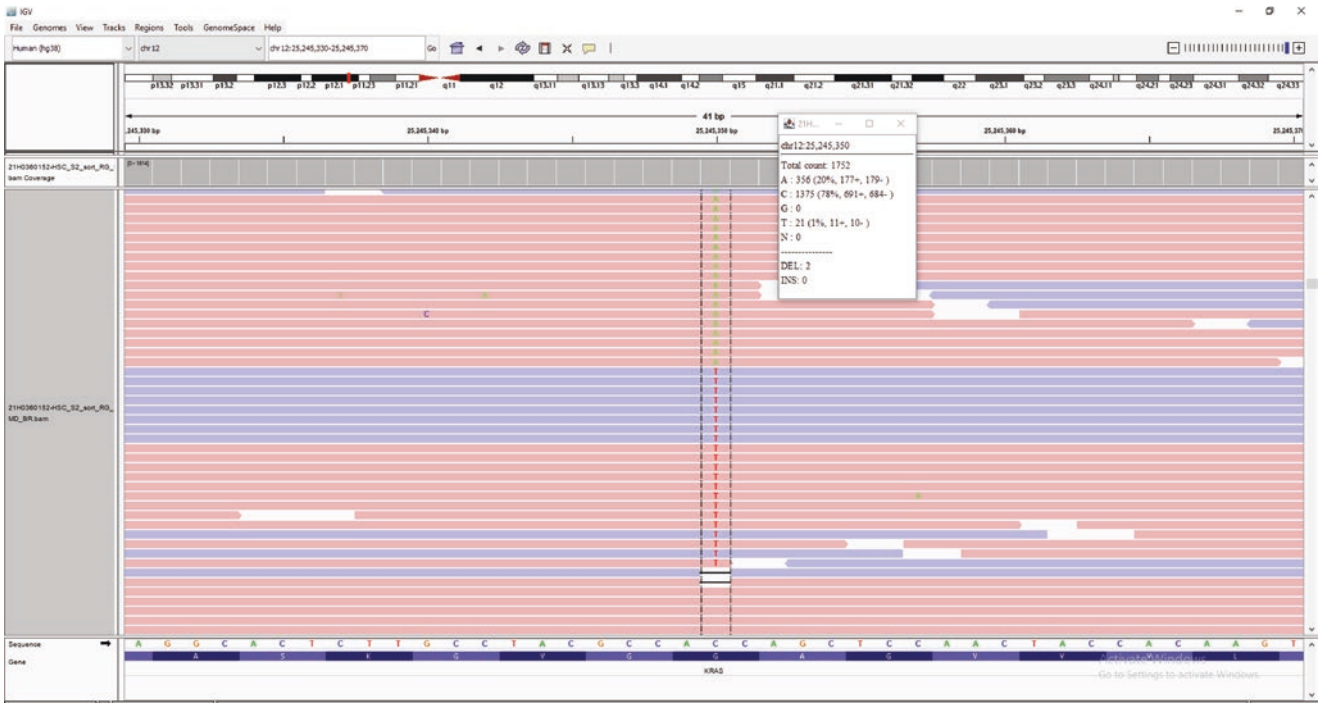

Fig. 3.3 Vertically complex variants in *KRAS*. At codon G12, the nucleotide position chr12:25245350 (GRCh38) has two nucleotide changes (i.e. C>A and C>T) on different reads, resulting in two types of amino acid change, namely G12D and G12V, in the *KRAS* gene

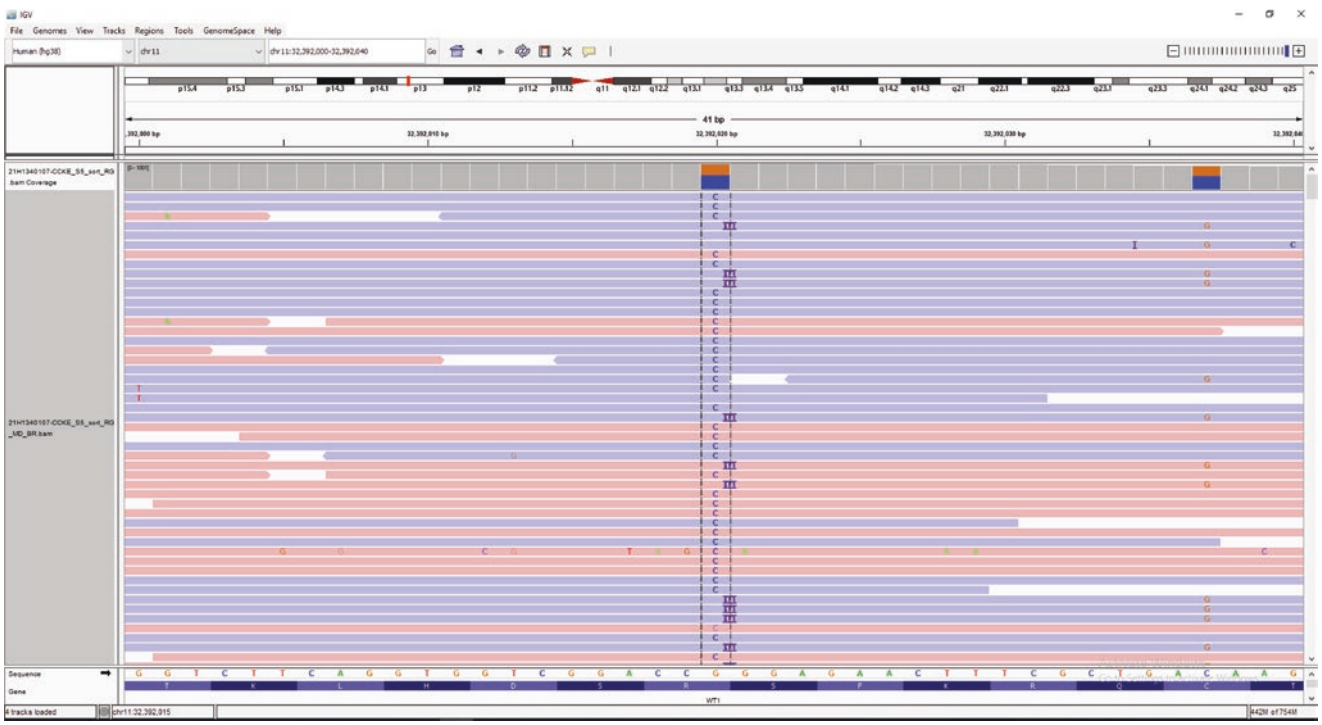

Fig. 3.4 Vertically complex and horizontally complex variants in *WT1*. At nucleotide position chr11:32392020 (GRCh38), the sequence reads show vertically complex variants that include a single nucleotide variant G>C or a 14-base pair insertion. The 14-base pair insertion is part of a horizontally complex variant that also includes a single nucleotide variant C>G at nucleotide position chr11:32392037 (GRCh38). Variants with such complex features are challenging for most variant callers, indicating the need for manual review for all potential variants to be reported

3.4.2.4 Variant Calling for Long Indels

Apart from typical SNVs and short indels, clinical laboratories should also review their bioinformatic strategies in the detection of longer indels that are clinically relevant in leukaemias. Typical examples of difficult-to-detect variants include *CALR* type 1 52 bp deletion in myeloproliferative neoplasms and *FLT3*-internal tandem duplication (ITD) in AML. In our experience, *CALR* type 1 deletion or similar variants can be reliably detected using variant callers that employ re-assembly strategy in regions near potential variants, e.g. Mutect2 or VarDict. For *FLT3*-ITD, as the size of the duplicated region ranges from 3 bp to over 200 bp, which can be longer than the typical read lengths in short-read sequencing, dedicated bioinformatic algorithms [48–50] are required to reliably detect the long ITD variants. Pindel [48] has a high sensitivity in the detection of *FLT3*-ITD in NGS assay using hybridization capture [51]. In our experience of paired-end sequencing after hybridization capture, Pindel can reliably detect ITD with length shorter than 100 bp using its short insertion (SI) algorithm, and also ITD with length longer than 100 bp using its long insertion (LI) or tandem duplication (TD) algorithms. *FLT3*-ITD allelic ratio from NGS has been demonstrated to be largely concordant with fragment length analysis in a recent study, with a minority of cases having a discordant assignment of prognostic group due to borderline low allelic ratio by NGS [52].

3.4.2.5 Detection of CNVs at the Gene Level

Panel sequencing can be used to detect CNVs at the level of the captured genes. The number of sequence reads for a given genomic region in the tested specimen is normalized against the number of sequence reads for the corresponding region in specimens of the same NGS batch or specimens collected from normal individuals. If the read count is significantly lower than the "normal" read count, deletion of the corresponding region can be concluded. On the contrary, if the read count is significantly higher than the "normal" read count, amplification of the corresponding region can be concluded. Examples of clinically relevant CNVs at the gene level include deletion of tumour suppressor genes, e.g. TP53, in myeloid neoplasms and detection of KMT2A-partial tandem duplication (PTD) in acute myeloid leukaemia (Fig. 3.5).

3.4.3 Specific Applications of DNA Sequencing Strategies in Leukaemias

3.4.3.1 Molecular Consensus Sequencing for Detection of Subclonal or Rare Variants

Despite the relatively high sequencing accuracy of short-read sequencing, the error rate is typically reported at 0.1% to 1%, depending on the genomic regions being tested. In the detection of somatic variants in cancers, subclonal or rare variants may present at low VAF, and such variants may become difficult to differentiate from errors or artefacts introduced during library preparation, sequencing, and bioinformatic procedures. Various error reduction methods are available to improve sequence accuracy, including computational method, biochemical method, and molecular consensus sequencing strategy [53].

During the library preparation for NGS, DNA library is usually PCR-amplified. For simple amplicon sequencing, it is impossible to know whether two sequence reads originate from the same DNA molecule. For hybridization capture with random shearing of input DNA, the origin of two sequence reads can be ascertained by comparing whether the start sites and end sites of the inserts are identical. If so, the two sequence reads can be assumed to originate from the same starting DNA molecule and the duplicated read will be discarded from downstream analysis. Single molecular consensus sequencing strategy involves the use of unique molecular identifier (UMI) to uniquely tag DNA molecule before PCR amplification [54], so that one can definitively conclude that identically tagged reads originate from the same starting DNA molecule. As all identically tagged reads should originate from a common DNA molecule, their sequences should be in consensus with each other. Any deviation from the consensus sequence would imply the deviates being technical errors [53]. Single molecular consensus sequencing requires the presence of duplicate reads so that it can make use of the duplicated information to verify the consensus sequence. While it can facilitate the elimination of technical errors, the requirement on generating duplicate reads implies a higher sequencing depth and therefore higher sequencing cost for each sample.

In view of the higher sequencing accuracy of single molecular consensus sequencing, it can be applied for detection of subclonal variants or rare variants, especially in the

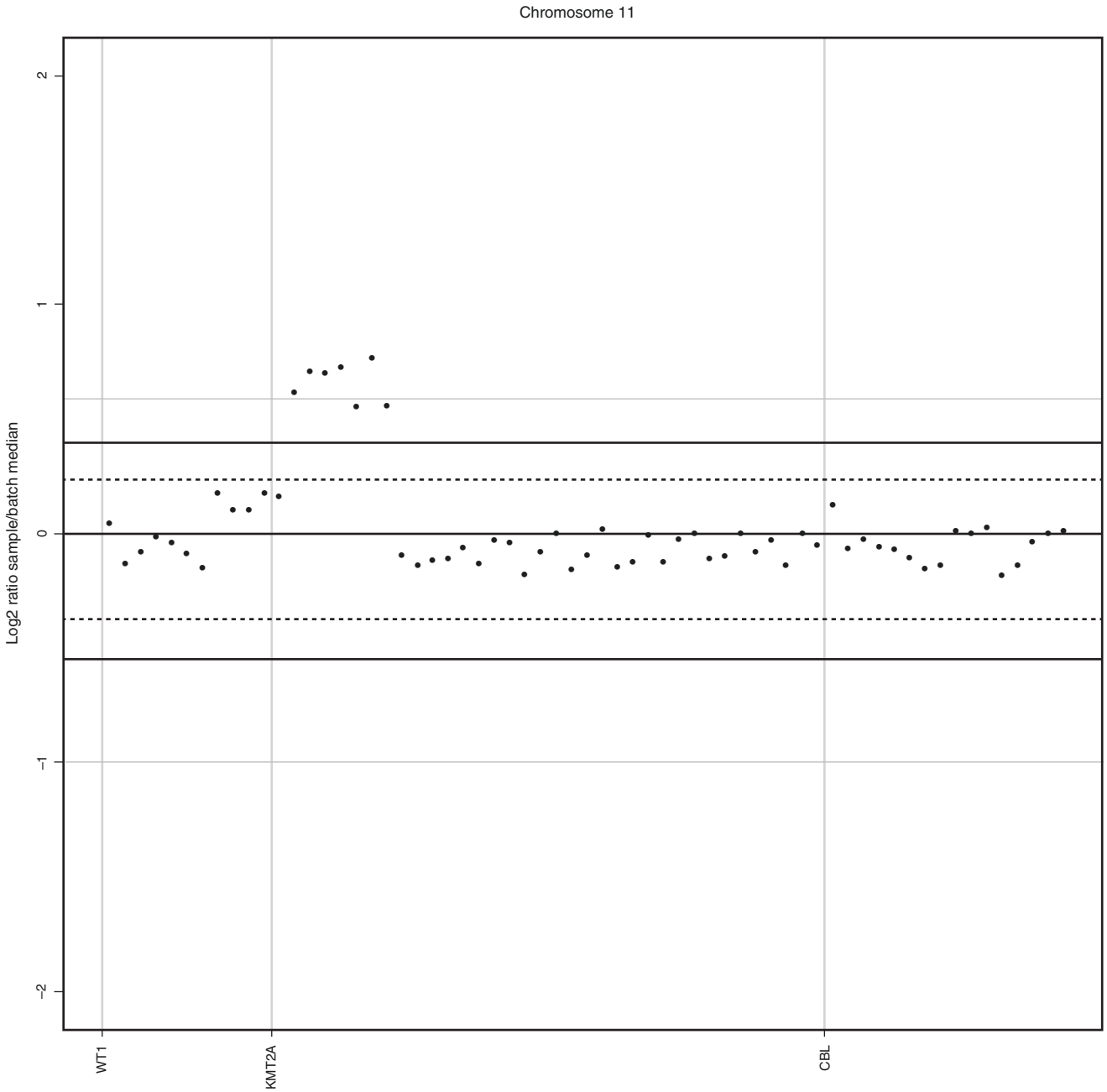

Fig. 3.5 Detection of *KMT2A*-partial tandem duplication (PTD) by detecting copy number changes. Visualization of copy number variants after normalization using VisCap software showed increased copy number for exons 2 to 8 in *KMT2A*, suggestive of *KMT2A*-PTD, a recurrently reported variant in acute myeloid leukaemia. Result has been confirmed by RT-PCR

setting of MRD monitoring. From the experience of MRD monitoring in AML, the use of such error-corrected techniques enabled the detection of variants with lower VAF [55]. Reported analytical sensitivities vary from 0.005% to 0.1% in MRD monitoring of AML, depending on the mean pre-deduplicate sequencing depths that range from 6100× to 200,000×, and downstream filtering strategies employed in various studies [56–58].

While the uniquely tagged DNA molecule derived from one strand fragments of DNA in single molecular consensus sequencing can correct for errors introduced in PCR amplification during library preparation, single molecular consensus sequencing has not harnessed all the sequence information from the double-stranded nature of DNA. Other types of errors may not be corrected by this technique, e.g. errors that are introduced at the first cycle of PCR can be propagated to

all copies in downstream PCR cycles and DNA damage that appears in one of the DNA double strands can affect all copies after PCR. To address this, duplex consensus sequencing has been proposed to independently tag and sequence each of the two strands of the DNA double strands [59], with the availability of a published protocol for wet-bench procedures and proposed bioinformatic analysis [60]. As the complementarity in DNA double strands dictates, DNA changes that occur in one strand will affect the complementary nucleotide(s) in the other strand. Such double-proof information is harnessed in duplex consensus sequencing to verify whether a change occurring in one sequence read on one DNA strand is associated with a complementary change in sequence reads on the corresponding opposite DNA strand. If so, the DNA change is supported by consensus reads and can be regarded as genuine. The theoretical error rate of duplex consensus sequencing is less than 10^{-9}, due to the low probability of an error occurring at the same position of both DNA strands [53]. Recent application of duplex consensus sequencing for the detection of *ABL1* tyrosine kinase domain mutation in DNA specimens has validated the sensitivity level at 0.005%, facilitating the early detection of potential clinically actionable resistant clones in B-lymphoblastic leukaemia with *BCR-ABL*1 [61].

3.4.3.2 Evaluation of Immunoglobulin/T-Cell Receptor Genes

Immunoglobulin (Ig) and T-cell receptor (TCR) gene rearrangement studies are conventionally performed using the BIOMED-2 PCR-based protocol [62] by fragment length analysis. Despite there being extensive efforts in standardizing the method and interpretation of Ig and TCR gene rearrangement assays [62, 63], the interpretation of clonality remains challenging, with many potential pitfalls [64]. Conventional MRD monitoring using Ig or TCR gene rearrangement by Sanger sequencing requires the prior design of patient-specific primers with individual validations, which is both labour-intensive and time-consuming. The recent applications of NGS for Ig and TCR gene rearrangement studies have addressed many of these major challenges, as NGS permits the characterization of the exact DNA sequences of the rearranged Ig or TCR genes and provides a reliable way to quantify the abundance of a given rearranged sequence among other rearrangements. NGS can adopt a universal amplicon-based approach for Ig and TCR sequence analysis, as developed by EuroClonality-NGS working group [65]. This is a one-design-serves-all model that can be adopted for clonality testing and MRD monitoring in various lymphoid neoplasms. NGS approach in Ig gene rearrangement testing has been shown to offer improved performance in clonality testing compared to the conventional approach [66, 67], with similar sensitivity on MRD testing when compared to high-sensitivity flow cytometry [68]. As the amplicon-based NGS includes the amplification of variable regions of the Ig and TCR genes, certain PCR products are relatively long. This requires the presence of long fragments for reliable testing, and only limited types of short-read NGS sequencers that permit read length of at least single-end 600 base pairs or paired-end 300 base pairs can be used for performing Ig and TCR gene rearrangements.

NGS has also been used to evaluate for the presence of somatic hypermutation (SHM) in chronic lymphocytic leukaemia (CLL), as the presence of SHM is associated with a more favourable prognosis in CLL. Readers are referred to a recent review for detailed discussion in this area [69].

3.4.3.3 Genome Sequencing for Cytogenomic Investigations

Cytogenomic investigations of haematological malignancies are currently performed by conventional karyotyping and fluorescence in-situ hybridisation (FISH) for the detection of CNV and SV at the genome level. The reliance on conventional techniques renders such cytogenomic investigations highly labour-intensive and of low "resolution" for detection of genomic abnormalities. Also, the use of conventional karyotyping requires the presence of mitotic cells after cell culture. The success of cell culture is not uniform across all subtypes of haematological malignancies. On the other hand, the use of FISH requires prior knowledge of the genetic abnormalities before targeted FISH probes can be applied for specific interrogations. Such prior knowledge may not always be possible and may limit the detection rate of driver variants in haematological malignancies.

Short-read sequencing has been evaluated for diagnostic evaluation in myeloid neoplasms [70, 71]. In one study [70], short-read GS detected all clinically significant abnormalities that were detected by conventional karyotyping and FISH in over 200 patients, with the additional benefits of providing risk-stratification information in culture-failure cases and detection of new abnormalities that were not present in karyotypic analysis in 25% of patients with acute myeloid leukaemia (AML). Streamlining of workflow with the use of automated data analysis facilitate a turnaround of 3 days [70]. However, this study adopted a highly focused approach to detect only pre-set clinically relevant mutations that may limit the comprehensiveness of diagnostic possibilities. In another study that investigated the performance of both GS and whole transcriptome sequencing (WTS) in AML, concordance between short-read GS and conventional karyotyping is 94%, while GS and WTS has 99% concordance [71]. It remains to be determined whether this technique can be reliably and efficiently introduced into the clinical setting, as there are many practical considerations before such dramatic migration of testing platforms can take place [72].

3.5 RNA Sequencing

3.5.1 Principles of Assay Design

RNA sequencing (RNA-seq) in NGS uses complementary DNA (cDNA) generated from reverse transcription (RT) of RNA as input for sequencing. Transcriptome sequencing is usually performed for comprehensive evaluation of gene expression profiles in various disease conditions. For haematology specimens, transcriptome sequencing is usually performed on mRNA after poly-A tail selection or on total RNA after ribosomal RNA and/or globin mRNA depletion. The former method investigates mRNA expression profiles, while the latter method investigates mRNA expression, along with expression profiles of regulatory noncoding RNAs, e.g. long intergenic noncoding RNAs, antisense RNAs, and circular RNAs. In the diagnostic setting, major applications of RNA-seq in clinical laboratories include the detection of fusion transcripts in leukaemias, while applications to measure gene expression profiles in specific categories of leukaemias and to detect SNVs and short indels are emerging using RNA-seq. Targeted RNA-seq is a more focused and less costly strategy for clinical application. The smaller number of enriched RNAs implies more sequencing throughput can be directed to sequence the enriched RNAs, therefore enabling a higher depth of coverage given the same RNA expression levels and therefore higher sensitivity in the detection of lowly expressed genes, lowly expressed isoforms or fusion transcripts, and also facilitating a higher precision in the profiling of the expression of the targeted genes, when compared to transcriptome sequencing [73]. The rest of this section will focus on the clinical application of targeted RNA-seq in haematological malignancies.

Common strategies of target enrichment of cDNA include anchored multiplex PCR (AMP) [74], single primer extension (SPE), and hybridization capture of cDNA [75]. An important application of targeted RNA-seq in haematological malignancies is to detect fusion transcripts that have diagnostic, prognostic, or therapeutic implications. The enrichment techniques for targeted RNA-seq should be able to detect both recurrently reported fusion transcripts and novel fusion transcripts with one novel gene partner fused with a known gene of clinical significance, e.g. a novel gene partner fused with RARA in acute promyelocytic leukaemia with variant RARA translocation, for more comprehensive coverage of fusion detection. In this aspect, simple amplicon sequencing after RT-PCR will not detect novel fusion transcripts, while SPE, AMP, and hybridization capture can enrich "unknown" DNA sequence that is fused with a known targeted DNA sequence.

AMP is based on 5′ or 3′ rapid amplification of cDNA ends (RACE). It utilizes a universal half-functional adapter that ligates to the 5′-end of cDNA fragments and then performs nested PCR, with both rounds using primers against the 5′ universal adapter and 3′ gene-specific primers. The two rounds of PCR have an enrichment effect by functionalizing the initially half-functional universal adapter for clonal amplification of fragments containing targeted sequences [74]. As the ligation of universal adapter will include novel fusion partners attached to targeted genes in the panel, it can detect fusion transcripts with novel gene partners. SPE uses the combination of a universal primer and a gene-specific primer to perform one round of PCR for enrichment of the target. Hybridization capture of cDNA utilizes capture probes that are complementary to sequences of targeted cDNA. In the event of fusion of a targeted gene with a novel partner gene, sequences of the novel partner gene that are fused to the targeted gene will be pulled down by the capture probes and enriched for sequencing. A recent study has demonstrated the increase in fusion gene diagnostic rate by using targeted RNA-seq after hybridization capture, when compared to conventional diagnostic approaches [76]. Efficiency of enrichment in hybridization capture can be assessed by the inclusion of RNA exogenous reference transcripts (e.g. ERCC) [75].

3.5.2 Bioinformatic Considerations for RNA Sequencing

The initial procedures of quality inspection, adaptor-trimming, and trimming of low-quality bases of RNA-seq data are the same as those in DNA sequencing. For investigation of gene expression profiles, sequence alignment for RNA-seq data should be performed by high-speed splice-aware aligners, such as STAR [77] and HISAT2 [78]. If RNA exogenous reference transcripts have been included as a quality control measure, the reference sequences of the transcripts should be incorporated into the reference genome and transcripts in the splice-aware aligners. Transcripts that align to various gene regions can be counted using feature Counts [79] or HTseq [80]. Transcript counts can then be normalized for gene length and library size using edgeR or DESeq2 [81]. The relative abundance of transcripts and differential expression of genes can then be used for downstream application to classifiers or calculation of diagnostic or prognostic scores.

Bioinformatic algorithms that detect fusions from RNA-seq data usually start with sequence alignment by splice-aware aligners (e.g. STAR) or genome aligners (e.g. Bowtie2) [82]. Chimeric reads from the former and discordant reads from the latter will be further processed to identify candidate gene fusions, with subsequent filtering process to remove probable artefacts. As different bioinformatic algorithms may give rise to different artefacts even after filtering process, it has been reported that the use of multiple algorithms may increase the specificity of fusion detection [76]. Laboratories should determine their limit of detection for the combined system of wet-bench procedures and bioinformatic algorithms. It is essential to routinely include a posi-

tive control with known fusion transcript(s), e.g. using diluted patient sample or cell line, at a level around the limit of detection to ensure the analytical sensitivity of the assay.

Variant detection using RNA-seq data in haematological malignancies has been investigated recently [83, 84]. GATK has a recommendation of best practice workflow for short variant discovery in RNA-seq. Variant detection using unaligned RNA-seq reads has also been reported [85]. While the initial results are encouraging, it remains to be determined in larger studies whether RNA-seq is sufficiently reliable to be used solely for variant detection in haematological malignancies.

3.6 Molecular Monitoring of Measurable Residual Disease

Measurable residual disease (MRD) monitoring using molecular methods is routinely performed in the clinical setting for many acute and chronic leukaemias, with many standardizations established in the field [65, 86–89]. Molecular MRD monitoring can be divided into allele-specific techniques, such as real-time quantitative PCR (RQ-PCR) and digital PCR (D-PCR), and multiplexed techniques, such as short-read NGS in MRD monitoring of AML, ALL, and myeloma. Allele-specific techniques typically target one to a few genetic abnormalities, e.g. fusion transcript or SNV, for disease monitoring, such that only a subset of patients harbouring the specific genetic abnormalities in a disease is eligible for monitoring. In contrast, multiplexed techniques can simultaneously measure many genetic abnormalities, e.g. SNV and short indels in AML, or Ig/TCR rearrangement in lymphoid neoplasms. The versatility of multiplexed techniques permits the application of testing platforms in most of the patients with a given disease. MRD monitoring using fusion detection by targeted RNA-seq in NGS is in development, with preliminary evidence showing its sensitivity may not be as high as conventional allele-specific techniques [90], though modification of wet-bench procedures has been proposed to boost the sensitivity of RNA-seq [91].

The principles of multiplexed techniques using NGS have been discussed in the section of short-read NGS. This section will consider allele-specific techniques, using CML as an illustrating example. Molecular monitoring in CML is one of the best established in terms of standardization. Reference to applications to other diseases will be provided where appropriate.

Common to all the molecular MRD strategies is that diagnostic sample testing is much preferred to ascertain the genetic variants that can be used for subsequent monitoring and to facilitate the detection of subsequent acquisition of additional genetic abnormalities by the tumour in the case of some multiplexed techniques. It is also important for clinical laboratories to establish standardized time points for disease monitoring after the administration of treatments, as standardized time points permit the establishment of consistent cut-off levels to inform the choice of clinical management, facilitating the clinical actionability of the MRD results.

3.7 Real-Time Quantitative PCR

3.7.1 Principles

RQ-PCR utilizes real-time measurement of PCR products to quantitate the abundance of DNA/RNA target in the initial starting material. The real-time measurement is performed either by hydrolysis probes or DNA intercalating dyes.

Hydrolysis probe, also known as TaqMan probe, is an oligonucleotide probe that has been dual-labelled with a reporter fluorochrome and a quencher fluorochrome. This oligonucleotide probe is designed to be complementary to the target sequence to be quantified and its annealing site is located between the pair of PCR primers used for the assay. The reporter fluorochrome in its intact form is quenched by the quencher fluorochrome due to fluorescence resonance energy transfer (FRET). During the process of RQ-PCR, the hydrolysis probe anneals to the target sequence, while a *Taq* DNA polymerase is used to perform PCR amplification. When the *Taq* DNA polymerase extends the PCR primers during the amplification process, the exonuclease activity of the *Taq* DNA polymerase cleaves the hydrolysis probe and detach the reporter fluorochrome from the quencher fluorochrome. The fluorescence signal of the reporter fluorochrome is no longer quenched and is released for measurement. The intensity of fluorescence signals collectively released by individual PCR amplification depends on the number of template available for PCR reaction to carry out. As the number of PCR cycles increases, the number of products accumulate, so does the intensity of fluorescence signal. The end-point of measurement happens when the number of PCR cycles raises the fluorescent intensity beyond a pre-set threshold. This number of PCR cycles is termed threshold cycle. The threshold cycle can be translated back to the initial quantity of the target sequence by constructing a standard curve using multiple dilutions of target sequence with known quantity.

Fluorescent DNA intercalating dyes, such as SYBR green, bind the minor grooves of double-stranded DNA and emit fluorescent signal. As the number of double-stranded DNA target increases during PCR, the fluorescent intensity will increase as well. The threshold cycle is determined similar to that using hydrolysis probe, with the initial quantity of DNA determined by a standard curve. Since intercalating dyes can potentially bind to non-specific double-stranded PCR-products, the specificity of RQ-PCR using intercalating dyes can be lower than that using hydrolysis probe, particularly when the PCR primer is not specific. Also, primer-dimers will also bind intercalating dyes leading to potential false positive signals.

3.7.2 MRD Monitoring in CML

The presence of *BCR::ABL1* fusion transcript is the defining feature in CML. Molecular monitoring of *BCR::ABL1* fusion transcript has become the standard of clinical practice in the era of tyrosine kinase inhibitors (TKI) for treatment, with established monitoring and treatment implications for various transcript levels at defined time points after commencement of treatment [89].

After the clinical and histological diagnosis of CML, the presence of *BCR::ABL1* transcript is demonstrated by RT-PCR, with the transcript subtype determined. The determination of transcript subtype is critical for subsequent molecular monitoring [92]. Common transcript subtypes include e13a2 and e14a2, in which *BCR* exon 13 or exon 14 is fused to *ABL1* exon 2, respectively. Most RQ-PCR assays are designed to monitor e13a2 and e14a2 in CML patients. If an uncommon transcript subtype (e.g. e1a2, e14a3) is detected in a patient, the monitoring strategy for the patient has to be individually devised.

During transcript measurement using RQ-PCR in CML, the transcript levels of *BCR::ABL1* and *ABL1* are individually measured in duplicate. The monitoring result is typically expressed as a *BCR::ABL1* to *ABL1* ratio. In this ratio, the *ABL1* in the denominator serves as a housekeeping control gene. The inclusion of a control gene serves as a compensatory mechanism for RNA degradation in the specimen and control for the efficiency of reverse transcription [93]. Acceptable level of *ABL1* is typically quoted at 10000 copies per reaction volume to prevent false negative results due to poor sample quality [94]. It is worth noting that the linearity of the *BCR::ABL1/ABL1* ratio maintains only at low levels (e.g. lower than 10%) of *BCR::ABL1* transcripts, as the primers used for quantification of *ABL1* usually amplify the *ABL1* portion in the *BCR::ABL1* transcript [95]. To facilitate the harmonization of RQ-PCR results across laboratories, an International Scale (IS) has been devised [93] to permit traceability of transcript levels to the original IRIS study [96] that demonstrated the clinical efficacy of imatinib, i.e. the first TKI for the treatment of CML. A transcript level of 100% in IS is arbitrarily defined by the median of pre-treatment transcript level in 30 selected CML patients recruited in the study. A 3-log (i.e. 0.1% in IS) or more reduction from this standardized baseline achieved after 12 months of TKI therapy is regarded as the most important milestone that is associated with a survival close to 100%, because disease progression is unlikely at this IS level [89]. This milestone is termed major molecular response (MMR). The latest ELN recommendation also stipulates that an IS ratio greater than 10% after 3 months of TKI treatment indicates treatment failure when confirmed, or after 6 months of TKI treatment [89]. Also, after 12 months of TKI treatment, if MMR is not achieved, an IS ratio of more than 1% denotes treatment failure. Patients with treatment failure should be assessed for the causes of treatment resistance, including analysis of tyrosine kinase domain mutations, with considerations for alternative treatment strategies [89].

The remarkable efficacy of TKI therapy in majority of CML patients is evidenced by a significant number of patients achieving molecular response (MR) deeper than MMR, i.e. 4-log (or IS <0.01%, also termed MR4), 4.5-log (or IS <0.0032%, also termed MR4.5), or 5-log (or IS <0.001%, also termed MR5) reduction from the IS baseline, after treatment with imatinib or other second-generation TKI. This has enabled CML patients with deep molecular response to attempt cessation of TKI therapy, termed treatment-free remission (TFR), in the clinical setting under strict inclusion criteria [97]. Clinical laboratories that monitor patients on TFR should have accurate and sensitive RQ-PCR IS standardized assay available for monitoring, with turnaround of test results within 4 weeks and testing intervals of 4–6 weeks [97]. The European Treatment and Outcome Study for CML (EUTOS) has provided laboratory recommendations for determining such deep molecular responses. EUTOS has consolidated the concepts of deep MR in the setting of detectable and undetectable disease. To facilitate the scoring of deep MR, EUTOS recommended summing the transcript levels of the fusion transcript and control gene individually before the calculation of the final *BCR::ABL1/ABL1* ratio and permitting the extrapolation below the level of the lowest plasmid standard to quantify very low level of *BCR::ABL1* transcripts [88]. In the context of limit of detection (LoD), the observation of one copy or two copies of *BCR::ABL1* transcripts should be rounded up to three copies in any replicate measurement. This is recommended on the basis that when three *BCR::ABL1* copies are observed, theoretically there is less than 5% chance of the sample genuinely containing no *BCR::ABL1* transcript at all assuming a Poisson distribution [88]. While some of the theoretical bases may be up to debate [98, 99], such standardization effort has greatly facilitated a harmonized method of determining deep MR to qualify CML patients for TFR in the field.

3.7.3 RQ-PCR Monitoring in Other Leukaemias

Similar to molecular monitoring in CML, potentially around 35–45% of AML and ALL have fusion genes as drivers that can serve as targets for molecular monitoring using RQ-PCR. For AML, ELN recommended routine monitoring of core-binding factor AML (i.e. AML with *RUNX1::RUNX1T1* fusion, AML with *CBFB::MYH11* fusion) and acute promyelocytic leukaemia with *PML::RARA* at informative clinical time points [86]. Primer design and experimental conditions for monitoring of common fusion transcripts in AML and ALL have been published by the Europe Against Cancer (EAC), including recommendations on reporting of MRD [100]. Apart from fusion genes, AML with mutated *NPM1* is the only short variant that has been included in the ELN recommendation [86].

3.8 Digital PCR

3.8.1 Principles

Digital PCR (dPCR) utilizes microfluidic technology to randomly distribute DNA molecules into thousands of partitions, such that most partitions contain no or one molecule. The method of partitioning varies by testing platforms. PCR is performed in each of the nano-litre reaction in each of the partition individually. The PCR products of targeted mutants, wild-type sequence, and/or control gene can then be measured by using fluorescent probes [101]. The actual number of molecules can be calculated from the number of positive and negative partitions after adjustment using the Poisson distribution. Since dPCR performs absolute quantitation of targeted genetic material, there is no need to construct a standard curve, in contrast to RQ-PCR. This has the advantage of reducing the number of reactions required to generate results, therefore saving costs potentially when compared to RQ-PCR. The quantitation in RQ-PCR is dependent on PCR efficiency. DPCR is an absolute quantitation method by endpoint PCR and the quantitation is not dependent on PCR efficiency. It is potentially a more precise method when compared to RQ-PCR, especially in the context of MRD monitoring.

Recent versions of dPCR analysers adopt an integrated and automated workflow that combines sample loading, sample partitioning, PCR amplification, and signal detection in partitions into one equipment. This improvement has greatly facilitated the introduction of dPCR technology into high-throughput clinical setting, as it permits a streamlined workflow and faster turnaround time, while reducing manual hands-on time and human handling errors. Newer equipment also supports the measurement of multiple fluorescent dyes, such that more gene targets can be monitored in one measurement, permitting the design of multiplexed assays for detection of multiple gene targets.

Since the component of PCR amplification in dPCR is similar to that of RQ-PCR, theoretically most primer designs for RQ-PCR TaqMan assays can be directly adopted for use in dPCR. In practice, validation runs should be performed using known positive samples and negative samples to determine if a distinct separation of signals can be observed between the positive and negative partitions and also to determine the false positive rate of measurement. If the preliminary result is suboptimal, the experimental conditions, in particular the primer annealing temperature denature/extension time, or primer design, may need to be further optimized.

3.8.2 Considerations on Analytical Sensitivity in DPCR

DPCR is generally shown to have higher analytical sensitivity than RQ-PCR in the context of MRD monitoring, mainly by its capability to provide quantitative results in cases scored as "positive but non-quantifiable" by RQ-PCR [102, 103]. In clinical practice, the analytical sensitivity of dPCR depends on multiple factors. As MRD monitoring by dPCR generally expresses results in the form of a ratio (i.e. mutant to control gene ratio), the determining factors of analytical sensitivity would include factors that affect the chance of false positives of the nominator (i.e. mutant) and factors that affect the magnitude of the denominator (i.e. control gene).

False positive is an important limiting factor for the analytical sensitivity of dPCR, as any false positives would result in a higher level of limit of detection (LoD) in a given assay. Special consideration should be exercised during primer design to ensure the specificity in amplifying the intended gene target. An increased level of false positivity would necessitate a higher cut-off for positive results, leading to a loss of sensitivity, as exemplified by a recent application of the EAC protocol for major *BCR::ABL1* transcripts in dPCR [104]. The inherent characteristics of gene targets would also affect the rate of false positivity in dPCR. A gene target with sequence more different from its wild-type counterpart would permit the design of primers for a more specific amplification process, therefore lowering false positivity during measurement. For example, a dPCR assay used to detect SNVs or short indels would be expected to yield higher false positive signals than a dPCR assay used to detect the presence of fusion transcripts, due to the more significant difference in genetic sequence in fusion genes compared to SNVs and short indels (Fig. 3.6). This has implications on the selection of gene targets when multiple potential targets are available for MRD monitoring, especially if high analytical sensitivity is a priority.

Apart from limiting false positivity, methods that increase the measured copy number of the control gene would contribute to a higher sensitivity. The most straightforward way to increase the measured copy number of control gene is to perform the test on more replicates. A higher number of replicates can increase the sensitivity of the assay, as this implies surveying an increased amount of sample for the gene target. The increased number of measured control gene serves as a surrogate for the increased surveying space of the MRD assay. In line with this principle, the EUTOS recommendation for CML monitoring requires laboratories reporting detectable and undetectable MRD to attain certain number of control genes as appropriate to the level of the MRD [88]. For example, the *ABL1* copy number should be higher than 10,000 to report MRD at MR4; higher than 32,000 to report MRD at MR4.5; and higher than 100,000 to report MRD at MR5. Despite the EUTOS recommendation being originally published for RQ-PCR, the general principles are broadly applicable to other molecular MRD assays in leukaemias. Increasing the number of replicates would increase costs for each test and require more patient samples, which may sometimes be limited in the clinical setting. Alternative method that may increase the measured copy number of con-

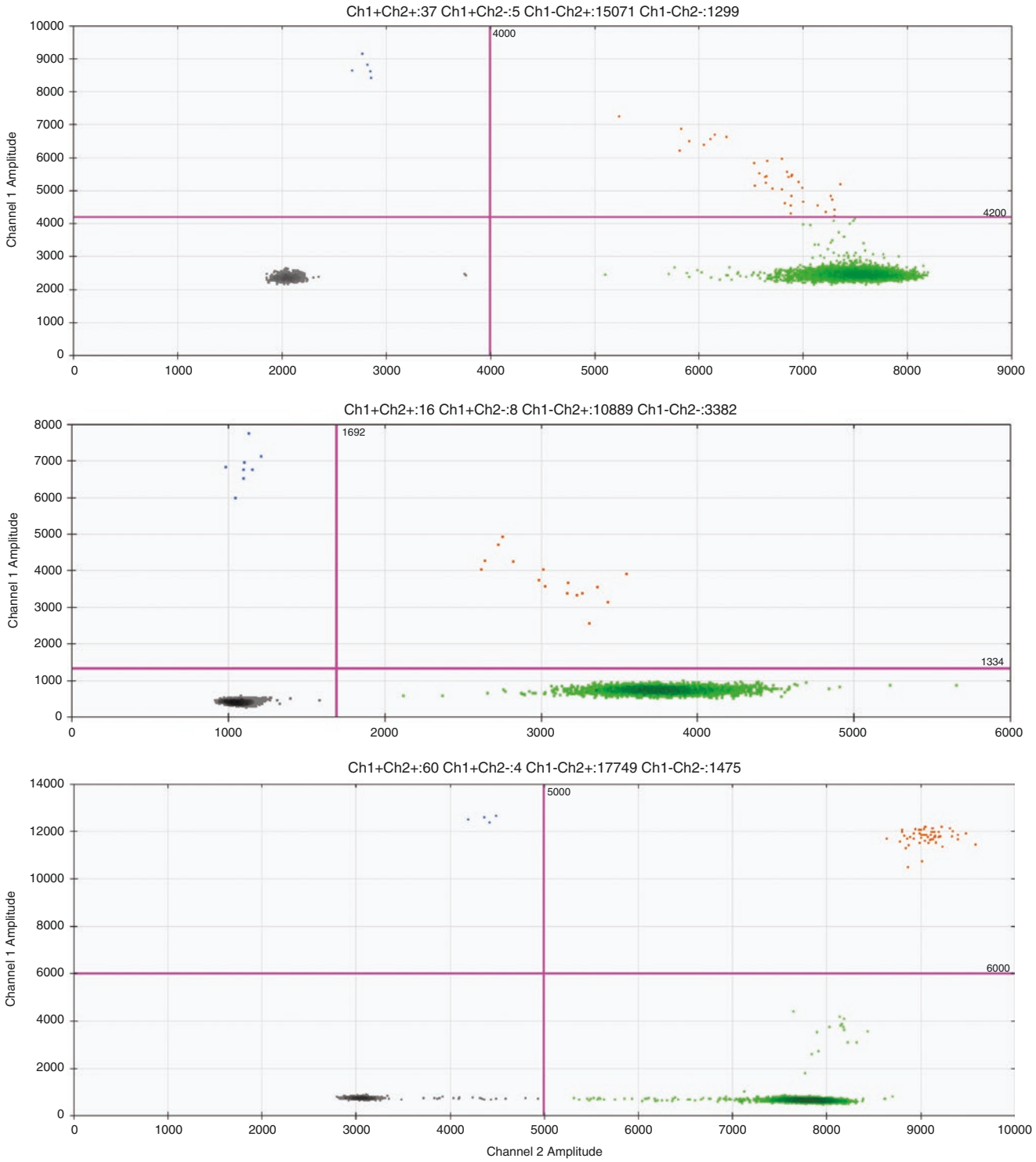

Fig. 3.6 Scatterplots showing how the signal specificity in digital PCR may depend on the inherent characteristics of gene targets. X-axis of plots denotes result of control gene. Y-axis of plots denotes result of gene target. (*Top plot*) This plot shows the result of digital PCR assay for *IDH1* p.R132H, which is a single nucleotide variant (SNV). The close similarity between the mutant and wild-type sequence, apart from the SNV, results in suboptimal separation between the *IDH1* p.R132H-positive droplets (orange dots) and p.R132H-negative droplets (green dots). (*Middle plot*) This plot shows the result of digital PCR assay for *NPM1* type A mutation. The higher degree of difference (i.e. 4-base pair duplication) between the mutant and wild-type sequence permits a better separation between the mutant-containing droplets (orange dots) and mutant-negative droplets (green dots). (*Bottom plot*) This plot shows the result of digital PCR assay for *BCR-ABL1* e1a2 fusion transcript. The significant difference in sequence between the fusion transcript and the wild-type transcript facilitates the tight clustering of fusion-positive droplets (orange dots) and fusion-negative droplets (green dots), facilitating the high specificity of the assay

trol gene includes selecting a reverse transcriptase that yields a higher copy number of the control gene [93, 105].

3.9 Gene Expression Profiling

The advent of microarray in the late 1990s has permitted high-throughput analysis of expression levels in thousands of genes by simultaneous measurement of many RNA transcripts. Gene expression profiling (GEP) has instilled significant biological insights in various haematological malignancies, providing important means for disease subtyping and prognostication in many leukaemias and lymphomas. Notwithstanding the promising roles of GEP in clinical studies, the introduction of GEP in clinical diagnostic laboratories has been disproportionately slow. One of the reasons may be due to the difficulty in harmonizing GEP results across laboratories, such that diagnostic or prognostic observations in clinical studies cannot be reproducibly translated into clinical application. Also, clinical laboratories are required to exert substantial efforts to re-validate GEP platforms or calculation schemes in their local settings, and such endeavours may not always be possible in clinical laboratories.

With the increasing number of GEP platforms available and, more importantly, the inclusion of disease categories that require GEP for definitive categorization in the latest WHO classification and International Consensus Classification [106, 107], it becomes more pressing for the field to devise diagnostic algorithms with incorporation of GEP features.

3.9.1 Brief Review of GEP Platforms

The application of RQ-PCR and dPCR to detect expression level(s) of one to a handful of genes has been discussed in previous sections. To test for the expression of more genes, RQ-PCR can also be upscaled to medium throughput GEP of dozens to hundreds of genes using 384-well plates in the form of a low-density array (LDA). If higher throughput GEP is required, microarray and RNA-seq are usually the platforms of choice.

Microarray-based GEP uses oligonucleotide probes that are complementary to thousands of targeted transcripts for the measurement of transcript expression profile. The expressed transcripts in the sample are labelled with fluorochrome and hybridized to the oligonucleotide probes on the microarray. The relative abundance of all targeted transcripts is measured by fluorescent signals on the microarray after high resolution image scanning. Due to the high-throughput nature of microarray platforms, bioinformatic analysis tends to be more complex, though many commercial or open-source solutions are readily available for such tasks nowadays.

Despite the popularity of microarray-based GEP in the 2000s, it is increasingly supplanted by RNA-seq in recent years. The principle of RNA-seq has been discussed in a preceding section. Comparisons between RNA-seq and microarray showed good correlation of GEP between the two platforms [108]. There are several advantages of RNA-seq over microarray-based platforms. First, the design of microarray probes requires the targets-to-be-investigated be known and the measurement may only cover part of the targeted transcript, while RNA-seq for transcriptomic studies does not require pre-designed probes, so RNA-seq can detect potential novel transcripts or alternatively spliced isoforms [109]. Second, RNA-seq permits high flexibility in sequencing throughput, such that high-throughput experiments can be designed to detect low abundance transcripts, e.g. long noncoding RNAs. Third, RNA-seq data can be used to study genetic variants in expressed genes. For clinical laboratories, the capability of NGS to measure GEP, in addition to DNA sequencing, represents an all-in-one solution for GEP. The drawback of RNA-seq includes the relatively complex downstream data analysis, but this difficulty is increasingly offset by the well-established bioinformatic solutions available in the field [110].

More recently, direct measurement of RNA by NanoString platform [111] represents an attractive solution for potentially reproducible GEP in clinical laboratories. NanoString is a hybridization-based platform. It co-hybridizes a biotin-labelled capture probe and a fluorescent barcode-labelled reporter probe to a target transcript. The capture probe immobilizes the target transcript to streptavidin-coated cartridge and the platform counts the immobilized transcript using the barcode. NanoString measurement does not require PCR amplification or reverse transcription, therefore eliminating result inaccuracies secondary to amplification bias. It can measure expression of up to 800 genes in a single reaction [112] and serves as an intermediate between LDA and higher throughput platforms like microarray and transcriptome study by RNA-seq. The availability of targeted RNA-seq presents another attractive solution for medium throughput GEP with higher sequencing depth, while harnessing the strengths of RNA-seq of nucleotide resolution and ability to detect novel transcripts.

3.9.2 Clinical Applications of GEP in Leukaemias

This section will provide a few recent examples in haematology that utilize GEP for diagnosis and prognosis, as a review of all GEP applications in haematology is beyond the scope of this chapter.

The WHO classification has recently included B-lymphoblastic leukaemia/lymphoma, *BCR::ABL1*-like (*BCR::ABL1*-like ALL) as a diagnostic entity [106]. *BCR-*

ABL1::like ALL was originally identified by GEP using microarray in the late 2000s [113, 114]. Subsequent characterization [114, 115] revealed the underlying genetic abnormalities giving rise to *BCR::ABL1*-like ALL signature, most commonly due to kinase-activating alterations, including rearrangements of *CRLF2*, *JAK2*, *ABL1/2*, *CSF1R*, *PDGFRB*, *EPOR*, *NTRK3,* etc. that jointly account for around 90% of *BCR::ABL1*-like ALL [115]. Despite a range of techniques, including flow cytometry, cytogenetics, fluorescence in situ hybridization, RT-PCR, and NGS for fusion detection [116], have been employed to detect clinically actionable genetic alterations associated with *BCR::ABL1*-like ALL signature, GEP remains the gold standard for the diagnosis of *BCR::ABL1*-like ALL. To facilitate the clinical application of GEP in the diagnostic setting, smaller GEP panels have been proposed using LDA platform with reported sensitivity of 93.0% and specificity of 89.7% when compared to one gold standard GEP assay [117, 118]. Notwithstanding the well-established role of GEP in the diagnosis of *BCR::ABL1*-like ALL, some major challenges of applying GEP assays in the clinical setting remain. These include the need for standardizing GEP platforms and wet-bench procedures; standardizing bioinformatic analysis of the GEP data; and local validation of the clinical sensitivity and specificity of such assay(s). It is worth noting that the two prototypical GEP signatures for *BCR::ABL1*-like ALL yield discrepant categorization in a significant proportion of patients, with only a subset of patients being categorized concordantly by both signatures [119]. Apart from *BCR::ABL1*-like ALL, it can be projected that there will be an increasing number of disease entities requiring GEP for diagnosis, e.g. B-ALL with *ETV6::RUNX1*-like features; B-ALL, *ZNF384* rearranged like; B-ALL, *KMT2A* rearranged-like, as stipulated in the new WHO and ICC classifications.

In the context of AML, many GEP-based prognostic scores have been reported [120–122]. Many of these GEP assays measure non-overlapping sets of genes. A more recent version of GEP prognostication in AML used genes differentially expressed in leukaemia stem cells for 17-gene signature (LSC17), with demonstrated prognostic effects independent of ELN risk stratification by genetics in AML [123]. The prognostic role of LSC17 has been validated using NanoString platform in both adult and paediatric AML patients to facilitate clinical application [124, 125].

3.10 Newer Techniques

3.10.1 Long-Read Sequencing

Long-read sequencing is a newer technological advance that enables single molecule sequencing with length usually in the range of at least several kilobases. Longer reads permit the investigations of large complex structural variants or repetitive regions in the genome using information obtained from single continuous reads. In the context of RNA, longer reads permit the investigations of full length RNA transcript sequences. There are two major technologies of long-read sequencing currently available, namely single-molecular real-time (SMRT) sequencing by PacBio and nanopore sequencing by Oxford Nanopore Technologies [18].

SMRT uses fluorescent signal to monitor the real-time incorporation of single nucleotide by polymerase. In contrast to sequencing-by-synthesis technology in short-read sequencing, which requires clonal amplification of sequence templates to generate fluorescent signals for detection, SMRT does not require clonal amplification of the sequence templates. Instead, each sequence template is distributed into picolitre wells that have a tethered polymerase at the transparent well bottom. The sequence template is then used for guiding the incorporation of single nucleotide. The minute fluorescent signal released during the single nucleotide incorporation is picked up by a specially devised system termed zero-mode waveguides [18]. To reduce sequencing error, SMRT platform uses hairpin adapters to generate circular templates for sequencing, so that the polymerase can loop through the sequence multiple times for sequencing. The multiple passes permit a consensus sequence to be produced.

Nanopore sequencing measures the changes in ionic current when nucleic acid is passing through a protein nanopore to determine the sequence of the nucleic acid. The pattern of current change is characteristic of short DNA sequences, termed k-mers. Nanopore sequencer can perform real-time selective sequencing, termed Read Until, by real-time analysis of sequencing signals generated from the initial portion of the input nucleic acid [126]. If the sequencing signals match the specified DNA molecules to be selected for sequencing, sequencing will proceed. If the sequencing signals do not match the specified DNA molecules, the driving voltage across the nanopore can be reversed to reject the current nucleic acid, so that a new molecule can be recruited for sequencing. With such selective sequencing technology, there is no need to customize library preparation procedures for target enrichment before sequencing. Only computational algorithms need to be customized for selective sequencing. Recent development of analysis algorithms permits the technique to be used with lower demand of computational resources and has been used to selectively sequence *PML::RARA* fusion in the NB4 cell line [127].

Preliminary application of long-read sequencing has seen promising diagnostic roles in the detection of structural variants in leukaemias. For example, nanopore sequencing coupled with AMP has been shown to be capable of rapid detection of gene fusions in samples with high level of fusion transcripts [128]. Despite the presence of studies reporting variant detection of SNV and short indels in leukaemias

using long-read sequencing [129], the major challenge of long-read sequencing is the higher sequencing error rate when compared to short-read sequencing and sequencing error around sites of homopolymers. These have adverse effects on the analytical sensitivity of the platform. As such, improvement in wet-bench procedures or bioinformatic algorithms is likely necessary to lower sequencing error and increase analytical sensitivity before widespread clinical use for the detection of SNV and short indels is possible.

3.10.2 Single-Cell Sequencing

Single-cell sequencing (SCS) provides a means to investigate tumour heterogeneity in leukaemia. While conventional bulk-sample sequencing captures a cumulative snapshot of the genetic status of all nucleated cells included in a given sample, SCS has enabled a dissected view of genetic status in individual cells. SCS requires the isolation of single cells for subsequent genetic testing. Common approaches for single-cell isolation in haematology include flow-activated cell sorting (FACS) and microfluidics, since large number of cells in suspension are usually available in peripheral blood or bone marrow specimens. In recent years, microfluidic-based platforms have become increasingly popular in the research field. Microfluidic-based platforms partition single cells into small reaction chambers or emulsion droplets. The nucleic acids within single cells are then barcoded using molecular indices to allow identification of the originating cells. The single cells can then be subjected to different types of downstream processing. For example, in single-cell DNA sequencing, DNA can be subjected to targeted regions or whole-genome PCR amplification before the performance of short-read sequencing; while in single-cell RNA sequencing, RNA in single cells can be subjected to reverse transcription and cDNA amplification before the performance of short-read sequencing. The details of single-cell processing and its related bioinformatic analysis are beyond the scope of this review. Readers are referred to excellent reviews for these topics [130, 131]. Advancement of technologies has permitted the simultaneous capture of multi-omic data from the same cell, for instance, the concurrent capture of gene expression data with spatial information of the same cell in tissues; mutational or gene expression data with antigenic expression of the same cell; and gene expression data with chromatin information of the same cell.

SCS has enabled many insights into the characteristics of haematological malignancies. To name a few, SCS of targeted genes in myeloid neoplasms has recently revealed the clonal architecture and patterns of clonal relationships between driver mutations in AML and MPN [132, 133]; single-cell RNA-seq coupled with single-cell targeted genotyping in AML has showed the cellular hierarchies of malignant cells and the transcriptional programmes in various cell types of AML [134]. Despite new biological insights have been enabled by SCS, it remains to be determined how SCS can be applied in clinical laboratories given its inherent difficulties of low amount of starting DNA or RNA material from single cells, coverage inconsistency, and relatively high cost. Future studies will need to address these issues before the widespread application of SCS in clinical diagnostics.

3.10.3 Optical Genome Mapping

Optical genome mapping (OGM) uses nicking enzymes that recognize specific sites in long DNA fragments up to megabases in length and label them with fluorescent probes to tag selective sequences in the DNA fragments. The optical signals released from the fluorescent probes are captured by fluorescence imaging, using a nanofluidic chip that can facilitate the long DNA fragments to attain elongated state. The relative locations of fluorescent probes in a given DNA fragment form patterns that can be recognized by comparison with a known reference genome map [135]. Such direct visualization of DNA permits the detection of structural variations that disrupt the relative locations of fluorescent probes, with information captured for haplotype blocks. Despite the technology does not yield information at the base pair level, it is complementary to massively parallel sequencing, especially for the interrogation of difficult regions in the genome. The ability of OGM to detect CNV and SV (including balanced translocations) places this technology as a promising platform to potentially transcend conventional cytogenetic tests, with good concordance when compared to conventional cytogenetic techniques, while demonstrating the capability of identifying additional clinically actionable abnormalities in various haematological malignancies.

3.10.4 Circulating Tumour DNA Testing in Leukaemia

Circulating tumour DNA (ctDNA) testing has enabled major advancement of cancer diagnostics for many solid cancers in recent years [136]. In the context of leukaemia, as there is usually the presence of circulating tumour cells at diagnosis, the diagnostic utility of ctDNA testing is limited. The value of ctDNA testing for disease monitoring is currently under active exploration. The current sample of choice for MRD assessment is bone marrow. As ctDNA testing only requires peripheral blood for testing, its less invasive nature compared to bone marrow examination presents an attractive alternative to the latter.

A recent study comparing the performance of ctDNA testing and MRD testing in bone marrow has shown that around

half of all mutations in AML were concordantly detected by both ctDNA testing and bone marrow MRD testing by NGS with UMI, though discordance between ctDNA testing and bone marrow MRD testing was also noted in a minor subset of mutations [137]. In the context of CLL, ctDNA testing using targeted NGS has been shown to reflect disease status across different disease compartments. After ibrutinib treatment in CLL, despite the initial increase in peripheral lymphocytosis, ctDNA levels have reduced, which is more in line with radiological assessment of disease status [138]. In the post-haemopoietic stem cell transplantation (HSCT) setting for AML and myelodysplastic syndrome (MDS), ctDNA testing by digital PCR at 1 and 3 months post-HSCT showed that persistence of mutations is associated with higher 3-year cumulative incidence of relapse, comparable to bone marrow MRD testing [139].

The promising preliminary results of ctDNA testing for MRD monitoring in leukaemias encourage further studies to determine how the clinical application of ctDNA testing can be translated into ascertainment and improvement in clinical outcomes in various leukaemias. Readers are referred to recent reviews on ctDNA testing in leukaemias for detailed discussion of the topic [140–142].

3.11 Conclusion

This chapter has depicted the compendium of current techniques for the diagnosis and monitoring of acute and chronic leukaemias in clinical laboratories. While conventional techniques of allele-specific PCR and Sanger sequencing will continue to serve as valuable tools for detecting a focused set of genetic variants at low costs, the expansion of diagnostic entities defined by genetic features in the latest WHO classification and ICC [106, 107] and the increasing number of genetic features in prognostic systems [123, 143–145] necessitate the use of high-throughput techniques for sustainable genetic investigations in routine diagnostic service. More genetic entities identifiable at diagnosis of leukaemias entails more molecular targets for disease monitoring. Such increase in the depth and breadth for molecular diagnosis and monitoring of leukaemias represents the major challenge of clinical laboratories in this genomic era.

The past decade has seen numerous exciting developments in molecular techniques and data analysis strategies that enable clinical laboratories to live up to the ever-increasing clinical demands on broader scope of genomic testing with shorter turnarounds. It is the endeavour of this chapter to provide a snapshot of these techniques. The future challenges of clinical genomic laboratories would be to harness such advanced techniques and refine the strategies of data analysis, while keeping abreast of the latest research development at the multi-omic levels, to extract a comprehensive set of clinically actionable genetic aberrations and inform clinical management in the age of precision medicine.

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Flow Cytometric Techniques in the Diagnosis and Monitoring of Acute Leukaemias

4

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Abstract

Flow cytometry has revolutionized the way acute leukaemias are diagnosed and monitored. This technique has enabled accurate diagnosis and classification of acute leukaemias. With more accurate methodology, flow cytometry allows detailed monitoring of acute leukaemias which has been shown to affect prognosis of the disease. In this chapter, we will discuss flow cytometry in the diagnosis of acute leukaemias touching on the technical aspects. We will then explore monitoring of acute leukaemias, especially in regard to minimal residual disease in acute lymphoblastic leukaemia and acute myeloid leukaemia.

Keywords

Flow cytometry · Minimal residual disease (MRD) Acute myeloid leukaemia · Acute lymphoblastic leukaemia

4.1 Introduction

As hematopoietic cells differentiate from stem cells to committed progenitors to later stage mature forms, they undergo a tightly regulated sequence of morphologic, immunophenotypic, and functional changes. As a result, there is a pattern of antigenic expression that is characteristic to the lineage and stage of maturation. In the 1980s and 1990s, the antigenic patterns of normal hematopoietic maturation were elucidated and found to be essentially constant between individuals. This led to the use of immunophenotyping techniques for classification of hematopoietic cells into their lineage and maturation stage with a high degree of specificity [1, 2]. Flow cytometry, a method of measuring characteristics of particles suspended in a liquid medium, is well suited for immunophenotyping of hematopoietic cells. The fluidics system of the flow cytometer transports particles in a fluid stream in single file through the path of a laser beam. The laser is used to excite fluorophores conjugated to reagent antibodies which are bound to molecules on or within the cell. The emission of light from the fluorophore thus excited by the laser is collected by the optics system and diverted onto photodiodes which convert the light emission into electric pulses. An analogue-to-digital converter then converts the electric pulse height into number, thereby producing the readout which correlates with the expression of molecules.

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4.2 Flow Cytometry in the Diagnosis of Acute Leukaemia

Multi-parametric flow cytometry (MFC) is a vital tool in the diagnosis and classification of haematolymphoid malignancies, and in particular acute leukaemias [3–5]. The sensitivity and specificity of flow cytometry in distinguishing myeloid from lymphoid leukaemia approach 100% [6, 7]. This technique has allowed accurate diagnosis of acute leukaemia and subtyping of the leukaemia.

The hematopoietic stem cell is characterized by expression of bright CD34 and low to absent CD38 with low CD13, CD33, CD117, CD133, and HLA-DR without lineage-defining antigens. In acute myeloid leukaemia (AML), subtypes of AML show different characteristics on flow cytometry [6]. For example, M0 and M1 blasts show low forward and side scatter and express CD13, CD33, CD117, and HLA-DR, with M0 showing more CD34 expression than M1. Aberrant cross-lineage CD7 is seen more frequent in M0 and is associated with CD34 expression. In M2, granulocytic maturation can be seen on flow cytometry and blasts typically express more CD15. The co-expression of CD19, and less often, CD56 in M2 is associated with the t(8;21). In M3, abnormal hypergranular promyelocytes show abundant side scatter, presence of MPO, CD13, and CD33, persistence of CD117 weakly, and absence of HLA-DR. CD2 cross-lineage expression is not infrequently seen in M3, especially the hypogranular variant. M4 and M5 blasts typically have more forward and side scatter than M0/1 blasts and on CD45-side scatter, the blasts may merge into the CD45+ monocytic region. Monocytic blasts are frequently CD34 negative, CD64 and CD33 tend to be brighter than CD13, and blasts may or may not express CD14 which is a marker of more mature monocytes. M6 and M7 AML are rare. M6 in flow cytometry typical shows prominent erythroid component, myeloid maturation disarray, and glycophorin antibodies may demonstrate erythroid differentiation on the blasts. Immunophenotyping is an important aspect of M7 diagnosis as neither morphologic nor cytochemical features are pathognomonic of megakaryoblasts. Megakaryoblasts are typically identified by CD61 (glycoprotein IIIa) and/or CD41 (glycoprotein IIb) expression, with cytoplasmic expression of these markers being more specific than surface expression, as platelets adherent on leukaemia blasts may lead to false-positive interpretation, necessitating careful analysis by an experienced operator.

In acute lymphoblastic leukaemia (ALL), immunophenotyping is well established as a routine part of diagnostic work-up as B and T-lymphoblasts are indistinguishable by morphology. Immunophenotyping provides unambiguous classification for B and T-ALL which are hence defined immunophenotypically [5]. B-ALL typically expresses CD19 (a very sensitive B-lineage marker expressed from even the earliest stages of B-cell commitment), cytoplasmic CD79a, cytoplasmic CD22, and dim surface CD22, with or without CD20. T-ALL is cytoplasmic CD3 positive and expresses CD7 and dim or absent membrane CD3. Of note, with the potential exception of cytoplasmic CD3 which is virtually specific to T-lineage ALL, there is no single marker that is entirely specific for lineage. Importantly, expression of combinations of lineage antigens, for example CD19 and cytoplasmic CD79a, cytoplasmic CD3 and membrane CD7, or expression at high intensity is considered to be complementary in support of lineage assignment [5, 7]. In T-ALL, the European Group of Immunophenotyping of Leukaemia (EGIL) proposed a classification in which T-ALL can be classified into subtypes corresponding to maturation stages of immature thymic T-cells, i.e. pro-T (cytoplasmic CD3+, CD2− CD1a−), pre-T (cytoplasmic CD3+, CD2+, CD1a−), cortical thymic-T (CD1a+, CD4/CD8 double positive), and the rare medullary/mature-T (membrane CD3+, CD4 or CD8 single positive, CD1a−) [5]. Cases of pro- and pre-T ALL correspond to the description of early T-cell precursor phenotype of CD1a and CD8 negativity, weak CD5 with co-expression of stem-cell or myeloid markers, and are associated with high remission failure and relapse risk [8]. Conversely, the CD1a cortical thymic T-ALL carries a relatively better prognosis [9, 10]. Similarly, in B-ALL, three broad maturation stages as determined by immunophenotyping are recognized, namely the early pro-B-ALL stage (CD34+, nuclear TdT+, CD10−), the intermediate or common B-ALL stage (CD10+, CD20− cytoplasmic m chain negative), and the pre-B-ALL stage (CD10+/−, cytoplasmic m chain+) [6]. Uncommonly, a B-ALL stage with surface immunoglobulin heavy chain without light chain expression, termed transitional pre-B-ALL, may be encountered. Maturation stage of B-ALL carries less of a prognostic significance, but has well-recognized clinical and genetic correlates. For example, the pro-B-ALL CD10− phenotype with CD15 and CD65 expression is associated with infant ALL and t(4;11), the CD9+, CD10+, CD34− phenotype is associated with t(1;19), and the CD10+, CD34+, CD13+, CD38 dim phenotype with Philadelphia positive ALL [11].

Acute leukaemias of ambiguous lineage are rare subtypes of leukaemia that show no clear evidence of differentiation along a single lineage (acute undifferentiated leukaemia) or one that expresses antigens belonging to more than one lineage (mixed phenotype acute leukaemia). In the former, a comprehensive panel of immunophenotyping antibodies fails to reveal lineage markers, and blasts typically bear a primitive phenotype of CD34, TdT, HLA-DR with or without CD38 expression. Blastic plasmacytoid dendritic cell neoplasm, NK-cell leukaemia, and rare AML subtypes such as basophilic leukaemia would need to be excluded prior to

making a diagnosis of acute undifferentiated leukaemia. In mixed phenotype acute leukaemia, the WHO definition for lineage assignment has greater specificity and has superseded the previous EGIL criteria [12].

4.3 Minimal Residual Disease Monitoring

Acute leukaemia is a heterogeneous disease in which outcome can be highly variable, depending on prognostic factors identified at diagnosis. These include patient-related factors, cytogenetics, and molecular genetics. For a few decades until now, the definition of complete remission (CR) has been based on morphology, where the bone marrow should contain less than 5% blasts. Assessment by morphology is limited by poor sensitivity as normal haematogones may be difficult to distinguish from abnormal leukaemia blast.

Minimal residual disease (MRD) denotes the presence of leukaemia cells below the threshold of 5% blasts used to define morphological remission. Numerous studies have consistently shown that presence of measurable MRD is associated with worse outcomes and is a powerful predictor of disease-free survival in acute leukaemia and a variety of other hematologic malignancies. The paradigm of MRD as a therapeutic goal and for guiding therapeutic decisions was first pioneered in the treatment of chronic myeloid leukaemia where the use of tyrosine kinase inhibitors was coupled with MRD detection. This MRD-directed treatment is also widely demonstrated in ALL and has been applied more belatedly to AML. The level of MRD which are measurable with current methods ranges from 1 leukaemia cell in 10^4 to 10^6 white cells. Methods for MRD quantification are based either on the detection of leukaemia cells by immunophenotyping by flow cytometry or on molecular genetic methods such as the detection of leukaemia-specific rearrangements of immunoglobulin and T-cell receptor genes, fusion gene transcripts by real-time quantitative polymerase chain reaction (RT-qPCR), and more recently, next-generation sequencing and digital droplet PCR. Each methodology has its own advantages and limitations as shown in Table 4.1. For AML and ALL, there is no one superior methodology for monitoring of disease, and they are usually dependent on availability.

The foundations provided by immunophenotyping of acute leukaemia at diagnosis serve as a starting point for MRD detection by flow cytometry. Notably, aberrant cross-lineage antigen expression (for example, expression of myeloid antigens on lymphoblasts), asynchronous antigen expression at odds with the expected pattern of appearance of antigens during normal maturation, and over or under-expression of antigens are hallmarks of leukaemia blasts.

Table 4.1 MRD methodology comparison

MRD method	Sensitivity	Advantages	Disadvantages
MFC–LAIP	10^{-3}–10^{-5}	• Sensitive	• Requires diagnostic sample
		• Widely available	• Requires fresh sample
		• Applicable to >90% of patients	• Extended antibody panel required
		• Rapid turnaround	• Does not take into account phenotypic shift
			• Limited standardization
MFC–DfN	10^{-3}–10^{-5}	• Sensitive	• Requires fresh sample
		• Widely available	• Significant operator experience required
		• No diagnostic sample required	• Limited standardization
		• Phenotypic shifts will not interfere with results	
		• Applicable to >90% of patients	
		• Rapid turnaround	
qPCR	10^{-4}–10^{-6}	• Sensitive	• Expertise required
		• Standardized	• Appropriate targets present in only approximately 60% of patients
			• Many mutations not suitable for MRD
			• Not readily available
			• Time-consuming
			• Labour-intensive
NGS	10^{-3}–10^{-5}	• Highly sensitive	• Confounded by pre-leukaemic mutations
		• Multiple mutations detectable	• Time-consuming
			• Not readily available
			• Expensive
			• Error rates lead to low sensitivity of mutated sequences
			• Not standardized

These qualitative and quantitative antigenic deviation of leukaemia blasts both in AML and ALL, which allow its differentiation from normal counterparts, form the principle of MRD detection by flow cytometry [13–15]. Two separate approaches have been used for assessing MFC MRD: (1) the leukaemia-associated immunophenotypes (LAIP) approach, which defines LAIPs at diagnosis and tracks these in subsequent samples; and (2) the different-from-normal (DfN) approach, which relies on the difference in immunophenotypes present in the remission sample as compared to a normal immunophenotype distribution. The advantages of DfN over the LAIP method are that it can be applied if information from diagnosis is not available and can detect new aberrancies, together with disappearance of aberrancies at diagnosis [16]. These 'immunophenotypic shifts' may emerge from leukaemia evolution or clonal selection. This ability to detect MRD without a baseline diagnostic sample is a less certain approach and in laboratories that use the DfN method, baseline LAIPs are also generally used for comparison. A combined 'LAIP-based DfN' approach has been advocated by the ELN MRD Working Party to evaluate MRD in acute leukaemia to obtain the highest degree of certainty [16].

In general, flow cytometry sensitivity ranges from 10^{-3} to 10^{-5} depending on the number of colours and reagent combinations used. Flow cytometry MRD sensitivity is on average 1 log lower than for molecular methods [17]. However, the sensitivity of flow cytometry can be increased to 1 in 10^5–10^6 by next-generation flow methods which in essence employ optimized reagent combinations to improve reagent performance and specificity, 10-fold increase of acquired cellular events for evaluation, and fully standardized sample processing and acquisition protocols which are reproducible between multi-center laboratories [18]. Next-generation flow has been exemplified in myeloma MRD, but its principles have been also employed in B-ALL MRD. As demonstrated elegantly by Theunissen et al. in B-ALL MRD [19] and reviewed by Donnenberg [20], one aspect of increasing the sensitivity of rare event MRD detection is by increasing the number of cellular events acquired for analysis, and a sensitivity of $\leq 10^{-5}$ can be reached when more than 4 million cells are acquired for analysis [19]. The caveat for sensitive flow cytometry MRD, if not all MRD studies, is the quality of the sample. Haemodiluted bone marrow samples could lead to false negative MRD results, and hence first draw bone marrow is recommended for MRD studies. The ELN MRD Working Party has recently published a technical guideline on MRD assessment in AML [16].

4.3.1 Flow Cytometry in ALL MRD

In the case of B-cell maturation, the immunophenotypic pattern of hematogones is tightly regulated, precise, and virtually invariant between individuals [13]. By application of several basic backbone markers used to delineate normal B-cell maturation, such as CD19, CD10, CD20, CD22, CD38, CD34, and CD45, more than 90% of B-ALL will show deviation from the normal pattern. Frequent aberrancies observed include overexpression of CD10, asynchronous expression of bright CD10 with CD20 which is a marker of relatively more mature B-cells, dim or absent CD38, absent CD45, and over-expression of CD19. Use of this "difference from normal" approach would help to overcome immunophenotypic shifts due to clonal emergence during therapy or relapse, which could lead to leukaemia blasts with an immunophenotype different from that at diagnosis. It would also enable MRD assessment even when the immunophenotype of the diagnostic sample is unknown or unavailable at the treating center. A standard panel which is applicable to the vast majority of ALL would obviate the need to tailor MRD panels to patient-specific immunophenotypic profiles which can be highly diverse, an obvious advantage to high volume clinical laboratories. Discovery of several discriminatory LAIP markers in addition to the aforementioned backbone markers has led to an increase in the applicability of flow cytometry to at least 98% of B-ALL [19, 21]. Chief among these are CD66c, CD58, CD123, CD73, CD86, and CD304 [21–23].

Ectopic presence of lymphoblasts in tissues that do not normally contain them is likewise an indication of residual disease. For example, in T-ALL, the presence of immature T-cells outside the thymus, such as the bone marrow or peripheral blood, is pathognomonic of residual disease, as marrow and blood compartments do not normally contain immature T-cells [11]. Immature B-cells are also not expected in the peripheral blood and hence finding of circulating immature B-cells is highly indicative of disease relapse or residual disease. In this regard, MRD assessment using peripheral blood for T-ALL may be of equivalent sensitivity as the use of bone marrow, as blood harbours similar levels of MRD as bone marrow in T-ALL [24]. However, this is not true of B-ALL, as the levels of MRD in blood in B-ALL are approximately 1–3 log lower than in the marrow [25].

The era of antibody-based targeted therapy has led to re-evaluation of strategies for flow cytometry MRD detection. Therapeutic antibodies targeting CD19, CD20, and CD22 typically lead to down-regulation and loss of membrane expression of these antigens. After treatment with anti-CD19

CAR-T cells for example, CD19 can no longer be used for gating for detection of B-lymphoblasts [26]. An alternative strategy using CD22, CD24, or CD79a as gating markers can be employed in order to avoid false negative MRD.

4.3.2 Flow Cytometry in AML MRD

To approach AML MRD by flow cytometry, an understanding of the disease biology, which differs somewhat from that of ALL, is required. AML is a heterogenous disease and each AML disease itself is comprised of heterogenous populations and complex genetic hierarchies that may not be tightly correlated to blast count [27]. The dominant leukaemia clone may be diminished after treatment, leaving other clones or subclones which were not overt at diagnosis. The leukemic clone may change between diagnosis and relapse due to genomic instability, or the clone responsible for relapse may be different from the one at diagnosis [27]. In terms of genetic MRD, it is recognized that certain mutations reflect the true AML cell burden and their persistence is associated with adverse outcome (for example *RUNX1*, *KIT*, *NRAS/KRAS,* and fusion transcripts), whereas other mutations may reflect a precursor clonal hematopoiesis rather than the AML clone itself and their persistence may not be associated with worse outcome (for example *DNMT3A*, *TET2*) [28].

As mentioned, there are two main approaches to flow cytometry AML MRD detection. The LAIP method, where LAIP are identified at diagnosis and then the leukaemia blast are tracked using these markers or the DfN method, whereby leukaemia cells are identified as cells that express antigen combinations not normally seen in normal marrows [16, 27]. In reality, there is little difference between the two approaches as LAIP are in fact different from normal. A combination of both methods is recommended [16]. With these approaches, flow cytometry can be applicable in up to 90% of AML [29].

Although there are numerous published studies on the prognostic value of flow cytometry MRD, these studies used a variety of different time point assessments, ranging from mid induction to post-induction, post-consolidation or pre-transplantation, different threshold MRD levels used to define MRD positivity ranging from 0.1–0.03%, and different outcome measures such as overall survival, event free survival, relapse free survival, and relapse rate. The main limitation in translating these studies to clinical practice was the lack of consensus as to the MRD threshold to define positivity and the clinically relevant time points at which action, that is intensification of therapy or subjecting a patient to transplant, should be taken for MRD positivity. The European LeukaemiaNet MRD working party in 2018 published a consensus document in an effort to standardize these issues [16]. A threshold of 0.1% was found to be relevant in most published studies and more broadly achievable and was hence recommended. Of note, this threshold is higher than the recommended threshold of 0.01% (10^{-4}) for ALL MRD. The optimal time points of MRD studies have not been established for flow cytometry, but extrapolated from the recommendations for molecular MRD; it is suggested after 2 cycles of chemotherapy and at end of treatment [16].

The approach to flow cytometry MRD for AML is evolving to include the analysis of the earliest stem cell compartment and to identify leukaemia stem cells which are thought to be quiescent cells responsible for disease relapse [16, 29–31]. Leukaemia stem cells are thought to express a immunophenotype akin to hematopoietic stem cells, i.e. CD34 positive, CD38 negative, CD117 dim, CD45RA negative, and CD133 positive, but with aberrancies not normally seen in their normal counterparts. Markers to detect and evaluate leukaemia stem cells are being incorporated into AML MRD panels [30, 32]. These will serve as good starting points in evaluating the prognostic significance of stem cell MRD persistence in AML [16].

4.3.3 Technical Considerations in Flow Cytometric MRD

The technique employed in flow cytometry MRD studies is complex and requires rigorous technical performance. Well-validated processing steps to avoid cell loss, careful antibody titration, compensation setup, consistent quality control, and daily instrument calibration must be performed. Consideration expertise is required when interpreting the results, as a thorough knowledge of the immunophenotypic patterns associated with normal haematopoiesis and therapy-related changes is essential before interpreting abnormal samples. In this regard, interpretation of AML MRD is considerably more challenging than ALL MRD as the marrow myeloid compartment shows greater complexity, inherently contain a multitude of different cell types, and myeloblasts themselves may show immunophenotypic heterogeneity and subclones of variable immunophenotype and genetic mutations. In addition, non-specific immunophenotypic changes related to non-malignant processes, such as CD56 expression on myeloid precursors post-G-CSF and in regenerating marrows, dim CD7 in small subsets of myeloid precursors post-chemotherapy, and therapy-induced changes such as glucocorticoid-induced loss of immature phenotype from loss of CD10 and CD34 on B-lymphoblasts may happen.

Research is focused on improving the method by defining threshold cut-off values as well as generating standards to equalize data among different instruments and software programs [33–35]. A study by Feller et al. further defined LAIPs and evaluated whether data from an established MRD monitoring laboratory could be replicated in four centres with no significant prior experience [36]. With discussion, the inex-

perienced laboratories had a success rate of 82% to 93% for defining at least one LAIP. When two LAIPS were present, an additional 9% to 20% of cases would have resulted in false negatives by the inexperienced centres. The authors proposed the design of redundant panels to account for immunophenotypic shift to minimize false negatives [36]. Inconsistencies in LAIPs with MRD of 0.1% or lower may be resolved with the use of a greater number of fluorochromes [35]. We would encourage MRD monitoring to be performed in core facilities to minimize variability and also encourage enrolment in clinical trials that provide MRD monitoring.

On the other hand, there are considerable advantages afforded by flow cytometry for MRD. Besides the obvious advantage of faster turnaround time, the advantage of flow cytometry relates to the fact that aberrancies can be mapped to the abnormal cell. In contrast, molecular and genetic MRD techniques interrogate the bulk marrow and may detect genetic changes in clones unrelated to the leukaemia clone [28].

4.4 Minimal Residual Disease Studies in Acute Lymphoblastic Leukaemia

Other than the conventional risk criteria for ALL, such as age, elevated white cell count at diagnosis, adverse immunophenotypic and cytogenetic features as well as late achievement of CR, the status of MRD has been considered as an additional prognostic marker. Multiple studies in paediatric and adult ALL patients have supported the significance of MRD negativity as a prognostic marker for CR duration, reduced risk of relapse, and HSCT success [37–39]. In recent years, the European Society for Medical Oncology (ESMO), European Leukemia Network, and National Comprehensive Cancer Network clinical practice guidelines for adult patients with ALL recommend the quantification of MRD to guide treatment [40–42]. MRD positivity post-treatment is recognized as an accepted risk factor and considered as an indication for HSCT as highlighted in the consensus paper of EWALL and AWLP EBMT experts [43].

Although MRD is recognized as being an important assessment tool in ALL, the practical implications have yet to be agreed upon and there is no consensus on the test methodology, the time points of testing, and the sensitivity of the assay used. The difficulty arises as the use of MRD testing is specific for each study protocol. ALL studies have utilized MFC or PCR to measure MRD. Most studies have utilized one or the other or a combination of both; for this chapter, we will mainly be focusing on studies that utilized MFC to measure MRD.

4.4.1 MRD Assessment in Childhood ALL

In a study in children with ALL, patients with detectable MRD (flow cytometry sensitivity level $< 1 \times 10^{-4}$) at the end of induction therapy had a significantly higher cumulative incidence of relapse (CIR) and a higher relapse rate than those who were MRD negative [44, 45]. Persistence of MRD through week 14 of continued treatment was associated with higher risk of relapse compared with patients who became MRD negative by 14 weeks (68% vs. 7%) [45].

MRD assessments at an earlier time point in the course of treatment (e.g. during induction therapy) have been shown to be highly predictive of outcomes in children with ALL. In one study, nearly 50% of patients had MRD clearance (MRD $<1 \times 10^{-4}$ by flow cytometry) before day 19 of induction therapy; the 5-year CIR was significantly higher among patients with MRD at day 19 of treatment than those without detectable MRD (33% vs. 6%) [46]. This early predictive value of MRD response was demonstrated in the AIEOP-BFM-ALL (Associazione Italiana Ematologia Oncologia Pediatrica and Berlin-Frankfurt-Münster Study Group) 2000 trial, where bone marrow MRD positivity at day 15 was the most powerful early predictor of relapse, applicable to virtually all patients [47]. This raises the possibility of identifying patients with high-risk disease who may potentially benefit from earlier intensification or tailoring of treatment regimens, or for potentially allowing less-intensive treatments to be administered in patients at low risk for relapse based on early MRD measurements.

4.4.2 MRD Assessment in Adult ALL

In an early small study looking at post-induction MRD (flow cytometry sensitivity level $< 0.05\%$) in adult patients with ALL, median RFS was significantly longer among patients with MRD less than 0.05% at day 35 compared with those with MRD of 0.05% or greater (42 vs. 16 months). This was also seen when only patients in morphological CR were analysed. Although this study used a cut-off level that we would now deem MRD positive, these early results highlighted the importance of MRD assessment [48].

Further studies evaluating post-induction MRD demonstrate that MRD negativity is an independent predictor of relapse even among adult patients considered to be standard risk based on traditional prognostic factors. In a study of adult patients with Ph-negative ALL ($n = 116$), MRD status after induction therapy ($<1 \times 10^{-3}$) was significantly predictive of relapse (9% vs. 71%, 3-year CIR) regardless of whether the patient was standard risk or high risk at initial evaluation [38]. In a prospective study from the MDACC,

340 adult patients with B-ALL were monitored for MRD by MFC (sensitivity level = ($<1 \times 10^{-4}$) at CR and at approximately 3-month intervals after CR. MRD negative status at CR significantly correlated with improved disease-free survival (DFS) and overall survival (OS) and was an independent predictor of DFS [49].

Different levels of detectable MRD may also have prognostic value, and risk of relapse is proportional to the quantity of MRD in several studies. For example, in one study, patients with lower detectable MRD (between 10^{-4} and 10^{-3}) by either ASO-PCR or MFC had significantly longer duration of remission, RFS, and OS than those with very high MRD ($\geq 10^{-1}$) [50].

In a meta-analysis involving 13,637 children and adults, the benefit of MRD negativity across disease subtypes (e.g. Ph-negative and Ph-positive, B-lineage and T-lineage), therapies, methods, timing of MRD assessment, and MRD cutoffs was demonstrated. In adults, the 10-year event-free survival (EFS) for patients who achieved MRD negativity was 64% compared with 21% for those with detectable MRD [hazard ratio (HR), 0.28; 95% confidence interval (CI): 0.24–0.33]. A significant OS benefit to achieving MRD negativity was also observed in children (HR, 0.28; 95% CI: 0.19–0.41) and adults (HR, 0.28; 95% CI: 0.20–0.39) [51]. A subsequent meta-analysis of 23 published articles reporting on MRD in adults with B-cell ALL confirmed an overall improvement in both RFS and OS with random effects HRs of 2.44 (95% CI: 1.91–2.86) and 2.19 (95% CI: 1.63–2.94), respectively, for patients achieving MRD negativity [52].

4.4.3 MRD Assessment in Hematopoietic Stem Cell Transplant for ALL

Most initial studies evaluating MRD as a prognostic factor in the pre-transplant setting utilized PCR-based techniques. Nonetheless, all these early studies demonstrated that in ALL [53–56], and subsequent studies, the presence of MRD resulted in impaired PFS. Several studies have suggested that allogeneic stem cell transplant (allo-SCT) in first CR is associated with lower risk of relapse and longer survival in patients with ALL who achieve a suboptimal MRD response [54, 57]. In the German Multicenter ALL Study Group (GMALL 07/03 trial), patients with persistent MRD ($\geq 10^{-4}$) measured by RT-qPCR after first consolidation (week 16) were considered high risk for relapse and were offered allo-SCT. Overall, 47% of patients with MRD persistence received allo-SCT in first CR. The 5-year continuous CR and OS was significantly higher for patients who received allo-SCT in first CR compared with those with chemotherapy alone (66% vs. 12% CR rates and 54% vs. 33% OS) [55].

Dhedin et al. demonstrated that allo-SCT improved relapse-free survival versus chemotherapy alone in patients with ALL who had MRD $\geq 10^{-3}$ after induction [54]. Utilizing MFC, Bar et al. and Brammer et al. demonstrated in both B and T-cell ALL, patients with MRD positivity prior to transplant had a significantly higher rate of progression compared to those with MRD negativity (61–76% vs. 34%) [58, 59]. The timing of allo-SCT affects survival as patients transplanted in first complete remission (CR1) had a 3-year OS of 62% versus those transplanted in CR2 or greater (24%) (hazards ratio 1.6, $p = 0.2$) [59].

Brammer et al. demonstrated in 102 T-cell patients who achieved early MRD negativity (week 16) had a 5-year continuous CR and 5-year OS rates of 74% and 81%, respectively, in the absence of allo-SCT [59]. A larger prospective study by PETHEMA ALL-AR-03 in adolescents or adults with high-risk Ph-negative ALL based on at least one high-risk disease feature (i.e. age between 30 and 60 years, white blood cells >30 × 109/l, or t(4;11) or other MLL rearrangements) was assigned to post-remission therapies based on early cytologic response (<10% blasts in bone marrow at day 14 of induction) and MRD status. Patient with favourable cytologic and MRD response continued to receive chemotherapy alone ($n = 108$) and those with poor cytologic response or suboptimal MRD response ($n = 71$) were assigned to receive allo-SCT. The 5-year DFS and OS were 32% and 37%, respectively, for patients assigned to allo-SCT, and 55% and 59% for those assigned to chemotherapy [60].

Together, these studies suggest that MRD assessment after initial chemotherapy can be used to identify patients most likely to benefit from allo-SCT in first remission, even among patients who appear otherwise high-risk based on pretreatment characteristics. Persistence of MRD at early evaluation should be a determining factor for proceeding with allo-SCT at first remission. They also highlight the relatively poor outcomes for patients with persistent MRD positivity, even when allo-SCT is performed.

4.4.4 MRD Assessment in Relapse ALL

MRD reappearance after initial chemotherapy or allo-SCT is also a sign of impending leukaemia relapse. Eighty percent of patients subsequently developed morphological relapse after a median of 3 months (range, <1–33 months) from detection of MRD recurrence. The 5-year CR duration and 5-year OS were 10% and 14%, respectively. Post-relapse, patients who underwent allo-SCT had a statistically significant advantage for both median DFS and median CR duration, but there was no survival benefit [61].

Despite good treatment outcome, approximately 20% of children treated with intensive therapies for ALL will ultimately experience disease relapse. In patients who experienced a second remission (morphologic CR) after reinduction

treatment, MRD positivity after reinduction was significantly associated with risks for relapse; the 2-year CIR was 70% among patients with MRD $\geq 1 \times 10^{-4}$ versus 28% among those with MRD $<1 \times 10^{-4}$. In addition, in the subgroup of patients who experienced first relapse after cessation of treatment, the 2-year CIR of second relapse was 49% in patients with MRD $\geq 1 \times 10^{-4}$ versus 0% for those with MRD $<1 \times 10^{-4}$. Both the presence of MRD at day 36 of reinduction therapy and at first relapse occurring during therapy were significant independent predictors of second relapse based on multivariate analysis [45].

With the advent of cellular therapy, MRD assessment remains a goal as in studies evaluating single novel agents (e.g. blinatumomab and inotuzumab ozogamicin); MRD negativity was associated with lower rates of relapse [62, 63]. In a single arm phase II study of 36 patients with R/R B-cell ALL treated with blinatumomab, 69% of patients achieved complete remission with full hematologic recovery (CR) or complete remission with partial hematologic recovery (CRh), and 88% of responders achieved a MRD response within the first 2 treatment cycles. MRD response was associated with significantly longer OS [62]. This led to the phase II BLAST study where adult patients with B-cell ALL in CR but with persistent or recurrent MRD at a level of $\geq 10^{-3}$ after intensive chemotherapy received up to 4 cycles of blinatumomab. Among 116 patients, 78% achieved complete MRD response after the first cycle. Despite the inclusion of higher-risk patients (35% of patients in second or later remission and 47% with MRD levels $\geq 10^{-2}$), the 18-month RFS rate was 54% and the median OS was 36.5 months. Patients who achieved complete MRD response had significantly longer RFS (23.6 vs. 5.7 months) and OS (38.9 vs. 12.5 months) compared with MRD non-responders [64]. Based on these results, blinatumomab was approved by the US FDA in March 2018 for the treatment of patients with B-cell ALL in CR, but with detectable MRD at a level of $\geq 10^{-3}$ [65].

In The INO-VATE trial comparing inotuzumab ozogamicin versus combination chemotherapy for patients with relapse B-cell ALL, MRD negativity was achieved in 63% of patients in the inotuzumab ozogamicin arm, and MRD response was associated with prolongation of both PFS and OS compared with MRD non-responders (median PFS: 8.6 vs. 5.4 months; median OS: 14.1 vs. 7.2 months) [63].

Other immunotherapeutic strategies include the use of CD19-directed chimeric antigen receptor (CAR) T cells for MRD eradication. In a phase I trial, 53 adult patients with relapse B-cell ALL received autologous CD19 CAR T cells and achieved a CR rate of 83%. Patients with low disease burden (defined as <5% bone marrow blasts) had significantly longer EFS and OS compared with patients with higher disease burden (defined as ≥5% bone marrow blasts or presence of extramedullary disease; median EFS: 10.6 vs. 5.3 months; median OS: 20.1 vs. 12.4 months, respectively). These findings suggest that CAR-T cells may play a particularly important role in the management of MRD positive disease, where such therapy may be curative for a subset of patients [66].

4.5 Minimal Residual Disease in Acute Myeloid Leukaemia

As with ALL, the presence of MRD has been shown in numerous studies to adversely affect the outcome of AML treatment in younger as well as older patients [31, 67–69]. The 2017 European LeukemiaNet guidelines suggest monitoring of MRD for AML and have proposed a response category of CR without minimal residual disease, which is associated with a lower risk of relapse. Similarly, to ALL, limitations include a lack of international consensus on the test methodology, the time points of testing, and the sensitivity of the assay used. The difficulty arises as the use of MRD testing is specific for each study protocol.

4.5.1 MRD Assessment in Adult AML

In studies of patients <65 years of age with AML who were fit to receive cytosine arabinoside plus anthracycline-based induction and consolidation chemotherapy, MRD negative status as detected by MFC was identified as the most important independent predictor of RFS and OS [31, 67, 68, 70]. The HOVON/SAKK groups looked at 398 AML patients between 18 and 60 years old, enrolled in the AML 42A study [68]. MRD was evaluated at three times points: after cycle 1, cycle 2, and consolidation therapy. At each time point, MRD negativity predicted lower relapse rate and better relapse-free survival and overall survival. Multivariate analysis after cycle 2 showed that MRD level $\geq 10^{-3}$ was associated with a higher risk of relapse.

The UK MRC group studied 472 patients older than 60 years old in the AML16 trial, who achieved complete remission [69]. MRD was measured by MFC after the first or second cycle of chemotherapy. MRD negative patients had a better 3-year survival from CR, compared to MRD positive patients (Cycle 1: 42% vs. 26%, Cycle 2: 38% vs. 18%). MRD positive patients had a higher risk of early relapse (median time to relapse 8.5 vs. 17.1 months). In multivariate analysis, MRD status was found to be an independent prognostic factor. A study published by the PATHEMA group included 306 patients with AML who underwent MRD monitoring, who were mostly younger than 65 years old [70]. The authors found that MRD levels defined the high-risk (≥ 0.1%), intermediate-risk (≥0.01–0.1%), and low-risk (<0.01%) categories, with RFS of 38%, 50%, and 71%,

respectively. Multivariate analysis identified age, MRD, and cytogenetics and independent variables. In a study by Ravandi et al., 166 AML patients younger than 65 years treated in two different trials underwent MRD assessment at various time points. At all-time points, MRD negative patients had better RFS and OS. In multivariate analysis, achieving MRD negative status was the most significant prognostic factor at all three time points studied [67]. A large study on 1076 patients with AML, from 60 hospital participating in the PATHEMA registry, showed that MRD level of $\leq 10^{-3}$ is predictive of CIR and OS [71]. However, the study found that decentralized MRD testing had limited prognostic value in multivariate analysis. This was attributed to heterogeneity of testing methodology and reporting among the different laboratories.

4.5.2 MRD Assessment in Paediatric AML

Similar to adult patients with AML, MRD has important prognostic implications in pediatric patients with AML. The AML-BFM study group analysed the MRD levels in 150 patients with AML enrolled in the BFM 98 study. The presence of MRD detected by flow cytometry was found to be predictive of EFS at all time points, but in multivariate analysis the presence of MRD was no longer statistically significant [72]. Van der Velden et al. analysed 94 children with AML enrolled in the UK MRC AML12 and the GCOG ANLL97 trials [73]. 3-year RFS was 85% for MRD negative patients (MRD < 0.1%), 64% for MRD low-positive patients (≥0.1 to <0.5%), and 14% for MRD-high-positive patients (≥ 0.5%), whereas OS was 95%, 70%, and 40%, respectively. Multivariate analysis showed that MRD after the first course on chemotherapy was an independent prognostic factor [73].

In larger studies comprising of more than 1000 patient samples, MRD monitoring is able to predict an unfavourable EFS that was statistically significant [74–76]. The COG AAML03P1 protocol demonstrated that at the end of the first induction, even at morphological CR, 24% of patients were still MRD positive [75]. MRD positive and negative patients had 3-year relapse risk of 60% and 29%, respectively, and the RFS was 30% and 65%. In multivariate analysis, persistent MRD positivity was an independent predictor of relapse [75]. Tierens et al. analysed the results of 101 patients in the NOPHO-AML 2004 study, who had MRD measured by flow cytometry at day 15 and before consolidation therapy [77]. EFS and OS were significantly different in patients with and without MRD. In multivariate analysis, MRD before consolidation therapy was the strongest independent prognostic factor for EFS [77]. These studies suggest that MRD persistent and the inability to obtain MRD negativity at earlier time points are associated with worse prognosis.

4.5.3 MRD Assessment in Hematopoietic Stem Cell Transplant for AML

In a study by the EORTC/GIMEMA group [78], 34 patients on the AML-10 (18 to 60 years old) protocol and 22 patients on the AML-13 (more than 60 years old) protocol were analysed. The AML-10 protocol included induction, consolidation, and autologous hematopoietic stem cell transplantation (ASCT). The AML-13 protocol included induction, consolidation, and consolidation. MRD positive status after consolidation was associated with shorter OS and RFS. It was also found that ASCT did not alter the prognostic effect of high MRD levels after consolidation, as the relapse rate after transplantation was 70%. In subsequent report [34] which included more patients on the same protocols, MRD positive patients either underwent autologous or allogenic stem cell transplant. Eighty-two percent of those who underwent ASCT experienced relapse as compared to 43% who had allo-SCT. This showed that MRD positive patients do not benefit from ASCT, but should instead receive allo-SCT.

Further evidence came from a series of studies conducted by Walter's group from Fred Hutchinson Cancer Research Center. In the first study [79], 99 patients who underwent myeloablative HCT in first CR were retrospectively studied for the MRD status before transplantation. Twenty-four out of the 99 patients were MRD positive. Two-year OS was 30.2% for MRD positive patients and 76.6% for MRD negative patients, while relapse rate was 64.9% for MRD positive patients and 17.6% for MRD negative patients. In multivariate analysis, MRD positive HCT was associated with increased overall mortality and relapse. In the second study [80], the authors examined the prognostic significance of MRD for AML patients undergoing myeloablative HCT in second complete remission (CR2) compared to those in first complete remission (CR1). The study found that the negative impact of pre-HCT MRD is similar for AML in CR1 or CR2. In the third study [81], the authors studied 86 AML patients who received non-myeloablative (NMA) conditioning and 115 who received myeloablative (MA) conditioning, who has MRD measured before HCT. The study concluded that the negative impact of MRD on relapse risk is similar regardless of conditioning chemotherapy. In a fourth study [82], the authors studied 359 AML patients who underwent HCT. Three-year relapse estimates were 67% in patients in MRD positive morphologic remission, 65% in active AML, and 22% in MRD negative remission. Three-year OS were 26%, 23%, and 73%, respectively. In multivariate analysis, MRD negative remission status was associated with longer OS and PFS, as lower risk of relapse. The outcomes were similar in the patients with MRD positive morphologic remission and those having active disease. Another study showed similar findings, where pre-transplant staging with flow cytometry demonstrated similar outcomes in 3-year OS

and PFS estimates between patients with MRD positive morphologic remission and patients with active disease (26% vs. 23% and 12% vs. 13%, respectively) when compared to patients in MRD negative remission (73% and 67%, respectively) [82].

The similarities in outcomes between patients in MRD positive morphologic remission and those with active disease at the time of HCT support the use of treatment algorithms that use MRD rather than morphology-based disease assessments.

4.5.4 MRD Assessment in Post Remission AML

At present, there are few published studies that have evaluated MRD stratification and therapeutic strategies to eradicate MRD in patients with AML. Other than recent trials using FLT3 inhibitors as a maintenance therapy [83, 84], it is unclear if further treatment is needed post consolidation or allo-SCT if MRD is still detectable.

Azacitidine has been shown to increase expression of epigenetically silenced leukaemia antigens and induce a $CD8^+$ T-cell response to tumour antigens post-transplant, potentially augmenting a graft-versus-leukaemia effect. At least two studies have evaluated pre-emptive use of azacitidine after allo-SCT based on detection of MRD. The RELAZA phase 2 study evaluated azacitidine after allo-HSCT in 20 patients with $CD34^+$ AML or myelodysplastic syndromes who are not in morphological remission but MRD positive [85]. After 4 cycles of azacitidine, 80% were MRD negative; with 4 out of 10 patients remaining MRD negative at a median follow-up of 347 days. The investigators noted that tracking MRD after allo-HSCT via peripheral blood $CD34^+$ donor chimerism monitoring allowed pre-emptive use of azacitidine only when MRD was detected, avoiding unnecessary toxicity in patients in CR at low risk of relapse.

With the advent of oral azacitidine, interest in maintenance therapy in AML was re-newed. In the phase III QUAZAR AML-001 trial, maintenance therapy was associated with prolonged OS and RFS in all patient subgroups when compared to placebo regardless of MRD status. However, after controlling for treatment arm, the presence of MRD at study entry was significantly associated with worse OS (HR, 0.74; $p < 0.0001$) and RFS (HR, 0.63; $p < 0.0001$) compared with MRD negativity [86].

4.6 Conclusion

Multi-parametric flow cytometry has revolutionized the diagnosis and monitoring of acute leukaemias. It has enabled accurate characterization of acute leukaemia at diagnosis and although a certain technical expertise is required, most laboratories are able to support the basic flow laboratory.

Minimal residual disease has been demonstrated to have prognostic significance in both ALL and AML. However, monitoring of acute leukaemias by flow cytometry relies heavily on expertise that may not be readily available. Other than technical limitations, certain leukaemias, such as acute promyelocytic leukemia, are better monitored using molecular techniques. For the majority of ALL and AML, flow cytometry is a recognized tool for diagnosis and monitoring for better management of disease.

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5 Genomic Landscape and Risk Stratification of Acute Myeloid Leukemia

Hsin-An Hou

Abstract

Acute myeloid leukemia (AML) is a heterogeneous hematologic malignancy based on its clinical features, underlying pathogenesis, and treatment outcomes. Recent advances in genomic sequencing have revealed the molecular complexity of AML leukemogenesis, thereby resulting in the refinement of risk stratification, prognostication, and personalized therapeutic strategies for patients with AML. Annotation of the mutational landscape in AML has markedly facilitated the refinement of the current classification and risk stratification systems. In this chapter, we summarize the most relevant genetic markers in AML, with a special focus on the prognostic relevance and risk stratification of these aberrations.

Keywords

Acute myeloid leukemia · Genomics · Leukemogenesis
Risk · Prognosis

Acute myeloid leukemia (AML) is a clonal hematological malignancy with wide variability in clinical features, pathogenesis, and treatment outcomes [1]. The incidence of AML has increased over time, with males at a higher risk of developing AML [2]. It is the most common form of acute leukemia affecting adults and accounts for the highest percentage of leukemia-related deaths [3]. The long-term survival in *de novo* patients younger than 60 years is approximately 30–50% and less than 10% in older patients and those with secondary or therapy-related AML [4–6], thereby highlighting the urgent need for better risk stratification and novel treatment strategies. Herein, we provide an overview of the most relevant genomic biomarkers in AML, the current genetic risk stratification systems, and their clinical implications.

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5.1 Classification of AML Changes and Advances in Biological Techniques

AML was first classified by the French-American-British (FAB) Cooperative Group in 1976 according to cell lineage and the extent of differentiation of leukemic cells based on the cell morphology and cytochemical staining of bone marrow (BM) cells [7]. However, the process of precise stratification and prediction of disease outcomes for this heterogeneous disease is still limited. The identification of recurrent cytogenetic abnormalities would facilitate a deeper understanding of AML biology and drive better decision making in risk stratification and treatment [8–10]. Nevertheless, 45–50% of patients with AML do not harbor any chromosomal aberrations at diagnosis and are classified as having cytogenetically normal (CN)-AML [10, 11]. In 2001, the World Health Organization (WHO) introduced a new classification system (WHO-2001), including recurrent cytogenetic abnormalities as an AML diagnostic and management criteria [12], which was subsequently revised in 2008 (WHO-2008) [13]. In WHO-2008, AML with 11q23 (*MLL*) abnormalities in WHO-2001 criteria had been redefined to focus on AML with t(9;11)(p22;q23); *MLLT3-MLL*. Translocations of *MLL* other than that involving *MLLT3* should be specified. Three new cytogenetically defined entities were added: (1) AML with t(6;9)(p23;q34); *DEK-NUP214*, (2) AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1*; and (3) AML (megakaryoblastic) with t(1;22)(p13;q13); *RBM15-MKL1*. Two provisional entities were also added: AML with mutated *NPM1* and AML with mutated *CEBPA*. AML with multilineage dysplasia was changed and expanded to AML with myelodysplasia-related changes (AML-MRC).

Advances in genomic techniques and researches have greatly improved our understanding of cancer biology. High-throughput next-generation sequencing (NGS), including whole-genome or whole-exon profiling, has revealed that more than 95% of patients with AML have driving and co-

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concurring mutations irrespective of the presence of cytogenetic abnormalities [14, 15]. The Cancer Genome Atlas (TCGA) first proposed nine subcategories of genetic alterations, comprising those involving transcription-factor fusions, myeloid transcription-factor genes, *nucleophosmin 1* (*NPM1*), tumor suppressors, signaling genes, DNA methylation, chromatin modifiers, cohesin complex, and splicing factors, in mutational patterns of AML leukemogenesis [14]. Accordingly, Dr. Papaemmanuil targeted the sequencing of 111 cancer genes in 1540 patients with AML and identified 11 classes with distinct clinical and prognostic features [15]. In addition to the nine AML subgroups, three heterogeneous genomic categories were identified: AML with mutations in chromatin and/or RNA-splicing regulators, AML with *TP53* mutations and/or chromosomal aneuploidies, and AML with *isocitrate dehydrogenase 2* (*IDH2*) R712 mutations [15]. Consequently, the genetic hierarchy of AML can be categorized according to the functional consequences of these molecular events [4, 15–17].

To improve diagnostic accuracy, the Society for Hematopathology and the European Association for Haematopathology in collaboration with the WHO released the fourth edition of the *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues* (the 2016 WHO classification, WHO-2016) as a part of the second volume of the "Blue Book" series, which was based on cytogenetic and genomic data. The WHO-2016 classification system defined six subtypes of AML and related neoplasms: AML with recurrent genetic abnormalities (either cytogenetic or molecular genetic), AML with MRC, therapy-related myeloid neoplasms, AML, not otherwise specified (NOS), myeloid sarcoma, and myeloid proliferations of Down syndrome (Table 5.1) [1].

Recent advances and breakthroughs in NGS techniques [18] have deepened our understanding of the pathobiology of myeloid neoplasms. In 2022, the fifth edition of the WHO classification system emphasized the integration of clinical, molecular, and pathologic parameters in AML diagnosis (WHO-2022; Table 5.2) [19]. This classification highlighted two points: (1) the elimination of the requirement of 20% blasts for diagnosing AML types with defined genetic abnormalities, except for AML with *BCR*::*ABL1* and *CCAAT/enhancer binding protein alpha* (*CEBPA*) mutations. AML with *NPM1* mutations can be diagnosed irrespective of the blast count; and (2) AML was divided into two families: AML with defined genetic abnormalities and AML defined by differentiation. AML, NOS was no longer used. The definition of AML with *CEBPA* mutations changed to include biallelic and single mutations located in the basic leucine zipper (bZIP) region of the gene. Concomitantly, the 2022 International Consensus Classification (ICC) recategorized myeloid neoplasms based on the introduction of new entities and a refined criteria of existing diagnostic categories (Table 5.3) [20]. ICC expanded the subtypes that can be diagnosed as AML with ≥10% blasts to encompass additional recurrent genetic abnormalities, in addition to the originally defined acute promyelocytic leukemia and core binding factor (CBF) leukemia. AML with a *TP53* mutant, including myelodysplastic syndrome (MDS) with mutated *TP53*, MDS/AML with mutated *TP53,* and AML with mutated *TP53*, is now recognized as a separate entity among myeloid neoplasms. Finally, diagnostic qualifiers such as therapy-related, progressing from MDS, or MDS/myeloproliferative neoplasm (MPN) should be used following a specific AML diagnosis to prevent confusion due to the substantial overlap of prior categories.

Table 5.1 WHO 2016 classification of AML and related neoplasms

AML with recurrent genetic abnormalities
AML with t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
APL with *PML-RARA*
AML with t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*
AML with t(6;9)(p23;q34.1); *DEK-NUP214*
AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2, MECOM*
AML (megakaryoblastic) with t(1;22)(p13.3;q13.3); *RBM15-MKL1*
AML with mutated *NPM1*
AML with biallelic mutations of *CEBPA*
AML with myelodysplasia-related changes
Therapy-related myeloid neoplasms
AML, NOS
AML with minimal differentiation
AML without maturation
AML with maturation
Acute myelomonocytic leukemia
Acute monoblastic/monocytic leukemia
Pure erythroid leukemia
Acute megakaryoblastic leukemia
Acute basophilic leukemia
Acute panmyelosis with myelofibrosis
Myeloid sarcoma
Myeloid proliferations related to down syndrome
Transient abnormal myelopoiesis (TAM)
Myeloid leukemia associated with down syndrome

NOS not otherwise specified
Adapted from Arber et al. [1]

Table 5.2 WHO 2022 classification of AML

Acute myeloid leukemia with defining genetic abnormalities
Acute promyelocytic leukemia with *PML*::*RARA* fusion
Acute myeloid leukemia with *RUNX1*::*RUNX1T1* fusion
Acute myeloid leukemia with *CBFB*::*MYH11* fusion
Acute myeloid leukemia with *DEK*::*NUP214* fusion
Acute myeloid leukemia with *RBM15*::*MRTFA* fusion
Acute myeloid leukemia with *BCR*::*ABL1* fusion
Acute myeloid leukemia with *KMT2A* rearrangement
Acute myeloid leukemia with *MECOM* rearrangement
Acute myeloid leukemia with *NUP98* rearrangement
Acute myeloid leukemia with *NPM1* mutation
Acute myeloid leukemia with *CEBPA* mutation
Acute myeloid leukemia, myelodysplasia-related
Acute myeloid leukemia with other defined genetic alterations
Acute myeloid leukemia, defined by differentiation
Acute myeloid leukemia with minimal differentiation
Acute myeloid leukemia without maturation
Acute myeloid leukemia with maturation
Acute basophilic leukemia
Acute myelomonocytic leukemia
Acute monocytic leukemia
Acute erythroid leukemia
Acute megakaryoblastic leukemia

Adapted from Khoury et al. [19]

Table 5.3 International Consensus Classification (ICC) of AML with percentage of blasts required for diagnosis

APL with t(15;17)(q24.1;q21.2)/*PML::RARA* ≥ 10%
APL with other *RARA* rearrangements ≥10%
AML with t(8;21)(q22;q22.1)/*RUNX1::RUNX1T1* ≥ 10%
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/*CBFB::MYH11* ≥ 10%
AML with t(9;11)(p21.3;q23.3)/*MLLT3::KMT2A* ≥ 10%
AML with other *KMT2A* rearrangements ≥10%
AML with t(6;9)(p23;q34.1)/*DEK::NUP214* ≥ 10%
AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/*GATA2; MECOM (EVI1)* ≥ 10%
AML with other *MECOM* rearrangements ≥10%
AML with other rare recurring translocations ≥10%
AML with t(9;22)(q34.1;q11.2)/*BCR::ABL1* ≥ 20%
AML with mutated *NPM1* ≥ 10%
AML with in-frame bZIP *CEBPA* mutations ≥10%
AML with mutated *TP53* (any somatic mutation, VAF > 10%) ≥20%
AML with myelodysplasia-related gene mutations ≥20% (defined by mutations in *ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, or ZRSR2*)
AML with myelodysplasia-related cytogenetic abnormalities ≥20%[a]
AML not otherwise specified (NOS) ≥20%
Myeloid sarcoma

Adapted from Arber et al. [20]

[a]Defined by detecting a complex karyotype (≥ 3 unrelated clonal chromosomal abnormalities in the absence of other class-defining recurrent genetic abnormalities), del(5q)/t(5q)/add(5q), 27/del(7q), 18, del(12p)/t(12p)/add(12p), i(17q),217/add(17p) or del(17p), del(20q), and/or idic(X)(q13) clonal abnormalities

5.2 Genomic Landscape in AML

In early 2000, a two-hit model suggested that AML development requires cooperation between at least two classes of gene mutations [21, 22]. Class I mutations that involve mutations in genes, such as *RAS, FMS-like tyrosine kinase 3* (*FLT3*), *KIT, PTPN11,* and *JAK2,* that are involved in the kinase signaling pathways result in cell survival and proliferation and Class II mutations, such as t(15;17)/*PML-RARA*, inv(16)/*CBFB-MYH11* and t(8;21)/*RUNX1-RUNX1T1* fusions, *MLL*/PTD, *CEBPA,* and *AML1/RUNX1* mutations, that involve transcription factors or cofactors resulting in impaired hematopoietic differentiation. In addition to genetic abnormalities, epigenetic dysregulation is critical to the pathogenesis of AML [23]. Compatible with these findings, several novel mutations involving genes related to epigenetic modifications, such as *IDH1*/*IDH2*, *ten-eleven translocation* 2 (*TET2*), *additional sex comb-like 1* (*ASXL1*), and DNA methyltransferase 3A *(DNMT3A)*, have been identified in AML [24–30].

Currently, mutations that have a putative role in AML pathogenesis are classified into eight categories based on their biological function, including those involving myeloid transcription factor genes, *NPM1*, tumor suppressors, signaling genes, DNA methylation, chromatin modifier, cohesin complex, and splicing factors (Table 5.4) [4, 14, 16, 17]. It is common for multiple mutations to occur concurrently in the same patient indicating a role of concerted interaction of mutations in the pathogenesis of AML [11, 31]. The identification of genetic alterations has led to the refinement of prognostication in AML.

The European LeukemiaNet (ELN) first published guidelines for the diagnosis and management of AML based on cytogenetic and genetic status in 2010 (ELN-2010), in which four subgroups (favorable risk, intermediate I risk, intermediate II risk, and adverse risk) were proposed [32]. In the revised version of the ELN recommendations for AML (ELN-2017) [33], AML is divided into three risk categories (favorable, intermediate, and adverse) (Table 5.5). ELN-2017 is probably the most widely used risk-stratification model in current clinical practice as it incorporates cytogenetic changes and gene mutation status, including the *FLT3*-ITD allelic ratio (AR), to propose a refined stratification model that largely enhances the stratification power compared to the ELN-2010 recommendations.

Since 2017, the accumulation of new data regarding the prognostic relevance of recurrent genetic alterations using NGS platforms and cytogenetic abnormalities has prompted the need to further refine the risk stratification. Accordingly, the ELN updated the latest risk stratification system in 2022 (ELN-2022, Table 5.6) [34], and the most important changes are briefly summarized here. First, biallelic *CEBPA* in-frame

Table 5.4 Functional categories of genes that are commonly mutated in AML

Functional category	Gene members	Role in AML Leukemogenesis
Transcription factor genes or fusions	*RUNX1*::*RUNX1T1*, *CBFB*::*MYH11*	Impaired transcriptional regulation and hematopoietic differentiation
	RUNX1, *CEBPA*, *GATA1*, *GATA2*, *ETV6*	
NPM1 gene	*NPM1*	Aberrant cytoplasmic delocalization of NPM1 and its partner proteins resulting in perturbated cellular function
Tumor suppressor genes	*TP53, WT1, PHF6*	Deregulation of transcriptional activity and impaired cellular degradation through the impairment of negative regulators
Signaling transduction genes	*FLT3*/ITD, *FLT3*/TKD, *KIT, PTPN11, JAK2*, *NRAS*, *KRAS*, *CBL*, *CSF3R*	Proliferative advantages through the RAS/RAF/MEK/REK, JAK/STAT, and PI3K/PTEN/AKT/mTOR signaling pathways
DNA methylation genes	*DNMT3A, TET2, IDH1, IDH2*	Deregulation of DNA methylation and impaired genome topology by oncometabolites production
Chromatin modifiers	*ASXL1, EZH2*, *KMT2A*/PTD, *KMT2A* fusions	Impairment of chromatin modification and abrogation of methyltransferases function
Cohesin complex genes	*STAG1, STAG2, RAD21, SMC1A, SMC3*	Impairment of chromosome segregation resulting in transcriptional deregulation
Splicing factor genes	*SF3B1, U2AF1, SRSF2, ZRSR2*	Deregulated RNA processing leading to aberrant splicing patterns
	U2AF2, SF1, SF3A1, PRPF40B, PRPF8, LUC7L2	

Table 5.5 2017 ELN risk classification based on genetics at initial diagnosis

Risk	Genetic abnormality
Favorable	• t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*
	• inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
	• Mutated *NPM1* without *FLT3-ITD* or with *FLT3-ITD*^{low}
	• Biallelic mutated *CEBPA*
Intermediate	• Mutated *NPM1* and *FLT3-ITD*^{high}
	• Wild-type *NPM1* without *FLT3-ITD* or with *FLT3-ITD*^{low} (without adverse-risk genetic lesions)
	• t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*
	• Cytogenetic abnormalities not classified as favorable or adverse
Adverse	• t(6;9)(p23;q34.1); *DEK-NUP214*
	• t(v;11q23.3); *KMT2A* rearranged
	• t(9;22)(q34.1;q11.2); *BCR-ABL1*
	• inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2,MECOM(EVI1)*
	• −5 or del(5q); −7; −17/abn(17p)
	• Complex karyotype, monosomal karyotype
	• Wild-type *NPM1* and *FLT3-ITD*^{high}
	• Mutated *RUNX1*
	• Mutated *ASXL1*
	• Mutated *TP53*

Low low allelic ratio (<0.5), *high* high allelic ratio (≥0.5)
Adapted from Döhner et al. [33]

mutations affecting the bZIP region of *CEBPA* are related to favorable prognosis, either monoallelic or biallelic [35–37]. Second, *FLT3*-internal tandem duplication (*FLT3*-ITD) is now considered an intermediate risk, regardless of *FLT3*-ITD AR, as kinase inhibitors have achieved promising results in either induction or salvage treatments [38–40] and because of the increasing role of measurable residual disease (MRD) in treatment decisions, including early transplantation in this subgroup [41, 42]. Third, mutations in MDS-related genes *ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, and ZRSR2* are thought to be associated with poor outcomes [43–46]. Two additional adverse cytogenetics, t(8;16)(p11;p13)/*KAT6A-CREBBP* and t(3q26.2;v)/*MECOM*(*EVI1*), have recently been identified [47–49]. Based on an integrated analysis of large cohorts of patients and patterns of mutual cooperativeness or exclusivity between cytogenetic and molecular genetic features, the majority of AML cases can be classified into a number of biologically and prognostically distinct subgroups [31]. Herein we provide an overview of the most relevant genetic markers of AML, highlighting its clinical features and risk stratification.

Table 5.6 2022 ELN risk classification based on genetics at initial diagnosis[a]

Risk category	Genetic abnormality
Favorable	• t(8;21)(q22;q22.1)/*RUNX1*::*RUNX1T1*[b,c]
	• inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/*CBFB*::*MYH11*[b,c]
	• Mutated *NPM1*[b,d] without *FLT3*-ITD
	• bZIP in-frame mutated *CEBPA*[e]
Intermediate	• Mutated *NPM1*[f] with *FLT3*-ITD
	• Wild-type *NPM1* with *FLT3*-ITD
	• t(9;11)(p21.3;q23.3)/*MLLT3*::*KMT2A*[b,g]
	• Cytogenetic and/or molecular abnormalities not classified as favorable or adverse
Adverse	• t(6;9)(p23;q34.1)/*DEK*::*NUP214*
	• t(v;11q23.3)/*KMT2A*-rearranged[h]
	• t(9;22)(q34.1;q11.2)/*BCR*::*ABL1*
	• t(8;16)(p11;p13)/*KAT6A*::*CREBBP*
	• inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/*GATA2,MECOM*(*EVI1*)
	• t(3q26.2;v)/*MECOM*(*EVI1*)-rearranged
	• −5 or del(5q); −7; −17/abn(17p)
	• Complex karyotype[h], monosomal karyotype[i]
	• Mutated *ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, or ZRSR2*[j]
	• Mutated *TP53*[k]

Adapted from Dohner et al. [34]

[a]Frequencies, response rates, and outcome measures should be reported based on risk category; if sufficient numbers are available, specific genetic lesions are indicated

[b]Mainly based on results observed in intensively treated patients. The initial risk assignment may change during the treatment course, based on the results of the analyses of measurable residual disease

[c]Concurrent *KIT* and/or *FLT3* gene mutations did not alter the risk categorization

[d]AML with *NPM1* mutation and adverse-risk cytogenetic abnormalities are categorized as adverse risk

[e]Only in-frame mutations affecting the basic leucine zipper (bZIP) region of *CEBPA*, irrespective of whether they occur as monoallelic or biallelic mutations, have been associated with favorable outcomes

[f]The presence of t(9;11)(p21.3;q23.3) takes precedence over rare concurrent adverse-risk gene mutations

[g]Excluding *KMT2A* partial tandem duplication (PTD)

[h]Complex karyotype: ≥3 unrelated chromosome abnormalities in the absence of other class-defining recurring genetic abnormalities; excludes hyperdiploid karyotypes with three or more trisomies (or polysomies) without structural abnormalities

[i]Monosomal karyotype: presence of two or more distinct monosomies (excluding loss of X or Y) or a single autosomal monosomy in combination with at least one structural chromosome abnormality (excluding core-binding factor AML)

[j]For the time being, these markers should not be used as adverse prognostic markers if they co-occur with favorable-risk AML subtypes

[k]*TP53* mutation at a variant allele fraction of at least 10%, irrespective of the *TP53* allelic status (mono- or biallelic mutation); *TP53* mutations are significantly associated with AML with complex and monosomal karyotypes

5.3 Mutations That Lead to Leukemia Cell Survival and Proliferation

5.3.1 *FLT3* Mutations

FLT3, located on chromosome 13q12, encodes a receptor tyrosine kinase that plays a major role in hematopoiesis regulation [50, 51]. There are two types of *FLT3* mutations; internal tandem duplication (ITD), mostly of the FLT3 juxtamembrane domain (JMD) which are gain-of-function mutations [52, 53], and tyrosine kinase domain (TKD) point mutations mainly at codon 835 or 836 within the activation loop of the second kinase domain [54, 55]. The majority (~70%) of ITD insertions occur within the JMD, whereas ITDs can be detected in approximately 25% of cases affecting the beta-1 sheet of TKD1 [56, 57]. The FLT3 mutant protein constitutively activates

FLT3 signaling in the absence of FLT3 ligands, promoting cell proliferation and decreasing apoptosis [58–60].

Mutations in *FLT3* are detected in approximately 30–35% of patients newly diagnosed with AML, with either ITD (20%) or point mutations in TKD (5–10%) [16, 61–63]. *FLT3*-ITD is associated with younger age, higher white blood cell (WBC) counts, normal karyotypes, and mutations in *NPM1, DNMT3A,* and *IDH* [15, 52, 64]. Of note, patients with this mutation have shorter disease-free survival (DFS), increased relapse rate, and poorer overall survival (OS) [63, 65, 66], particularly patients with a high AR [67] or absence of *NPM1* mutation [66, 68, 69]. In addition, the insertion site and ITD length of FLT3, as well as concomitant mutations, also appear to influence prognosis [57, 67, 70, 71]. For these reasons, patients with *FLT3*/ITD are frequently referred for early allogeneic hematopoietic cell transplantation (allo-HSCT) in first complete remission (CR). Accordingly, the ELN-2017 and National Comprehensive Cancer Center Consensus panels designated *FLT3*/ITD with high AR as an unfavorable prognostic subgroup [33, 72]. Interestingly, *FLT3*/ITD with AR $\geq$ 0.35 also independently predicted poor OS in patients with t(8;21) AML [73]. Nevertheless, some controversy remains, as some studies have shown a negative impact of low AR of *FLT3*-ITD (*FLT3*-ITD^{low}) in patients with AML [42, 74, 75]. The reasons underlying the higher relapse rate in patients with *FLT3*-ITD^{low} need to be explored; however, they could possibly be due to the significant perturbations in the RAS pathway and concurrent *NRAS* mutations as well as in *MLL*-PTD in these patients [42]. On the other hand, AML patients with *FLT3*-TKD manifest specific clinicopathological features, such as an elevated WBC count at diagnosis, higher frequency of a normal karyotype, and mutations in *NPM1*, *CEBPA*, and *NRAS,* compared with patients without the mutation [76–78]. Nonetheless, the prognostic impact of *FLT3*-TKD is not well defined [76–79].

Up to one third of AML patients with *FLT3*-ITD or *FLT3*-TKD could lose the mutation at relapse, whereas the acquisition of novel *FLT3* mutations was detected in 20% of the patients exhibiting disease progression [80–82]. It is clinically important to retest the *FLT3*-ITD or *FLT3*-TKD status at all subsequent treatment decision points in every patient regardless the *FLT3* status at the time of diagnosis [83]. Given the adverse prognostic effect of *FLT3*-ITD and the higher frequency of *FLT3* mutations in AML, FLT3 inhibitors have emerged as an important part of therapy for patients with mutated *FLT3* with both frontline and relapsed/refractory status [40, 84, 85]. In a retrospective exploratory analysis of the CALGB 10603/RATIFY trial, the beneficial effect of midostaurin was only identified in patients with only JMD insertions [86]. TKD1 insertion is still known to have a negative impact on prognosis even under conditions of treatment with a multi-kinase inhibitor. In ELN-2022, *FLT3*-ITD is considered an intermediate risk, regardless of *FLT3*-ITD AR, as kinase inhibitors have achieved promising results either as induction or salvage treatments [38–40]. Further, the role of MRD monitoring in making treatment decisions, including early transplantation, in the *FLT3*-ITD subgroup is also increasing [41, 42].

5.3.2 *RAS* Mutations

RAS proteins are a large superfamily of low-molecular-weight guanine nucleotide-binding proteins that are activated by cytokine receptors in response to ligand stimulation and therefore control cell proliferation and survival of hematopoietic progenitors [87–90]. Three members of the RAS family, HRAS, KRAS, and NRAS, are activated in response to mutations in human cancers, including AML [90, 91]. Almost all *RAS* mutations are single nucleotide substitutions in codons 12, 13, and 61 [92–96]. *NRAS* mutations occur more frequently than *KRAS* mutations, and *HRAS* mutations rarely happen (<1%). *NRAS* and *KRAS* mutations are found in approximately 10.3–30% and 9–14% of patients with AML, respectively [92–96]. In a large cohort study involving 2502 patients with AML, *NRAS* mutations were found to be prevalent in patients with inv(16)/t(16;16) and inv(3)/t(3;3), but were seldom found in those with t(15;17) and complex karyotypes [92].

However, the prognostic relevance of *RAS* mutations in AML remains unclear. Some studies showed that *RAS* mutations predicted poor prognosis [97–99], while others showed no impact on the clinical outcomes [92, 100–102], whereas *RAS* mutations were found to be associated with a favorable prognosis in other studies [103, 104]. A systemic review and meta-analysis showed that *RAS* mutations were not associated with a poor prognosis in AML [105]. Further analysis of a subgroup of children indicated that patients with *NRAS* mutations had an adverse prognosis [hazard ratio (HR): 1.55, 95% confidence interval (CI): 1.13–2.12, $p = 0.007$], but not those with *KRAS* mutations (HR: 1.51, 95% CI, 0.34–6.73, $p = 0.59$).

5.3.3 *KIT* Mutations

KIT, also known as stem cell factor receptor (cluster of differentiation 117, CD117), is a member of the type III receptor tyrosine kinase family and is involved in the regulation of survival and proliferation of hematopoietic progenitor cells [106, 107]. KIT is highly expressed in most leukemic blasts [108, 109], and *KIT* mutations that most commonly affect exons 8 and 17 (especially D816 mutations) are identified in approximately 25% of the patients with CBF AML [73, 110–112], but infrequently found in patients with other AML sub-

types [15]. The prognostic impact of *KIT* mutations in AML is uncertain; some studies suggested that *KIT* mutations, especially the D816 mutant, were associated with inferior outcome in CBF AML [113–116], while others did not [117, 118]. A systemic review and meta-analysis showed that a *KIT* mutation was associated with increased relapse risk and shorter OS in t(8;21), but not in inv(16) [119]. The targeted high-throughput sequencing in 331t(8;21) patients showed that *KIT* mutation, especially in cases with a higher AR (a mutant level ≥ 25%), was independently associated with increased relapse rate and reduced OS [73].

5.3.4 *PTPN 11* Mutations

SHP-2 is encoded by *PTPN11*, located on chromosome 12q24. It is a non-receptor tyrosine phosphatase that is involved in intracellular signaling elicited by a number of growth factors, cytokines, hormones, and adhesion molecules [120, 121]. Germline *PTPN11* mutations were first reported by Tartaglia et al. in patients with Noonan syndrome [122–124]. Subsequently, somatic *PTPN11* mutations were also identified in patients with juvenile myelomonocytic leukemia and MDS [125–128]. *PTPN11* mutation is not a frequent molecular event (4–8%) in AML [129–132]. Importantly, *PTPN11* mutations are closely associated with older age, French-American-British (FAB) M4/M5 subtypes, a normal karyotype, and an *NPM1* mutation [131–133]. Alfayez et al. further showed that *PTPN11* mutations co-occur more commonly with *FLT3*/ITD and less commonly with mutations in *IDH2* and a complex karyotype [132]. Compared with wild-type, mutant *PTPN11* was independently associated with lower CR rates and shorter OS [132, 133]. In a study by Stasik et al., the deleterious effect of *PTPN11* mutations was confined predominantly to the ELN-2017 favorable-risk group and patients with subclonal *PTPN11* mutations (HR, 2.28; $p < 0.001$), but not found with dominant *PTPN11* mutations (HR, 1.07; $p = 0.775$), presumably because of significant differences within the rate and spectrum of concurrent mutations [133]. Further, Loh et al. and Tartaglia et al. revealed that *PTPN11* mutations had no prognostic implications for pediatric patients with AML [129, 130].

5.3.5 *JAK2* Mutations

The *JAK2* V617F mutation, first described in 2005, results in a valine-to-phenylalanine substitution in codon 617 of the JAK2 protein [134–136]. This mutation induces activation of the JAK2-STAT5 pathway and substantially alters the proliferation and self-renewal of hematopoietic precursors [137, 138]. It is characteristic of the majority of Philadelphia chromosome-negative MPNs. [135, 136, 139, 140]. In contrast to patients with secondary AML transformed from an underlying MPN, patients with *de novo* AML rarely harbor *JAK2* V617F (<1–2%) [141–144]. Further, patients with a *JAK2*-mutated AML-MPN were more likely to have splenomegaly, MPN-like megakaryocytes, and a higher mean allelic frequency of *JAK2* V617F at the time of diagnosis than patients with *de novo JAK2*-mutated AML [145]. Mutations in genes affecting DNA methylation are more common in *de novo JAK2*-mutated AML, whereas a complex karyotype is more frequent in *JAK2*-mutated AML-MPN cases. Illmer et al. showed that 3.6% of the patients with CBF AML had a *JAK2* V617F mutation, and these patients had an aggressive clinical course and a poor outcome [144]. In an international study of 331 patients with t(8;21), a *JAK2* mutation was identified as a significantly poor prognostic factor for OS in addition to age, WBC count, and high AR of *FLT3*/ITD and *KIT* mutations in multivariate analysis [73].

5.4 Mutations That Impair Hematopoietic Cell Differentiation

5.4.1 *CEBPA* Mutations

CCAAT/enhancer binding protein α (C/EBPα), located on chromosome 19q13.1, is a 42 kDa transcription factor that possesses a DNA-binding basic leucine zipper domain (bZIP) at the COOH terminus and two transactivation domains (TAD), TAD 1 and TAD 2, at the NH_2 terminus [146]. As a transcription factor, it plays a crucial role in granulocytic differentiation, and a decreased C/EBPα activity contributes to myeloid progenitor transformation [147–149]. *CEBPA* mutations are observed in 5.1–18.9% of patients with AML; a higher incidence rate is observed in patients with AML from Asia than in Western countries [36, 150–155]. Two major types of *CEBPA* mutations have been identified, one alters the COOH terminal bZIP of *CEBPA*, resulting in decreased DNA-binding and/or dimerization activity, and the other disrupts the translation of the C/EBPα NH_2 terminus, thereby upregulating the expression of an alternative 30 kDa isoform that exhibits a dominant-negative effect on the full-length wild-type C/EBPα [156–158].

Most patients with *CEBPA* mutations harbor biallelic mutations involving both the NH2-terminal TAD region and the COOH-terminal bZIP domain [36, 151, 153, 154, 159–161]. *CEBPA* mutations occur most frequently in patients with the FAB M2 subtype and are closely associated with the expression of CD7, CD15, CD34, and HLA-DR on the surface of leukemic cells, higher counts of circulatory blasts, and normal cytogenetics and mutations in *TET2*, *GATA2, WT1,* and *CSF3R* [36, 151–154, 162–164]. Importantly, germline *CEBPA* mutations are highly penetrant, causing

early-onset *de novo* AML without a preceding dysplastic or cytopenic phase and are associated with favorable survival outcomes [165]. This mutation seems quite stable during disease course and may be a potential marker for monitoring MRD. The fact that none of the patients with AML who did not have *CEBPA* mutations at the time of diagnosis acquired the mutation at relapse suggests that this mutation may not play a major role in the progression of AML [151, 166].

Mutant *CEBPA* predicts favorable outcomes in AML patients with an intermediate-risk or normal cytogenetics [150, 152, 167, 168]. The favorable impact of *CEBPA* mutations in AML patients was only observed in the absence of *FLT3*/ITD or other associated cytogenetic abnormalities [159]. Moreover, only double *CEBPA* mutations, but not single *CEBPA* mutations, were associated with better prognosis and defined as distinct genetic entities [160, 161, 169, 170]. Concomitant *WT1* mutation or *CSF3R* mutation helped predict poor prognosis in patients with AML with double mutant *CEBPA* [157, 158].

Recently, the clinical and molecular features (younger age, higher WBC counts, the presence of *GATA2* mutations) and favorable survival are confined to patients with in-frame mutations in bZIP ($CEBPA^{bZIP\text{-}inf}$); in other words, only patients bearing $CEBPA^{bZIP\text{-}inf}$ exhibited superior to other subtypes [36]. These findings impact the current WHO-2022 [19], ELN-2022 [34], and ICC [20], resulting in the changing of the category, "AML with biallelic mutations of *CEBPA*" into "AML with in-frame bZIP mutations of *CEBPA*."

5.4.2 *AML1/RUNX1* Mutations

The AML1/RUNX1 gene (hereafter referred to as *RUNX1*) [171], consisting of 10 exons (exons 1–6, 7A, 7B, 7C, and 8), is one of the most frequently deregulated genes—through chromosomal translocations and point mutations—in leukemia [172–175]. RUNX1 is required for definitive hematopoiesis, and its functional dysregulation leads to leukemia [174, 175]. Monoallelic germ-line mutation of *RUNX1* occurs in rare cases of familial platelet disorder with a predisposition to AML (FPD/AML) [176]. Acquired *RUNX1* mutations have been frequently reported in therapy-related MDS and MDS/AML [177].

The incidence of *RUNX1* mutations in *de novo* AML varies from 2.9% to 46% depending on the population selected, the regions of *RUNX1* screened, and the methods used [178–184]. *RUNX1* mutations were detected in 13.2% [179] of 470 and 5.6% [181] of 945 non-M3 cases. *RUNX1* mutations have been associated with older age, undifferentiated/immature FAB subtypes (M0/M1), and specific cytogenetic abnormalities, such as trisomy 8 (+8), +13, or +21 [178, 179, 181–185]. None of the patients with t(8;21), inv(16), t(15;17), or 11q23 translocation have been reported to possess a *RUNX1* mutation [179]. Further, *RUNX1* mutations are closely associated with *MLL*/PTD, *ASXL1* mutations, and *IDH1/IDH2* mutations, but negatively associated with *CEBPA* and *NPM1* mutations [179, 181, 183–185]. *RUNX1* mutation-associated expression signatures featured upregulation of lymphoid regulators and B-cell linker, but downregulation of promoters of myelopoiesis [183, 184].

RUNX1 mutations predicted resistance to chemotherapy and represented an independent risk factor for poor DFS/EFS and OS in most studies [179, 183–185], but for shorter EFS only in one study [181]. A meta-analysis of four previously published studies involving 1581 patients demonstrated that HRs for OS and DFS were 1.55 [95% CI, 1.11–2.15; $p = 0.01$] and 1.76 (95% CI, 1.24–2.52; $p = 0.002$), for patients with *RUNX1* mutations [186]. Notably, allo-HSCT ameliorated the poor survival impact of *RUNX1* mutations [179, 181]. A *RUNX1* mutation is defined as an adverse genetic subset in ELN-2017 and ELN-2022 and listed as one of the myelodysplasia-related gene mutations in ICC [20]. However, in the WHO-2022 classification, AML with somatic *RUNX1* mutation is not recognized as a distinct disease subtype owing to the lack of sufficient unifying characteristics [19].

5.5 Mutations Involving *NPM1*

5.5.1 *NPM1* Mutations

The *NPM1* gene encodes a ubiquitous multifunctional protein with prominent nucleolar localization that shuttles between the nucleus and cytoplasm [187]. *NPM1* mutations in AML were first identified by Dr. Falini's group, which noticed that some patients with AML leukemia cells exhibited aberrant cytoplasmic localization of the NPM1 protein, which is normally located in the nucleoli of non-mitotic cells [188]. Subsequent investigations revealed a tetra-nucleotide insertion near the C-terminal end of the coding sequence of *NPM1*. Importantly, *NPM1* mutations are always heterozygous and are mostly restricted to exon 12 [189]. The most frequent form of *NPM1* mutations is the duplication of TCTG (type A, c.860_863dupTCTG), resulting in the alteration of the peptide sequence from DLWQWRKSL* to DLCL AVEEVSLRK*. *NPM1* mutations occur in approximately one third of patients with AML, are more frequent in elderly patients [187, 188, 190], and are highly associated with a normal karyotype, *FLT3*/ITD, *DNMT3A* mutation, and *IDH* mutation, but are significantly exclusive to *CEBPA* mutations, favorable karyotypes, and expression of CD34 and HLA-DR [15, 187, 188, 191–197]. It must be noted that a diagnosis of AML with AML-MRC should be made if typi-

cal cytogenetic alterations and/or a previous history of MDS are documented, even in the presence of *NPM1* mutations [198].

NPM1 mutations generally predict better prognosis [188], especially when *FLT3*-ITD is absent [69, 199] and refinement of patient groups revealed three groups with distinct prognoses, i.e., good (*NPM1*$^+$/*FLT3*-ITD$^-$), intermediate (*NPM1*$^+$/*FLT3*-ITD$^+$ or *NPM1*$^-$/*FLT3*-ITD$^-$), and poor (*NPM1*$^-$/*FLT3*-ITD$^+$) [200]. Based on the ELN-2017 recommendations, *NPM1* mutations have a relatively favorable prognosis only in the absence of *FLT3*-ITD or a low AR (<0.5, *FLT3*-ITD^{low}). However, recent studies have not supported the good prognosis of *NPM1*-mutated/*FLT3*-ITD^{low} AML [42, 201, 202]. Accompanying mutations other than *FLT3* mutations and differences in treatment settings, such as use of FLT3 inhibitor and/or allo-HSCT, truly impact the survival in these patients.

NPM1 mutations seem to be consistent with the disease status and are usually not found in individuals with clonal hematopoiesis [203–208]. Serial analyses of *NPM1* mutations showed that the mutation disappeared at CR, but the same mutation usually reappeared at relapse. This feature makes the *NPM1* mutation an ideal marker for MRD monitoring [209]. Studies have shown that *NPM1* mutation levels measured by real-time quantitative polymerase chain reaction (RQ-PCR), digital droplet PCR, or NGS reflect disease status, predict impending relapse, and have prognostic implications [203, 204, 209–215]. MRD negativity in peripheral blood (PB) or BM at multiple time points after chemotherapy predicts a low risk for leukemia relapse and better survival [187, 209].

5.6 Mutations in Tumor Suppressor Genes

5.6.1 *TP53* Mutations

Somatic mutations in the tumor suppressor gene *TP53*, located at 17p13, are frequently detected in patients with therapy-related AML [216, 217] or AML with a complex or monosomal karyotype (53–73%) [218–221]. In contrast, *TP53* mutations are found in only 7–8% of patients with *de novo* AML [14, 221]. Notably, nuclear p53 expression, measured by immunohistochemical staining, strongly correlates with *TP53* mutational status and variant allelic frequency (VAF), and individuals with increased nuclear p53 level showed more frequent complex karyotypes, especially 17p abnormalities and 17p and 5q deletions, high blast counts, and inferior OS [222]. In general, *TP53* mutations independently predict lower CR rates, higher relapse rates, and shorter EFS and OS. AML with mutated *TP53* is a new subset of ICC [20] and is categorized as an adverse subgroup in ELN-2017 and ELN-2022 [33, 34].

Accumulating evidence supports that *TP53*-mutated MDS and AML represent a unique continuum of myeloid neoplasms with an overall aggressive course, irrespective of the BM blast percentage [43, 223–225]. The allelic status of *TP53*, either monoallelic or biallelic, exerts a strong impact on the prognosis of patients with MDS [226–228]; however, data from patients with AML have been inconclusive [229, 230]. Prochazka et al. reported subclonal *TP53* mutations to be a novel prognostic parameter in a cohort of 1537 patients with AML and emphasized the usefulness of NGS technologies for risk stratification in this disorder [229]. In a study by Short et al., *TP53*-mutated VAF influenced clinical outcomes in patients treated with a cytarabine-based regimen (median OS, 4.7 vs. 7.3 months for VAF >40% vs. 40%; $p = 0.006$), whereas VAF did not significantly affect OS in patients treated with hypomethylating agents (HMA) [230]. In other words, among *TP53*-mutated patients with low VAF, OS was higher in those treated with a high-dose cytarabine, whereas patients with high VAF showed poor OS, regardless of the therapeutic regimen.

5.6.2 *WT1* Mutations

The Wilms' Tumor 1 (*WT1*) gene, located on chromosome 11p13, encodes a zinc-finger transcription factor that is physiologically expressed in hematopoietic stem cells and is involved in the regulation of cell survival, proliferation, and differentiation [231, 232]. *WT1* was initially identified as a tumor suppressor gene [233], but was later found to be overexpressed in AML as well as other cancers, leading to its classification as a potential oncogene [234–236]. Mutations in *the WT1* gene are found in approximately 7–13% of CN-AML patients, with hotspots in the four Cys-His zinc finger domains on exons 7 and 9 [235, 237–240]. The majority of *WT1* mutations are frameshift mutations occurring in exon 7, followed by single amino acid substitutions in exon 9; frameshift mutations in exon 9 are rare. *WT1* mutations occur at similar frequencies in patients with normal karyotypes and abnormal cytogenetics [240]. The chromosomal abnormality t(7;11)(p15;15), a translocation resulting in *NUP98/HOXA9* fusion, is closely associated with *the WT1* mutation [240]. *WT1* mutations were positively associated with *FLT3*/ITD and *CEBPA* mutations [239, 241]. Paschka et al. showed that patients with *WT1* mutations exhibited higher expression of *ERG* and *BAALC* than those without the mutations [237].

WT1 mutation is an independent poor prognostic factor in patients with CN-AML as well as in AML [237, 238, 240, 241]; however, different results have been reported [239,

242]. Outcomes of patients with co-mutated *NPM1*/*WT1* resembled those of patients with adverse-risk ELN-2017 [243]. Notably, concomitant *WT1* mutations predicted poor outcomes in patients with AML with double-mutant *CEBPA* [158]. On the other hand, the prognostic implications of *WT1* mutations have not been well clarified and remain unclear in pediatric AML [244, 245]. Recently, *WT1* mutations were found to exert an independent adverse effect on EFS and OS in a cohort of 870 pediatric patients [246].

5.7 Mutations in Genes Related to DNA Methylation

5.7.1 *DNMT3A* Mutations

The enzyme DNMT3A, a 130 kDa protein encoded by the *DNMT3A* gene on chromosome 2p23, catalyzes 5-methylcytosine methylation [247]. DNMT3A is important in embryonic and hematopoietic stem cell differentiation and interacts with DNMT3B to regulate stem cell function [248–250]. *DNMT3A* mutations include missense, nonsense, frameshift, or in-frame alterations, with a hotspot in exon 23 at arginine 882 (*DNMT3A*^{R882}) [26]. Most *DNMT3A* mutations in AML patients have been found to be heterozygous and associated with changes in DNA methylation [247, 251–253]. All nonsense, frame-shift, and in-frame mutations generate truncated peptides with complete or partial deletion of the MTase domain and are suggested to abolish the catalytic activity of this enzyme. *DNMT3A*^{R882} results in impaired enzyme activity and acts as a dominant-negative inhibitor of wild-type DNMT3A enzymatic activity [254, 255]. A study on a conditional knockout mouse model showed that Dnmt3a loss leads to aberrant methylation pattern and significant expansion of hematopoietic stem cells with differentiation defect over serial transplantation [249]. Genes associated with stem cell self-renewal, such as homeobox A9 (*Hoxa9)* and Meis homeobox 1 (*Meis1)*, were upregulated, whereas differentiation factors were downregulated; however, no overt leukemia phenotype was identified [249].

Mutations in *DNMT3A* were first detected by NGS in up to 22.1% of 281 adult patients with AML in 2010 [26, 256, 257]. Since then, *DNMT3A* mutations have been found in 12.0–23.1% of unselected AML cohorts, 19.5–33.7% in patients with intermediate-risk cytogenetics, and 22.9–37.1% in the CN-AML group [26, 258–265]. Different from myeloid malignancies, *DNMT3A* mutations in lymphoid malignancies are predominantly non-R882 mutations and are usually biallelic [266–268].

DNMT3A mutations are closely associated with intermediate-risk and normal cytogenetics. In contrast, none of the patients with t(8;21), t(15;17), or inv(16) mutations harbored *DNMT3A* mutations [26, 258, 259, 261]. *DNMT3A* mutations occur more frequently in elderly individuals [26, 258, 259, 263–265]. The relationship of this mutation with advanced age is consistent with the recent findings that *DNMT3A* mutation is the most common clonal change in hematopoietic cells in an aged population without evident myeloid malignancies [207, 269]. *DNMT3A* mutation occurs in human hematopoietic stem cells (HSCs), in which they can act as a preleukemic lesion, and mutant HSCs persist in CR. In AML, *DNMT3A* mutations are associated with higher WBC and platelet counts, higher BM and PB blast counts, and FAB M4/M5 subtypes. *DNMT3A* mutations rarely occur alone, and frequent concurrent mutations include *FLT3*/ITD, *NPM1*, and *IDH* mutations [26, 258, 259, 263, 264]. Notably, a comprehensive analysis of genetic and epigenetic landscapes in TCGA Research Network revealed AML with concomitant mutations of *NPM1*, *DNMT3A,* and *FLT3*/ITD as a novel subtype of AML that was closely associated with distinct clusters in mRNA, microRNA, and DNA methylation. [253].

The prognostic impact of *DNMT3A* mutations in AML is well-studied. Ley et al. first demonstrated that *DNMT3A* mutations are associated with increased risk and poor outcomes in patients with AML [26]. The negative prognostic impact of *DNMT3A* mutations was confirmed in subsequent large-scale studies; mutations resulted in poor OS among all patients with AML [26, 258, 259, 263, 265], those with a normal karyotype [26, 258, 259, 261, 262], and those with *FLT3*/ITD [258–260]. Similarly, the presence of a *DNMT3A* mutation may facilitate leukemia transformation in patients with MDS and MPN [247]. However, Gaidzik et al. reported that the association of *DNMT3A* mutation with poor prognosis was only observed in patients with unfavorable-risk CN-AML, defined by ELN-2017, but not in the total cohort comprising 1770 young adult patients [264]. Whether patients with R882 and non-R822 mutations have different outcomes remains unclear [259, 262, 264]. Results of two meta-analyses showed that the presence of mutant *DNMT3A* predicted poor outcome in patients with AML except in the favorable genotype subgroup [270, 271].

5.7.2 *IDH* Mutations

IDH1 and *IDH2* encode two isoforms of isocitrate dehydrogenase that catalyze the oxidative decarboxylation of isocitrate to α-ketoglutarate (α-KG) [272]. Mutant IDH proteins convert α-KG to 2-hydroxyglutarate (2-HG), an oncometabolite that contributes to tumor growth or malignant transformation [273, 274]. In addition, 2-HG promotes cytokine independence and epigenetic alterations and blocks the differentiation of hematopoietic cells [275, 276]. IDH1 is found in the cytoplasm and peroxisomes, whereas IDH2 resides in the mitochondria. Mutations in *IDH1* and *IDH2*

were first identified in patients with glioblastoma multiforme [277]. Later, an *IDH1* mutation was found in a patient with AML by whole-genome sequencing [25], and subsequent studies showed that *IDH* mutations are recurrent in patients with AML [25, 274, 278–280]. *IDH1* mutations affect the arginine residue at position 132 (R132H, R132C, R132G, and R132S) and *IDH2* mutations affect arginine 140 (R140Q and R140W) or arginine 172 (R172K and R172G) of exon 4 [273, 274]. Notably, R132C is the most common *IDH1* mutation in AML, whereas R132H occurs predominantly in gliomas [281].

In *de novo* AML, *IDH1* mutations occur in 1.7–13.1% of the total patients and 7.8–16.0% in those with CN-AML [25, 68, 197, 274, 278–280, 282–293], while *IDH2* mutations occur in 2.2–15.0% of the total cohorts and 10.0–19.0% of those with CN-AML [68, 197, 265, 274, 278, 279, 282, 285–289, 291, 293–296]. Occasionally, *IDH* mutations are detected in healthy older individuals with age-related clonal hematopoiesis, suggesting that *IDH* mutations occur early in hematopoiesis [297]. Both *IDH1* and *IDH2* mutations are associated with a normal karyotype, older age, *NPM1* mutation, and *DNMT3A* mutation, but are mutually exclusive with favorable-risk cytogenetics and *WT1* and *TET2* mutations [278, 294]. However, differences exist in the clinical presentations between patients with *IDH2* R140 and R172 mutations [296]. Compared with the *IDH2* R140 mutation, the *IDH2* R172 mutation is associated with younger age and lower WBC count and is inversely correlated with *NPM1* mutation. Epigenetic profiling of a large cohort of patients with AML demonstrated the mutual exclusivity of *IDH1/2* and *TET2* mutations, suggesting that these genes have similar functional roles in epigenetic regulation [298].

The impact of *IDH* mutations on prognosis remains unclear. Some studies reported that *IDH1* mutations did not predict patient survival [265, 274, 280, 282, 283, 286, 289]; however, Patel et al. reported a favorable outcome in patients with *IDH1* mutation among *FLT3*/ITD patients [68], while others showed that *IDH1* mutation predicted an adverse effect only in selected populations, such as younger patients [290], *NPM1*-wild patients [25, 291], patients with AML1/ETO [292], or molecular low-risk subgroups [197, 278, 279, 284, 285, 287, 288, 293]. In a meta-analysis of 8121 patients from 15 studies, an *IDH1* mutation appeared to have a moderate adverse prognostic impact (hazard ratio, HR, 1.17; 95% CI, 1.02–1.36) [299]. Similarly, studies on the prognostic relevance of *IDH2* mutations have yielded conflicting results. Some studies showed that the mutation was a favorable prognostic factor [68, 274, 294], while others did not find a difference in the survival of patients with and without the mutation [197, 265, 278, 279, 282, 286, 288, 289, 295], some even showed dismal outcome in *IDH2*–mutated patients [285, 287, 291, 293, 296]. A meta-analysis suggested that *IDH2* mutations confer a favorable prognosis with longer OS (HR, 0.80; 95% CI, 0.67–0.95; $p = 0.01$) and better EFS (HR, 0.83; 95% CI, 0.63–1.09; $p = 0.18$) [300]. In an analysis of 33 reports, *IDH2* mutation was associated with improved OS (HR, 0.78; 95% CI, 0.66–0.93; $p = 0.0053$), particularly in patients with intermediate-risk cytogenetics (HR, 0.65; 95% CI, 0.49–0.86; $p = 0.0026$) [301]. Notably, some studies reported differences in the clinical outcomes between patients with *IDH2* R172Q and R140Q: poorer induction response or worse prognosis was observed in the former group of patients [278, 285, 296], while better prognosis was found in the latter group of patients [68, 284, 301]. The reason behind mutations in different loci of the same gene rendering distinct clinical and prognostic features remains unclear.

5.7.3 *TET2* Mutations

The *ten-eleven* translocation (TET) family of proteins, including TET1, TET2, and TET3 proteins, can catalyze the conversion of 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC) in DNA, with ferrous iron and α-KG as cofactors [302]. Enrichment of 5-hmC in CpG dinucleotides within exons and near transcriptional start sites is associated with increased gene expression in embryonic stem cells, possibly due to the inhibition of active DNA methylation [303]. *TET2* mutations usually cause loss of function. Mutations in *TET2* result in global DNA hypermethylation as well as lower 5-hmC levels; however, conflicting results have been reported [298, 304, 305]. Deletion of TET2 in mice leaded to dysregulated hematopoietic stem cells and subsequent development of myeloid malignancies, resembling chronic myelomonocytic leukemia (CMMoL), MPN, and MDS in humans [306]. Further, cooperative *TET2* and *FLT3*/ITD mutations altered site-specific changes in DNA methylation and gene expression, which were not observed with either mutation alone, eventually inducing the AML phenotype [307].

The *TET2* mutation was first identified in myeloid malignancies via single nucleotide polymorphism analysis and comparative genomic-hybridization array, which revealed a common deletion of this gene in chromosome 4q24 [29, 308]. Subsequent studies confirmed that the mutations are common in MDS, MPN, CMMoL, and secondary AML, with frequencies of 10.0–26.0% [29, 308–311], 2.0–20.0% [29, 312–314], 22.0–58.0% [310, 315–317], and 24.0–32.0% [318–320], respectively. In *de novo* AML, *TET2* mutations are detected in 7.6–27.4% of total patients, 8.9–30.8% of those with intermediate-risk cytogenetics, and 6.0–30.1% of those with CN-AML [24, 321–329]. *TET2* mutations are closely associated with older age, higher WBC counts, normal karyotypes, and intermediate-risk cytogenetics. Some patients harbor more than one *TET2* mutation at diagnosis,

and *TET2*-mutated patients frequently have other concurrent genetic alterations. This mutation is associated with mutations in *NPM1* [322, 323, 326–329], *DNMT3A* [326–328], *ASXL1* [323], and *RUNX1* [328], but is mutually exclusive with *IDH* mutations in the vast majority of cases [24, 323–325, 328].

Studies on the prognostic implications of *TET2* mutations in patients with AML have yielded inconsistent and conflicting results. Several reports have shown that *TET2* mutations are associated with poor OS [321, 326]. However, no survival difference could be demonstrated in other reports [322, 325]. The prognostic impact of *TET2* mutations differed in AML subgroups. For example, in the ELN 2010 favorable-risk group (patients with CN-AML with mutated *CEBPA* and/or mutated *NPM1* without *FLT3*/ITD) [32], but not the intermediate-1 risk group (CN-AML with wild-type *CEBPA* and wild-type *NPM1* and/or *FLT3*/ITD), *TET2*-mutated patients had a shorter DFS, RFS, and OS than *TET2*-wild patients [24, 324, 328]. On the contrary, Chou et al. reported that a *TET2* mutation was an unfavorable prognostic factor in patients with intermediate-risk cytogenetics, and its negative impact was further enhanced when the mutation was combined with *FLT3/*ITD, *NPM1*-wild, or unfavorable genotypes. Similar findings have been reported in another study [327]. Using a multivariate analysis, Ahn et al. showed that homozygous *TET2* mutations that were detected in 25.9% of *TET2*-mutated patients, but not heterozygous mutations, independently predicted the risk of relapse [329]. In a meta-analysis of two cohorts, including 2552 and 4378 patients with *de novo,* secondary, and therapy-related AML, *TET2* mutation appeared to be an adverse prognostic factor for OS in all patients and those with CN-AML [330, 331].

5.8 Mutations of Genes Related to Histone Modification

5.8.1 *ASXL1* Mutations

ASXL1, a human homologue of the additional sex combs (*Asx*) gene of *Drosophila*, is mapped to chromosome 20q11, a region predominated by cancer-related genes [332]. Fisher et al. showed that *ASXL1* knockout mice exhibited defects in the differentiation of lymphoid and myeloid progenitors, but exhibited only mild phenotypes, possibly because other *ASXL* genes have redundant functions with *ASXL1* [333]. *ASXL1* mutations result in the loss of polycomb repressive complex 2 (PRC2)-mediated H3K27 trimethylation and a global decrease in histone H3K27 activity. *ASXL1* knockdown in mice, in collaboration with $NRAS^{G12D}$, promoted myeloid leukemogenesis [334]. C-terminal-truncating *ASXL1* mutations, or a deletion or loss of *ASXL1,* lead to an MDS-like disease in mouse models [335–337]. Furthermore, BAP1, a nuclear-localized deubiquitinating enzyme, formed a core complex with host cell factor–1 and O-linked *N*-acetylglucosamine transferase, which can preferentially recruit ASXL1 to regulate gene expression and, thus, preserve normal hematopoiesis. BAP1 deficiency in mice resulted in a CMMoL-like phenotype [338].

Mutations in the *ASXL1* gene have been identified in a substantial proportion (11.0–25.0%) of patients with MDS [28, 311, 339–342] or MPN [343, 344] and are correlated with unfavorable outcomes [311, 341, 342]. The incidence of *ASXL1* mutations among the total cohort of patients with AML and CN-AML is 3.0–30.0% and 5.3–12.5%, respectively [27, 68, 265, 340, 345–349]. *ASXL1* mutations are more frequent in AML from antecedent hematologic disorders than primary AML. *ASXL1* mutations are always located on exon 12. Almost all of these mutations are heterozygous, either nonsense or frameshift mutations, leading to the disruption of the C-terminal PHD finger, which is involved in chromatin modification and is well conserved among different species [332, 344, 350–352]. More than half of patients with *ASXL1* mutations exhibit c.1934dupG, which results in G646WfsX12.

ASXL1 mutation is closely associated with older age, male sex, isolated trisomy 8, and *RUNX1* mutations, but is inversely associated with t(15;17), complex cytogenetics, *FLT3/*ITD, and mutations in *NPM1* and *WT1* [27, 346–349, 353]. *ASXL1* mutations are recognized as a stratification criterion for AML in the ELN-2017 and ELN-2022 because AML patients with *ASXL1* mutations had a shorter OS compared to those without; however, most studies did not prove the mutation to be an independent adverse prognostic factor in multivariate analysis [27, 346, 348, 349]. A study of 1047 patients showed that hyperleukocytosis, presence of *FLT3*-ITD or *RUNX1* mutations, and absence of *AML1-ETO* fusion gene further led to risk stratify *ASXL1*-mutated AML [353]. In a meta-analysis of 4143 patients, the *ASXL1* mutation predicted shorter EFS and OS in patients with AML (HR, 1.63, 95% CI = 1.27–2.08, $p < 0.0001$; and HR, 1.59, 95% CI = 1.34–1.88, $p < 0.00001$, respectively) [354].

5.8.2 *KMT2A*-Rearrangement

The mixed-lineage leukemia (*MLL*) gene (now renamed histone-lysine N-methyltransferase 2A, or *KMT2A*), located on chromosome 11q23, encodes a DNA-binding protein that methylates histone H3 lysine 4 (H3K4) and positively regulates Hox gene expression [355]. Two major rearrangements have been identified in leukemia: *KMT2A* fusion and partial tandem duplication (PTD). *KMT2A* fusions result from reciprocal translocations, whereas PTD is an intragenic mutation.

5.8.2.1 *KMT2A* Fusion Protein

To date, at least 80 *KMT2A* fusion partner genes have been identified [355–357]. The six most common *KMT2A* fusions, accounting for more than 80% of all MLL translocation-bearing leukemias, are: t(4;11)(q21;q23)/*KMT2A::AF4*; t(9;11)(p22;q23)/*KMT2A::AF9*; t(11;19)(q23;p13.3)/*KMT2A::ENL*; t(11;19)(q23;p13.1)/*KMT2A::ELL*; t(10;11)(p12;q23)/*KMT2A::AF10*; and t(6;11)(q27;q23)/*KMT2A::AF6* [356]. Among them, *KMT2A::*AF4 is predominantly associated with lymphoid malignancies, whereas *KMT2A::*AF9 often causes myeloid malignancies [356]. Several of these MLL translocations may activate leukemogenic processes via alterations of histone methylation [355, 357]. The incidence of *KMT2A* fusion in AML is approximately 5.0–12.0% [358–361]. RAS signaling pathway mutations are the most frequently concurrent genetic alterations in patients with *KMT2A* fusions [362, 363]. More than 50% of patients with therapy-related leukemias secondary to topoisomerase II inhibitors harbor MLL translocations [357]. However, the relationship between *KMT2A* rearrangements and AML outcomes exhibits substantial differences based on the fusion partner [364, 365].

5.8.2.2 *KMT2A*/PTD

KMT2A/PTD usually results from an in-frame repletion of *KMT2A* genes encompassing exons 5 through 11/12 and insertion of the duplicated segment into intron 4 of the full-length *KMT2A* gene [366]. The duplication involves a portion of the gene corresponding to the amino terminus of the KMT2A protein, comprising of "AT hooks" DNA-binding motifs and CXXC domains [367, 368]. The mechanism by which *KMT2A*/PTD contributes to the leukemic phenotype remains unclear, but it may act through silencing of the wild-type *KMT2A* and increasing the transactivation potential [355, 369]. KMT2A/PTD alone in a knock-in mouse model showed an increased number of hematopoietic precursors capable of self-renewal and *Hoxa9* expression, which was not sufficient to induce leukemia [370]. Notably/, $KMT2A^{PTD/WT}$ and $FLT3^{ITD/WT}$ knock-in mice developed AML with 100% penetrance [371].

The incidence of *KMT2A*/PTD in AML is 5.0–10.0% [358–361, 372]. *KMT2A*/PTD occurred frequently in patients with CN-AML or trisomy 11 [372, 373]. The presence of extra copies of chromosome 11 is associated with early relapse of disease following initial remission [359]. Compared with patients without *KMT2A*/PTD, patients with this mutation more often had the FAB M2 subtype and wild-type *NPM1* and showed CD11b expression and high *BAALC* expression, but had lower WBC counts, less frequent extramedullary involvement, and FAB M4/M5 subtype at diagnosis [360, 374].

Regarding prognostic relevance, the presence of *KMT2A* rearrangement usually predicts distal outcome;[356, 359–361, 364]. The prognostic implication of KMT2A fusion is heterogeneous and highly dependent on its associated partner genes. The OS was much better in patients with t(9;11) and t(11;19) than in those with other *KMT2A* fusions [375–378]. Allo-HSCT in first CR may overcome the poor prognosis conferred by the *KMT2A* rearrangement [374, 375, 379]. Nevertheless, t(6;11) and t(10;11) are still associated with a dismal outcome, thus, highlighting the unmet need of a novel targeted therapy.

5.8.3 *EZH2* Mutations

Polycomb group (PcG) proteins initiate and maintain transcriptional silencing through posttranslational histone modifications. PRC2, formed by PcG proteins, is crucial for transcriptional regulation through nucleosome modification, chromatin remodeling, and interaction with other transcription factors [305, 380]. PRC2 comprises EZH2 or EZH1 as the catalytic subunit and other components, such as embryonic ectoderm development (EED), suppressor of zeste 12 homologue (SUZ12), and RBAP48, functioning to maintain stable enzymatic structures [381]. EZH2 is located on chromosome 7q, which is a site frequently detected in myeloid malignancies [380]. EZH2 serves as an H3K27 methyltransferase and is essential for fetal hematopoiesis, whereas EZH2 deletion in adult BM only disturbs lymphopoiesis [382, 383]. *EZH2* overexpression is frequently found in prostate, breast, endometrial, and other cancers, and increased *EZH2* expression promotes tumor progression. This implies that EZH2 and PRC2 are attractive targets for cancer therapy [384–386].

In hematologic malignancies, gain-of-function somatic mutations in codon 641 (Tyr641) of the catalytically active SET domain of EZH2 are frequently detected in patients with follicular lymphoma and germinal center diffuse large B-cell lymphoma [387]. Furthermore, Sneeringer et al. demonstrated that the malignant phenotype required the coordination of the activities of an H3K27 monomethylating enzyme (wild-type EZH2 or EZH1) and mutant PRC2s for the augmented conversion of H3K27 to the trimethylated form [388]. In contrast, loss-of-function mutations at diverse sites in *EZH2* have been detected in myeloid malignancies. Loss of H3K27 trimethylation via *EZH2* inactivation due to mutations may contribute to MDS, MPN, or MDS/MPN overlap syndrome [389, 390]. Therefore, EZH2 may serve dual functions as an oncogene and tumor-suppressor gene in malignant hematopoiesis [380].

EZH2 mutations are found in 2.5–5.9%, 3.0–13.0%, and 8.0–12.0% of patients with MDS, MPN, and MDS/MPN, respectively, and mutant *EZH2* confers a poor prognosis in progression-free survival and OS [390–392]. However, this mutation is rarely detected in patients with AML (0–2.0%)

[390, 391, 393, 394]. Wang et al. studied a cohort of 714 *de novo* AML patients and showed that *EZH2* mutations (1.8%) were associated with male sex, lower BM blast percentage, and chromosomal changes of −7/del(7q) [394]. Similarly, *EZH2* mutations seldom occur in pediatric patients with AML [395, 396]. Loss-of-function mutations in *EZH2* provided leukemia cells with a selective growth advantage, which mediated chemotherapy resistance [397]. Due to the small number of patients harboring *EZH2* mutations, the clinicopathological features and prognostic relevance of this alteration in AML remain largely unknown.

5.9 Mutations Involving Splicing Complex Factor Genes

5.9.1 Splicing Factor Mutations

RNA splicing, a crucial posttranscriptional process, plays an important role in gene regulation and increases genomic diversity [398]. However, aberrant splicing pathologically drives the initiation and progression of cancer, including that of hematological malignancies. Somatic mutations involving the core components of the RNA splicing machinery were first detected in MDS [399, 400]. Mutations of the splicing factor (SF) genes occur most often in *SRSF2, U2AF1, SF3B1,* and *ZRSR2,* but infrequently in *U2AF2, SF1, SF3A1, PRPF40B, PRPF8,* and *LUC7L2* [399]. Similar to the MDS data, the majority of SF mutations occurred in hotspot areas of the representative gene: K666N and K700E in *SF3B1*; S34, and Q157 in *U2AF1*; and P95 in *SRSF2* [44, 46, 401–403]. SF mutations tend to be mutually exclusive and exhibit a strong genotype and phenotype association: *SF3B1* is mostly mutated in MDS-RS, *SRSF2* mutations occur mostly in CMMoL, and *U2AF1* mutations occur in secondary AML. [401–403]. The reported incidence of SF mutations in AML varies from 4.5–22% in different studies [14, 44, 46, 404–406], due to differences in ethnic background, the patient population information analyzed (age range, FAB subtypes and karyotypes, etc.), regions and number of SF genes screened, and experimental methods used. SF mutations are closely associated with intermediate-risk cytogenetics and *RUNX1*, *ASXL1*, *IDH2,* and *TET2* mutations [44, 46].

The presence of SF mutations independently predicts a lower CR rate and shorter DFS and OS [44, 46]. *SF3B1* mutation was also associated with a lower CR rate and shorter survival [44], even though *SF3B1* mutations predict better OS in patients with MDS [400, 407–409]. It would be beneficial to incorporate SF mutations in the 2017 ELN risk classification [46]. Lindsley et al. were the first to show that SF (*SF3B1, SRSF2*, *U2AF1,* and *ZRSR2*) as well as *ASXL1*, *EZH2*, *BCOR*, and *STAG2* mutations were highly specific for secondary AML and were secondary-type mutations in therapy-related AML and elderly *de novo* AML and defined a distinct subgroup of patients with a poor outcome [43]. Consequently, AML with myelodysplasia-related gene mutations is now categorized as a separate entity in the 2022 WHO classification (including the eight genes shown above) [19] and ICC (including the eight genes and *RUNX1* mutation) [20]. The discovery of somatic mutations in the spliceosome and/or aberrant splicing in cancer has prompted research interest in novel therapeutic approaches involving targeted splicing catalysis, splicing regulatory proteins, and individual key altered splicing events [410–412].

5.10 Mutations Involving Cohesin Complex Genes

5.10.1 Cohesin Mutations

Cohesin is a multimeric protein complex that was discovered in yeast [413]. In vertebrae somatic cells, the cohesin complex comprises four core subunits, including SMC1A, SMC3, RAD21, and either STAG1 or STAG2 proteins [414]. They form a ring-shaped structure [415] and mediate sister chromatid cohesion and segregation during mitosis and meiosis [416–418]. The cohesin complex is also involved in DNA repair [419], three-dimensional chromatin looping [420], and gene transcription regulation [421, 422].

Cohesin gene mutations have been reported in myeloid neoplasms, at a frequency of 6.3–13.3% in *de novo* AML [14, 15, 43, 404, 423–428]. The presence of a mutation in cohesin genes is highly specific for t(8;21) AML; and *RAD21* mutation predominated in this subgroup, whereas *STAG2* mutation predominated in CN-AML [429]. Cohesin gene mutations are mostly mutually exclusive, and numerous studies have implied that mutations in one subunit could lead to the abrogation of the cohesin complex [430–434]. Therefore, the cohesin complex should be considered as a whole rather than as separated subunits while investigating its role in AML. Cohesin gene mutations significantly co-occurred with mutations in *TET2* (p = 0.027), *ASXL1* (p = 0.045), and *EZH2* (p = 0.011) in myeloid neoplasms [426]. The most common concurrent molecular events in cohesin-gene-mutated AML were mutations in *FLT3*/ITD (21.6%) and *NPM1* (21.6%), followed by mutations in *TET2* (18.9%), *CEBPA* (18.9%), *DNMT3A* (18.9%), *IDH2* (13.5%), and SF genes (11.1%) [428]. Several factors affect the prognostic significance of cohesin gene mutations [429], thus, the prognostic significance of cohesin gene mutations in AML is still poorly understood. Thol et al. showed that cohesin gene mutations did not exhibit any association with clinical outcomes [424]. In contrast, Tsai et al. showed that

cohesin gene mutations had a favorable effect on both OS and DFS in a cohort of 391 patients with non-M3 AML [428].

5.11 Conclusion

Recent advances in genomic sequencing have revealed the molecular heterogeneity of AML leukemogenesis and have further refined risk stratification and prognostication. However, the complex pattern of cooperation and mutual exclusivity among different mutations remains a challenge. Therefore, it is clinically relevant to comprehensively elucidate molecular signatures to better characterize AML biology, precisely predict prognosis, and tailor treatment strategies with targeted agents.

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6 Frontline Management of Acute Myeloid Leukaemia Eligible for Intensive Chemotherapy

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Abstract

The phrase 'Panta Rhei—everything flows' by the Greek philosopher Heraclitus, a purported reference to a constantly changing flowing river, or more philosophically, 'continual transformation', can be aptly extended to describe the evolution of treatment strategies for many human diseases. In disappointing contrast, the drug treatment for patients with non-promyelocytic, acute myeloid leukaemia (AML) has remained essentially unchanged for over 50 years, with improved outcomes over this period, largely, a consequence of incremental improvements in supportive care and the application of allogeneic stem cell transplantation. The anti-leukaemic effectiveness of single-agent daunorubicin (D) or cytarabine (Ara-C) was first recognised over half-a-century ago, and intensified leukaemic cell kill with these genotoxic drugs (DA) became the standard approach for treating newly diagnosed AML patients. At the time of writing, induction therapy combining these two pharmacological classes of drugs, followed by intensified consolidation or allogeneic stem cell transplantation, remains the only proven strategy for curing AML. Here, through a review of the development of different anti-leukaemic drug combinations, we evaluate the effectiveness of various intensive chemotherapy platforms and the evidence for using adjunctive or sequential therapy with newer, genotoxic or non-genotoxic agents.

Keywords

Acute myeloid leukaemia · Induction chemotherapy · Consolidation chemotherapy · AML trials

6.1 Intensive Chemotherapy in AML: A Historical Perspective

There is little evidence to support the recognition of AML as a unique cancer until the early twentieth century [1, 2]. Developments in the late nineteenth century that established the relevance of the bone marrow to blood cell production, and improvements in staining techniques enabled the recognition of the myeloblast as a granulocyte precursor cell. The distinction between myeloblasts and lymphoblasts, led to the identification of sub-types of acute leukaemia by Reschad and Schilling-Torgau in 1913 including AML and monocytic leukaemia [1, 2]. Compared to chronic leukaemias, this delayed recognition of AML may, in part, have been due to a rapidly fatal clinical course, with limited diagnostic or therapeutic opportunities. Thus, descriptive reports on arsenic and x-ray treatment for 'leukaemia' at the turn of the nineteenth century included patients with likely chronic myeloid leukaemia (CML) or chronic lymphoid neoplasms, but not AML [3, 4]. Following the identification of AML as a subtype of acute leukaemia [1, 2, 5], early attempts to achieve disease control in these patients probably relied on similar approaches used to treat CML and acute lymphoblastic leukaemia (ALL) and included the use of urethane [6]. Notwithstanding limited scope for rigorous scientific scrutiny, an occasional, durable response following combination therapy with radiation, arsenic and thorium-X (RAT), was suggested [7], but in most patients, survival remained unaltered despite a modest, transient reduction in disease burden.

Research into nitrogen mustards during the second World War benefitted patients with lymphoma more than leukaemia; an indirect consequence was the development of alkyl-

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ating agents, subsequently incorporated into induction or maintenance regimens for AML [1, 2]. Important discoveries crucial to the successful pharmacotherapy of ALL followed, and included folate antagonists, prednisone and 6-mercaptopurine (6-MP), leading to an investigation of their effectiveness in AML [8, 9]. In a series of 15 patients with acute leukaemia including 11 with AML, treatment with 6-MP (2.5 mg/kg/day for 3 weeks) resulted in clearance of marrow leukaemic infiltrate and blood count recovery. This state of complete remission (CR) was maintained for 3 months and improved survival [9]. It would be easy to underplay the significance of these and other observations of the time, or the seemingly 'negative' results of older studies, but two principles that would form the cornerstone of future therapies, in particular intensive chemotherapy (IC) of AML, began to emerge. These included the recognition of 'remission' as a critical pre-requisite for improving survival, and the existence of unique differences between the drug susceptibility of ALL and AML blasts that necessitated a different pharmacological approach to improve the durability of responses.

By the early1960s, amidst the acknowledgment that extending the drug-therapy of ALL to AML could at best achieve CR rates of 20% [10, 11], pre-clinical and clinical studies of two drugs began, which would subsequently alter the treatment paradigm for AML. One of these, arabinosylcytosine or cytarabine, a cytidine analogue exhibited anti-leukaemic properties in murine models of the disease [12], with additive effects in combination with thioguanine [13]. In human studies, as a single agent, CR rates with cytarabine were not dissimilar to other forms of monotherapy of the time and approached 30% [14], but multi-agent therapy with methylglyoxal-bis-guanilhydrazole [15], 6-MP [16] and cytarbine increased CR rates to 40% [17]. In separate studies [18], a role for daunorubicin (daunomycin) in the therapy of AML was beginning to be recognised: starting with a dose of 2 mg/kg/day (60–80 mg/m^2 as the maximum daily dose), titrated to changes in circulating and marrow blasts each week, pronounced leucopaenia was observed in most patients within 3–5 successive days of treatment. Remarkably, over half the patients achieved CR after 2–8 weeks, with marrow aplasia around day 8, a consistent feature. Treatment with methotrexate and 6-MP maintained remission and was interrupted at intervals for re-induction with daunorubicin and methyl-GAG. The average duration of response was 155 days, with some patients maintaining remission even after a year. Based on this study, a cumulative daunorubicin dose ceiling of 750 mg/m^2 was suggested, but concerns around the drug's narrow therapeutic index [19], and acquired drug resistance with monotherapy meant that the subsequent focus was combination approaches incorporating daunorubicin at lower doses. By the late 1960s, cytarabine in combination with 6-MP, thioguanine, or daunorubicin was considered standard remission-induction therapy in newly diagnosed AML patients, based more on the clinical impression of efficacy than statistical significance [20, 21]. Studies relating changes in marrow cellularity to DNA synthesis in patients receiving 6-MP and 6-methylmercaptopurine highlighted the attainment of early post-treatment aplasia as a pre-requisite for CR and improved survival [22], thus establishing the importance of intensified induction therapy, despite its potential for toxicity.

Earlier studies by the Acute Leukemia Group B (renamed Cancer and Acute Leukemia Group B, CALBG in 1976) suggested the utility of two daily doses of daunorubicin with a 5 day schedule of cytarabine, administered as two 5 day courses [20]. Following a pilot study with a 7-day course of infusional cytarabine (100 mg/m^2/day) combined with daily daunorubicin (45 mg/m^2/day) for the first 3 days of treatment ('DA 3 and 7'), a multicentre randomised investigation of 'DA 3 and 7', compared to the 5-day schedule (DA 2 + 5), was undertaken in 352 previously untreated AML patients, including 247 < 60 years old [23]. The impact of the mode of cytarabine delivery on outcomes was also investigated: during induction, 24-h continuous intravenous infusion of the drug (100 mg/m^2/day) was compared with bolus injections (100 mg/m^2) administered 12-h apart and in 4-monthly cycles of maintenance treatment that combined lower-doses of cytarabine (by subcutaneous or intravenous bolus route) with thioguanine, cyclophosphamide, lomustine or daunorubicin. The results were clear: a superior rate of CR and reduction in induction deaths with the 7-day induction schedules was observed in patients lt;60 years and older patients in whom, until then, the potential toxicity of intensifying induction therapy was believed to negate the anti-leukaemic benefits. The numerical difference in CR rates between cytarabine as an infusion (56%) and bolus (49%) may have been skewed by an unexplained 29% decrease in CR rates in the bolus arm during the latter part of the study, but did not meet statistical significance even after adjustment for age and time-of-entry to study. Whether overall survival (OS) is affected by infused or bolus cytarabine is difficult to ascertain since survival in responding patients was analysed in sub-groups categorised by the route of cytarabine administration in induction and maintenance and included patients receiving DA 2 + 5. Nevertheless, by confirming the importance of CR to prolongation of survival, 'DA 3 and 7'-containing infusional or bolus cytarabine became an accepted standard for intensive induction therapy in many centres, to this day.

The British approach to induction IC had subtle differences: intravenous cytarabine (100 mg/m^2) was administered as 12-hourly bolus doses for 10 days with daunorubicin (50 mg/m^2/day on days 1, 3 and 5) and oral 6-thioguanine (100 mg/m^2 12-hourly, days 1–10), i.e. DAT 3 + 10 [24]. Although differences in CR rates were not statistically significant over the lower intensity schedule (DAT 1 + 5), time to CR was a median of 34 versus 46 days and associated with supe-

rior survival at 5 years (25% vs. 18%, *p* lt; 0.05). Thus DA 3 + 10 (with thioguanine subsequently omitted due to non-availability, hepatotoxicity, and questionable benefit) became an alternative standard to 'DA 3 and 7' against which investigative pharmacological approaches continue to be compared.

Over the last 30 years, strategies to optimise outcomes of IC have included changes to dosing intensity [25], conventional genotoxic drugs [26], drug-delivery systems [27], and the inclusion of small molecule drugs to target AML characterised by unique gene variants [28]. A meaningful comparison of results across studies presents challenges: fundamental differences exist in the design of induction and subsequent treatments and dosing-schedules. For example, the administration of a second course of anthracycline-containing induction therapy is standard practice following DA 3 + 10 in some countries [29]; in others, this approach is reserved only for patients with detectable disease following one course [30]. German study groups tend to use a double-induction strategy in patients <60 years old, but to achieve early treatment intensification, the second cycle is administered at the pre-defined time-point of day 21 of cycle 1 [31]. In addition to this variation between IC protocols, as the biology of AML advances [32–37], the possibility that outcomes following the same medicinal agent in contemporaneous studies may be confounded by unrecognised heterogeneity in disease sub-types also requires consideration.

6.2 Intensifying Induction Chemotherapy: Looking beyond 'DA'

6.2.1 Optimising Cytarabine

Over time, improvements in supportive care have enabled better management of chemotherapy-associated toxicity [38], facilitating the investigation of the effects of dose-intensification in induction. Initial studies compared the effects of increasing the duration or dose of cytarabine within the 'conventional' range of 100–200 mg/m^2/day. Extending the duration of 'DA 3 and 7' to 10 days, with additional days of cytarabine (100 mg/m^2/day) as an infusion, did not impact on remission-rates or duration [39]. Likewise, response-rates, relapse, and deaths in remission remained unaffected despite doubling the bolus cytarabine dose, from 100 to 200 mg/m^2 in DAT 3 + 10 [40]. This led to randomised comparisons of considerably higher doses of cytarabine in induction: conventional protocols containing 100–200 mg/m^2/day for 7–10 days, and thus a cumulative dose of 0.7–1.4 g/m^2 were compared against schedules containing cumulative doses of 5–24 g/m^2 [41–45]. Confounding a comparison of these studies are differences in concomitant therapy, including daunorubicin (40–50 mg/m^2/day for 3 days), idarubicin or mitoxantrone, with additional drugs given in some protocols [41, 44, 45]. Post-remission strategies between studies too differed, including the application of autologous or allogeneic stem cell transplantation, known to impact survival outcomes [46–48], and maintenance treatment. These confounders notwithstanding, the improvement in remission-duration or disease-free survival (DFS) with higher doses of cytarabine in induction regimens did not improve OS in most studies, including those investigating dose-dense regimens [31]. Exceptions include patients <46 years in the EORTC-GIMEMA AML-12 study [44] that investigated the effects of increased cytarabine dosage (3 g/m^2 every 12 h by 3-h infusion on days 1, 3, 5 and 7) and more recently, in a Chinese study, with patients receiving relatively lower doses [100 mg/m^2/day, days 1–4 followed by 1 g/m^2 every 12 h, days 5–7 (cumulative dose of 6.4 g), with 40 mg/m^2 of daunorubicin and omacetaxine mepesuccinate] [45]. In the EORTC-GIMEMA study [44], the survival benefit with higher dose cytarabine (42.5% vs. 38.7%) was not statistically significant, except in patients aged 15–45 years. In the study from China [45] that included a second randomisation to different cytarabine doses (3 vs. 1.5 g/m^2) in consolidation, a DFS (67% vs. 54%, $p = 0.005$) and OS (68% vs. 59%; $p = 0.014$) benefit was observed with the higher dose of cytarabine in induction, even after censoring for transplantation. While the second randomisation did not affect outcomes, the survival benefit with the higher cytarabine dose in induction was restricted to patients who received 3 g/m^2 in consolidation.

Since some protocols investigating higher-dose cytarabine in induction (3 g/m^2) had de-escalated drug-doses [41, 44] or avoided cytarabine [43] during consolidation, the sequential administration of higher-intensity cytarabine may achieve the depth of remission need for cure. This hypothesis was investigated through the randomisation of patients who had achieved remission following higher-dose cytarabine (at a cumulative dose of 24 g/m^2), etoposide and daunorubicin (ICE) induction to a further cycle of ICE or two courses of attenuated ICE that contained a lower, cumulative cytarabine dosage of 500 mg/m^2 [49]. Sequential intensified chemotherapy was more toxic without affecting relapse-free or OS. These results, supported by subsequent HOVON/SAKK [43] and AML-CG [50] studies, indicate that intensified cytarabine-induction is unlikely be advantageous to patients receiving second induction or consolidation at the same cytarabine dose.

6.2.2 Optimising Anthracyclines

Initial studies on daunorubicin investigated a dose schedule of 60–80 mg/m^2/day, with a mean of 7 doses (range 2–17) [18], but concerns regarding toxicity resulted in a reduction to 45 mg/m^2/day for 3 consecutive, or alternate days in many protocols, including in the landmark 'DA 3 and 7' study [23]. More recently, four randomised controlled trials [51–56] have investigated whether a higher dose of daunorubicin (90 mg/m^2) can optimise outcomes (Table 6.1). In a

Table 6.1 Summary of randomised clinical trials investigating the effects of daunorubicin-intensification in induction therapy

Study	Medianage (range)	*n*	Median follow-up (months)	End-point(s)	Drugs	CR	EFS	OS	Sub-group benefit
HOVON-SAKK-AMLSG [51]	67 (60–83)	813	40	EFS	**D**45 mg/m^2 d1–3	64% vs. 54% ($p = 0.002$)	2 years	–	Age (60–65 years)
					A200 mg/m^2 inf. d1–7		20% and 17% ($p = 0.12$)		Favourable risk CG
ECOG E1900 [52, 53]	48 (17–60)	657	80.1	OS	**D**45 mg/m^2 d1–3	70.6% vs. 57.3% ($p < 0.001$)	–	4 years	Age (<50 years)
					A100 mg/m^2 inf. d1–7			39% vs. 41% ($p = 0.001$)	All CG risk-groups
									NPM1^{mut}
									DNMT3A^{mut}
									FLT3 ITD
Cooperative Study group A for hematology [54]	43 (15–60)	383	52.6	CR, OS, EFS and RFS	**D**45 mg/m^2 inf. d1–3	82.5% vs. 72% ($p = 0.014$)	5 years	5 years	Intermediate-risk CG
					A200 mg/m^2 inf. d1–7		46.8% vs. 34.6% ($p = 0.03$)	40.8% vs. 28.4% ($p = 0.03$)	
							RFS unchanged		
NCRI AML17 [55, 56]	53 (16–72)	1206	28	OS	**D**60 mg/m^2 d1,3,5	73% vs. 75% ($p = 0.6$)	–	2 years	*FLT3*-ITD
					A100 mg/m^2/12 h d1–10			59% vs. 60% ($p = 0.16$)	

The daunorubicin (**D**) dose in the experimental arm of each study was 90 mg/m^2, with the cytarabine (**A**) doses and administration schedules ('inf.' indicating continuous intravenous infusion over 24 h) between the control (shown here) and experimental arms were identical

OS overall survival, EFS event-free survival, CR complete remission rate, RFS relapse-free survival, CG cytogenetic risk

comparison of 90 mg/m^2 with 45 mg/m^2, a significant improvement in CR rates was observed following the higher dose, with a similar incidence of haematological toxicity or induction deaths, with improvement in survival outcomes in three studies [51–54]. However, in the UK NCRI AML17 trial [55, 56] comparison of 60 mg/m^2 with 90 mg/m^2 in younger patients, a higher 60-day mortality was observed with daunorubicin dose-intensification, without an improvement in OS. Unlike the other studies, AML17 incorporated 2 cycles of anthracycline-containing induction, and therefore, the cumulative daunorubicin dose in the 'lower-dose' cohort was 330 mg/m^2, exceeding that in the high-dose, single-induction schedules (270 mg/m^2) of the other trials. Nevertheless, and sub-group analysis notwithstanding, a survival benefit was evident with 90 mg/m^2 of daunorubicin in patients with *FLT3* internal tandem duplications (ITD) in AML17 [56], commensurate with the ECOG study [53], which additionally reported a benefit in disease with *NPM1* and *DNMT3A* gene variants. Thus, daunorubicin could be useful at 60–90 mg/m^2 in induction, with a reduction in dose (45 mg/m^2), should re-treatment be required for persistent disease [30].

Idarubicin as a synthetic anthracycline analogue has been of interest as an alternative to daunorubicin [57], but a direct comparison of the two drugs across studies is challenging due to variation in dosage, concomitant, and subsequent therapies. A meta-analysis of 1052 patients treated in five randomised trials from the 1980s suggests an advantage with idarubicin over daunorubicin in younger patients [58], but the applicability of the conclusions to modern practice is questionable. More recently, higher doses of daunorubicin have been compared with idarubicin: in the JALSG AML201 Study [59], an additional 2 days of daunorubicin, i.e. 50 mg/m^2/day for 5 days, did not improve remission or survival outcomes over idarubicin (12 mg/m^2/day for 3 days). The ALFA-9801 study [60] randomised patients aged 50–70 years to daunorubicin at 80 mg/m^2 for 3 days or idarubicin, 12 mg/m^2 for 3 or 4 days, with a 7-day course of cytarabine. Idarubicin for 3 or 4 days produced superior remission rates (83% and 78% respectively) compared to daunorubicin (70%), but without a benefit to event-free survival or OS. Finally, a Korean study [61] of 3 consecutive days of idarubicin (12 mg/m^2/day) and daunorubicin (90 mg/m^2/day) too did not identify differences in remission or survival, except in patients with *FLT3*-ITD in whom daunorubicin appeared superior.

Studies of mitoxantrone have included its randomised comparison in combination with cytarabine and etoposide (MAE), against cytarabine, daunorubicin and etoposide (ADE) in the MRC AML12 trial that showed no differences response-rates or early mortality; while the relapse-risk was reduced with MAE (48% vs. 57%; $p = 0.006$), DFS survival and OS remained unaffected [40]. A similar lack of difference in OS at 5-years was observed by EORTC-GIMEMA investigators comparing outcomes following daunorubicin, idarubicin or mitoxantrone in induction and consolidation in patients <60 years [62]. Studies in older patients too have been inconclusive: randomised studies by EORTC-HOVON [63] and SAL [64] report improved response-rates with mitoxantrone compared to daunorubicin containing combination induction, but with many confounding variables, no benefit on survival outcomes has emerged.

6.3 Three Drug Combinations

6.3.1 Etoposide

Whether a 3-drug combination could lead to a superior and clinically meaningful anti-leukaemic effect was investigated by the Australian Leukemia Study Group in patients aged 15–70 years through the addition of etoposide to 'DA 3 and 7' [65]. Since recruitment started in 1984, data on cytogenetic risk groups were not routinely collected and variables within the two arms of the study were not evenly matched: the etoposide cohort contained a higher number of patients with APL, more patients with the FAB sub-type M1 received 'DA 3 and 7' and patients in this arm had a higher circulating leukaemic load. With these caveats, the addition of etoposide did not improve CR rates (59% vs. 56%), but benefited remission-duration (18 vs. 12 months, $p = 0.01$) without impacting OS. In patients <55 years, the improvement in remission-duration was associated with improved OS, suggesting the utility of etoposide in younger patients. The role of etoposide when added to DA (ADE) was evaluated against DA and FLAG-Ida in the NCRI AML15 trial in patients under 60 years [66]. As in the Australian study, the overall response-rates including CR and CR with incomplete count recovery (CRi) were similar in the randomised comparison of double-induction with ADE (86%) and DA (84%), although more patients achieved CR/CRi after the first course of ADE (70%) than DA (63%, $p = 0.002$). In a separate comparison, overall response-rates after one course of FLAG-Ida (see 6.3.2) (77%) were higher than ADE (67%, $p < 0.001$), but similar following the second course (86% and 85% respectively). As with etoposide in the Australian study [65], adverse events affecting the gastrointestinal tract were more frequently reported with ADE, but survival outcomes were not different.

6.3.2 Purine Analogues

Based on the in vitro potentiation of cytarabine cytotoxicity in blasts pre-treated with the purine nucleoside analogue fludarabine [67], and the clinical effectiveness of fludarabine-containing salvage regimens in relapsed or refractory AML [68, 69], the MRC AML15 trial investigated fludarabine, cytarabine, granulocyte colony-stimulating factor and idarubicin (FLAG-Ida) in induction for patients <60 years of age, compared to ADE [66]. Although a 10% improvement in CR/CRi was observed following 1 cycle of FLAG-Ida (77%), this difference was no longer evident after the second treatment-cycle of a double-induction schedule, with overall response-rates approaching 85% with both regimens. No differences in toxicity were evident after cycle 1, but haemopoietic recovery was delayed following the second cycle of FLAG-Ida, with more supportive care requirements. This excessive toxicity resulted in less than half the patients entering subsequent randomisation, with 54 patients discontinuing therapy entirely. Of interest, the survival of these patients receiving truncated therapy was similar to those treated with ADE or DA and a further two courses of HDAC. Others who completed the entirety of intended consolidation following double-induction with FLAG-Ida experienced superior survival (74% vs. 54%, $p < 0.001$) even after adjustment for age, white count, cytogenetics and secondary disease. For the entire cohort of FLAG-Ida recipients, at a median follow-up 5.6 years, the reduction in relapse-risk (38% vs. 55% $p < 0.001$,) however failed to translate into an OS benefit over standard treatment (44% vs. 37%, $p = 0.2$) due to excessive deaths in remission (17% vs. 11%, $p = 0.02$).

The effects of cladribine as a third drug with DA during induction (and consolidation) therapy in younger patients have been investigated by the Polish Adult Leukaemia Group (PALG) [70, 71]. With cladribine added to a double-induction schedule consisting of 'DA 3 and 7' (60 mg/m^2 daunorubicin and 200 mg/m^2 cytarabine administered as an infusion) CR rates were equivalent to 'DA 3 and 7', but a greater proportion of patients achieved CR after 1 cycle of cladribine-containing treatment (64% vs. 47%, $p = 0.0009$). The difference in CR rates did not translate into improvements in OS at 3-years, although leukaemia-free survival improved (44% vs. 28%, $p = 0.05$) in patients >40 years of age [70]. A follow-up study compared the addition of fludarabine or cladribine to 'DA 3 and 7' [71]. Here, superior remission rates with cladribine (67% vs. 56%, $p = 0.01$) were associated with better survival (45% vs. 33%, $p = 0.02$) at a median of 2.8 years, benefitting patients with poor-risk features: those older than 50 years, with high white counts or adverse karyotype. Although no obvious imbalance in patient demographics or disease characteristics was evident between the study cohorts, outcomes with DA were lower than in comparable studies [55, 56]. The addition of fludarabine (25 mg/m^2 daily for 5 days) to DA suggested an OS benefit to patients with an adverse karyotype, with no other difference in outcomes.

Clofarabine (2-chloro-2′-fluoro-deoxy-9-β-darabinofuranosyladenine), a second-generation purine nucleoside analogue, has been investigated as a 'third drug' in induction. As with fludarabine in AML15 [66], the addition of clofarabine (10 mg/m^2 daily for 5 days) to cytarabine (200 mg/m^2 infusion) and idarubicin (cycle 1) and amsacrine (cycle 2) in a HOVON-SAKK study [72] increased the speed of remission and reduced relapse-risk in adults <65 years old, without improving survival outcomes, except in patients with intermediate-risk disease [73]. Greater toxicity was observed in recipients of clofarabine. Clofarabine-containing IC and FLAG-Ida have only been directly compared as second induction in younger patients with high-risk disease following induction cycle one; here, the relapse-free survival and OS favoured FLAG-Ida over clofarabine and daunorubicin [74]. In patients >60 years old, the combination of daunorubicin and clofarabine (20 mg/m^2 daily for 5 days) as induction chemotherapy delayed haemopoietic recovery, but did not improve remission rates or survival over daunorubicin and cytarabine [75].

6.4 Gemtuzumab Ozogamicin (GO, GO)

The frequent expression of CD33 on AML blasts and rapid internalisation of antibodies targeting this antigen provided novel opportunities for therapeutic antibody-cytotoxic drug conjugates. Mylotarg/gemtuzumab ozogamicin (GO), a humanised anti-CD33 monoclonal IgG4 antibody conjugate with calicheamicin, an antibiotic that cleaves DNA at specific sequences [76], produced response-rates of 30% as monotherapy in relapsed AML [77] and received accelerated approval for managing disease-relapse in patients unsuitable for IC. In the registration study, 9 mg/m^2 of GO was administered intravenously, 2 weeks apart, based on >75% of target sites being saturated at this dose [76, 77]. In the post-marketing study commitment, the effects of adding GO to IC were investigated with a single dose of 6 mg/m^2 in 'DA 3 and 7' (45 mg/m^2 of daunorubicin) [78]. The control arm consisted of 'DA 3 and 7' (60 mg/m^2 of daunorubicin). Significant differences in toxic death during induction treatment in the experimental arm (17/296 vs. 4/300 with DA, $p = 0.0062$) were observed due to haemorrhage and pulmonary events, leading to pre-mature termination of the study. No difference in survival outcomes was evident between the

treatment-arms; in subset analysis relapse-free survival in patients with favourable cytogenetics was improved. Concerns regarding the toxicity of GO at doses ≥6 mg/m^2 were heightened further by Grade 3 or 4 hyperbilirubinemia (29%) reported in the final analysis [79] of the licensing study. This, combined with the rapidity of CD33 re-expression [80], made investigators study lower, or fractionated doses of GO in combination with IC [81]. The UK NCRI AML15 study in patients <60 years of age randomised patients to 3 mg/m^2 of GO in induction (DA, ADE or FLAG-Ida) and/or consolidation. GO did not impact on remission-rates or 30-day mortality, but supportive care requirements in recipients of GO were greater despite the equivalence in times to haemopoietic recovery [82]. There was a significant reduction to the relapse-risk, and a survival advantage emerged when the follow-up was extended to 8 years [83]. When risk groups were analysed, an OS benefit was evident in patients with favourable cytogenetics, and to a lesser extent, in intermediate-risk disease. In a separate NCRI trial, AML16 [84], a single dose of GO (3 mg/m^2), combined with DA 3 + 10 or daunorubicin-clofarabine-based induction therapy for patients gt;60 years, improved relapse-free survival (21% vs. 16% at 3 years, $p = 0.04$), as well as OS at 2 (35% vs. 29%) and 3 years (25% vs. 20%, $p = 0.05$). This improvement in outcomes was not replicated in an EORTC-GIMEMA study investigating the sequential administration of a single-dose of GO (3 mg/m^2), followed by intensive induction with mitoxantrone, etoposide and cytarabine in patients over 61 years of age, and excessive toxicity observed in older patients [85]. In the context of the ALFA-0701-based DA induction schedule, however, fractionated doses of GO (3 mg/m^2 with a maximum dose of 5 mg given on days 1, 4 and 7) positively affected event-free survival (13.6 vs. 8.5 months, $p = 0.006$) in patients between 50–70 years (who received an additional single dose during consolidation cycles), despite the absence of an OS benefit (HR: 0.81, $p = 0.16$) [86, 87]. The survival benefit in favourable and intermediate-risk AML including *NPM1* and *FLT3* mutated disease in sub-analysis, but not adverse cytogenetic risk AML, was confirmed in a meta-analysis of five frontline trials in which GO was combined with induction IC [83]. However, no improvement in event-free survival was evident in *NPM1* mutant disease with a single dose of GO (3 mg/m^2) added to induction (and consolidation) therapy in a German study of sufficient statistical power that included older patients [88]. Here, the reduction in cumulative incidence of relapse was negated by a higher induction-death rate with GO that disproportionately affected older patients (20.4% vs. 4%), possibly due to toxicity with idarubicin, cytarabine, etoposide-containing chemotherapy and all-trans retinoic acid (ATRA) in patients >70 years old. The reduction in relapse in recipients of GO was associated with a greater suppression of *NPM1* mutant transcript levels suggesting a potent, deeper anti-leukaemic effect [89].

The toxicity of doubling the dose of GO (6 vs. 3 mg/m^2) during induction with DA in younger patients was observed in the NCRI AML17 trial [90]. Near-doubling of mortality with the higher dose was observed at 30 days (7% vs. 3%, $p = 0.02$) and 60 days (9% vs. 5%, $p = 0.01$), with more veno-occlusive disease (5.6% vs. 0.5%, $p < 0.0001$) but unchanged response-rates or longer-term survival outcomes [30].

6.5 Modulators of Chemotherapy

Optimisation of induction chemotherapy has also been investigated through the addition of drugs with differentiation-inducing or cytotoxicity-potentiating capabilities. The use of ATRA [91, 92] with chemotherapy failed to improve outcomes in randomised studies [93–95], except in sub-analysis of patients with *NPM1*^{mut} disease [94] or low *MN1* expression [96]. These results are however not supported by other studies [97] or meta-analysis [98]. Pharmacological inhibition of the membrane transporter P-glycoprotein (P-gp) to reduce chemotherapy efflux from leukaemic cells [99, 100] using valspodar (PSC-833) has been attempted, but the toxicity was problematic and disease control remained unchanged [101–103]. The use of G-CSF either as a priming agent [104], or to ameliorate the toxicity of chemotherapy has also yielded disappointing results: the benefits restricted to DFS survival [105] have not been reproducible [106], and in the post-treatment phase, infection-related complications remain unchanged despite faster neutrophil recovery and reduced hospitalisation [107]. A meta-analysis of 19 trials including colony-stimulating factor therapy too supports the limited utility of G-CSF in unselected patients receiving IC [108].

6.6 Post-Remission Strategies in AML: Consolidation and Maintenance

6.6.1 Consolidation-Intensity: A Determinant of Outcomes

The re-emergence of AML in patients achieving CR with induction IC had indicated the need for additional treatment to eliminate persisting leukaemic cells [22, 109], now identifiable as measurable residual disease (MRD). Based on studies of the kinetics of leukaemic cell reduction and proliferation in patients achieving remission with 6-MP and 6-methylmercaptopurine in the 1960s, an additional year-and-a-half of therapy of similar intensity had been suggested for disease eradication [22]. Post-remission strategies

between 1960 and 1980 generally consisted of combinations of drugs and doses, including those used in remission-induction, in cyclical rotation and with variable treatment-intensity [20, 21]. Toxicity and supportive care requirements were the surrogates that defined the ceiling of treatment-intensification, with consolidation courses arbitrarily accepted as more intensive than maintenance treatment. The benefits of post-remission therapies administered as 'consolidation' or 'maintenance', or both, in sequence, on the prolongation of remission and survival, however, remained uncertain.

In 1980, in an ECOG study [110], 146 AML patients (including APL) achieving remission after DAT 3 + 5 (daunorubicin 60 mg/m^2/day, infusional cytarabine 200 mg/m^2/day) were randomised to 2-years of maintenance therapy with cytosine arabinoside and 6-thioguanine, or two courses of DAT (daunorubicin 45 mg/m^2/day × 2 days, single bolus dose of 100 mg/m^2 of cytarabine) as consolidation, followed by the same maintenance schedule. Sequential consolidation and maintenance therapy was associated with greater, non-fatal, haemopoietic toxicity and a non-significant improvement in 2-year DFS (28% vs. 14%), but not OS. Thus, while consolidation therapy had the potential to improve disease control, survival was unlikely to improve through mere repetition of previous drugs at attenuated doses. The possibility of acquired cytarabine resistance with conventional-dose (100–200 mg/m^2/day) schedules [111–113] provided further impetus for the investigation into alternative, intensified consolidation strategies.

6.7 Identifying the Standard for Intensive Consolidation

6.7.1 High-Dose Cytarabine: Studies Defining the 'Optimal' Dose in Consolidation

Studies in relapsed acute leukaemia, including AML, suggested the feasibility of administering single doses of cytarabine at up to 7.5 g/m2 at weekly or 4-weekly intervals [114]. Subsequently, trials on dose and duration were undertaken [115]: beginning with 3 g/m^2 of cytarabine (termed high-dose cytarabine, HDAC) administered 12 hourly for 2, 4, 6 or 12 days, followed by 50% increments in drug-dose. Thus, 3 g/m^2 12 hourly for 6 days was identified as the maximum tolerated treatment, but concerns regarding neurotoxicity in subsequent CALGB studies [116] led to the schedule being revised to 6 doses of 3 g/m^2/dose administered over 3 h, 12 h apart on alternate days, a cumulative dose of 18 g/m^2. The dose-finding studies further identified 400 mg/m^2/day dose for 5 days as the maximum tolerated cytarabine 24-h infusional schedule [116]. These doses were then compared against conventional schedules (100 mg/m^2/day as a continuous infusion for 5 days) as post-remission consolidation in a randomised study (n = 693), with eligibility not restricted by age [117]. Four cycles of consolidation at the 3 different doses (3 g/m^2, 400 or 100 mg/m^2) were followed by 4 cycles of daunorubicin (day 1) and subcutaneous cytarabine (100 mg/m^2/day twice daily) for 5 days in all patients. Tolerance of the 3 g/m^2 by older patients was poor with frequent treatment discontinuation, but DFS at 4-years, 39% (3 g/m^2), 25% (400 mg/m^2) and 21% (100 mg/m^2) favoured the highest dose, even after adjusting for age (p = 0.003). The OS was 46%, 35% and 31%, respectively (p = 0.04), with patients <60 years experiencing particular benefit (52%, 40% and 35%, p = 0.02). These results supported the use of HDAC as consolidation strategy, particularly in patients receiving conventional-dose cytarabine during induction.

However, since the effects of graded increments in cytarabine dose (i.e. between 400 mg/m^2 and 3 g/m^2) were not investigated, the necessity of 3 g/m^2 as the standard-defining dose of cytarabine in consolidation has been questioned [25, 118]. In addition, whether survival could be improved using multi-agent rather than single agent consolidation and the optimal number of consolidation courses required clarification.

Retrospective sub-analysis of the CALBG study [117] suggested that the survival advantage with HDAC was limited to patients with core-binding factor (CBF) AML (n = 57) and AML with *RAS* mutations (n = 34) [119]. A benefit to younger patients with favourable cytogenetics (n = 218) has also been suggested in a Japanese study comparing 2 g/m^2 of cytarabine for 5 days with multi-agent chemotherapy p = 0.050) [120]. In the entire patient cohort, however, survival outcomes following the administration of three courses of higher-dose cytarabine did not differ from four courses of multi-agent chemotherapy.

Results from a German SAL trial further questioned the use of HDAC in consolidation: in this study [121], cytarabine administered 12 hourly for 6 days at 1 g/m^2 (at a cumulative dose of 12 g/m^2) or 3 g/m^2 (36 g/m^2 cumulatively), both with mitoxantrone showed no differences in survival outcomes, including in CBF AML. It would be important to highlight that patients in the SAL study received double-induction therapy including 1 g/m^2 of cytarabine (12 hourly for 5 days, i.e. a cumulative dose of 10 g/m^2) in cycle 2, and therefore, the exposure to cytarabine by the end of consolidation cycle one was 22.8 g/m^2 cumulatively in the 'lower' dose cohort, compared to 19.4 g/m^2 in the CALBG study [117]. In addition, the cumulative amount of cytarabine per 'high-dose' consolidation cycle (36 g/m^2) in the SAL study was double that in the CALGB study (18 g/m^2). The permissibility of risk-adapted approaches including autologous and allogeneic stem cell transplantation and sub-optimal compli-

ance with protocol-directed therapy further compromised evaluation of the SAL study.

Whether an 'intermediate' dose of cytarabine was as effective as HDAC from the CALGB study was investigated in the prospective randomised MRC AML15 trial in younger patients [66]. Here, the results showed no survival difference regardless of whether 3 or 1.5 g/m^2 of cytarabine was used; a trend towards a reduction in relapse-risk was observed with the higher dose, but with greater supportive care requirements. The 'intermediate' dose of cytarabine is therefore an attractive option, particularly for older patients [30].

6.7.2 Defining the Optimal Drug Combination

Given the risks of resistance with repeated cytarabine exposure [113], the advantages of multi-agent non-cross-resistant post-remission therapy have been of interest. The MRC AML9 trial between 1984–1990 recruited patients aged between 1–79 years to compare 2 cycles each of cyclophosphamide, vincristine, prednisolone and 5 days of conventional-dose cytarabine, with amsacrine, azacytidine (substituted subsequently with 200 mg/m^2 intravenous cytarabine days 1–5) and etoposide (MAZE/MACE) [24]. The relapse-risk at 5 years was reduced with MAZE (66% vs. 74%, $p = 0.030$), but supportive care requirements and toxic deaths were higher, with no OS benefit (37% vs. 31%). In a CALGB study involving older patients, adding mitoxantrone to cytarabine administered by intravenous infusion resulted in greater toxicity than single-agent cytarabine [122]. The dose of cytarabine (500 vs. 100 mg/m^2), number of cycles (2 vs. 4) and inter-cycle interval (60 days and 1 month) differed between the combination and monotherapy arms, and no difference in OS was evident with the more intensive approach.

With the emergence of HDAC (3 g/m^2) as a 'standard of care', this regimen formed the comparator against which multi-agent consolidation was studied. In the CALGB 9222 study, patients (15–59 years, including APL) in remission after conventional-dose cytarabine-containing induction were randomised to 3 cycles of HDAC, or in sequence, HDAC, followed by cyclophosphamide with etoposide, and finally, mitoxantrone and diaziquone [123]. Multi-agent consolidation was associated with greater non-haematological toxicity, but did not confer a benefit on survival outcomes, overall or in any cytogenetic sub-group. A similar lack of benefit and greater toxicity including delayed haematological recovery was observed in the MRC AML15 study [65] that randomised 1445 adults <60 years old to cytarabine (3 or 1.5 g/m^2) or MACE (containing 200 mg/m^2/day infusional cytarabine for 5 days), followed by mitoxantrone with cytarabine (1 g/m^2 given as a 2 h infusion twice daily for 3 days). In sub-analysis of patients with adverse karyotype ($n = 54$) however, a survival benefit with multi-agent consolidation (39% vs. 0%, $p = 0.0004$, with $p = 0.003$ for interaction) emerged, despite higher levels of toxicity. The AML15 trial also investigated whether the randomised addition of GO as a single 3 mg/m^{2}dose in consolidation (course 3) improved relapse-risk or survival, but no difference in outcomes was observed [82]. This negative result remains the only randomised study of GO in conjunction with chemotherapy in consolidation, despite its approval for use in this setting. More recently, a French phase 2 randomised trial has compared the effects of consolidation with HDAC against clofarabine and 'intermediate-dose' cytarabine (1 g/m^2/day for 5 days) (CLARA) on relapse-free survival in younger patients with intermediate or poor-risk cytogenetics ($n = 223$) [124]. Originally intending to exclusively recruit patients without stem cell donors, the subsequent availability of donors and transplantation confounded interpretation of the results; nevertheless, combination therapy improved 2-year relapse-free survival (53.3% vs. 31%, $p = 0.043$), even after adjustment for stem cell transplantation. However, CLARA was associated with more adverse events, and despite the absence of toxicity-related deaths, conferred no OS benefit, particularly in allograft recipients. The exclusion from the trial, of patients with favourable-risk cytogenetics, who would normally not receive an allograft in first CR, meant that the relapse-risk following CLARA could not be investigated in this sub-group of patients.

6.7.3 Defining the Optimal Number of Consolidation Courses

While intermediate or HDAC is now an established consolidation therapy particularly in younger patients, the optimal number of courses remains less well-defined. An alternative approach has been to include autologous stem cell transplantation, usually undertaken after a single consolidation course, a strategy probably as effective as repetitive courses of consolidation, at least in patients without adverse-risk cytogenetics [46]. In sequential NCRI trials, AML12 [40], AML15 [66] and AML17 [125] in patients <60 years old, the number of consolidation courses for optimal outcomes following double-induction therapy was investigated. In AML12, of a total of 992 patients completing MACE as first consolidation course, those randomised to a total of two consolidation courses went on to receive a higher-dose cytarabine-containing regimen (MidAc). In patients receiving five courses, conventional-dose cytarabine with idarubicin and etoposide, followed by MidAc, was administered as cycles 4 and 5, respectively. The results demonstrated no difference in relapse-free or OS with the additional course of consolidation; of concern, survival in patients older than 40 years was adversely affected. The absence of benefit with a fifth cycle,

consisting of single agent cytarabine (1.5 g/m^2), was confirmed in the follow-up MRC AML15 study, further suggesting capping intensive consolidation chemotherapy to a maximum of 2 cycles in younger patients, a pragmatic decision that CALGB had reached [118], prior to the availability of data from these studies.

With risk-stratification of AML, it became important to determine whether consolidation treatment could be de-escalated further in disease-sub-types, without impacting on relapse. In the NCRI AML17 trial [125], patients <60 years in remission after 2 cycles of induction, and classed as having 'favourable'- or 'intermediate'-risk disease (n = 1017) based on a weighted scoring-system [126, 127], were randomised to either 1 or 2 courses of consolidation predominantly with HDAC (3 g/m^2), although a minority received multi-agent chemotherapy. In the entire cohort, the relapse-free survival at 5-years favoured the use of two consolidation cycles (43% vs. 36%, p = 0.030). Furthermore, a trend towards improved OS (63% vs. 56%, p = 0.090) was apparent in analysis confined to those receiving HDAC consolidation. These results have to be interpreted on the basis that all patients in this study had received 2 cycles of induction chemotherapy prior to randomisation, but suggest that at least when HDAC consolidation is used, 2 cycles are to be recommended in those not being considered for allogeneic transplantation.

6.7.4 Consolidation Therapy in Older patients

Identifying the optimal strategy to consolidate remission in older patients (>60–70 years old) has proven more challenging than in younger patients due to poor tolerance of treatment-intensification [24, 117]. In addition, whether the frequent presence of poor-risk features including adverse cytogenetics and secondary AML in older patients can be overcome through repeated administration of less, or more intensive post-remission strategies requires clarification.

In the MRC AML11 trial [128], older patients achieving remission after two courses of DAT (2 + 7 and 2 + 5) were randomised to stopping after a further cycle of (DAT 2 + 7), i.e. a total of 3 cycles, or continue thereafter with 2 cycles of COAP containing cyclophosphamide, vincristine, conventional dose subcutaneous cytarabine and prednisolone, with an intervening cycle of DAT 2 + 7, i.e. a total of 6 treatment cycles. Disease control and OS were not improved with additional treatment. Subsequent studies failed to demonstrate any advantages with 4 cycles of multi-agent chemotherapy compared to 3 (AML14) [103], or indeed 3 cycles over 2 (AML16) [26]. As mentioned previously [122], treating older patients with 2 cycles of intensified multi-agent consolidation with higher doses of cytarabine compared to 4 cycles of cytarabine monotherapy at conventional (100 mg/m^2) confers no survival advantage. Whether doses of 1 g/m^2 of cytarabine will be more effective than standard doses, or no consolidation therapy in older patients, is unclear.

6.7.5 Maintenance Therapy

The intensity of therapies aiming to 'maintain' remission is operationally less than with consolidation regimens. Traditionally, maintenance genotoxic treatments were scheduled to commence at the time of marrow regeneration between courses of intense regimens, or as 'stand-alone', long-term treatment following the completion of intensive therapies but fell out of favour due to no definitive improvement in OS (reviewed in [129]). An interest in maintenance therapies has been re-invigorated recently with new approaches including modulators of immune function, hypomethylating agents or as will be described subsequently, kinase inhibitors.

One of the earlier approaches to maintenance following IC was immunotherapy in CR with subcutaneous injections of irradiated autologous blasts and BCG injections [130, 131]. Although statistically underpowered, these studies promoted enthusiasm for immunomodulatory drugs such as interferon or interleukin-2. Interferon as maintenance therapy [128, 132], however, failed to improve disease control or survival. In contrast, low-dose IL-2 plus histamine dihydrochloride improved DFS [133] and received regulatory approval, but uptake remains limited. Additional agents of promise include the androgen norethandrelone [134]. Given concomitantly with IC and with 6-MP and methotrexate maintenance, 5-year OS in older patients improved with norethandrelone (26.3% vs. 17.2% respectively). The mechanism of action is unclear, and the limited availability of the drug precludes widespread use. Recently, the oral formulation of the hypomethylating agent azacitidine (CC-486), administered as maintenance therapy following IC in patients ≥55 years of age, has demonstrated an OS improvement (24.7 months compared to 14.8 months with placebo; $p < 0.001$), with an acceptable safety profile [135].

The success of CC-486 is not unexpected: the NCRI AML16 trial in patients >60 years had investigated 12 months of subcutaneous azacitidine (75 mg/m^2 daily as 5-day cycles at intervals of 6 weeks) following IC [26]. Although no improvement in OS was evident for the entire cohort, azacitidine was associated with improved 5-year survival in two patient sub-groups: (1) those in whom MRD was undetectable by flow-cytometry following intensive induction [136] and (2) patients who had been randomised to just two courses of chemotherapy [26]. Patients receiving three courses had no benefit from azacitidine maintenance. The HOVON97 trial too has reported improved DFS (64% vs. 42% at 12 months) with subcutaneous azacitidine (50 mg/m^2 for 5 days) as 1-year of maintenance therapy following IC in older patients [137]. The differences in pharmacokinetic and pharmacodynamic properties between CC-486 and paren-

teral azacytidine [138] may confer greater potency to the oral drug which translates into a survival benefit.

6.7.6 Risk-Adapting Intensive Therapy in AML

Although the existence of AML sub-types was recognised over 100 years ago [1, 2, 5], the heterogeneity in post-treatment responses among disease sub-groups was described in the 1960s [139, 140]. Advances in conventional karyotyping provided in part, a biological rationale for the variation in outcomes [32–34], with the identification of the clinical efficacy of retinoic acid in APL with t(15;17) [91], highlighting the need to adapt therapeutic strategies to AML sub-type. With recurrent molecular genetic variants now recognised in AML, disease re-classification continues to evolve alongside efforts to identify drugs against 'actionable' targets [35–37]. Therapeutic decisions can thus be adapted to biological information at diagnosis [28]. Another strategy for risk-stratifying AML relies on measuring treatment-responses at sensitive levels in patients in CR: unique gene or protein-expression for detecting MRD at pre-defined time-points during treatment can serve as a surrogate for longer-term responses [141–143]. Thus, in patients predicted to have a higher relapse-risk based on MRD, treatment-intensification could enable a 'real-time' risk-adapted approach. There, however, remain challenges to the standardisation of techniques in characterising and quantifying MRD; these, combined with differences in treatment protocols, limit the generalisation of results across studies [141–143].

6.7.7 Intensive Combinations and 'Actionable' Genetic Sub-Types of AML

Examples of adapting intensive strategies to improve outcomes in sub-types of AML include the use of GO and HDAC in patients with CBF-AML described previously. In these patients, the quantification of disease-transcripts following therapy can be used to predict the relapse-risk [144, 145] and molecular stratification through *KIT* or *FLT3* analysis [146–149] can inform decisions regarding integrating tyrosine kinase inhibition with IC [149–151].

The utility of identifying 'actionable' AML sub-types has been confirmed by the superior survival observed in patients with AML with *FLT3* variants (ITD or tyrosine kinase domain mutations) treated with midostaurin, a small molecule multi-kinase inhibitor administered in sequence with 'DA 3 and 7' and HDAC [28]. Midostaurin-containing treatment resulted in a 22% reduction in death (hazard ratio for death, 0.78; one-sided $p = 0.009$) compared to placebo, in patients <60 years old, regardless of the mutant to wild-type *FLT3* ratio. The statistical significance for difference in survival was lost when patients were censored for allogeneic stem cell transplantation (4-year OS 63.7% vs. 55.7% with placebo); nevertheless, midostaurin has been approved for use in all age-groups in conjunction with anthracycline and cytarabine-containing chemotherapy, and as maintenance treatment.

More recently, drugs with the ability to attenuate signalling pathways critical to leukaemia cell survival have been shown to result in clinically and statistically meaningful improvements in survival, when combined with non-intensive chemotherapy or hypomethylating agents. The newer drugs include inhibitors of the pro-survival protein Bcl-2 (venetoclax) [152] and oncometabolite-generating mutant IDH1 (ivosidenib) [153] and IDH2 (enasidenib) [154] proteins and appear to benefit distinct genetic AML sub-types. A logical extension of these data is to investigate outcomes after combining these drugs with IC. An early-phase investigation of the dosing schedule of venetoclax with cytarabine and idarubicin containing IC (CAVEAT) in older patients has highlighted the potential for haemopoietic toxicity, particularly affecting the platelet count [155]. Overall response rates were 72% and 97% in de novo AML indicating potential anti-leukaemic benefits of administering venetoclax around IC. Likewise, the use of ivosidenib or enasidenib with IC in younger patients with mutant *IDH1* or *IDH2* AML was associated with manageable toxicity, with no excess non-haematological adverse events [156]. Thus, the incorporation of small molecule drugs [155–157] with IC could be a promising biomarker-based curative strategy in AML.

6.7.8 Intensive Drug-Delivery Platforms for Secondary AML

Secondary AML evolving from an antecedent myelodysplastic syndrome or myeloproliferative neoplasm or occurring after previous genotoxic therapy (t-AML) is poorly responsive to conventional IC [73, 139, 140]. In a minority of t-AML patients with CBF lesions, disease control and survival following conventional intensive treatment is comparable to de novo CBF AML; in others, the outlook remains dismal [30]. Recently, CPX-351 (VYXEOS), a liposomal encapsulation of cytarabine and daunorubicin (in a 5:1 synergistic molar ratio), was compared to 'DA 3 and 7' in patients aged 60–75 years with secondary or t-AML [27]. CPX-351 improved remission rates (47.7% vs. 33.3%; two-tailed $p = 0.016$), early mortality, and OS (9.56 vs. 5.95 months, one-tailed $p = 0.003$), with no excess non-haematological toxicity despite delayed haemopoietic recovery. Estimated OS at 1- and 2- years (41.5% and 31.1% with

CPX-351 vs. 27.6% and 12.3% with DA 3 and 7, respectively) favoured CPX-351, and the statistical difference in survival was maintained at 5-years. A greater proportion of patients treated with CPX-351 was able to receive allogeneic stem cell transplantation, with exploratory analysis suggesting a post-transplant survival benefit in these patients. Whether CPX-351 will confer superior outcomes in other AML sub-types in older patients compared to GO-containing intensive induction therapy is currently being investigated.

6.8 Measurable Residual Disease (MRD)-Adapted Therapy: Genetic-MRD-Based Strategies

The use of MRD measurement to identify patients at higher risk of relapse despite morphological remission potentially enables the risk-adaptation of subsequent treatments [141–143]. The utility of MRD monitoring in CBF-AML has been mentioned previously, but this disease sub-type constitutes a relatively small proportion of AML cases. Defining a reliable molecular genetic marker for MRD detection and its standardised measurement can be difficult but in younger patients with AML characterised by the nucleophosmin1 (*NPM1*) mutation [158], the persistence of *NPM1*-mutated transcripts in blood after the second cycle of anthracycline-containing induction chemotherapy associates with a higher relapse-risk (82% vs. 30%, $p < 0.001$) and lower survival (24% vs. 75%, $p < 0.001$) at 3-years, even after adjustment for concomitant genetic drivers of prognostic significance [159]. Thus, based on MRD analysis, majority of younger patients with *NPM1*-mutant disease are likely to be cured with standard HDAC consolidation, without the need for treatment intensification and allogeneic stem cell transplantation.

6.9 Measurable Residual Disease (MRD)-Adapted Therapy: Multi-Parametric Flow-Cytometry (MFC)-MRD-Based Strategies

The frequency and fidelity of genetic markers to reliably inform relapse-risk is currently restricted to a small proportion of patients, but the ability to identify a unique leukaemia-associated immunophenotype (LAIP) in almost all AML patients provides an alternative strategy for MRD detection [141–143]. In patients <60-years old with AML and wild-type *NPM1*, detection of MRD by MFC after the second cycle of induction therapy confers a higher relapse-risk (HR 1.88, $p < 0.001$) and lower survival (HR 1.77, $p < 0.001$) [160]. In patients >60 years, the detection of MRD by MFC after the first cycle of induction therapy is predictive of a 12% higher relapse-risk and 16% difference in OS at 3 years [161]. In both patient groups, MRD measurements provide opportunities to select patients for early treatment intensification, novel therapies or allogeneic stem cell transplantation.

6.10 Too Early to Draft the Obituary for IC?

The identification of prognostic and predictive biomarkers in AML, and newer anti-leukaemic therapies, has renewed optimism for prolonging survival and cure in patients. The early attainment of CR is critical for better survival and potentially cure, and for an overwhelming majority of patients, IC currently represents the best chance of achieving rapid CR. The success of tyrosine kinase inhibition in CML [162], or arsenic trioxide-ATRA combinations in APL [163], has led to enthusiasm for therapeutic strategies not reliant on IC to cure AML. The greater repertoire of cellular and molecular drivers in AML relative to CML or APL, however, appears to confer context-dependent redundancy that facilitates disease-escape, to explain the absence of durable responses to monotherapy with current small molecule drugs against 'actionable' targets in AML. Thus, while it may become possible to cure subsets of AML, for example, patients with *NPM1* mutant disease using non-intensive non-genotoxic therapy [164, 165], analogous to the current therapy of low-risk APL [163], for the foreseeable future, a backbone of IC will remain the mainstay of cure for most AML patients.

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7 Frontline Management of Elderly Acute Myeloid Leukemia Ineligible for Intensive Treatment

Yin-Jun Lou, Jie Jin, and Hong-Hu Zhu

Abstract

With the improvement of basic science and clinical translation research, the outcome of elderly patients with AML has potentially improved. Novel therapeutic regimens not only improve survival, but also increase the quality of life. Particularly, recent trials have established treatment with venetoclax in combination with HMAs or LDAC as the new standard of care for frontline management in older and unfit patients with AML who are considered unsuitable for intensive chemotherapy. Target drugs such as FLT3-inhibtors, IDH-inhibitor, or Glasdegib also bring new hope for this population. Here, we review the current treatment of elderly AML ineligible for intensive treatment.

Keywords

Acute myeloid leukemia · Older patients · Unfit
Targeted therapies · Venetoclax

7.1 Introduction

Acute myeloid leukemia (AML) is the most common form of acute leukemia in adults, with a crude incidence of approximately 4 cases per 100,000 people per year in the United States. Moreover, AML is disease more common occurring in older population with a median age of about 68 years and about one third of patients with AML are aged >75 years at diagnosis [1–3].

Historically, older patients with AML usually show a dismal outcome with a median survival of only 5–10 months [4]. Poor outcomes in older and unfit patients are likely due to host factors, poor performance status, preexisting medical comorbidities, and adverse biological characteristics of their disease, such as adverse cytogenetic, adverse molecular risk, and secondary or therapy-related AML [2, 5].

Traditionally, hypomethylating agents (HMA) or low-dose cytarabine (LDAC) were used as low-intensity therapies for the treatment of AML in older and unfit patients [6]. However, the outcomes with LDAC or HMAs (azacitidine or decitabine) monotherapy are disappointing, with an overall response rate 22% and a median overall survival (OS) of 6–8 months [6]. Moreover, the proportion of older patients receiving antileukemia therapy is only 40% in the United States in a population-based study between 2000 and 2009 [7]. The scenario had been changing in recent years due to the introduction of novel low-intensity regimens. Here, we have reviewed the current evidence regarding the treatment strategies of AML in older and unfit populations.

7.2 Diagnosis and Risk Classification

AML is a highly heterogeneous disease. Accurate diagnosis and precise risk stratification may help plan the best therapeutic options for each patient. In general, diagnostic procedures included bone marrow aspiration and/or biopsy, morphology, immunophenotyping, conventional cytogenetic, and molecular mutational analysis. The primary diagnosis of AML is established by the presence of ≥20% blasts in the bone marrow or peripheral blood according to the latest World Health Organization classification [8]. The diagnosis can be made regardless of blast count for AML with t(8;21), inv. [9], t(16;16), and t(15;17). Cytogenetic and molecular profiles are the most important independent prognostic factors in AML [10–12]. The updated National Comprehensive Cancer Network (NCCN) and European Leukemia Net 2017 risk stratification have proposed categorizing patients into favorable, intermediate, and adverse risk groups based on cytogenetic and molecular profiles [13, 14]. Compared to younger patients with AML, older adults with

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AML are more likely to be categorized into the adverse risk groups [15].

Molecular mutations of leukemic cells could be useful for identifying patients who are likely to benefit targeted therapies. With the advancement of next-generation sequencing technologies, targeted panel sequencing of AML-related gene seems feasible for obtaining gene mutational results in a timely manner. The Beat AML Master Clinical Trial suggested that there is a very low risk of delay within 1 week of induction for comprehensive molecular analysis in most older patients with AML [16]. It is reasonable to delay initiation induction therapy while waiting for the molecular information.

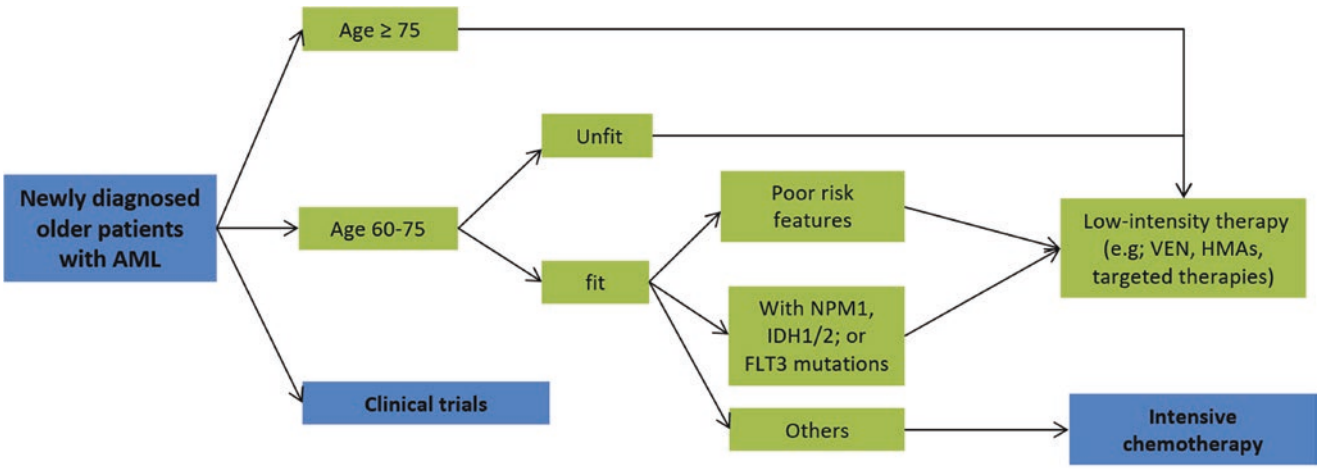
7.3 Definition of "Ineligible"

After the diagnosis of AML, the next step is to choose the available treatment strategies for each patient. Currently, the established selection criteria for "unfit" patients with AML are lacking. In many situations, physicians often use an empiric approach to identify older patients who may benefit from less-intensive approach. Figure 7.1 shows our preliminary proposed work flow for selecting older patients with AML in low-intensive therapies.

Since most older patients with AML are more than 70 years of age, they have a poor prognosis even with intensive chemotherapy [9]. In our clinical practice, we proposed that patients aged ≥75 years or those aged ≥60 years with poor Eastern Cooperative Oncology Group Performance Status (ECOG PS) ≥3 should be considered for low-intensity therapies. Further, low-intensity regimens are considered for patients aged 60–75 years, with one or more comorbidities or organ dysfunction that preclude ineligibility intensive induction, such as cardiac disease, chronic lung disease, and hepatic or renal dysfunction.

Importantly, with the recently developed of B-cell lymphoma-2 inhibitor venetoclax, low-intensity approaches have shown similar response rates comparable to those with standard induction therapy. The medical decision to discriminate fit and unfit seems not as critical as previous studies [17]. In real-world practice, for patients with high-risk features (e.g., TP53-mutated AML) or those with nucleophosmin (NPM) 1 and, isocitrate dehydrogenase (IDH) 1/2 mutations, physicians may prefer to select venetoclax-based regimens. Thus, previous scoring tools for unfitness for patients with AML will need to be redefined in the current venetoclax era.

The goal of treatment is also crucial in decision making in older patients with AML. In general, the therapeutic goal is to achieve remission, prolonged survival, and improvement of quality of life. Based on the accurate diagnosis and geriatric assessment, in our center, we usually have a discussion on the disease with the patient and/or the family members. The topics include the patients' expectations, individual preference, socioeconomic status, financial burdens, and the benefit/risk ratio of antileukemia therapy.



Abbreviations: FLT3, Fms-like tyrosine kinase 3; HMAs, hypomethylating agents; IDH, isocitrate dehydrogenase; NPM1, nucleophosmin 1; VEN, venetoclax.

Fig. 7.1 Proposed for elderly patients with AML to low-intensive therapies. FLT3, Fms-like tyrosine kinase 3; HMAs, hypomethylating agents; IDH, isocitrate dehydrogenase; NPM1, nucleophosmin 1; VEN, venetoclax

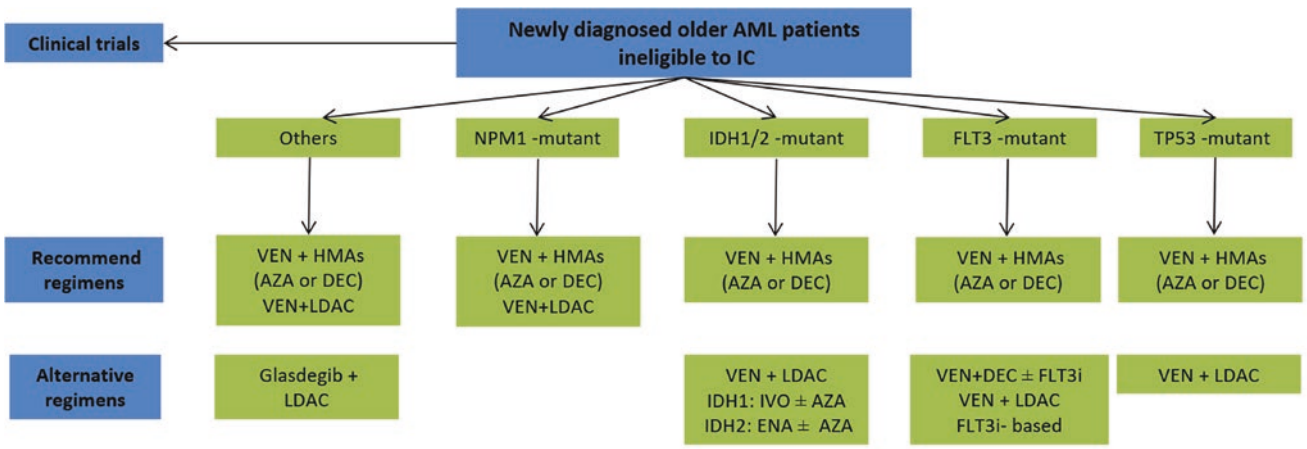
7.4 Frontline Therapeutic Strategies

More recently, several clinical trials have demonstrated promising results with novel low-intensity regimens in older patients with AML. Here, Table 7.1 summarizes the recently published clinical studies on patients with AML ineligible for intensive treatment. Figure 7.2 shows our preliminary proposed treatment algorithm for elderly and unfit patients with AML.

Table 7.1 Clinical trials for the management of newly diagnosed elderly unfit AML patients

Study	Phase	Regimen	No. of Patients	Median age (range), y	CR/CRi rate, %					Median OS, mo
					Total	NPM1	IDH1/2	FLT3	TP53	
DiNardo et al. Blood (2019)	1b/2	VEN + DEC or AZA	145	74 (65–86)	73	92	71	72	47	17.5
Wei et al. JCO (2019)	1b/2	VEN + LDAC	82	74 (63–90)	54	89	72	44	30	10.1
DiNardo et al. NEJM (2020)	3	VEN + AZA vs. AZA	286[a]	76 (49–91)	66[a]	67	75	72	55	14.7[a]
Wei et al. Blood (2020)	3	VEN + LDAC vs. LDAC	143[a]	76 (36–93)	48[a]	79	57	45	18	8.4[a]
DiNardo et al. Lancet Haematol (2020)	2	VEN + DEC	70	72(70–78)	84	95	84	NA	69	18.1
Cortes et al. Leukemia (2019)	2	Glasdegib+LDAC vs. LDAC	88[a]	77 (63–92)	17[a]	NA	NA	NA	NA	8.3[a]
Roboz et al. Blood (2020)	1	Ivosidenib	34	77 (64–87)	42	NA	42	NA	NA	12.6
DiNardo et al. JCO (2021)	Ib	Ivosidenib+AZA	23	76 (61–88)	70	NA	70	NA	NA	NE
Pollyea et al. Leukemia (2019)	1/2	Enasidenib	39	77 (58–87)	21	NA	21	NA	NA	11.3
Stein et al. Blood (2020)	2	Enasidenib	60	75 (60–89)	47	NA	47	NA	NA	24.4
DiNardo et al. Blood (2019)	2	Enasidenib+AZA vs. AZA	68[a]	74 (62–85)	50[a]	NA	50	NA	NA	22[a]

AZA azacitidine, *CR* complete remission, *CRi* CR with incomplete count recovery, *DEC* decitabine, *FLT3* Fms-like tyrosine kinase 3, *HMAs* hypomethylating agents, *IDH* isocitrate dehydrogenase, *LDAC* low-dose cytarabine, *mo* months, *NPM1* nucleophosmin 1, *TP53* tumor protein 53, *VEN* venetoclax, *OS* overall survival, *NA* not applicable, *NE* not estimable, *y* year
[a]Considering experimental arm only



Abbreviations: AZA, azacitidine; DEC, decitabine; ENA, enasidenib; FLT3, Fms-like tyrosine kinase 3; HMAs, hypomethylating agents; IC, intensive chemotherapy; IDH, isocitrate dehydrogenase; IVO, ivosidenib; LDAC, low-dose cytarabine; NPM1, nucleophosmin 1; TP53, tumor protein 53; VEN, venetoclax;

Fig. 7.2 Frontline treatment paradigm of elderly patients with AML ineligible for intensive therapy. AZA, azacitidine; DEC, decitabine; ENA, enasidenib; FLT3, Fms-like tyrosine kinase 3; HMAs, hypomethylating agents; IC, intensive chemotherapy; IDH, isocitrate dehydrogenase; IVO, ivosidenib; LDAC, low-dose cytarabine; NPM1, nucleophosmin 1; TP53, tumor protein 53; VEN, venetoclax

7.4.1 Venetoclax Plus HMAs

The recent incorporation of the venetoclax into frontline treatment may be the most significant progress for older patients with AML. In 2013, Konopleva et al. conducted a single-arm study evaluating venetoclax (800 mg, daily) in patients with high-risk relapsed/refractory or unfit AML. The study found that venetoclax monotherapy demonstrated a 19% (6/32) objective response rate, with 6% ($n = 2$) patients achieving complete remission (CR) and 13% ($n = 4$) patients achieving a CR with incomplete recovery (CRi). In terms of toxicity, venetoclax monotherapy had a manageable safety profile.

Based on the preliminary safety and efficacy data, a prospective phase 1b/II trial was conducted to evaluate the safety and efficacy of venetoclax in combination with HMAs (azacitidine, 75 mg/m^2, days 1–7, or decitabine, 20 mg/m^2, days 1–5) in newly diagnosed older and unfit patients with AML [18]. This trial enrolled 145 patients with a median age of 74 years. Venetoclax (400 mg, daily) was recommended as the standard dose based on its clinical activity and safety. The overall response rate (ORR) in the venetoclax (400 mg) plus HMAs group was 73%. The most common toxicities were hematological grade 3/4 adverse events. The time to response was very rapid, 1.2 months with venetoclax plus azacitidine and 1.9 months with venetoclax plus decitabine.

In a landmark randomized placebo-controlled phase 3 trials (VIALE-A trial) [19], patients received azacitidine (75 mg/m^2, days 1–7) with or without oral venetoclax (400 mg, daily) in a 28-day cycle. The primary endpoint was OS. The median OS was 14.7 months in the venetoclax plus azacitidine group and 9.6 months in the control group ($p < 0.001$). The CR/CRi rate was significantly higher in the venetoclax plus azacitidine group than in the azacitidine group (66.4% vs. 28.3%; $p < 0.001$).

7.4.2 Venetoclax Plus LDAC

Dr. Wei et al. conducted a phase Ib/II study in newly diagnosed older AML patients with AML ineligible for intensive chemotherapy, to evaluate the safety and efficacy of venteoclax (600 mg, daily) administered in combination with LDAC (20 mg/m^2, days 1–10) in a 28-day cycle. Eighty-two patients were enrolled, with a median age of 74 years. The CR/CRi rate was reported in 54% patients. The median OS was 10.1 months. For patients without prior HMAs therapy, the CR/CRi was achieved in 62% and the median OS was 14.8 months.

Subsequently, the authors conducted an international phase 3 randomized placebo-controlled trial (VIALE-C trial), which randomized patients to receive either venetoclax ($n = 143$) or placebo ($n = 68$) in 28-day cycles, plus LDAC on days 1–10. The median patient age was 76 years, and 20% patients had received prior HMAs treatment. The median OS, primary endpoint was 7.2 and 4.1 months in the venetoclax plus LDAC and LDAC groups, respectively ($p = 0.11$), which failed to meet the primary endpoint. However, the additional 6-month follow-up demonstrated a median OS 8.4 months and 4.1 months in the venetoclax plus LDAC and LDAC groups, respectively ($p = 0.04$). The CR/CRi rates were 48% and 13% in the venetoclax plus LDAC and LDAC groups, respectively. Thus, the study confirmed that the venetoclax plus LDAC regimen significantly improved in CR/CRi rate and OS compared with LDAC alone.

Thus, the efficacy and safety of venetoclax in combination with HMAs or LDAC has been well established. Venetoclax in combination with HMAs or LDAC is currently recommended as the frontline regimens for unfit patients with AML according to the guidelines [14].

7.4.3 Glasdegib Plus LDAC

Another interesting agent is glasdegib, which is a selective oral inhibitor of Hedgehog signaling pathway through binding to Smoothened. To evaluate the efficacy and safety of glasdegib plus LDAC compared to those of LDAC alone, a phase II, randomized, multicenter study (BRIGH trial) was performed in patients with AML or high-risk MDS who were ineligible for intensive chemotherapy. Glasdegib was orally administered (100 mg, daily) continuously in 28-day cycles and LDAC (20 mg, subcutaneously, twice daily, days 1–10) was administered every 28 days [20]. In this trial, 132 patients were randomized to receive either glasdegib plus LDAC ($n = 88$) or LDAC alone ($n = 44$). The median OS was 8.8 and 4.6 months in the glasdegib plus LDAC and LDAC alone groups ($p < 0.001$) [20]. In a subset of patients with AML, the ORR was 26.9% (21/78) with glasdegib plus LDAC therapy and 5.3% (2/38) with LDAC monotherapy. The addition of glasdegib to LDAC was generally well tolerated, with a manageable safety profile. The trial confirmed that the glasdegib plus LDAC regimen demonstrated a statistically significant survival benefit compared to LDAC alone.

An ongoing clinical trial (NCT03416179) is evaluating the safety and efficacy of glasdegib in combination with azacitidine in patients with AML and high-risk MDS; its results are eagerly awaited.

7.5 Therapeutic Strategies in Specific Molecular Subsets

Although recent studies did not aim to assess the impact of mutations on patient prognosis, subset analysis provides valuable information of the prognostic relevance of molecular aberrations in patients receiving venetoclax-based therapy. Moreover, promising drugs targeting driver mutations were incorporated into the current treatment paradigm for specific AML patients. Thus, the gene mutations, such as NPM1, FLT3, and TP53 mutations can not only affect risk classification, but also assist clinicians to choose the optimal treatment.

7.5.1 AML with NPM1 Mutations

NPM1 mutations are one of the most frequent recurrent genetic aberrations in AML and the incidence of NPM1 mutations is not age-dependent. NPM1-mutated AML is recognized as a distinct entity in the 2016 WHO classification of tumors of hematopoietic and lymphoid tissues [8]. NPM1-mutated AML without FLT3-internal tandem duplicate (ITD) mutations or with low ratio FLT3-ITD mutations (ratio < 0.5) has a relatively favorable prognosis [13]. Although therapies specifically targeted NPM1 mutant AML are not available, a subgroup analysis of recent clinical studies has identified that venetoclax-based regimens (plus HMAs or LDAC) are highly effective in NPM1-mutated AML. The CR/CRi rate was 67–92%. Although comparisons between trials should be performed with caution due to potential bias, venetoclax plus HMAs or LDAC regimens showed favorable response for NPM1-mutated AML. In addition, since NPM1 mutations frequently co-occur with FLT3 mutations, venetoclax in combination with gilteritinib (NCT03625505) and quizartinib (NCT03735875) studies are currently ongoing; the results of these clinical trials are eagerly awaited.

7.5.2 AML with FLT3 Mutations

Mutations in FLT3 occur in 20–30% of adult patients across the entire age spectrum. FLT3 mutations can be subdivided into ITDs and point mutations in the tyrosine kinase domain. FLT3 is usually considered an ideal molecular target for antileukemic therapy. However, monotherapy with FLT3 inhibitors showed only a transient reduction of blast counts. Interestingly, the addition of FLT3 inhibitors to venetoclax plus decitabine regimen for the treatment of FLT3-mutated AML was feasible and could lead to improved durability of responses in this high-risk population [21]. In an exploratory subgroup analyses that tested the addition of FLT3 inhibitors to the venetoclax plus 10-day decitabine regimen, 10 patients who received FLT3 inhibitors simultaneously showed a CR/CRi rate of 86%, with 80% patients achieving measurable residual disease (MRD) negativity. The median OS was not reached. The results shed light on a possible successful strategy of the venetoclax plus 10-day decitabine ± FLT3 inhibitor regimen for patients with FLT3-mutated AML. In addition, a phase 3 randomized trial investigating gilteritinib plus azacitidine versus azacitidine alone is ongoing (NCT02752035).

7.5.3 AML with IDH Mutation

IDH1 and IDH2 mutations occur in 6–16% and 8–19% patients with AML, respectively. IDH-mutated AML is characterized by preferential occurrence in older patients. Ivosidenib is an oral, targeted, small-molecule inhibitor of IDH1 mutation. In a phase I study, 34 patients with newly diagnosed AML ineligible for intensive therapy were enrolled. The median age was 76.5 years. Patients were treated by ivosidenib (500 mg, daily). The CR/CRi rate was 42.4%. During a median follow-up of 23.5 months, the median OS was 12.6 months. The study suggested that ivosidenib single agent was safe and induced durable response.

Recently, a phase Ib trial administered oral ivosidenib 500 mg once daily in combination with azacitidine (75 mg/m^2, days 1–7) in 28-day cycles in newly diagnosed unfit patients with IDH1-mutated AML [22]. The CR rate was 61%, with ORR of 78.3%. The 1 year survival rate was 82%. The ivosidenib plus azacitidine combination was well tolerated in patients with IDH1-mutated AML.

Of note, enasidenib, an oral targeted small-molecule inhibitor of IDH2 mutation, was also developed. The initial phase 1/2 single-arm trial evaluated the safety and efficacy of enasidenib as a single agent in patients IDH2-mutated AML who were not candidates for intensive treatments [23]. Thirty-nine patients with newly diagnosed AML were enrolled in the trial. The median age of patients was 77 years. The ORR was 30.8% (12/39 patients), with a CR/CRi rate of 21%. The median time to best response was 3.7 months.

In a subsequent multicenter randomized phase II study, adult patients with newly diagnosed IDH2-mutated AML were randomized in a 2:1 ratio to receive either enasidenib plus azacitidine (n = 68) or azacitidine only (n = 33) in repeated 28-day cycles [24]. Patients received azacitidine 75 mg/m^2/day (days 1–7 of each cycle) with or without enasidenib (100 mg/day). In the interim analysis, the response rates were significantly higher in the enasidenib plus azacitidine group than in the azacitidine-only group (ORR, 71% vs. 42%; CR 53% vs. 12%). The median OS was 22 and

22.3 months in the enasidenib plus azacitidine and azacitidine-only groups, respectively ($p = 0.97$). The OS was not statistically different between the groups, possibly due to the excellent OS in the azacitidine-only group compared to that reported previously. There were significant improvements in ORR in the enasidenib plus azacitidine group compared to those in azacitidine-only group.

Although the promising results of IDH1/2 inhibitors for the treatment of IDH1/2-mutated AML were confirmed, ivosidenib and enasidenib were not approved for the treatment of newly diagnosed AML yet. Moreover, in our experience, excellent response rates (CR/CRi rate > 70%) were achieved following upfront venetoclax plus HMAs therapy in older adults with newly diagnosed IDH1/2-mutated AML. In practice, clinicians may prefer to choose venetoclax-based regimens over IDH1/2 inhibitors. In the near future, the outcomes of clinical trials of triple combinations such as IDH1/2 inhibitors combined with venetoclax and/or azacitidine are eagerly awaited.

7.5.4 AML with TP53 Mutations

Patients with TP53-mutated AML are roughly 10–20% of de novo and 30–40% of patients with second AML and are categorized into the unfavorable risk group [11]. These subsets of patients tend to be older and have median OS of only 4–6 months when treated with standard cytotoxic chemotherapy [25]. Previous studies have reported that the 10-day decitabine approach improved the response rate in patients with an unfavorable cytogenetic risk and TP53 mutations [26]. Interestingly, the venetoclax with 10-day decitabine approach provided further superior outcomes (CR/CRi = 69%) in patients TP53-mutated AML [21].

More recently, a TP53-modulating agent, eprenetapopt (APR-246), was developed. Sallman DA et al. presented results of a phase Ib/II study of eprenetapopt plus azacitidine in patients with TP53-mutated MDS or oligoblastic AML [27]. Of the patients with AML ($n = 11$) cohort, the ORR was 64%, and the CR rate was 36% ($n = 4$). The regimen showed a safety profile. However, the number of patients with AML enrolled in the trial was very small.

7.6 Post-Remission Therapy

Although about 60–70% elderly patients with AML have a response to nonintensive therapy, most elderly patients experience relapse, become refractory, and eventually succumb to their disease. Improving the duration of response and preventing relapse are important treatment goals for patients after attainment of a CR/CRi. The optimal post-remission therapy is not well established for the treatment of AML in elderly patients. Currently, in most clinical trials, the same low-intensity regimens are recommended until disease progression or development of unacceptable toxicity. In our practice, despite poor initial performance status at diagnosis, for those patients with improving performance status after CR, an alternative intensified post-remission strategy can be considered. For eligible candidates, the use of allogeneic stem cell transplantation with non-myeloablative conditioning could also represent an attractive option for high-risk and/or MRD-positive patients. In near future, combination of new therapeutic targets or immune therapy for MRD-positive patients may be amenable to further improve outcomes.

7.7 Supportive Care

During induction, despite the less intensive therapies showing efficacy and tolerability with a favorable safety profile, supportive care is crucial for older patients. Infectious complications and bleeding are the major causes of death during induction. Supportive therapies such as human recombinant granulocyte-colony-stimulating factor, blood component transfusion, and antimicrobial prophylaxis antibiotics are necessary when significant adverse events occur. Ideally, patients should be treated at a specialized cancer center during induction.

Post-remission therapy is well tolerated and allowed outpatient therapy. Careful consideration of patient quality of life, management of early and late toxicity, and psychological factors are essential for treatment adherence. On the other hand, patients' familiar members should be educated about the common adverse effects during administering medications and generally take active roles in the patients' treatment.

In countries or local communities where coronavirus disease is widespread, we have to care for our patients by minimizing their risk of infection, especially in elderly and unfit patients. Social media platforms may also help in medical communication. To reduce clinic visits, oral agent-based regimens (e.g., venetoclax, gilteritinib, enasidenib, and ivosidenib) should be considered. Recently, a phase 3 trial confirmed the safety and efficacy of oral formulation of azacitidine (CC-486) [28].

7.8 Conclusions and Prospects

In summary, with the improvement of basic science and clinical translation research, the outcome of AML in older patients has potentially improved with the adoption of novel agent combination regimens. Such therapeutic regimens not only improve survival but also increase the quality of life. Particularly, recently trials have established treatment with

venetoclax in combination with HMAs or LDAC as the new standard of care for frontline management in older and unfit patients with AML who are considered unsuitable for intensive chemotherapy. Notably, despite the biological complexity of AML, venetoclax-based regimens suggested the outstanding results in all risk subgroups. AML with NPM1 or IDH1/2 mutations had a more favorable outcome with venetoclax-based regimens in subgroup analyses.

However, novel agents are not available or high cost in most treatment centers in developing countries. The relapse is still high after achieving CR/CRi. The future challenge is the optimal combination and schedule with existing agents and incorporation of novel molecular-targeted therapies. Triplet regimens involving venetoclax plus HMAs in combination with target agents (such as FLT3 or IDH1/2 inhibitors and APR-246) are underway. In the near future, the treatment paradigm of elderly patients with AML will enter a new venetoclax ± HMAs + X era. Next-generation inhibitors, CD47 targeting agents (such as magrolimab), antibody-drug conjugates, and immunotherapeutic approaches, including bispecific antibodies and chimeric antigen receptor-modified T-cell therapy, are being explored for the treatment of AML.

Conflict of Interest Statement The authors declare no conflicts of interests.

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8 Management of Acute Myeloid Leukemia with Myelodysplasia-Related Changes and Therapy-Related Acute Myeloid Leukemia

Jan Philipp Bewersdorf and Amer M. Zeidan

Abstract

Acute myeloid leukemia with myelodysplasia-related changes (AML-MRC) and therapy-related AML (t-AML) are often combined under the term secondary AML (sAML) and are characterized by distinct historical, clinical, and molecular features that distinguish them from de novo AML. AML-MRC and t-AML patients tend to be older and more frequently harbor adverse cytogenetic features such as complex or monosomal karyotypes and high-risk mutations (e.g., *TP53*) leading to higher rates of resistance to conventional chemotherapy and an adverse prognosis.

CPX-351, a liposomal formulation of cytarabine-daunorubicin, has been approved for the frontline treatment of AML-MRC and t-AML in the United States and Europe based on a randomized phase III clinical trial that showed an overall survival benefit compared to standard 7 + 3 induction chemotherapy in patients with AML-MRC and t-AML aged 60–75 years of age. However, given that patients with sAML tend to be older, lower-intensity treatment alternatives such as azacitidine/venetoclax or molecularly targeted agents (e.g., gilteritinib, enasidenib, ivosidenib) are an important addition to the treatment landscape, although subgroup analyses in patients with sAML as well as comparative data with intensive chemotherapy are limited. With advances in molecular testing, an increasingly individualized and genetically driven treatment approach seems to be possible.

Keywords

Acute myeloid leukemia (AML) · Secondary AML
AML-MRC · Therapy-related AML · CPX-351
Hypomethylating agent · Venetoclax

8.1 Introduction

Acute myeloid leukemia (AML) is the most common form of acute leukemia in adults and can arise either de novo or as a secondary form [1, 2]. Among secondary AML (sAML), 60–85% of cases have been reported to originate from an antecedent myelodysplastic syndrome (MDS) [1]. Besides this etiologic classification, the World Health Organization (WHO) has published a revised classification scheme in 2016 that includes cytogenetic, morphological, and clinical features to categorize patients with AML into various groups; namely AML with recurrent genetic abnormalities, AML with myelodysplasia-related changes (AML-MRC), therapy-related myeloid neoplasms (t-AML), AML not otherwise specified, myeloid sarcoma, and myeloid proliferation related to Down Syndrome [3]. While most patients with AML-MRC have a known antecedent MDS and all patients with a prior diagnosis of MDS who progress to AML fall under this category, this is not a requirement and the diagnosis of AML-MRC can also be based on characteristic cytogenetic abnormalities unless they constitute a recurrent genetic abnormality such as *RUNX1-RUNX1T1*, *CBFB-MYH11*, *NPM1,* or biallelic *CEPBA* mutations [3]. Additionally, patients with a history of myelodysplastic/myeloproliferative neoplasm (MDS/MPN) are also included in the AML-MRC category [3]. Therapy-related (t)-AML, on the other hand, is defined as any AML arising from prior leukemogenic chemo- or radiation therapy and t-AML is effectively a subgroup of sAML [3, 4].

Despite the potential overlap, making the accurate distinction between AML-MRC, t-AML, and other forms of AML can have important prognostic and potentially therapeutic implications. In contrast to patients with de novo

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AML, patients with sAML tend to be older and to have a higher burden of comorbidities contributing to the adverse prognosis that has been associated with sAML [5, 6]. Another notable feature is the higher rate of resistance to intensive induction chemotherapy and an enrichment of adverse genetic features in older patients with de novo and sAML in general and t-AML in particular [7, 8].

8.2 Diagnosis of AML-MRC and T-AML

Based on the current 2016 WHO classification, AML-MRC is diagnosed based on the presence of ≥20% myeloid blasts in the peripheral blood or bone marrow and at least one of the following characteristics: [1] History of MDS or MDS/MPN, [2] an MDS-related cytogenetic abnormality, or [3] multilineage dysplasia in >50% of at least two cell lineages in the absence of *NPM1* or biallelic *CEBPA* mutations [1, 3, 9]. The latter is an important change from the 2009 WHO classification based on data showing that the presence in multilineage dysplasia lost its prognostic impact in *NPM1*-mutant AML [10, 11]. Additionally, patients with recurrent cytogenetic abnormalities such as core-binding factor leukemias (*RUNX1-RUNX1T1; CBFB-MYH11)* or acute promyelocytic leukemia (PML-RARA) would be classified as AML with recurrent genetic abnormalities [3].

T-AML has been defined by the WHO as a distinct entity of myeloid neoplasms that arises after previous exposure to cytotoxic therapy [3]. While t-AML and AML-MRC are often combined under the umbrella of sAML, the WHO classifies those as distinct entities and the distinction between both is based primarily on patient history as well as histopathology, though there could be a substantial overlap [3].

Emerging data also highlight the potential of molecular testing using next-generation sequencing to not only diagnose, but also potentially risk-stratify patients with AML-MRC [12]. Mutations enriched in patients with AML-MRC include spliceosome mutations (*SRSF2*, *SF3B1*, *U2AF1*, *ZRSR2*), chromatin modifiers (*ASXL1*, *EZH2*, *BCOR*), and *STAG2*, which are also frequently encountered in MDS patients [13–15]. For example, Devillier et al. showed in a study of 125 patients with AML-MRC that mutations in *ASXL1* or *TP53* were independently associated with adverse outcomes, while other MDS-related cytogenetics or presence of multilineage dysplasia were not [16]. However, it is important to note that *TP53* mutations are also frequently found in t-AML and making a diagnosis of AML-MRC versus t-AML based on molecular testing alone is not sufficiently validated at this time [9, 17–19].

Commonly encountered cytogenetic abnormalities in t-AML include complex and monosomal karyotypes, which are driven by the genomic instability conferred by *TP53* mutations and have been associated with an adverse prognosis [20–22]. It has also been shown that small clonal populations with somatic mutations in genes associated with myeloid neoplasms (e.g., *DNMT3A*) are common in older, otherwise healthy, individuals; a condition referred to as clonal hematopoiesis of indeterminate potential (CHIP) [23, 24]. However, CHIP has also been associated with a higher-risk of development of an overt myeloid neoplasm, especially if clonal populations with *TP53* and *IDH* mutations are present or if such individuals are exposed to subsequent cytotoxic therapies [25–28]. Despite these associations, molecular testing is not part of the diagnostic criteria of t-AML at this time, although it can be a suggestive feature with prognostic and potentially therapeutic implications.

With the approval of novel treatment options (e.g., CPX-351) for AML-MRC and t-AML, a timely diagnosis is important to enable initiation of appropriate therapy, but turnaround times for both karyotype and NGS panel testing remain long (sometimes up to weeks), which highlights the importance of a history of prior cytotoxic therapy, MDS or MDS/MPN, and the assessment for multilineage dysplasia by a skilled hematopathologist in establishing a diagnosis [9].

8.3 Treatment of AML-MRC and T-AML

Treatment of patients with AML-MRC and t-AML poses unique challenges related to the older age of many patients, the higher frequency of adverse cytogenetic features such as complex karyotype, and the lower frequency of targetable driver mutations (e.g., *FLT3)* [9, 29–31]. A major branch-point in the approach to treatment of AML patients is whether the individual patient is a candidate for intensive chemotherapy or if a lower-intensity approach is warranted [2, 32]. Figure 8.1 outlines our approach to the treatment of patients with AML-MRC and t-AML. The treatment options for AML in both the frontline and relapsed/refractory setting have expanded significantly over recent years and results of the large clinical trials underlying the approval of those novel agents with a focus on patients with sAML are summarized in Table 8.1.

8.3.1 Intensive Chemotherapy

8.3.1.1 CPX-351

Cytarabine-anthracycline-based intensive chemotherapy, also known as "7 + 3", has been the standard of care for newly diagnosed, chemotherapy-eligible AML patients for decades [40, 41]. Attempts to optimize the activity of this regimen have led to the development of CPX-351, which is a liposomal formulation of cytarabine and daunorubicin in a 5:1 molar ratio [42, 43]. Although CPX-351 failed to achieve

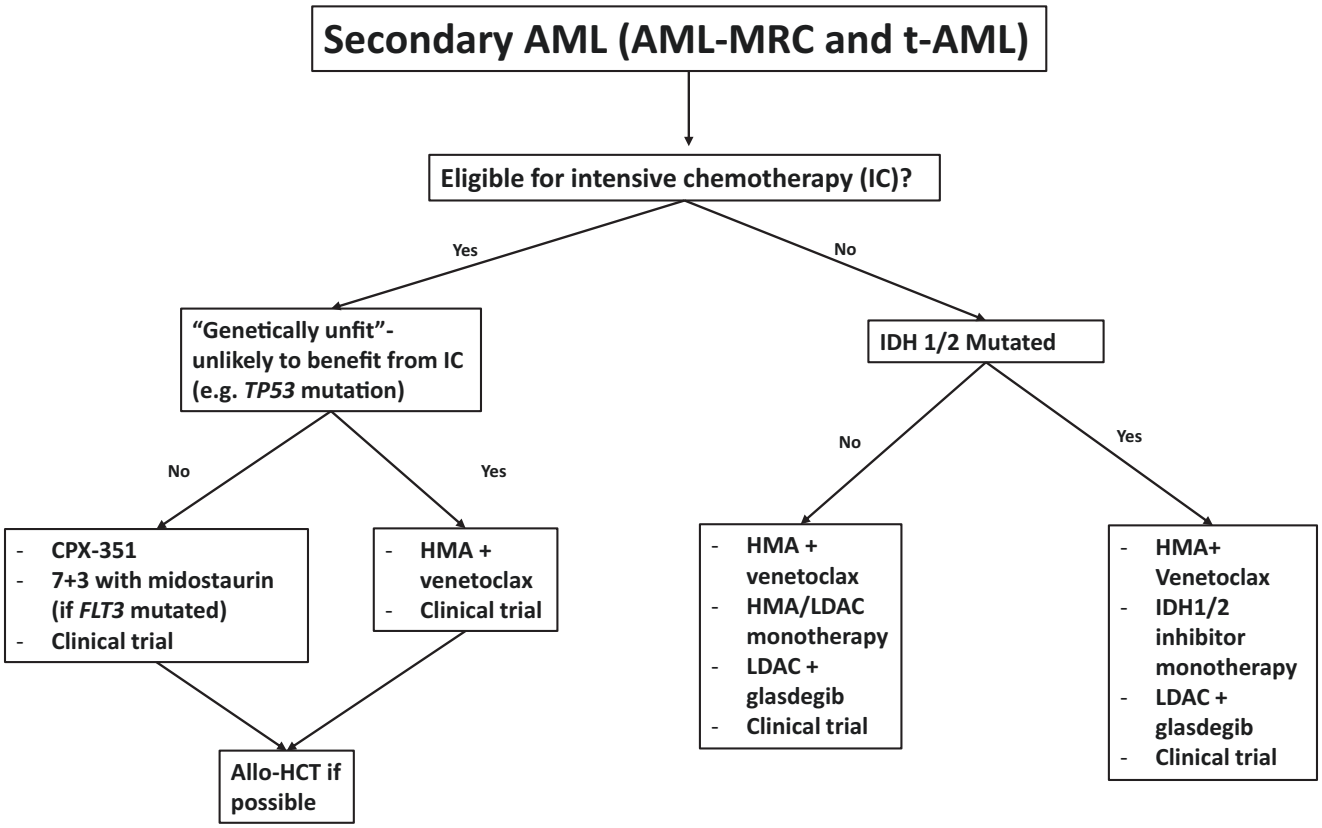

Fig. 8.1 Potential treatment approach to AML-MRC and t-AML. Therapy selection for a newly diagnosed patient with AML-MRC or sAML is an individualized decision that is made by the treating physician and the patient based on the patient's age, comorbidities, performance status, molecular and cytogenetic data, patient's wishes, and goals of therapy. For patients with newly diagnosed sAML who are candidates for intensive chemotherapy, CPX-351 has been shown to be superior to standard 7 + 3 induction chemotherapy. For patients with *FLT3* mutations who are candidates for intensive therapy, 7 + 3 + midostaurin has shown improvement in survival over 7 + 3, but has not been compared to CPX-351 for patients with AML-MRC with *FLT3* mutations. There are limited data currently on combining CPX-351 with FLT3 inhibitors and we would recommend against this approach based on current data. Patient should also be evaluated for allogeneic hematopoietic cell transplant (allo-HCT) as this remains the treatment modality with the only chance of cure for most patients with AML-MRC or sAML. If patients are not candidates for intensive chemotherapy or are considered "genetically unfit" and unlikely to benefit from such intensive therapy such as those with TP53 mutations, venetoclax with hypomethylating agents (HMA) or low-dose cytarabine (LDAC) is a preferred option in absence of clinical trials. Patients with mutations in *IDH1* and *IDH2* could also be considered for IDH1 or IDH2 inhibitor monotherapy, respectively. Clinical trial enrollment is always the preferred option in any setting when feasible

an overall survival (OS) advantage in a phase II trial of newly diagnosed AML patients 60–75 years of age compared to standard 7 + 3, subgroup analyses suggested promising activity in the subgroup of patients with secondary AML in terms of response rate (57.6% vs. 31.6%, $p = 0.06$) as well as event-free survival (EFS; hazard ratio [HR]: 0.59, $p = 0.08$) and OS (HR: 0.46, $p = 0.01$) [43]. This led to a randomized phase III trial comparing CPX-351 with 7 + 3 for induction and consolidation chemotherapy in 309 patients aged 60–75 years with newly diagnosed therapy-related AML or AML-MRC [33]. The trial met its primary endpoint of an improvement in median OS with CPX-351 (9.56 vs. 5.95 months; HR: 0.69; 95% CI: 0.52–0.90; one-sided $p = 0.003$), which led to the approval of CPX-351 by the United States Food and Drug Administration (FDA) for the treatment of newly diagnosed t-AML and AML-MRC [33]. Of note, despite the limited age group enrolled in the phase III trial of CPX-351, the FDA label for CPX-351 is age-agnostic. Subgroup analyses from this trial showed similar efficacy of CPX-351 in AML-MRC and t-AML as well as its safety and efficacy in patients younger than 60 years who were treated outside of the clinical trial protocol [44]. The benefit in terms of median OS and rate of allo-HCT with CPX-351 compared to 7 + 3 also appears to apply to patients with prior HMA exposure who achieved CR/CRi in the randomized phase III trial [45].

A concern with CPX-351 is the potential for extended myelosuppression and higher rates of treatment-related mortality. Although time to neutrophil and platelet count recovery in the CPX-351 arm was longer compared to standard 7 + 3, the rates of ≥grade 3 adverse events were similar in both groups and 30-day and 60-day mortality showed a sta-

Table 8.1 A summary of recent selected clinical trials for patients with

Author (reference)	Agent	Clinical trial design	Patient population	Outcomes in sAML
Lancet et al. [33]	CPX-351	Phase III randomized CPX-351 vs. standard 7 + 3	309 patients age 60–75 years with newly diagnosed therapy-related AML, AML with antecedent MDS or CMML, or de novo AML with MDS-related cytogenetic abnormalities	– CR/CRi rate: 47.7% with CPX-351 vs. 33.3% with 7 + 3; two-sided $p = 0.016$ – Median OS: 9.56 months with CPX-351 vs. 5.95 months with 7 + 3; HR: 0.69; 95% CI, 0.52 to 0.90; one-sided $p = 0.003$
DiNardo et al. [34]	AZA/VEN	Phase III randomized AZA/VEN vs. AZA/placebo	431 patients with untreated AML ineligible for standard induction therapy because of comorbidities or age ≥ 75 years; 72 and 35 sAML patients in AZA/VEN and AZA/placebo group	Median OS in sAML subgroup: 16.4 months (95% CI: 9.7–24.4) and 10.6 months (4.9 to 13.2), (HR: 0.56; 95% CI, 0.35 to 0.91)
Wei et al. [35]	LDAC/VEN	Phase III randomized LDAC/VEN vs. LDAC/placebo	211 patients with untreated AML ineligible for standard induction therapy because of comorbidities or age ≥ 75 years; 58 and 23 sAML patients in LDAC/VEN and LDAC/placebo group	– CR/CRi: 29% with LDAC/VEN vs. 4% with LDAC/placebo – OS: LDAC/VEN vs. LDAC/placebo: HR 0.71 (0.40–1.2)
DiNardo et al. [36]	Ivosidenib	Phase I, single arm	179 IDH1-Mut R/R AML patients (33% sAML); 125 patients in primary efficacy population (34% sAML)	Response of sAML patients not reported
Stein et al. [37]	Enasidenib	Phase I, single arm	176 IDH2-Mut R/R AML (26% AML-MRC, 1% t-AML)	Response of sAML patients not reported
Cortes et al. [38]	LDAC/glasdegib	Phase II randomized, open label LDAC/glasdegib vs. LDAC alone	132 patients with ≥55 years with newly diagnosed, previously untreated AML or high-risk MDS ineligible for intensive chemotherapy because of comorbidities or age ≥ 75 years	Response of sAML patients not reported
Perl et al. [39]	Gilteritinib	Phase III randomized, open label gilteritinib vs. salvage chemotherapy	371 FLT3-Mut R/R AML patients	Response of sAML patients not reported

AML acute myeloid leukemia, *AML-MRC* AML with myelodysplasia-related changes, *AZA* azacitidine, *CMML* chronic myelomonocytic leukemia, *CR* complete remission, *CRi* CR with incomplete count recovery, *LDAC* low-dose cytarabine, *MDS* myelodysplastic syndrome, *OS* overall survival, *R/R* relapsed/refractory, *sAML* secondary AML, *t-AML* therapy-related AML, *VEN* venetoclax.

tistically nonsignificant trend favoring CPX-351 (30-day: 5.9% and 10.6% [two-sided $p = 0.149$]; 60-day: 13.7% and 21.2% [two-sided $p = 0.097$]) [33].

However, it is important to note that long-term survival or even cure in the majority of this high-risk patient population is only realistic with a subsequent allogeneic hematopoietic cell transplant (allo-HCT). An OS landmark analysis of patients proceeding to allo-HCT in the phase III trial showed that CPX-351 improved 3-year OS compared to 7 + 3 (56% vs. 23%) [46]. Interestingly, this OS difference was largely driven by a reduction in non-relapse mortality rates (HR: 0.42 [95% CI: 0.21–0.86]) and lower rates of graft-versus-host disease, which suggests that CPX-351 is not only bridging more patients to allo-HCT in CR/CRi, but is also associated with better tolerability [46]. Further support for the safety and efficacy of CPX-351 as a bridge to allo-HCT comes from a retrospective analysis of CPX-351-treated patients in Canada [47]. In this study, 50% (25 out of 50) of patients achieved a CR/CRi and 36% (18 patients) proceeded to allo-HCT in CR1, which was associated with a statistically significant advantage in OS at 18 months (62% vs. 14.5%; $p = 0.0008$) [47]. Similar data have been reported from a compassionate use program of CPX-351 in Italy [48].

8.3.1.2 Combination Therapy

Based on the randomized, phase III RATIFY trial, addition of midostaurin to intensive induction chemotherapy for patients with *FLT3* mutations has become standard of care [49]. However, *FLT3* mutations appear to be less common in patients with AML-MRC compared to an unselected AML patient population (13.5% vs. 20–30%) [20, 50]. This is likely explained by the low frequency of *FLT3* mutations in MDS, although acquisition of driver mutations such as *FLT3* has been implicated in the evolution from MDS to AML [14, 30, 51]. In the absence of subgroup analyses from the RATIFY trial and with the caveat that only patients aged 18–59 years were enrolled in the trial, [49] addition of midostaurin to intensive chemotherapy for newly diagnosed patients with AML-MRC appears reasonable based on the documented advantage in OS (HR for death: 0.78; one-sided

$p = 0.009$) and EFS (HR: 0.78; one-sided $p = 0.002$) compared to placebo [49]. Data on the combination of *FLT3* inhibitors with CPX-351 are limited, but clinical trials such as the open-label, non-randomized, multi-arm phase IB V-FAST trial (#NCT04075747) are ongoing to evaluate the safety and efficacy of CPX-351 in combination with targeted agents (venetoclax, midostaurin, enasidenib) [52].

Besides *FLT3* mutations, mutations in *IDH1* and *IDH2* can be targeted by the specific inhibitors ivosidenib and enasidenib, which have been approved as single agents for relapsed or refractory (R/R)-AML [36, 37]. However, their role in the frontline setting in chemotherapy-eligible, newly diagnosed patients is not defined yet. A recent phase I study combining either ivosidenib or enasidenib with induction and consolidation chemotherapy in newly diagnosed *IDH1/2*-mutant AML showed rates of CR/CRi/CRp of 77% (ivosidenib) and 74% (enasidenib) with 39% and 23% of patients, respectively, achieving mutation clearance by digital PCR [53]. Although 30% and 38% of patients treated with ivosidenib and enasidenib, respectively, had sAML in this trial, the outcomes for patients with AML-MRC versus t-AML have not been reported separately and *IDH1/2* mutations occur in only 6–14% of patients with AML-MRC [30, 53].

Finally, the antibody-drug conjugate gemtuzumab ozogamicin which targets CD33 has been approved for the treatment of newly diagnosed and R/R CD33-positive AML [40]. While CD33 expression has been documented in 69% of cases of AML-MRC and appears similar to de novo AML, survival benefit with the addition of gemtuzumab ozogamicin to intensive chemotherapy has been shown in patients with favorable and – to a lesser extent – intermediate disease risk and patients with prior MDS and MDS/MPN had been excluded from the ALFA-0701 trial but not those with adverse risk karyotypes (which includes many patients with sAML, t-AML and AML-MRC) [54–57]. Currently, the National Comprehensive Cancer Network (NCCN) is recommending addition of gemtuzumab ozogamicin to intensive chemotherapy only in CD33-positive patients with favorable and intermediate-risk cytogenetics [32]. However, this will only apply to a minority of sAML patients.

8.3.2 Treatment Options for Chemotherapy-Ineligible Patients

8.3.2.1 Hypomethylating Agent Monotherapy

Prior to the approval of venetoclax, monotherapy with the hypomethylating agents (HMA) azacitidine or decitabine has been the standard of care for chemotherapy-ineligible patients with AML. In the randomized AZA-AML-001 trial of patients with newly diagnosed AML, azacitidine had a statistically nonsignificant OS advantage of 10.4 versus 6.5 months with standard of care ($p = 0.101$) [58]. However, in the subset of patients with AML-MRC (as defined by central review; 262 patients or 54% of the initial AZA-AML-001 trial population), treatment with azacitidine appeared to lead to an OS improvement compared to standard of care, although interpretation is limited due to post-hoc nature of the analysis and the absence of correction for multiplicity of testing (median OS: 8.9 vs. 4.9 months; HR: 0.74 [95% CI: 0.57–0.97]) [29]. Similarly, a subset analysis from the AZA-001 trial of patients with 20–30% blasts supported the use of azacitidine in this setting with a median OS of 24.5 months with azacitidine compared to 16.0 months with conventional care (HR: 0.47; 95% CI: 0.28–0.79; $p = 0.005$) [59].

Decitabine has been evaluated in AML patients ≥65 years in a randomized, open-label, phase III trial compared to treatment choice (low-dose cytarabine [LDAC] or supportive care) in 485 patients [60]. Decitabine showed a nonsignificant improvement in median OS compared to treatment choice (7.7 months [95% CI: 6.2–9.2 months] vs. 5.0 months [95% CI: 4.3–6.3]; $p = 0.108$; HR: 0.85 [95% CI: 0.69–1.04]) [60]. However, several unplanned subgroup analyses supported the use of decitabine with larger benefits seen in patients ≥75 years of age, de novo AML, or bone marrow blasts >30% [60]. Patients with sAML did not appear to experience a statistically significant survival benefit and outcomes for AML-MRC patients were not reported separately [60]. Additionally, there does not appear to be a significant difference between azacitidine and decitabine neither in registry studies nor in a subgroup analysis of patients enrolled in the phase III ASTRAL-1 trial (NCT02348489) that compared guadecitabine to physician choice of azacitidine, decitabine, or LDAC in treatment-naïve AML patients ineligible for intensive chemotherapy [61–63].

More recently, Stahl et al. reported an international, retrospective analysis of patients with R/R-AML treated with HMA [64]. Among 655 patients (57% treated with azacitidine and 43% with decitabine), 11% achieved a CR and median OS was 6.7 months (95% CI: 6.1–7.3 months) in the entire cohort. 26.9% of patients in this cohort were classified as AML-MRC, which was not statistically significantly associated with response or OS [64].

8.3.2.2 Venetoclax-Based Combinations

Registry studies have shown that a substantial proportion of older patients with AML are not receiving any active AML-directed therapy [65]. Given that sAML is enriched among older patients, this highlights an area of unmet need [9]. With the FDA approval of the BCL-2 inhibitor venetoclax in combination with HMA or LDAC, the treatment options for chemotherapy-ineligible patients have significantly expanded [34, 35]. VIALE-A patients with newly diagnosed AML who were 75 years or older or ineligible for intensive induction chemotherapy were randomized to either venetoclax +

azacitidine or azacitidine + placebo showing a mortality benefit for the combination arm (HR for death: 0.64; 95% CI: 0.50–0.82) [34]. Keeping limitations of small sample sizes in mind as only 25% of patients enrolled had sAML, and among those, 64% had a prior MDS or chronic myelomonocytic leukemia (CMML), it is encouraging to note that the combination therapy arm appeared superior in terms of mortality (HR for death: 0.56; 95% CI: 0.35–0.91) and median OS (16.4 months [95% CI: 9.7–24.4] vs. 10.6 months [95% CI: 4.9–13.2], HR: 0.56 [95% CI: 0.35–0.91]) compared to azacitidine monotherapy [34]. It is also important to note that patients with prior HMA exposure were excluded from this trial.

Regarding patients with prior HMA exposure, the VIALE-C trial of venetoclax + LDAC showed that response rates in this patient subgroup were substantially lower compared to HMA-naïve patients (composite of CR/CRh: 18% with prior HMA vs. 54% without prior HMA) [35]. Similarly, responses in patients with sAML (90% with prior hematologic disorder) with venetoclax + LDAC were less frequent compared to patients with de novo AML (CR/CRh: 59% vs. 29%) [35]. However, CR/CRi and OS still appeared numerically higher in the venetoclax + LDAC arm compared to LDAC alone in the subgroup of patients with sAML (CR/CRi: 29% vs. 4%; HR for death: 0.71 [95% CI: 0.40–1.2]) [35].

While cross-trial comparisons are inherently limited and the number of patients with AML-MRC in VIALE-A and VIALE-C was small, results appear comparable to superior in terms of median OS to historic controls from patients with AML-MRC treated with azacitidine monotherapy [29]. This highlights that especially the patient population with prior HMA exposure continues to be a clinically challenging patient subgroup with limited options. While criteria to define ineligibility for intensive chemotherapy have been developed and used in clinical trials, this remains a challenging and highly individualized decision for many older patients [7, 34, 35, 66, 67]. To add to this complexity, "biologic or genetic unfitness"-such as those with *TP53* mutations- is being increasingly used to describe patients who are physically fit for intensive chemotherapy, but are thought to derive minimal clinical benefit for such intensive therapies and therefore often receive HMA-based combination therapies. Clinical trials to compare intensive therapies to HMA-venetoclax are urgently needed for such physically fit but "genetically unfit" patients.

8.3.2.3 Single-Agent Targeted Therapies

As discussed above, treatment with targeted therapies as single agent has been approved for the treatment of patients with R/R-AML and those who are ineligible for intensive chemotherapy. For patients with *FLT3* mutations, gilteritinib has shown a survival advantage in a randomized phase III trial of 247 R/R AML patients compared to salvage chemotherapy (median OS: 9.3 vs. 5.6 months; HR for death: 0.64; 95% CI: 0.49–0.83; $p < 0.001$) [39]. However, outcomes in patients with AML-MRC were not reported separately.

For patients with *IDH1* and *IDH2* mutations, ivosidenib and enasidenib monotherapy was demonstrated in single-arm trials to lead to meaningful survival prolongation in subsets of patients, especially if a CR is achieved [37]. Stein et al. showed in a phase I trial of the IDH2 inhibitor enasidenib an overall response rate (defined as CR, CRi, CRp, partial remission, or morphologic leukemia-free state) of 40.3% among R/R-AML patients with a median OS of 9.3 months (19.7 months among patients with CR) [37]. Similarly, DiNardo et al. reported outcomes from a phase I study of the IDH1 inhibitor ivosidenib from 179 R/R-AML patients (125 in the primary efficacy cohort) with ORR of 41.6% (95% CI: 32.9–50.8%; CR 21.6%) and a median OS of 8.8 months (95% CI: 6.7–10.2; 50.1% 18-month survival probability in patients with CR) [36]. Again, no separate data for the AML-MRC subgroup from either trial are available and only 20% of the patients in the ivosidenib study had a prior MDS or MPN as well as 26% of patients with AML-MRC in the enasidenib study [36, 37].

8.3.2.4 Glasdegib

The smoothened inhibitor glasdegib has been approved by the FDA in combination with LDAC for the treatment of AML in patients 75 years or older or otherwise unfit for intensive chemotherapy. This approval was based on a randomized open-label study of glasdegib + LDAC vs. LDAC monotherapy in patients with newly diagnosed AML or higher-risk MDS, which showed a median OS benefit for the combination arm compared to LDAC monotherapy (8.8 months [80% CI: 6.9–9.9] vs. 4.9 months [80% CI: 3.5–6.0]; HR: 0.51 [80% CI: 0.39–0.67; $p = 0.0004$) [38]. However, given that LDAC monotherapy is rarely used for treatment of AML, the role of this combination in the treatment landscape of AML is poorly defined and likely limited to patients with prior exposure to HMA and/or venetoclax.

8.4 Future Directions

Two major trends have the potential to impact the general treatment landscape of patients with AML-MRC and t-AML, namely the greater individualization of treatment concepts based on genetic disease characteristics and the emerging evidence on the safety and efficacy of maintenance therapy for patients in CR following induction and consolidation chemotherapy.

As noted before, AML-MRC and t-AML are heterogenous disease entities and the prognosis and treatment of patients should be individualized based on molecular disease

characteristics. For example, *TP53* mutations are enriched in patients with sAML and have been associated with other cytogenetic abnormalities such as complex and monosomal karyotypes [18, 68]. More importantly for clinical practice, *TP53* mutations have also been shown to confer a higher rate of resistance to standard cytotoxic chemotherapy and an overall adverse prognosis [18–20]. However, there are limited, retrospective data that suggest that CPX-351 can induce deep remissions even in patients with poor-risk mutations such as *TP53*, *ASXL1*, *RUNX1,* and *EVI1* in some studies abrogating the adverse prognosis [69, 70] but not in others, [71, 72] highlighting the need for additional validation. Assuming that chemotherapy resistance of *TP53* mutations also applies to CPX-351 raises the question if alternative therapeutic strategies such as HMA-based combinations could be more effective. While decitabine was able to achieve high rates of remission in *TP53*-mutated patients, responses were short-lived, [73] and it remains to be seen what the long-term outcomes in HMA/venetoclax-treated patients without subsequent transplant will be. Therefore, the development of novel targeted agents such as the anti-CD47 antibody magrolimab or the mutant p53-refolding agent APR-246 is important. Although early results have been encouraging, those should be interpreted cautiously and both longer follow-up and data from the ongoing phase III trials in newly diagnosed patients with higher-risk MDS and low-blast count (≤30%) AML are needed before solid conclusions can be drawn [74–76]. Given the association of sAML with *TP53* mutations, those agents could also become a therapeutic option for these patients as well.

Although speculative at this point, it is an intriguing question whether older patients with sAML—or at least subsets of patients, e.g., with *TP53* mutations—would be as effectively managed with azacitidine + venetoclax combination as with CPX-351. While no prospective clinical trial data exist comparing azacitidine + venetoclax with intensive chemotherapy, retrospective series suggested comparable survival and early mortality rates for CPX-351 and azacitidine + venetoclax-treated patients [77, 78]. Additional options to improve responses to CPX-351 are combinations with either targeted agents (e.g., FLT3 inhibitors or gemtuzumab ozogamicin) or venetoclax [79, 80]. The combination of CPX-351 with venetoclax is currently being evaluated in a phase II clinical trial in both newly diagnosed and R/R AML patients (94% R/R) [79]. Among the 16 evaluable patients, 6 patients (37%) achieved CR/CRi (1 MRD-negative CR) with a median OS of 6.4 months and 6 out of 7 responders proceeding to allo-HCT [79]. However, three grade 5 adverse events were reported highlighting the need for careful patient selection [79]. Although encouraging, additional results from ongoing clinical trials will be essential to confidently compare safety, efficacy, and ideally quality-of-life outcomes for patients treated with intensive chemotherapy and venetoclax-based regimens.

Besides its prognostic and predictive potential, mutation analyses supplemented by machine learning algorithms could also be useful to distinguish patients with AML-MRC from other AML subtypes based on unique mutational patterns similar to what has already been demonstrated in MDS [68, 81]. For example, Baer et al. demonstrated in a cohort of 739 AML patients (22% AML-MRC) that a combination of genetic testing and patient history accurately identified 96–99% of AML-MRC cases [68]. Additionally, they described a subset of patients (11–14%) with a molecular "AML-MRC-like" phenotype that was not classified as AML-MRC based on morphology but had a comparably poor outcome [68]. However, whether these patients would also benefit from treatment with, e.g., CPX-351, needs to be determined through clinical trials.

For patients who achieve CR/CRi with induction chemotherapy but are unable to proceed to allo-HCT, maintenance therapy with the oral azacitidine analogue CC-486 is a potential option that has recently received approval by the FDA based on data from the randomized phase III QUAZAR AML-001 trial (NCT01757535) [82]. In QUAZAR AML-001, 472 patients with newly diagnosed, intermediate or poor-risk cytogenetics AML in CR/CRi following induction +/− consolidation chemotherapy were randomized to placebo or CC-486 (on days 1–14 of each 28-day cycle) [82]. CC-486 let to statistically significant improvements in both median OS (24.7 vs. 14.8 months; $p < 0.001$) and RFS (10.2 vs. 4.8 months; $p < 0.001$) with a safety profile comparable to injectable azacitidine (neutropenia 41%, thrombocytopenia 22%, and anemia 14% being most common grade 3/4 AEs) [82]. Although the patient population meeting the inclusion criteria for QUAZAR AML-001 is small and 91% of patients in the trial had de novo AML, [82] CC-486 in those patients could be a valid option if allo-HCT is not available or feasible but outcomes for patients with sAML specifically are not available.

8.5 Conclusion

AML-MRC and t-AML have historical, clinical, and genetic features distinct from other forms of AML and are associated with an adverse prognosis. For patients eligible for intensive chemotherapy, CPX-351 is the preferred frontline treatment based on randomized phase III trial data showing superiority over standard 7 + 3 induction, though benefit in *TP53* mutated patients seems to be lacking. Given that AML-MRC and t-AML patients tend to be older, lower-intensity treatment alternatives such as azacitidine + venetoclax or molecularly targeted agents (e.g., gilteritinib, enasidenib,

ivosidenib) are important additions to the treatment landscape for unfit patients. Comparative trials of these agents with intensive chemotherapy are urgently needed for patients with AML-MRC, t-AML, and sAML, given they are often resistant to intensive therapies. With increasing number of available therapeutic options and advances in molecular testing, an increasingly individualized and genetically driven treatment approach is becoming the standard approach for decision making to optimize clinical outcomes.

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Management of Relapsed or Refractory AML

9

Harinder Gill

Abstract

Standard induction chemotherapy for acute myeloid leukemia (AML) results in a remission rate of around 70%. Up to 30% of patients do not achieve a remission and approximately 50% of patients who achieve complete remission after standard frontline induction chemotherapy relapse. Outcome with conventional approaches in relapsed or refractory AML is poor and only a minority of patients achieve long-term cure. Results from various novel agents and combinations in relapsed or refractory AML have only shown response rates between 30% and 55%. In patients achieving remission after re-induction and undergoing allogeneic hematopoietic stem cell transplantation (HSCT), overall survival rates range between 20% and 55% at 2 years. In this chapter, we outline the current and upcoming approaches in optimizing the management of patient with relapsed or refractory acute myeloid leukemia.

Keywords

Acute myeloid leukemia · Relapsed or refractory · Novel therapy

9.1 Young or Fit Patients with Relapsed or Refractory AML with Salvage Chemotherapy

The current treatment paradigm for treatment of relapsed or refractory (R/R) AML is shifting towards a targeted approach (Fig. 9.1). Nevertheless, allogeneic hematopoietic stem cell transplantation (allo-HSCT) remains the therapy with maximal antileukemic effect in R/R AML and should be considered in young or fit patients with suitable donors. Though a small subgroup of patients with primary refractory AML (i.e., patients who do not achieve complete remission after two courses of induction chemotherapy) may benefit for an early upfront allo-HSCT [1, 2], salvage therapy is generally recommended as a bridge to allo-HSCT. None of the commonly used intensive chemotherapy regimens has been found to superior over each other in a randomized controlled trial setting and the choice of therapy depends on the patient age, comorbidities, prior treatment toxicities, salvage status, duration of previous responses (if any), genomic characteristics of AML, the availability of novel agents, and patient preferences [3–7]. Most salvage regimens comprise purine analogues, medium or high dose cytarabine (AraC) with or without anthracyclines or topoisomerase II inhibitors. Common salvage regimens used include FLAG +/− Ida (Fludarabine, AraC, Growth colony-stimulating factor with or without Idarubicin), CLIA (Cladribine, Idarubicin, AraC), CLAG +/− Ida (Cladribine, AraC, Growth colony-stimulating factor with or without Idarubicin), and MEC (Mitoxantrone, Etoposide, AraC) [4, 5, 8–12]. A second complete remission (CR2) is achieved in 20–50% of patients treated with these regimens [5–7]. A recent salvage regimen designed in Hong Kong combined the use of Clofarabine, AraC, and Mitoxantrone (CLAM) [13] for patients with AML as first salvage. The composite complete response (CR) rate after the first cycle of CLAM was 90.4% (complete remission, CR: 69.2%; CR with incomplete hematologic recovery, CRi: 21.2%) and toxicities were manageable [13]. CLAM was thus a safe and effective bridge to allo-HSCT. In an effort to improve responses to FLAG-Ida, venetoclax was combined to FLAG-Ida both in newly diagnosed and relapsed or refractory AML [14]. In the phase IB (16 patients) and phase IIB (23 patients) R/R AML cohorts, the composite CR rate was 75% and 61%, respectively [14]. A measurable residual disease (MRD)-negative composite CR was 69% in the R/R AML cohorts [14] and 46% of patients with R/R AML were bridged to allo-HSCT [14].

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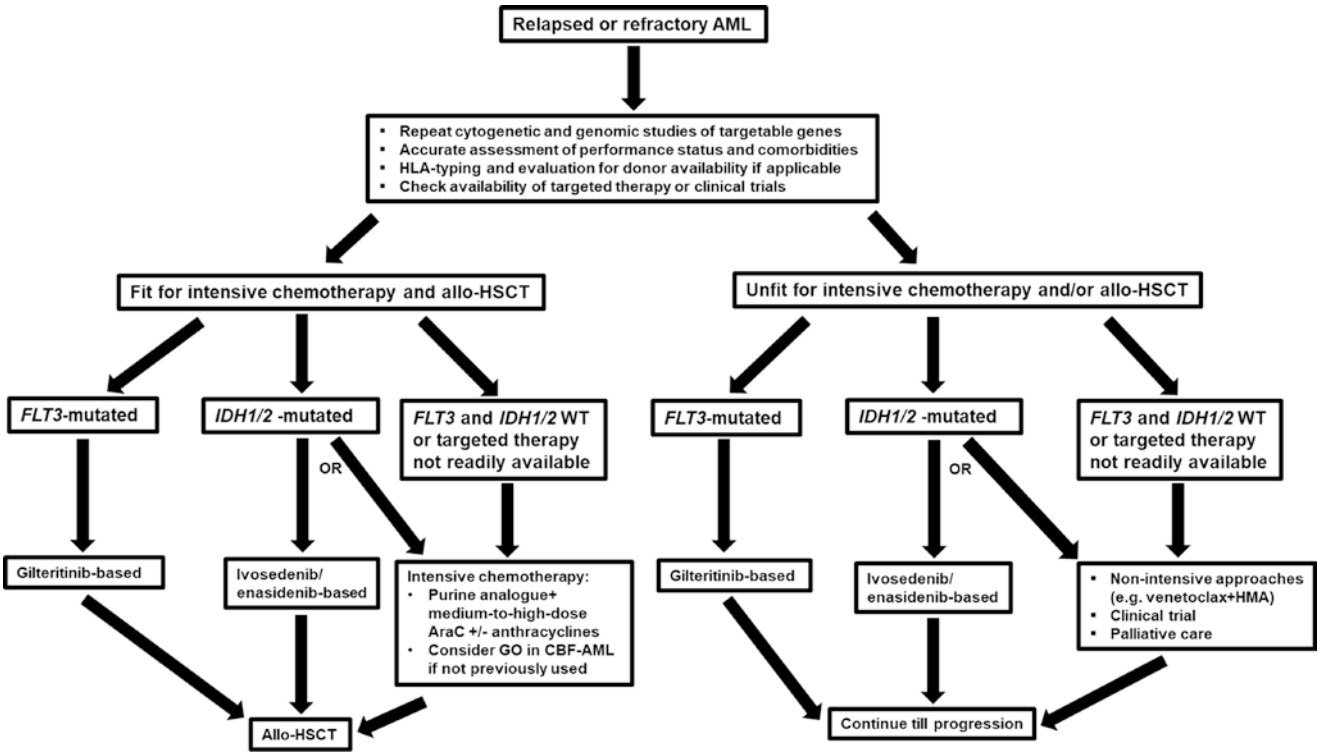

Fig. 9.1 Treatment algorithm in patients with relapsed or refractory acute myeloid leukemia (AML). *HLA* human leucocyte antigen, *Allo-HSCT* allogeneic HSCT, *FLT3* fms-like tyrosine kinase-3, *IDH1/2* isocitrate dehydrogenase 1/2, *AraC* cytarabine, + with, +/− with or without, *GO* gemtuzumab ozogamacin, *CBF* core-binding factor, *HMA* hypomethylating agents

9.2 Relapsed or Refractory *FLT3*-Mutated AML

While *FLT3*-internal tandem duplication (*FLT3*-ITD) and *FLT3*-tyrosine kinase domain (*FLT3*-TKD) mutations occur in approximately 30% and 5% of AML at diagnosis, respectively [15], their occurrence at relapse can be variable due to the instability of these mutations during clonal evolution. Recent studies have shown that up to 40% of *FLT3*-mutated AML treated with frontline midostaurin plus induction chemotherapy have undetected *FLT3* mutations at relapse [16]. New *FLT3*-ITD clones were also observed in approximately 10% of these patients [16]. The phase III randomized ADMiRAL trial showed the improvement in median overall survival (OS) with the second-generation FLT3-inhibitor Gilteritinib compared with salvage chemotherapy (median OS: 9.3 months in gilteritinib arm vs. 5.6 months) [17, 18]. A composite CR rate of 34% was achieved compared with salvage chemotherapy [17, 18]. In a recent phase IB open-label dose-escalation/dose-expansion study, Gilteritinib was combined with venetoclax in patients with relapse or refractory AML [19]. In 56 *FLT3*-mutated patients, the modified composite CR rate was 75% (CR: 18%; CR with incomplete blood count recovery: 4%; CR with incomplete platelet recovery: 18%; morphologic leukemia-free state: 36%) [19]. Of note, 64% of these patients had prior exposure to FLT3 inhibitors [19]. In the QuANTUM-R trial that recruited 367 R/R FLT3-ITD positive patients, patients were randomized to the second-generation FLT3 inhibitor quizartinib versus salvage chemotherapy. The median OS was 6.2 months in the quizartinib arm versus 4.7 months (hazards ratio 0.67, $p = 0.02$) [20]. Sorafenib, another multikinase inhibitor with FLT3 inhibitory activity, has shown activity in combination with azacitidine in patients with R/R secondary AML and relapsed AML post-allogeneic HSCT [21, 22]. The activity of sorafenib post-allogeneic HSCT may partly be explained by its graft-versus-leukemia effect [23, 24]. Gilteritinib is the only FLT3 inhibitor that is FDA- and EMA-approved for the treatment of R/R *FLT3*-mutated AML, while quizartinib is only approved in Japan for this indication as the time of writing of this chapter.

9.3 Relapsed or Refractory *IDH1* or *IDH2*-Muated AML

The use of the IDH1/2 inhibitors ivosidenib and enasidenib as single agents has resulted in an overall response rate of approximately 40% with a median OS of approximately 9 months in phase II studies [25, 26]. The randomized phase

III study of enasidenib versus conventional care in R/R *IDH2*-mutated AML failed to show an overall survival benefit [5]. Trials of combinatorial regimens with IDH1/2 inhibitors in combination with venetoclax and azacitidine are currently ongoing [5].

9.4 Nonintensive Approach in Unfit Patients

Response rates to hypomethylating agents (HMA) are generally worse with R/R AML compared with newly diagnosed AML. In a large international multicenter retrospective analysis, the CR and CRi rate in R/R AML (including patients relapsing after allogeneic HSCT) was 11% and 5.3%, respectively, with a median OS of 6.5 months [27]. In patients achieving CR or CRi, the median OS was 21 months [27]. In patients failing prior treatment with venetoclax plus HMA, the outcome is dismal with a median OS of 2.9 months [28]. Venetoclax plus HMA or low-dose cytarabine has been evaluated in patients with R/R AML, including those receiving prior allogeneic HSCT. The CR rates range between 38% and 46% with a median OS of 5.6 to 7.8 months. Durable responses have been seen in patients with *IDH1/2* or *NPM1*-mutated R/R AML without prior venetoclax exposure, while *FLT3* mutations, *RAS* mutations, and *TP53* multi-allelic mutations generally confer resistance to venetoclax in the R/R setting [29, 30]. In a prospective evaluation of R/R AML treated with venetoclax and 10-day decitabine, the overall response rate was 62% with a median OS of 7.8 months [31]. While nonintensive approaches may play a role in the relapsed or refractory setting, long-term remissions are rare and cure is not the aim. Early integration of palliative and supportive care should be instituted to improve or maintain the quality-of-life of these patients [32, 33]. In patients with adequate performance status, every effort should be made in identifying patients suitable for clinical trials involving novel agents.

9.5 Relapse After Allogeneic HSCT

Outcome of AML after relapse from allogeneic HSCT is generally poor with 2-year OS of approximately 14–25% [34]. There is no approved standard-of-care for the management of relapsed AML post-allogeneic HSCT. A second allogeneic HSCT may provide some benefit in patients relapsing from than 6 months after the first HSCT and in those achieving a remission before a second allogeneic HSCT is done. Donor lymphocyte infusion (DLI) is another option with outcomes similar to second allogeneic HSCT. The 5-year overall OS with second allogeneic HSCT and DLI were 19% and 15%, respectively [35]. A second allogeneic HSCT is also associated with a significantly higher risk of transplant-related mortality [35].

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The Role of BCL-2/MCL-1 Targeting in Acute Myeloid Leukemia

10

Kenny Tang and Steven M. Chan

Abstract

The B cell lymphoma-2 (BCL-2) family of proteins plays a critical role in the intrinsic pathway of apoptosis. It is therefore not surprising that this pathway is frequently dysregulated in numerous malignancies, including acute myeloid leukemia (AML), in order to evade apoptosis. In the last 25 years, research into the pathobiology of AML has focused intensely on the antiapoptotic proteins, BCL-2, and myeloid cell leukemia-1 (MCL-1), whose overexpressions are associated with enhanced survival and chemoresistance of leukemic cells. In light of this, BCL-2 and MCL-1 have been attractive targets in the development of novel agents to treat AML. Many BCL-2 and MCL-1 inhibitors have yielded promising results in preclinical trials and are currently undergoing evaluation in clinical trials. Recently, venetoclax, a first-in-class selective oral BCL-2 inhibitor, was approved for upfront treatment of AML in the unfit or elderly population and had revolutionized the therapeutic landscape of AML. In this chapter, we will review the role of BCL-2 and MCL-1 in AML as well as the preclinical and clinical data supporting the use of BCL-2 and MCL-1 inhibitors in AML treatment. Furthermore, we will discuss the mechanisms of resistance to BCL-2 inhibitors and highlight ongoing clinical trials of combination therapies aimed at overcoming such resistance pathways.

Keywords

BCL-2 · MCL-1 · Acute myeloid leukemia · Apoptosis Resistance · Venetoclax

10.1 Role of the BCL-2 Family of Proteins in Apoptosis

Programmed cell death, or apoptosis, is a tightly regulated process critical to the maintenance of cellular homeostasis through carefully orchestrated elimination of senescent and genetically aberrant cells [1]. It is governed by two interconnected pathways, the extrinsic pathway, which is activated by external signalling proteins such as TNF-α and FAS-L, and the intrinsic pathway, which is strictly regulated by the BCL-2 family of proteins [1, 2]. In the resting state, suppressor proteins (BCL-2, MCL-1, BCL-xL, and BCL-w) bind to effector (BAX and BAK) and activator proteins (tBID and BIM) and inhibit their activity, thereby preventing downstream apoptotic signalling. Conversely, in response to cellular stress signals, such as DNA damage from cytotoxic agents, the intrinsic apoptotic pathway is activated, leading to production of sensitizers and activators. Sensitizer proteins (e.g., PUMA, NOXA, and BAD) antagonize the actions of antiapoptotic BCL-2 proteins through interactions with their BCL-2 homology 3 (BH3) domains [3]. Thus, increased expression of sensitizers liberates activators and effectors from the inhibitory effects of antiapoptotic BCL-2 proteins. Consequently, activators bind to effectors and induce a conformational change, resulting in the creation of pores in the outer mitochondrial membrane. This causes mitochondrial outer membrane permeabilization (MOMP) and release of cytochrome C from the intermembrane space into the cytoplasm. Cytochrome C complexes with Apaf-1 to form an apoptosome, which recruits and activates downstream caspases to initiate apoptosis (Fig. 10.1) [1, 3].

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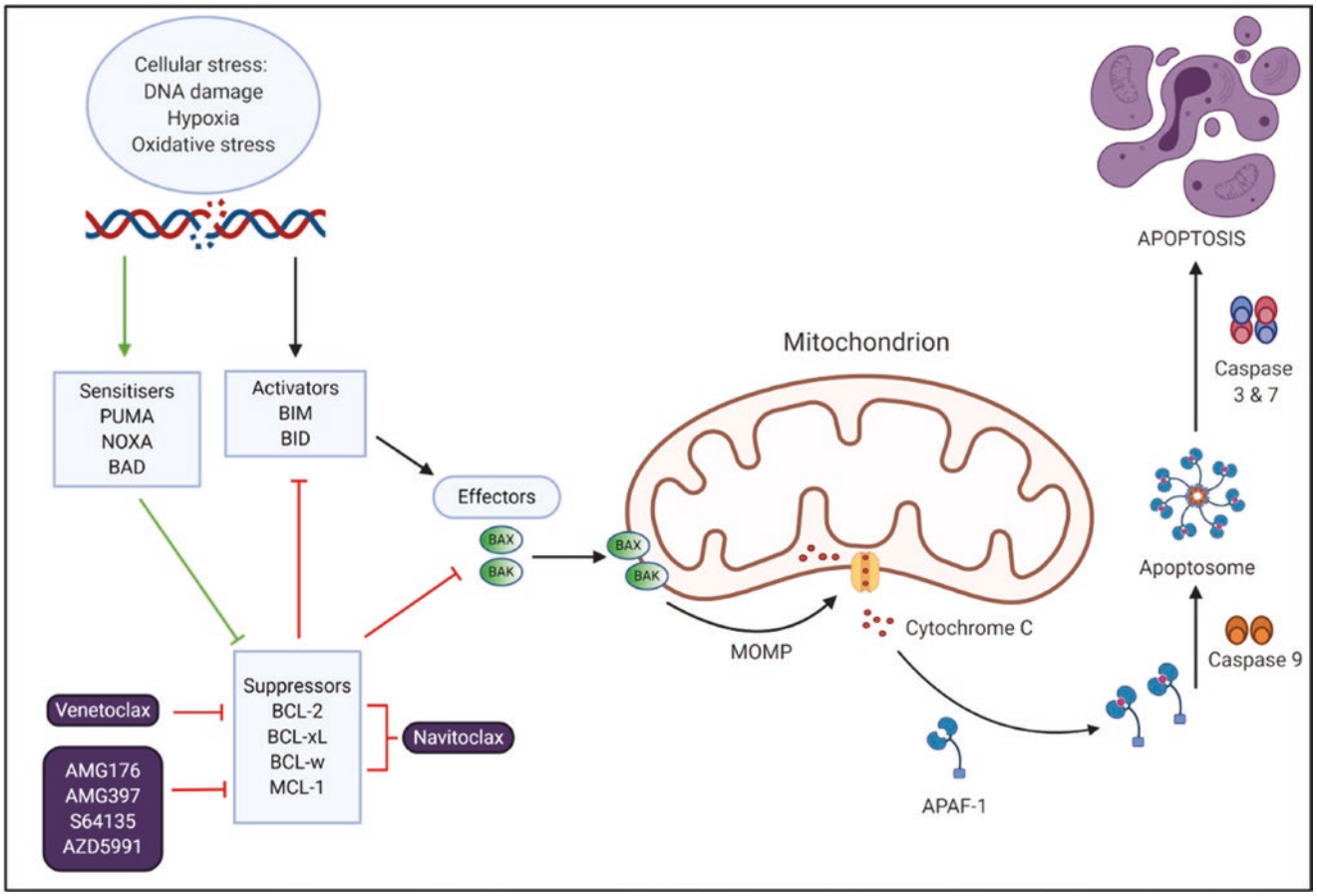

Fig. 10.1 Role of BCL-2 in apoptosis. In the resting state, suppressor proteins (BCL-2, BCL-xL, BCL-W, and MCL-1) bind to and inhibit effectors and activators, thus preventing apoptosis. Cellular stressors activate the intrinsic pathway and induce the production of sensitizers (PUMA, NOXA, BAD) and activators (BIM, BID). Sensitizers inhibit suppressor proteins, thus freeing activators from their inhibitory effects. This allows activators to bind to and activate effectors (BAX, BAK), resulting in mitochondrial outer membrane permeabilization (MOMP) and release of cytochrome C. This binds to Apaf-1 to form an heptameric complex that recruits caspase 9 to become an apoptosome. This, in turn, activates effector caspases 3 and 7 to induce apoptosis. Key BCL-2 family inhibitors (within purple boxes) and their respective targets are highlighted by the red brackets

10.2 Role of BCL-2 in AML

BCL-2 was first discovered as a partner of the immunoglobulin heavy chain in the translocation of chromosome 14 and 18, which is the oncogenic hallmark of follicular lymphoma [4]. However, its role in AML was not established until the 1990s, when a number of key studies confirmed that BCL-2 overexpression promotes leukemogenesis [5, 6], therapeutic resistance and poor responses to chemotherapy in AML [7], and leukemic stem cells (LSC) [8]. These findings led to the development of an array of BCL-2 inhibitors. The following section will expand on their development, efficacy, and usage in AML, with a focus on those in clinical use or undergoing clinical trial.

10.2.1 Oblimersen

Oblimersen was the first anti-BCL-2 agent explored in AML. It is an antisense oligonucleotide that targets human *BCL2* mRNA and leads to decreased BCL-2 protein expression. In preclinical models, this was shown to increase apoptosis of leukemic cells [9, 10]. This finding led to a Phase 1 trial of oblimersen in combination with fludarabine, cytarabine, and granulocyte colony-stimulating factor (FLAG) salvage chemotherapy in patients with relapsed and refractory AML or acute lymphoblastic leukemia (ALL) [11]. Overall, 20 patients were recruited, of which 17 had AML. Complete responses (CR) were observed in 5 of 17 (29%) AML patients, with two additional patients (12%)

achieving CR with incomplete hematological responses (CRi). Another Phase 1 trial performed by the same group combined oblimersen with chemotherapy in previously untreated older patients with AML [12]. Fourteen of 29 patients (48%) achieved a CR, with a decrease in *BCL2* mRNA copies observed in responders. The drug was considered tolerable compared to standard induction therapy.

Following on from these Phase 1 trials, the Cancer and Leukemia Group B (CALGB) performed a Phase 3 randomized controlled trial in treatment-naïve patients with AML who were older than 60 years of age [13]. Patients were treated with standard "3 + 7" (daunorubicin + infusional cytarabine) induction chemotherapy followed by high-dose cytarabine (HiDAC) consolidation with or without oblimersen. Unfortunately, there was no difference in CR rate (48% vs. 52%; $p = 0.75$) or overall survival (OS). Thus, no further trials of this drug were carried out in AML.

10.2.2 Obatoclax

Obatoclax was the first BH3-mimetic to enter clinical trials for AML. Preclinical models confirmed its ability to inhibit BCL-2 and related family members including BCL-xL, MCL-1, BCL-w, A1, and BCL-B [14]. In AML cell lines and primary AML samples, obatoclax was shown to induce apoptosis and impair leukemic proliferation [15]. In a Phase 1 trial of 44 patients with advanced hematological malignancies, including patients with refractory AML (57%), myelodysplastic syndrome (MDS) (32%), chronic lymphocytic leukaemia (CLL) (9%), and ALL (2%), obatoclax monotherapy was well tolerated but responses were modest given only one case of refractory AML achieved CR [16]. A follow-up Phase 1/2 study using obatoclax in older patients with untreated AML was performed based on the safety profile in the previous trial [17]. Nineteen patients were recruited with a median age of 81 years. None of the patients achieved a CR, and only four patients showed stable disease. Based on these disappointing responses, the drug was not further developed in AML.

10.2.3 ABT-737/ABT-263 (Navitoclax)

ABT-737 is another BH3-mimetic that inhibits BCL-2, BCL-xL, and BCL-w with high potency. Preclinical studies demonstrated that ABT-737 was effective in inducing apoptosis of AML cell lines and LSCs, inhibiting the growth of AML progenitor cells, and reducing leukemia burden in murine xenograft models of AML [18]. High MCL-1 expression was shown to confer resistance to ABT-737, with restoration of drug activity upon MCL-1 knockdown [18, 19]. Interestingly, this study also demonstrated that AML cells that were exquisitely sensitive to the pharmacological blockade of BCL-2 were those already "primed" for apoptosis. That is, effector proteins such as BAX were already assembled on the outer mitochondrial membrane, but were kept in check by inhibitory BCL-2 proteins. Thus, once the inhibitory effects of these proteins were neutralized by ABT-737, these cells were rendered exquisitely susceptible to apoptosis. BH3 profiling demonstrated widespread dependence on BCL-2 in the mitochondria of blasts from AML patient samples. Importantly, normal hematopoietic stem and progenitor cells were found to be less reliant on BCL-2, suggesting an exploitable therapeutic index for the inhibition of BCL-2 in AML. Despite these promising data, its lack of oral bioavailability and water insolubility hampered the translation of this drug into clinical practice. These limitations led to the development of ABT-263 (navitoclax). Navitoclax is an orally bioavailable BH3 mimetic with a similar spectrum of inhibitory activity as ABT-737 and induces apoptosis through disruption of interactions between BCL-2/BCL-xL and proapoptotic proteins [20]. As with ABT-737, navitoclax demonstrated preclinical efficacy in AML models [21–24]. However, no clinical trials with navitoclax have been undertaken in AML, in part, because it can potentially worsen thrombocytopenia through its 'on-target' inhibitory effects on BCL-xL which is required for platelet survival.

10.2.4 Venetoclax

Venetoclax is a BH3-mimetic that was engineered based on the structure of navitoclax. Critical modifications were made to enhance selectivity for BCL-2 while decreasing its affinity for BCL-xL and BCL-w, thus sparing platelets while maintaining antileukemic activity [25]. The efficacy of venetoclax was demonstrated in AML cell lines and in primary AML samples treated ex vivo and in murine xenograft models [26, 27]. Based on these preclinical findings and early safety data in CLL patients [28], venetoclax was swiftly transitioned to the clinical arena for treatment of AML.

10.2.4.1 Venetoclax Monotherapy in Relapsed/Refractory Patients

The first trial of venetoclax in AML was conducted in 32 patients with R/R AML or treatment-naive patients unfit for intensive chemotherapy [29]. In those with R/R AML, venetoclax monotherapy demonstrated only modest activity with a CR/CRi rate of 19% (6% CR and 13% CRi). However, this response was achieved rapidly, within 4 weeks in all but one patient, with a median duration of CR of 48 days. Moreover, those with *IDH1 or IDH2* mutations achieved a higher CR/CRi rate of 33%. This is consistent with preclinical studies showing that *IDH1/2* mutations, via the oncometabolite (R)-2-hydroxyglutarate, inhibit the activity of cytochrome C

oxidase in the mitochondrial electron transport chain, which in turn lowers the mitochondrial threshold to trigger apoptosis upon BCL-2 inhibition [30]. Importantly, venetoclax was well tolerated, with the most common Grade 3/4 adverse events (AEs) being febrile neutropenia, hypokalemia, pneumonia, hypotension, and urinary tract infections, all of which would be expected in this cohort of patients [31, 32]. There were no reported episodes of tumor lysis syndrome.

10.2.4.2 Venetoclax + Hypomethylating Agents (HMA) in Treatment-Naïve Patients

Venetoclax was subsequently studied in combination with HMAs, either azacitidine or decitabine, in treatment-naïve elderly patients or those unfit for intensive chemotherapy. This was driven by preclinical studies demonstrating a synergistic effect between BH3-mimetics and HMAs in AML cell lines and AML patient samples [33]. Furthermore, azacitidine has been shown to reduce MCL-1 levels, an anti-apoptotic protein not targeted by venetoclax, and thus, potential source of drug resistance. A phase Ib/II escalation and expansion study was conducted in 145 patients with untreated AML over the age of 65 years (median age 74 years). Venetoclax (400 mg once daily) in combination with either azacitidine or decitabine demonstrated remarkable CR/CRi rates of 71% and 74%, median duration of response of 21.2 and 15.0 months, and median OS of 16.9 and 16.2 months, for azacitidine and decitabine, respectively [34]. Efficacy was observed among all AML subgroups, including patients with secondary AML, those with adverse-risk cytogenetics, and across the genomic landscape of the disease [35].

These findings were confirmed by results of the Phase III VIALE-A trial published in 2020. In this trial, 433 patients (median age 76 years) underwent a 2:1 randomization to either azacitidine and venetoclax (400 mg once daily) (AZA + VEN) or azacitidine and placebo. Median OS was 14.7 months in the AZA + VEN group, compared with 9.6 months in the control group ($p < 0.001$). CR and CR/CRi rates were superior in the treatment arm at 36.7% versus 17.9% ($p < 0.001$) and 66.4% versus 28.3% ($p < 0.001$), respectively. Responses were both rapid and durable. Median time to first response was 1.0 month in the AZA + VEN group compared with 2.6 months in the control arm. Similarly, median duration of response was superior with AZA + VEN (17.5 vs. 13.4 months). Notably, the CR/CRi was improved across all AML genomic risk groups, including patients with adverse cytogenetic risk, secondary AML, and high-risk molecular mutations. These improvements in responses also translated into an increased OS in many of the evaluated subgroups, most notably among patients with either de novo or secondary AML, intermediate cytogenetic risk, and *IDH1* or *IDH2* mutations [36].

10.2.4.3 Venetoclax + Low Dose Cytarabine in Treatment-Naïve Patients

Venetoclax was also shown to be safe and effective in combination with low-dose cytarabine (LDAC) in upfront treatment of AML [37]. The rationale for this combination emerged from preclinical studies demonstrating venetoclax/LDAC synergy and reduced MCL-1 protein levels with combination therapy compared to venetoclax monotherapy [38, 39]. A Phase III, randomized, double-blinded trial (VIALE-C) examined this combination in 211 patients (median age 76 years) with treatment-naïve AML ineligible for intensive chemotherapy or ≥75 years of age, or both [40]. Subjects were randomized in a 2:1 fashion to receive either venetoclax 600 mg once daily or placebo plus LDAC (20 mg/m^2 subcutaneously daily on days 1–10). Prior HMA exposure was permitted unlike the VIALE-A trial. In total, 38% had secondary AML, 20% had prior HMA treatment, and 32% had poor-risk cytogenetic features. Patients who received venetoclax and LDAC showed an improved composite CR rate of 48% compared with 13% in those who received LDAC alone. This translated to an improved median OS of 8.4 versus 4.1 months and median event-free survival (EFS) of 4.7 versus 2.0 months in the venetoclax + LDAC and control arms, respectively. Similar to the VIALE-A trial, responses were also achieved more rapidly with the addition of venetoclax, with CR/CRi before initiation of cycle 2 observed in 34% of patients in the venetoclax arm, compared with only 3% of patients in the control arm. Venetoclax + LDAC was also associated with a higher rate of red cell and platelet transfusion independence (37% vs. 16%), as well as superior patient-reported outcomes, especially regarding fatigue and quality of life. Again, subgroup analyses showed superior rates of composite CR in those treated with venetoclax + LDAC compared with those who received LDAC alone. In addition, survival outcome was particularly promising for subgroups with *NPM1c* (median OS not reached) and *IDH1/2* mutations (median OS 19.4 months). Toxicities were primarily hematological, as expected, with febrile neutropenia, neutropenia, and thrombocytopenia representing the most common Grade ≥ 3 AEs. Although these were numerically higher in the venetoclax group, the rates of AEs leading to discontinuation (24% vs. 25%) and the rates of serious AEs such as pneumonia (13% vs. 10%) or sepsis (6% each arm) were nearly identical between the venetoclax and control arms, respectively.

In summary, these promising results have led to the FDA approval of venetoclax in combination with HMAs or LDAC as therapeutic options for treatment-naïve AML in elderly patients or those unfit for intensive chemotherapy.

10.2.4.4 Venetoclax + HMA/LDAC in Relapsed/Refractory Patients

Although it has not been directly tested in a clinical trial, there are a number of retrospective studies examining the role of venetoclax + HMAs or LDAC in R/R AML. In a series of 33 patients who received prior HMAs (61%) or allogeneic stem cell transplants (39%), the combination of venetoclax and either azacitidine or decitabine produced a CR/CRi rate of 33% [41]. In another series of 24 patients treated with the combination of venetoclax + HMA ($n = 8$) or venetoclax + LDAC ($n = 16$), the composite CR rate was 24% [42]. In yet another series of 43 patients with R/R myeloid neoplasms of which 91% had AML, the CR/CRi rate was a dismal 12%, with a median OS of only 3 months [43]. This is comparable to the 19% CR/CRi observed with single-agent venetoclax [29]. Hence, with the available data, the value of venetoclax as a salvage therapy, either alone or in combination with HMAs/LDAC, appears comparable with standard salvage regimens albeit with likely reduced toxicities [44].

10.3 Current Clinical Trials of Venetoclax in AML

10.3.1 Venetoclax + Intensive Chemotherapy

Trials are currently underway combining venetoclax with intensive chemotherapeutic regimens, including FLAG-IDA (fludarabine, cytarabine, filgrastim, idarubicin) (NCT03214562), "3 + 7" (NCT03709758), and CPX-351 (NCT03629171) (Table 10.1). Only preliminary data are available for the FLAG-IDA + venetoclax trial (FLAG-V-I) [45], which is recruiting fit patients with R/R AML over the age of 18. In an interim analysis of 11 patients, 8 patients (73%) achieved a CR/CRi. The safety profile was acceptable with no early mortality or severe AEs expected for such an intensive regimen. The median time to neutrophil recovery was 28 days, which is comparable to the recovery time for FLAG-IDA alone.

The efficacy of combining venetoclax with intensive chemotherapy in the elderly AML population has also been studied. In a phase Ib trial (CAVEAT) by Wei and colleagues [46], patients were treated with venetoclax and a modified cytarabine and idarubicin induction and consolidation regimen. Patients received 14 days of venetoclax with each cycle of chemotherapy, followed by 7 cycles of venetoclax monotherapy as maintenance. The overall CR/CRi rate was 71%, with an impressive 95% rate observed in de novo AML cases. The best responses were observed in patients with *NPM1* (100%), *RUNX1* (90%), *IDH1/2* (89%), and *RAS* (90%) mutations, while those with *TP53* (33%) mutations fared the worst. Remarkably, *NPM1* MRD negativity was demonstrated in 83% of patients with *NPM1* mutations. However, the question that remains is whether such high-intensity treatment is required, given the high responses observed by combining venetoclax with lower-intensity therapies, such as HMAs and LDAC.

10.3.2 Venetoclax + FLT3 Inhibitors

Venetoclax is also being explored in combination with FLT3 inhibitors. The rationale for this combination arises from preclinical models in which a synergistic effect was seen between BCL-2 inhibitor, ABT-737, and the FLT3 inhibitors, sunitinib and SU5614, in AML cell lines and primary AML blasts [47]. Ongoing trials include a Phase 1 study combining venetoclax with gilteritinib in R/R FLT3-mutated AML patients (NCT03625505) and a Phase 1/2 trial combining venetoclax with quizartinib in a similar cohort (NCT03735875).

In addition, a recent paper highlighted the synergistic effect of venetoclax combined with midostaurin or gilteritinib in vivo using a murine *FLT3*-ITD AML cell line-derived xenograft model [48]. Midostaurin and gilteritinib were shown to downregulate MCL-1 expression, which may, in part, explain the synergistic cytotoxicity observed. The combination of quizartinib and venetoclax has also been explored, with increased survival observed in a murine *FLT3*-ITD AML model [49]. The authors demonstrated reduced expression of MCL-1 and BCL-xL, but not BCL-2, in *FLT3*-ITD cell lines following treatment with quizartinib. In summary, the combination of venetoclax and FLT3 inhibitors is in early development, with preliminary safety data being awaited. Given that *FLT3* mutations have been associated with an inferior response to HMA/LDAC + venetoclax combinations, it will be interesting to see if exchanging an HMA/LDAC for a FLT3 inhibitor will result in improved responses.

10.3.3 Venetoclax + IDH1/2 Inhibitors

IDH1- and *IDH2*-mutant primary AML cells are more sensitive to venetoclax inhibition compared with wild-type *IDH1/2* cells due to the accumulation of 2-hydroxyglutarate [30], with durable responses and superior OS seen in *IDH*-mutated patients treated with venetoclax-based regimens [29, 36]. Preclinical studies using patient-derived xenograft AML models have demonstrated that concurrent therapy

Table 10.1 Ongoing clinicals involving BCL-2 inhibitors in AML

Clinical Trials.gov identifier	Target population	Phase	Trial
NCT03484520	R/R AML (age ≥ 18)	I	A study of Venetoclax and Dinaciclib (MK7965) in patients with R/R AML
NCT03441555	R/R AML (age ≥ 18)	I	A study of Venetoclax and Alvocidib in patients with R/R AML
NCT03874052	R/R AML (age ≥ 18)	I	Ruxolitinib in combination with Venetoclax for the treatment of R/R AML
NCT03625505	R/R AML (age ≥ 18)	I	A study to assess safety and efficacy of Venetoclax in combination with Gilteritinib in subjects with R/R AML
NCT03672695	R/R AML (age ≥ 18)	I	Phase I dose escalation study of intravenously administered S64315 (selective MCL-1 inhibitor) in combination with orally administered Venetoclax in patients with AML
NCT03844815	High risk AML (R/R AML, TP53 mutant, adverse cytogenetics); age ≥ 18	I	Study of Venetoclax in combination with Decitabine in subjects with AML
NCT03709758	AML (age 18–75); treatment-naïve	I	Venetoclax in combination with intensive induction and consolidation in treatment-naïve AML
NCT03613532	High-risk AML, MDS, MDS/MPN (age ≥ 18) proceeding to allogeneic SCT	I	Venetoclax added to Fludarabine + Busulfan prior to transplant for AML, MDS, and MDS/MPN
NCT04330820	R/R AML (age 18–75)	I	Trial for relapsed or refractory AML patients combining Cytarabine and Mitoxantrone with Venetoclax (RELAX)
NCT04017546	R/R AML or MDS (age ≥ 18)	I	CYC065 (Fadraciclib) CDK inhibitor and Venetoclax study in R/R AML or MDS
NCT04070768	R/R CD33+ AML (age ≥ 18)	I	Study of the safety and efficacy of Gemtuzumab Ozogamicin and Venetoclax in patients with R/R CD33+ AML: Big 10 cancer research consortium BTCRC-AML 17–113
NCT03113643	CD123+ AML (R/R or treatment-naïve and unfit for intensive chemotherapy; age ≥ 18)	I	SL-401 (anti-CD123) in combination with Azacitidine or Azacitidine/Venetoclax in AML or high-risk MDS
NCT03214562	Treatment-naïve and R/R AML (age ≥ 18)	I/II	Study of the BCL-2 inhibitor in combination with standard intensive AML induction/consolidation therapy with FLAG-IDA in patients with newly diagnosed or R/R AML
NCT03735875	R/R AML (age 18–65)	I/II	Venetoclax and Quizartinib in treating patients with FLT3-mutated R/R AML
NCT02670044	R/R AML (age ≥ 60)	I/II	A study of Venetoclax in combination with Cobimetinib and Venetoclax in combination with Idasanutlin in patients aged ≥60 years with R/R AML who are not eligible for cytotoxic therapy
NCT03862157	Treatment-naïve secondary AML with a history of MDS, MPN, MDS/MPN, CNL, aCML, CEL (age ≥ 18)	I/II	Azacitidine, Venetoclax, and Pevonedistat in treating patients with newly diagnosed AML
NCT03471260	R/R AML, high risk MDS, MPN (age ≥ 18)	I/II	Ivosidenib and Venetoclax with or without Azacitidine in treating participants with IDH1 mutated hematologic malignancies
NCT03867682	R/R AML (age ≥ 18; CD33 positive AML)	I/II	Venetoclax and Lintuzumab-Ac225 in AML patients
NCT03176277	R/R AML or high-risk MDS unfit for intensive chemotherapy (age ≥ 18)	I/II	A study of ONO-7475 (AXL/MERTK inhibitor) in patients with acute Leukemias
NCT04435691	Phase 1b: R/R AML (age ≥ 18) Phase 2: Treatment-naïve AML (age ≥ 18; primary or secondary AML; unfit for intensive chemotherapy)	I/II	Magrolimab, Azacitidine, and Venetoclax for the treatment of AML

NCT04092179	IDH2-mutated AML (age ≥ 18; treatment-naïve unfit for intensive chemotherapy or R/R;); IDH2-mutated high-risk MDS; IDH2-mutated MPN	I/II	Study of Enasidenib and Venetoclax in IDH2-mutated blood cancers
NCT04266795	AML (de novo or secondary; age ≥ 18; unfit for intensive chemotherapy	II	Triple combination of Pevonedistat and Venetoclax plus Azacitidine in adults with AML who are unfit for intensive chemotherapy (PEVENAZA)
NCT04150029	AML (age ≥ 18; treatment-naïve; unfit for intensive chemotherapy)	II	A study of MBG453 (Sabatolimab; anti-TIM-3 inhibitor) in combination with Azacitidine and Venetoclax in AML patients unfit for chemotherapy (STIMULUS-AML1)
NCT03573024	Treatment-naïve AML (age 18–59)	II	Venetoclax and Azacitidine for non-elderly adult patients with AML
NCT03466294	Treatment-naïve AML (age ≥ 60)	II	Azacitidine and Venetoclax as induction therapy with Venetoclax maintenance in the elderly with AML
NCT03629171	R/R AML (age ≥ 18) with dose expansion cohort for treatment-naïve	II	Liposome-encapsulated Daunorubicin-Cytarabine and Venetoclax in treating participants with R/R or untreated AML
NCT03586609	Treatment-naïve AML (age ≥ 60)	II	Venetoclax, Cladribine, low dose Cytarabine, and Azacitidine in treating patients with previously untreated AML
NCT04487106	Newly diagnosed o or R/R AML, R/R CMML-2 or R/R high-risk MDS (age ≥ 18)	II	Azacitidine, Venetoclax, and Trametinib (MEK1/2 inhibitor) for the treatment of relapsed or refractory AML or higher-risk MDS
NCT04161885	AML (age ≥ 12)	III	A study evaluating safety and efficacy of Venetoclax in combination with Azacitidine versus standard of care after allogeneic stem cell transplantation in participants with AML (VIALE-T)
NCT04102020	Newly diagnosed AML (age ≥ 18; intermediate or adverse cytogenetics; achieved CR/CRi within 4 months of enrolment)	III	A safety and efficacy study of Oral Venetoclax tablets and injectable Azacitidine versus best supportive care as maintenance therapy in adult participants with AML in first remission after conventional chemotherapy to evaluate improvement in relapse-free survival (VIALE-M)

R/R relapsed/refractory, *AML* acute myeloid leukemia, *MCL-1* myeloid cell leukemia-1, *MDS* myelodysplastic syndrome, *MPN* myeloproliferative neoplasm, *CNL* chronic neutrophilic leukemia, *aCML* atypical chronic myeloid leukemia, *CEL* chronic eosinophilic leukemia, *IDH* isocitrate dehydrogenase, *TIM-3* T-cell immunoglobulin domain and mucin domain-3, *CMML* chronic myelomonocytic leukemia, *MEK* mitogen-activated protein kinase/extracellular signal-regulated kinase kinase, *DLCBCL* diffuse large B cell lymphoma, *NHL* non-Hodgkin lymphoma

with enasidenib and venetoclax is superior to monotherapy in *IDH2*-mutated AML [50], with efficacy achieved through enasidenib-induced differentiation and venetoclax-mediated reduction in BCL-2. Building on these promising results, a phase Ib/II study of venetoclax in combination with enasidenib in *IDH2*-mutated AML (ENAVEN-AML; NCT04092179) is currently ongoing. Similarly, there is a separate phase Ib/II study investigating the combination of venetoclax and ivosidenib (with or without the incorporation of azacitidine) for patients with *IDH1*-mutated MDS and AML in both the R/R and treatment-naïve setting [51]. To date, 19 patients have been enrolled and interim results show an impressive composite CR (CR/CRi/CRh) of 78%, of which 50% achieved MRD negativity by flow cytometry.

10.3.4 Venetoclax + JAK Inhibitors (Ruxolitinib)

A preclinical study by Karjalainen et al. analyzed the ex vivo responses of primary AML blasts to various agents, including venetoclax and ruxolitinib, incubated in either bone marrow stroma-derived or standard culture conditions [52]. The authors demonstrated that bone marrow stroma-derived conditions protected AML blasts from the effects of BCL-2 inhibition, while this cytoprotection was reversed in the presence of ruxolitinib. Mechanistically, the bone marrow stroma appears to confer venetoclax resistance by reducing the BCL-2 dependency of primary AML cells through downregulation of BCL-2 expression, while increasing the expression of other antiapoptotic proteins such as BCL-xL and BCL-xS. The upstream effectors of this appear to be G-CSF and GM-CSF secreted from the stromal cells, which leads to increased phosphorylation of STAT5, and consequently, activation of JAKs. JAK inhibition with ruxolitinib, therefore, maintains BCL-2 dependency in AML blasts through suppression of the JAK-STAT pathway. Based on this preclinical work, a Phase I trial is currently exploring the effectiveness of this combination in R/R AML (NCT03874052).

10.3.5 Venetoclax + MCL-1 Inhibitors

Venetoclax is being trialled in combination with novel direct MCL-1 inhibitors, S64315 (NCT03672695) and AMG 176 (NCT03797261), in R/R AML patients. It is also being investigated in combination with indirect MCL-1 inhibitors, including the MEK inhibitor, cobimetinib (NCT02670044), and the cyclin-dependent kinase (CDK) inhibitors, dinaciclib (NCT03484520) and alvocidib (NCT03441555). Given its selectivity for BCL-2, an intrinsic mechanism of venetoclax resistance is due to increased AML blast dependency on other antiapoptotic proteins, such as MCL-1 and BCL-xL. In a Phase II trial of venetoclax monotherapy in R/R AML, increased BCL-xL and MCL-1 expression levels negatively correlated with response to venetoclax [29]. A number of novel therapies tested in preclinical models in combination with venetoclax have demonstrated synergistic effects by downregulating MCL-1 expression [21, 53–55]. Indeed, azacitidine has also been shown to reduce MCL-1 expression [56]. Hence, direct targeting of MCL-1 makes logical sense in combination with venetoclax in AML. As proof of concept, a recent study investigated the role of BCL-2 and MCL-1 in AML survival by combining inducible lentiviral vectors expressing BH3-only proteins [38]. Targeting BCL-2 and MCL-1 improved survival in a mouse xenograft model, whereas other combinations including BCL-2/BCL-xL/BCL-w or MCL-1 alone did not. Hence, combining venetoclax with an MCL-1 inhibitor is an exciting prospect for the treatment of patients with AML. Preliminary results from the Phase Ib trial combining cobimetinib and venetoclax showed overall responses of 18% in a heavily pretreated population, with gastrointestinal toxicity being the major adverse toxicity [57]. There are no preliminary results from the combination of the direct MCL-1 inhibitors, S64315 and AMG 176, and venetoclax, to date.

10.3.6 Venetoclax + MDM2 Inhibitors

Idasanutlin is a novel MDM2 inhibitor that is being tested in combination with venetoclax (NCT02670044). MDM2 is a negative regulator of wild-type p53 (WT-p53). In AML, *TP53* mutations occur in only 7–8% of de novo cases, whereas inactivation of WT-p53 occurs in almost all subsets, making disruption of the MDM2 and WT-p53 interaction a promising target [58]. Preclinical models have shown that approximately two thirds of AML cell lines and primary AML blasts respond to MDM2 inhibition, with resistance observed in the *TP53* mutant samples, as expected [59, 60]. The combination of venetoclax and MDM2 inhibitors has exhibited synergistic responses in vitro and in vivo models of AML [55, 61]. Preliminary results from the Phase Ib trial, which combines venetoclax and Idasanutlin, demonstrated a 38% overall response in the higher dose cohort, while no responses were seen in patients with the*TP53* mutation [57].

10.4 Role of MCL-1 in AML

MCL-1 is an antiapoptotic protein that binds to the proapoptotic effectors, BAK and BAX, to prevent cell death. In AML cell lines, MCL-1 has been shown to play an important role in cell survival [62–64]. In the clinical setting, overexpression of MCL-1 in human leukemia cells has been demonstrated in

nearly all bone marrow samples from patients with newly diagnosed AML [65]. This has been implicated in resistance to chemotherapy [66] and BH3-mimetics targeting BCL-2/BCL-xL [67, 68], as well as in the setting of relapsed AML [64]. Hence, targeting MCL-1 represents a promising, novel approach in the treatment of AML.

10.4.1 MCL-Inhibitors

Several Phase I clinical trials of MCL-1 inhibitors in AML are ongoing: AZD5991 [NCT03218683], S64315 [NCT02979366, NCT03672695], AMG 176 [NCT03797261, NCT02675452], and AMG 397 [NCT03465540]. Despite evidence of their efficacy in preclinical studies, the search for a safe, effective, and selective MCL-1 inhibitor has proven formidable for two reasons: (1) MCL-1 plays an important physiologic role in normal cells, including cardiac and hepatic tissues [69, 70], pluripotent stem cells [71], and brain cells [72]. Thus, it has been challenging to create an inhibitor with a sufficiently wide therapeutic index that does not cause unacceptable side effects; (2) The key binding site on MCL-1 is shallow and relatively inflexible compared with the binding sites on BCL-2 and BCL-xL. Thus, early MCL-1 inhibitors lacked specificity and were ineffective. Nonetheless, a number of selective MCL-1 inhibitors have been developed and are currently in various stages of clinical development [62, 63, 73, 74].

AMG 176 is a potent and selective MCL-1 inhibitor that has been shown to induce rapid and robust apoptosis in tumor xenografts after a single dose [75]. Similarly, it resulted in a dose-dependent reduction in tumor burden in an orthotopic model of AML in mice [73]. These data led to the initiation of two Phase I trials. The first trial examined the safety and tolerability of AMG 176 monotherapy in R/R multiple myeloma and AML (NCT02675452), while the second trial studied AMG 176 in combination with venetoclax in patients with R/R AML, non-Hodgkin lymphoma (NHL), or diffuse large B cell lymphoma [NCT03797261].

Similar to AMG 176, AMG 397 is an oral small-molecule inhibitor of MCL-1. It is the only oral MCL-1 inhibitor to reach the clinic thus far [76]. Preclinical data in the literature are sparse; however, clinical evaluation is underway. Unfortunately, the phase I dose-finding clinical studies involving AMG 176 (NCT02675452) and AMG 397 (NCT03465540) in patients with multiple myeloma, NHL, or AML are currently on hold for investigation of AEs related to cardiac toxicity [77]. The dose-finding combination trial of AMG 176 and venetoclax (NCT03797261) is also currently suspended based on this safety signal [78].

Another MCL-1 inhibitor, S64315, is also under clinical evaluation in a Phase I trial in patients with AML or MDS (NCT02979366). This non-randomized, non-comparative study aims to investigate the safety, tolerability, and incidence of dose-limiting toxicities of the drug. The study started in March 2017 and is estimated to finish in October 2020. Another study is also planned to assess S64315 in combination with venetoclax in patients with AML (NCT03672695) [79].

In addition to compounds that cause apoptosis through direct MCL-1 inhibition, there is an array of compounds that cause apoptosis, in part, through a reduction in MCL-1 cellular levels by reducing expression of *MCL1* or by increasing protein degradation. Therefore, in addition to direct MCL-1 inhibition, disruption of key proteins involved in MCL-1 regulation may offer potential therapeutic targets for cancer treatment. Among these indirect MCL-1 inhibitors, CDK9 inhibitors have most recently entered the clinic. CDK9 is an enzyme critical for transcriptional activation of MCL-1. Dinaciclib, a new generation CDK9 inhibitor, has demonstrated efficacy in hematological malignancies [80–82] and is currently being studied in combination with venetoclax in R/R AML in a Phase I trial (NCT03484520).

In summary, therapies targeting MCL-1 could offer a novel treatment approach for patients with disease resistant to other therapies. MCL-1 inhibitors could potentially synergize with other targeted agents or conventional chemotherapeutic agents to enhance their antileukemic efficacy. However, it remains to be seen if this can be achieved without causing unacceptable levels of toxicities to normal tissues.

10.5 Resistance Mechanisms to BCL-2 Inhibitors

Although venetoclax-based regimens have become a powerful addition to the AML treatment armamentarium, drug resistance remains a veritable barrier to maintaining durable responses. Therefore, understanding the mechanisms that lead to BCL-2 resistance is crucial to the development of strategies in overcoming this problem. The following section will highlight the salient mechanisms underpinning resistance to BCL-2 inhibition.

10.5.1 Increased Expression of MCL-1

The best described mechanism of venetoclax resistance is through increased expression of antiapoptotic proteins other than BCL-2, most notably, MCL-1. This has given rise to numerous clinical trials investigating the effect of direct and indirect MCL-1 inhibition on overcoming resistance to BCL-2 inhibition. As direct MCL-1 inhibitors have been discussed previously, this section will elaborate on the role of indirect MCL-1 inhibitors.

Indirect MCL-1 inhibitors comprise a large group of agents with a diverse range of mechanisms. The vast majority of these are still being evaluated in preclinical studies. These include: MEK1/2 inhibitors, which subvert the MAPK pathway that stabilizes MCL-1. These have been shown to synergistically enhance the proapoptotic effects of venetoclax in AML cell lines and reduce leukemia burden in AML xenograft models through increased levels of BIM [83]. Similarly, bromodomain extra-terminal protein inhibitors (BETi) reduce MCL-1 and BCL-xL levels while increasing BIM levels. They also synergize with venetoclax to induce apoptosis in AML cell lines, reduce leukemia burden, and improve survival in AML-engrafted mice [84]. Other indirect MCL-1 inhibitors include: CDK9 inhibitors, which impair the transcription of *MCL-1* [85]; FLT3 inhibitors, which downregulate MCL-1 to increase venetoclax activity [48]; CUDC-907, a dual PI3K and histone deacetylase inhibitor that downregulates MCL-1 while upregulating BIM to cause apoptosis [86]; MDM2 inhibitors, which restore TP53 activation and downregulation of MCL-1 through inhibition of the MAPK pathway [87]; PI3K inhibitors, which induce BAX-dependent mitochondrial apoptosis in AML cells when coadministered with venetoclax [88]; selinexor, an XPO1-selective inhibitor [89]; inhibitors of the Nedd8-activating enzyme and 3-hydroxy-3-methylglutaryl coenzyme A reductase, which lead to upregulation of NOXA and PUMA, respectively, resulting in neutralization of MCL-1 and increased activity of venetoclax [21, 90]; ibrutinib, a Bruton tyrosine kinase inhibitor, and ArQule 531, a multi-kinase inhibitor of SRC family kinases, have also been shown to synergize with venetoclax and overcome BCL-2 inhibition through MCL-1 inhibition [91, 92].

10.5.2 Dysregulation of Mitochondrial Energy Metabolism

One mechanism by which venetoclax kills AML cells is through inhibition of mitochondrial respiration. Thus, disruption of mitochondrial energy metabolism is implicated in the resistance of AML to venetoclax. Using a genome-wide CRISPR knockout screen, Sharon et al. [93] found that inactivation of genes involved in mitochondrial protein synthesis restored sensitivity of resistant AML cells to venetoclax. Pharmacologic inhibition of mitochondrial protein synthesis with antibiotics that target the ribosome, including tedizolid and doxycycline, can enhance the anti-AML effect of venetoclax and azacitidine in vivo and in vitro, thus potently reversing venetoclax resistance [93, 94]. In leukemic stem cells, mutated *TP53* disrupted mitochondrial homeostasis by dysregulating activation of transcription factor, DP-1, and translocation of PMAIP1 into the mitochondria, hence impairing the effector function of BAX and BAK [95]. Moreover, *TP53* mutation also impedes BCL-2 expression, thus directly decreasing the target of venetoclax and leading to drug resistance [95].

10.5.3 Disruption of Mitochondrial Architecture

The mitochondrial architecture plays an important role in apoptosis. Its organization and function are maintained by various proteins, including the mitochondrial chaperone, CLPB, whose function is to maintain mitochondrial cristae structure through interaction with the cristae-shaping protein, OPA1 [96]. When this interaction is disrupted, the structural integrity of the mitochondria is damaged, leading to stress responses and induction of apoptosis. Preclinical studies by Chen et al. demonstrated that CLPB is upregulated in human AML cells and its expression is induced upon acquisition of venetoclax resistance. Using a genome-wide CRISPR screen, they found that inactivation of this gene sensitized AML cells to venetoclax, and thus to apoptosis [96]. Thus, targeting the mitochondrial structure represents another novel approach of disarming venetoclax resistance.

10.6 Conclusion

Upregulation of antiapoptotic proteins in the BCL-2 family as a means of evading apoptosis is a key mechanism of treatment resistance and disease relapse in AML. Therefore, targeted inhibition of these proteins, especially BCL-2 and MCL-1, represents a compelling therapeutic approach in the management of AML. While this strategy has shown promising antileukemic activity in preclinical studies, only venetoclax has demonstrated efficacy in the clinical setting and is approved for use in AML. As confirmed by two recent Phase III trials, venetoclax in combination with a HMA or LDAC improves OS in treatment-naïve elderly patients, or patients unfit for intensive chemotherapy. Unfortunately, MCL-1 inhibitors have not yet generated the same success in clinical trials, in part due to their on-target but off-tissue toxicities.

While BCL-2 inhibition with venetoclax has revolutionized the therapeutic landscape in a cohort of AML patients who would otherwise have limited treatment options, there are a number of obstacles that remain. Overcoming resistance to BCL-2 inhibitors will be crucial in prolonging responses, and therefore, long-term survival. Although the mechanisms of resistance are being characterized in preclinical studies, the primary mechanisms of resistance in vivo remain unclear. Current research is focusing on combination strategies and appear promising. However, determining which drug combinations will provide optimal clinical efficacy and in which clinical setting will be an important goal moving forward.

The role of venetoclax and other BH3 mimetics in standard induction and consolidation therapy in younger patients or the fit elderly is also of great interest. The use of venetoclax combination therapies could replace "3 + 7" as the new standard induction regimen for all AML patients. Furthermore, there are ongoing clinical trials looking at the use of venetoclax and HMAs as maintenance therapy after consolidation chemotherapy or allogeneic stem cell transplant (Table 10.1). Determining the subgroups of patients who are most likely to benefit from BH3 mimetics is also of vital pertinence and requires further investigation.

In summary, it is currently an exciting time to be treating AML, especially with the approval of venetoclax, which represents a promising and significant advance in targeted treatment approaches in AML. Targeting other antiapoptotic proteins, such as MCL-1 and BCL-xL, in combination with venetoclax, is also an exciting prospect, and if successful, will be key in overcoming resistance to BCL-2 inhibition.

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Role of IDH1/IDH2 Inhibitors in AML

11

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Abstract

Mitochondrial activity is fundamental to supporting cellular metabolisms of almost all types of body cells. This carries paramount significance for the treatment of AML due to the presence of mitochondrial abnormalities, which can be exploited for selective AML cells targeting. In addition, other aberrant metabolic pathways discovered in LSCs are also being explored as targets for LSC eradication. In this chapter, we discuss the roles of IDH1 and IDH2 inhibitors in targeting the metabolic pathways in AML.

Keywords

Isocitrate dehydrogenase 1 · Isocitrate dehydrogenase 2
Acute myeloid leukaemia

11.1 IDH Inhibitors

Isocitrate dehydrogenase (IDH) 1 and 2 are ubiquitously expressed metabolic enzymes predominantly located in the cytoplasm and mitochondria, respectively. They mediate the tricarboxylic acid (TCA) by catalysing the conversion of isocitrate to α-ketoglutarate (α-KG) [1, 2]. In the process, NADPH, a crucial reducing agent with protective functions against oxidative damage, is also generated [2]. In AML, *IDH1* mutations occur at the R132 codon, while *IDH2* mutations occur in either the R172 or R140 codon [2]. Together, these mutations are found in 10–20% of patients [3, 4]. Mutant IDH1 and 2 are neomorphic enzymes which convert α-KG into 2-hydroxyglutarate (2-HG) with the consumption of NADPH as a co-factor [2]. The oncometabolite 2-HG inhibit enzymes for the regulation of DNA epigenetic status, such as histone and DNA demethylase, resulting in DNA hypermethylation, defective cellular differentiation, and subsequent leukaemogenesis [2]. IDH inhibitors exert anti-leukaemic activity via inhibition of mutant IDH1/2 and induction of leukaemic cell differentiation. Therefore, IDH differentiation syndrome is a distinct and significant adverse effect of these agents. Other major side effects include cytopenias, leucocytosis, QT prolongation, pneumonia, and gastrointestinal disturbances [5–15].

11.2 IDH1 Inhibitors

Ivosidenib (AG-120) is an orally available inhibitor of mutant IDH1. In 2019, it was approved by the FDA for the treatment of r/r AML with IDH1 mutation. It exhibited impressive activity against IDH1-mutant AML with tolerable side effects in a phase I trial among r/r AML patients with mutant *IDH1* [5]. In another trial among newly diagnosed AML patients, ivosidenib monotherapy was safe and effectively induced prolonged remissions [7]. Its combination with azacitidine also showed impressive responses among patients [11]. Subsequent clinical trials for ivosidenib as monotherapy or in combination with chemotherapy or other agents, such as hypomethylating agents, in newly diagnosed and r/r AML patients are ongoing (NCT04176393, NCT03839771. NCT04250051, NCT04493164, NCT04774393, NCT02074839, NCT03471260). It will also be compared with placebo in combination with azacitidine in a phase III trial (NCT03173248).

Olutasidenib (FT-2102) is another potent inhibitor of mutant IDH1 which demonstrated impressive clinical efficacy and safety in r/r IDH1-mutant AML, both as monotherapy and in combination with azacitidine [12, 13]. It is currently evaluated in combination with cytarabine or azacitidine in another study (NCT02719574). LY3410738 is a

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covalent inhibitor IDH1-R132 mutations which showed superior efficacy in IDH1-mutant AML compared to ivosidenib in murine xenograft models [15]. A trial of its use in r/r IDH1-mutant advanced haematological malignancies is ongoing [14]. Another potent and selective mutant IDH1 inhibitor with central nervous system (CNS) penetrating properties, IDH305, yielded promising results in preclinical and phase I clinical studies and will be further evaluated in subsequent clinical trials (NCT02381886) [16, 17].

BAY1436032, a pan-mutant-IDH1 inhibitor, showed promising efficacy and synergistic activity with azacitidine in preclinical studies. Disappointingly, a subsequent phase I trial failed to replicate these results and this agent was deemed not worthy of further clinical investigations in AML [18–20].

11.3 IDH2 Inhibitors

Enasidenib (AG-221) is an orally available specific inhibitor of mutant IDH2 which received FDA approval for the treatment of r/r IDH2-mutant AML in 2017. In a phase I/II clinical trial, it demonstrated clinical efficacy in IDH2-mutant AML patients by inducing differentiation of AML blasts, along with a favourable safety profile [8, 9]. Subsequent reports of enasidenib monotherapy in newly diagnosed older AML patients also showed prolonged clinical responses and tolerable adverse effects [6]. Combination therapy with azacitidine was also clinically effective [10]. Multiple trials regarding its use as monotherapy, in combination with other agents, or as post-HSCT maintenance therapy in r/r and newly diagnosed IDH2-mutant AML patients are underway (NCT04203316, NCT03825796, NCT03839771, NCT03720366, NCT03728335, NCT03683433, NCT02577406, NCT04774393, NCT03383575, NCT04092179, NCT03515512, NCT04522895).

11.4 IDH1/2 Inhibitors

Vorasidenib (AG-881), a dual inhibitor of mutant IDH1 and 2, is currently evaluated in a phase I trial among patients with advanced *IDH1/2*-mutant haematological malignancies (NCT02492737) [21].

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Next-Generation FLT3 Inhibitors for the Treatment of FLT3-Positive AML

12

Harinder Gill

Abstract

Tyrosine kinases regulate a wide range of cellular pathways and are crucial to signal transduction. Their aberrant activities can contribute to leukaemogenesis via promoting proliferation, impeding differentiation, and inhibiting apoptosis. In this chapter, we discuss the role of FLT3 inhibition in acute myeloid leukemia (AML) and the use of FLT3 inhibitors in AML.

Keywords

Acute myeloid leukemia · Fms-like tyrosine kinase 3 Targeted therapy

12.1 Introduction

Fms-like tyrosine kinase 3 (FLT3) is a class III receptor tyrosine kinase (RTK) which serves as a transmembrane cytokine receptor on the surface of early haematopoietic progenitors. It contributes to normal haematopoiesis by promoting survival and proliferation of haematopoietic cells. Activation of FLT3 is mediated by the binding of FLT3 ligand (FL), which induces FLT3 dimerization and activation [1–3]. FLT3 mutations are among the most common genetic mutations in AML, occurring in 30% of patients. FLT3 mutations can be divided into two types. FLT3 internal tandem duplication (ITD), which accounts for 75% of FLT3 mutations, occurs at the juxtamembrane domain of FLT3 and impairs its auto-inhibitory function. On the other hand, mutations in the tyrosine kinase domain (TKD) are less common, comprising the remaining 25% of FLT3 mutations. Both aberrations induce the constitutive activation of FLT3 receptor. Although downstream signalling events differ, both types of mutation involve aberrant signalling of the PI3K/AKT, STAT5, and RAS/RAF/MEK/ERK pathways, resulting in uncontrolled proliferation, reduced differentiation, and impaired apoptosis of leukaemic cells (Fig. 12.1) [4]. Unfortunately, the presence of FLT3 mutations in AML confers inferior prognosis and aggressive disease phenotype [2, 3, 5, 6]. The remarkable prevalence and inferior outcome of FLT3-mutant AML necessitate the development of novel therapeutic agents against this entity. Multiple FLT3 inhibitors have been developed and clinically tested in AML patients. There are two major ways of classifying these agents. The first divides FLT3 inhibitors into first and second generation according to their specificity towards FLT3. This will be covered more in detail subsequently. The second classification is based on their mechanisms of FLT3 inhibition. Type I inhibitors directly bind to the ATP-binding site of FLT3 receptor, while type II inhibitors bind to a specific site in the activation loop of the TKD during its inactive conformation. Ultimately, both types of receptors exert anti-leukaemic effects by inhibiting the binding of ATP to the TKD of FLT3 receptor, which halts downstream signalling pathways and leukaemogenesis [7, 8] (Fig. 12.2). Moreover, FLT3-TKD mutations generally confer resistance towards type II FLT3 inhibitors. This is because mutations occurring in the activation loop of the TKD, most commonly at the D835 residue, result in failure of maintaining the inactive conformation of FLT3 for their binding [2, 9]. However, certain point mutations in the TKD of FLT3, such as those involving the F691 residue, can cause resistance against both type I and type II inhibitors [2]. The characteristics of major novel FLT3 inhibitors are summarized in Table 12.1.

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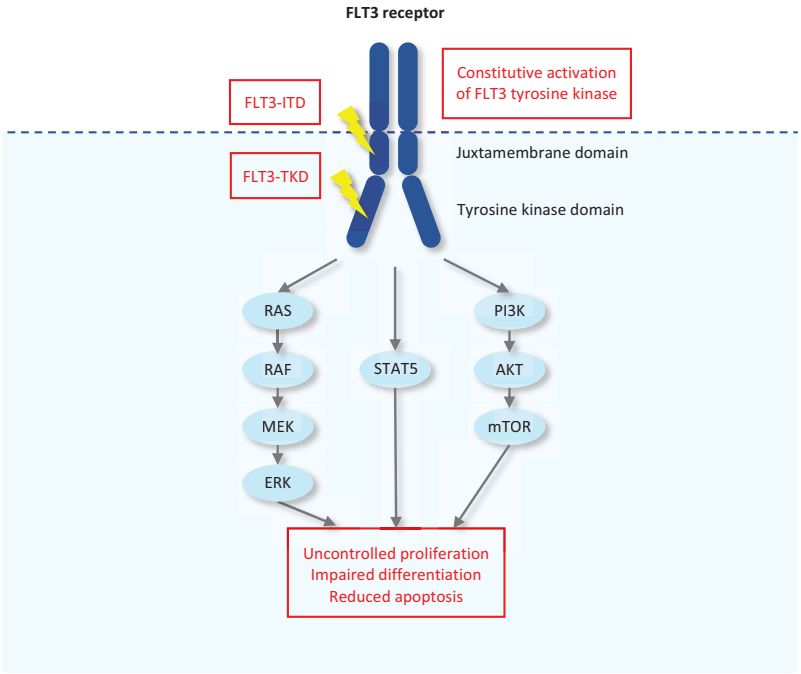

Fig. 12.1 Downstream events of aberrant FLT3 signalling. *AKT* protein kinase B, *ERK* extracellular-signal-regulated kinase, *FLT3-ITD* FLT3 internal tandem duplication, *FLT3-TKD* FLT3 tyrosine kinase domain mutations, *FLT3* Fms-like tyrosine kinase 3, *MEK* mitogen-activated protein kinase kinase, *mTOR* mammalian target of rapamycin complex, *PI3K* phosphoinositide 3-kinase, *RAF* rapidly accelerated fibrosarcoma, *Ras* rat sarcoma viral oncogene homolog, *STAT5* signal transducer and activator of transcription 5

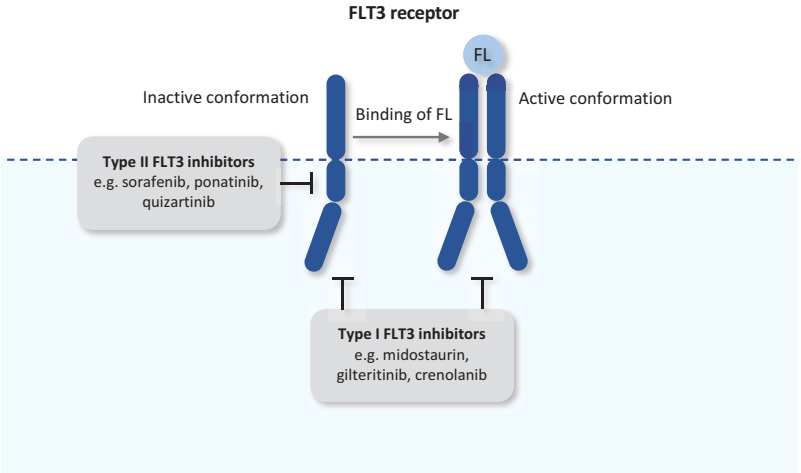

Fig. 12.2 Actions of type I and type II FLT3 inhibitors. *FL* FLT3 ligand, *FLT3* Fms-like tyrosine kinase 3

Table 12.1 Summary of major FLT3 inhibitors [11]

Agent	Stage of development in AML	Generation	Type	Off-target activity	Side effects
Sorafenib	Phase III	First	II	RAF, PDGFR, VEGFR, c-KIT, RET	Dermatological reactions (e.g. hand-foot-skin reaction, skin rash, mucositis), bleeding, cardiac events, febrile neutropenia, GI disturbance
Midostaurin	FDA-approved	First	I	PKC, SYK, SRC, c-KIT, VEGFR, PDGFR, AKT	Pulmonary toxicity (e.g. drug-induced pneumonitis), febrile neutropenia, QT prolongation, edema, bruising, GI disturbance
Sunitinib	Phase II	First	I	VEGFR, PDGFR, c-KIT	Dermatological reactions (e.g. hand-foot-skin reactions, erythema multiforme), myelosuppression, GI disturbances
Ponatinib	Phase II	First	II	RET, c-KIT, FGFR, PDGFR, BCR-ABL	Cardiovascular ischemic events, myelosuppression, febrile neutropenia, hepatotoxicity, skin rash, GI disturbances

Table 12.1 (continued)

Agent	Stage of development in AML	Generation	Type	Off-target activity	Side effects
Gilteritinib	FDA-approved	Second	I	AXL, ALK, LTK	Febrile neutropenia, liver toxicity, GI disturbance, fatigue
Quizartinib	Phase III	Second	II	c-KIT, RET, PDGFR, CSF1	Nausea, febrile neutropenia, sepsis or septic shock, QT prolongation
Crenolanib	Phase II	Second	I	PDGFR, c-KIT	Skin rash, GI disturbance, febrile neutropenia, elevation of transaminases

AKT protein kinase B, *ALK* anaplastic lymphoma kinase, *AXL* AXL receptor tyrosine kinase, *BCR-ABL1* breakpoint cluster region-Abelson murine leukaemia viral oncogene homolog 1, *c-KIT* tyrosine-protein kinase KIT, *CSF1* colony stimulating factor 1, *FDA* U.S. Food and Drug Administration, *FGFR* fibroblast growth factor receptor, *FLT3* Fms-like tyrosine kinase 3, *GI* gastrointestinal, *LTK* leucocyte tyrosine kinase receptor, *PDGFR* platelet-derived growth factor receptor, *PKC* protein kinase C, *RAF* rapidly accelerated fibrosarcoma, *RET* rearranged during transfection, *SRC* proto-oncogene tyrosine-protein kinase SRC, *SYK* tyrosine-protein kinase SYK, *VEGFR* vascular endothelial growth factor receptor

12.2 First-Generation FLT3 Inhibitors

First-generation FLT3 inhibitors are multi-targeted tyrosine kinase inhibitors which are often developed initially to target other kinases. Examples include sorafenib, midostaurin, sunitinib, and ponatinib [6]. Among them, midostaurin was approved by the U.S Food and Drug Administration (FDA) in 2017 for the treatment of newly diagnosed FLT3-mutant AML in combination with chemotherapy [10]. Owing to their non-specific actions and off-target actions against other tyrosine kinases, they typically carry higher risks of toxicity and undesirable side effects [2, 5, 6]. Nevertheless, some of them are still clinically relevant and widely used against FLT3-mutant AML.

Sorafenib

Sorafenib (DB00398) is one of the first pan-kinase inhibitors to be employed in clinical use and has received FDA approval in treating a number of solid organ malignancies, including renal cell carcinoma, hepatocellular carcinoma, and differentiated thyroid carcinoma [2, 5]. In addition to being a type II FLT3 inhibitor, it also possesses broad-spectrum activity against RAF, vascular endothelial growth factor receptors 1, 2, 3 (VEGFR), platelet-derived growth factor receptor β (PDGFRβ), c-KIT, and RET [11, 12]. Sorafenib inhibits FLT3-ITD intrinsically and is also metabolized by CYP3A4 into a more potent active metabolite, sorafenib N-oxide [13, 14]. Therefore, the concomitant use of CYP3A4 inhibitors reduces its efficacy and requires dose adjustment. Sorafenib is orally available and given at a dose of 200–400 mg twice daily [13]. Its broad-spectrum activity entails higher risks of adverse effects, including fever, dermatological reactions (hand-foot-skin reaction, skin rash, mucositis), gastrointestinal disturbances, bleeding, and cardiac events [15, 16]. The use of sorafenib in AML has been extensively studied in multiple phase I and II clinical trials with mixed results. Several phase I and II trials reported impressive complete remission rates and fair tolerability in paediatric and adult patients with newly diagnosed or r/r disease, both as a monotherapy and in combination with other agents [14, 16–22]. However, the single agent activity of sorafenib only induced minimal responses in a phase I trial and provided no benefit in treating relapse after HSCT [13, 23]. In the phase II randomized controlled SORAML trial, addition of sorafenib to conventional induction chemotherapy with cytarabine and daunorubicin (7 + 3) was found to improve clinical responses, but provided doubtful long-term survival benefits [15, 24]. Since early trials suggested the potential role of sorafenib as maintenance therapy after HSCT [19], the multi-center randomized controlled Sormain trial was subsequently carried out, which confirmed the decrease in risk of relapse and mortality with sorafenib maintenance [25, 26]. Several trials regarding the use of sorafenib in treating newly diagnosed or r/r AML either as a single agent or in combination with various agents, as well as its use as post-HSCT maintenance, are ongoing (NCT 02156297, NCT04752527, NCT01253070, NCT03132454, NCT02728050, NCT01578109, NCT03247088).

Midostaurin

Midostaurin (PKC412) is a staurosporine analogue which acts as a type I FLT3 inhibitor. Apart from FLT3, its therapeutic targets include PKC, SYK, SRC, c-KIT, VEGFR, PDGFR, and AKT. Important side effects of midostaurin include hand-foot-skin reaction, skin rash, mucositis, bleeding, cardiac events, febrile neutropenia, and gastrointestinal disturbances. Dose adjustment should be performed upon administration of CYP3A4 inhibiting agents due to the metabolism of midostaurin by CYP3A4. Although midostaurin is an FDA-approved FLT3 inhibitor, previous clinical trials regarding its use in AML had mixed results. In two phase II studies of midostaurin as monotherapy in r/r AML patients, varying degrees of haematological responses were observed but no complete remissions (CRs) were achieved

[27, 28]. However, the combination of midostaurin with either azacitidine or chemotherapy regimens (7 + 3; cladribine, cytarabine and granulocyte colony stimulating factor (G-CSF) (CLAG); bortezomib with mitoxantrone, etoposide and cytarabine (MEC)) was deemed effective and generally tolerable in subsequent trials [29–33]. In the multi-center randomized placebo-controlled phase III RATIFY trial, addition of midostaurin to 7 + 3 provided significant survival benefits and was tolerable, except for the increased dermatological toxicities and nausea [34]. The addition of midostaurin to 7 + 3 and its use as single agent maintenance therapy following HSCT were also proven to be effective and safe in the phase II AMLSG 16–10 trial [35]. Midostaurin will undergo further investigations as monotherapy and in combination with chemotherapy or other agents, such as decitabine (NCT04097470), gemtuzumab ozogamicin (GO) (NCT04385290, NCT03900949), and siremadlin (HDM201) (NCT04496999), among others (NCT03512197, NCT03280030, NCT03591510, NCT03379727, NCT00651261, NCT03686345, NCT00819546, NCT03092674). It will also be compared with crenolanib as post-consolidation maintenance therapy and with gilteritinib as adjuncts to standard induction and consolidation chemotherapy or post-consolidation maintenance therapy (NCT03258931, NCT03836209, NCT04027309).

Sunitinib

Sunitinib (SU11248) is another multi-kinase inhibitor approved for the treatment of multiple solid organ malignancies, including renal cell carcinoma, gastrointestinal stromal tumour, and well-differentiated pancreatic neuroendocrine tumour [36]. It acts primarily against VEGFR, PDGFR, and c-KIT and is a potent type I FLT3 inhibitor [37]. Significant side effects include myelosuppression, GI disturbances, and dermatological reactions such as hand-foot-skin reactions and erythema multiforme [38]. In preclinical studies, sunitinib demonstrated impressive anti-tumour efficacy in murine models [37]. Its activity against FLT3 in AML was elucidated in subsequent phase I clinical trials, where inhibition of FLT3 autophosphorylation was achieved in both FLT3-mutant and FLT3-wild-type (WT) patients, with the former achieving faster responses and higher remission rates [39–41]. In another phase I/II trial, combination of sunitinib and chemotherapy was shown to be effective in inducing remissions, but incidences of dose limiting toxicities were reported [38]. Despite these promising results, no phase III trials are currently planned for this agent.

Ponatinib and Related Compounds

Ponatinib (AP23534) is a broad-spectrum tyrosine kinase inhibitor with efficacy against FLT3, RET, c-KIT, FGFR, and PDGFR, BCR-ABL. Due to its ability to target abnormal BCR-ABL protein, it has been FDA-approved for the treatment of chronic myeloid leukaemia (CML) and Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukaemia [42]. Despite being a type II FLT3 inhibitor, ponatinib is active against the F691 FLT3-TKD mutation and may be useful in multi-drug-resistant AML patients [43]. However, the high risk of cardiovascular ischemic events associated with ponatinib therapy is a substantial concern [44, 45]. Other important side effects include myelosuppression, febrile neutropenia, gastrointestinal disturbances, hepatotoxicity, and skin rash [44, 45]. Dose reduction of ponatinib is required during concomitant administration of CYP3A4 inhibitors [46]. Preclinical studies and phase I trials on the use of ponatinib in AML showed promising results, though the cardiovascular risks of ponatinib in AML patients were still alarming [43, 47, 48]. Further clinical trials evaluating its role as monotherapy or in combination with other agents, such as venetoclax and decitabine (NCT04188405), in paediatric and adult AML patients, as well as in post-HSCT maintenance therapy, are underway (NCT03934372, NCT02428543, NCT03690115). HSN748, an analogue of ponatinib synthesized by replacing its benzamide moiety with nicotinamide, showed improved potency against FLT3-ITD and F691 mutations while reducing off-target kinase inhibitions in a preclinical study [49]. This suggests a potentially improved safety profile compared to ponatinib, which warrants confirmation in subsequent trials.

Other First-Generation FLT3 Inhibitors

Lestaurtinib (CEP-701) is broad-spectrum kinase inhibitor and type I FLT3 inhibitor. Despite its potent activity against FLT3 in preclinical studies, its clinical development has been discontinued due to the lack of positive results in subsequent clinical trials [50, 51]. Tandutinib, a type II FLT3 inhibitor, is also no longer in development in spite of the positive results from early studies [52].

12.3 Second/Next-Generation FLT3 Inhibitors

In contrast to first-generation FLT3 inhibitors, second- or next-generation FLT3 inhibitors are developed to specifically target FLT3. Compared to their predecessors, they are superior in potency and effectiveness and have improved toxicity profiles [2, 5, 6]. Examples of agents include gilteritinib, quizartinib, and crenolanib, with gilteritinib being an FDA-approved agent for the treatment of r/r AML with FLT3 mutations.

Gilteritinib

Gilteritinib (ASP2215) is an FDA-approved second-generation type I FLT3 inhibitor. It is highly specific for

FLT3 and has off-target activity only against AXL, ALK, and LTK. Significant adverse effects include febrile neutropenia, anaemia, thrombocytopenia, liver toxicity, GI disturbance, and fatigue [53–56]. Gilteritinib monotherapy demonstrated remarkable response rates, prolonged FLT3 inhibition, and a favourable safety profile in a number of early phase trials [53, 54]. This prompted the multi-centre randomized phase III ADMIRAL trial, where gilteritinib was shown to be superior in efficacy and tolerability compared to salvage chemotherapy [55, 56]. Ongoing trials will further study gilteritinib as single agent maintenance therapy, and in combination with other agents such as azacitidine, venetoclax and immune checkpoint inhibitors (NCT02927262, NCT02997202, NCT02310321, NCT02236013, NCT04240002. NCT03625505, NCT03730012). Trials comparing gilteritinib with salvage chemotherapy or midostaurin are also underway (NCT02421939, NCT03182244, NCT03836209, NCT04027309, NCT02752035, NCT04140487).

Quizartinib

Quizartinib (AC220) is a potent type II FLT3 inhibitor [2]. It is highly specific towards FLT3-ITD, with off-target activity primarily against PDGFR, c-KIT, RET, and CSF1R, all with at least 10-fold lower potency [57]. With a long duration of action at a low dosage and high oral availability, quizartinib has a compelling pharmacokinetic profile [57, 58]. Major side effects of quizartinib include QT interval prolongation, febrile neutropenia, thrombocytopenia, infections, and nausea [59]. The only major drug-drug interaction occurs with strong CYP3A4 inhibitors, where dose reduction of quizartinib is required [60, 61]. A number of phase II clinical trials with quizartinib illustrated its remarkable activity and tolerability for treating FLT3-ITD-positive AML in newly diagnosed and r/r patients, both as a single agent and in combination with azacitidine and various chemotherapeutic agents [62–69]. Interestingly, quizartinib also induced remissions in patients without FLT3-ITD, though with a lower response rate [62–65]. In a subsequent multi-center randomized controlled phase III trial (QuANTUM-R), quizartinib demonstrated superiority against salvage chemotherapy in terms of survival benefits. An expecting increase in risk of QT prolongation was seen in the quizartinib arm, but adverse events were generally tolerable [59]. More clinical trials evaluating the efficacy of quizartinib as monotherapy and in combination with chemotherapy or other agents, such as venetoclax, in newly diagnosed or r/r patients are currently underway (NCT02668653, NCT02984995, NCT03135054, NCT03723681, NCT03793478, NCT03989713, NCT04676243, NCT04107727, NCT03552029, NCT02668653, NCT04112589, NCT03735875, NCT03661307, NCT04128748, NCT04209725, NCT04047641, NCT01892371, NCT04687761).

Crenolanib

Crenolanib (CP-868-596) is another second-generation FLT3 inhibitor. Being a type I inhibitor, it retains efficacy against FLT3-TKD mutations in addition to FLT3-ITD [70]. Although less potent than quizartinib, crenolanib is superior in specificity, with off-target activity primarily against PDGFR and much lower potency against c-KIT [70, 71]. Major side effects associated with crenolanib use include skin rash, nausea, vomiting, diarrhoea, febrile neutropenia, and elevation of transaminases [72–74]. Only minimal QT prolongation and low degree myelosuppression are seen due to the reduced activity of crenolanib towards c-KIT [70, 72]. The safety and efficacy of crenolanib has been evaluated in multiple phase I and II studies. In summary, crenolanib showed efficacy against both FLT3-ITD and FLT3-TKD-positive AML in newly diagnosed and r/r patients, either as a monotherapy or in combination with chemotherapy. It also had an exceptional safety profile, causing only mild side effects in most patients [72–77]. Although no results from any phase III trials are available at the moment, multiple phase II and III clinical trials regarding the use of crenolanib in FLT3-mutant AML are underway, including comparison with midostaurin and with salvage chemotherapy, as well as its use as maintenance therapy after HSCT (NCT03258931, NCT03250338, NCT02400255).

12.4 Other Novel FLT3 Inhibitors

A674563, an orally available dual inhibitor of AKT and FLT3-ITD, has been shown to selectively promote apoptosis and cell cycle arrest in AML cells with FLT3-ITD in a preclinical study. It may also have a role in overcoming FL-mediated drug resistance in FLT3-positive AML [78]. In another preclinical study, FF-10101, a potent and irreversible FLT3 inhibitor, showed selective inhibition of leukaemic cells harbouring FLT3 mutations, including both FL3-ITD and FLT3-TKD mutations [79]. In addition, LT-171-861 demonstrated remarkable anti-leukaemic activity against FLT3-ITD and FLT3-TKD mutations in both in vitro and in vivo models [80]. A derivative of oxazol-2-amine, known as compound 7, also exhibited anti-leukaemic efficacy against both types of FLT3 mutations in murine models and showed synergism with Olaparib [81]. Similarly, HM43239 exhibited potent activity against FLT3-ITD and FLT3-TKD in murine models [82]. Two other novel FLT3 inhibitors, G-749 and MZH29, showed potent activity against AML cell lines carrying FLT3 mutations, including ones that commonly confer drug resistance to other FLT3 inhibitors [83, 84].

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Allogeneic Hematopoietic Stem Cell Transplantation for AML

13

Yu-Qian Sun and Xiao-Jun Huang

Abstract

Acute myeloid leukemia (AML) is the main indication of allogeneic hematopoietic stem cell transplantation (allo-HSCT). Even with the great advances of targeted drugs in last decades, allo-HSCT is still the most powerful curative method. The major advances in this field include several aspects. First, the accurate diagnosis and the dynamic minimal residual disease (MRD) monitoring lead to more precise risk stratification like who will benefit from allo-HSCT and non-transplantation, therefore redefined the allo-HSCT indication. Second, the great advance in allo-HSCT has further expanded the transplant-eligible population, including less toxic conditioning regimen, better prophylaxis of GVHD, and infection. Third, the advances in alternative donor transplantation have led to an era of "everyone has donor", and even challenged the classical rule "matched sibling donor is always preferred over alternative donor".

Keywords

Acute myeloid leukemia · Minimal residual disease
Allogeneic stem cell transplantation · Haploidentical donor

13.1 Introduction

Acute myeloid leukemia (AML) is one of the most common hematological malignancies and mainly developed in old age (median age at diagnosis of 70 years) [1, 2]. AML progressed rapidly, and the prognosis is very poor if without therapy. Before the 1960s, AML was regarded as an incurable disorder. In the last 6 decades, the prognosis of AML has improved significantly with the great advance in pathogenesis understanding, the supportive therapy, the development of chemotherapy, and allogeneic stem cell transplantation [2].

Generally, the treatment of AML includes remission-induction therapy and post-remission therapy. Achieving long-term complete remission is important when treating AML. Complete remission (CR) rates with induction therapy in AML patients <60 years and >60 years were around 60–85% and 40–60%, respectively [2]. The approval of several new drugs and the use of targeted drugs based on genomic analysis (such as FLT3-ITD mutation) have further improved the CR rates [2]. Post-remission therapy is necessary since most patients will relapse if without further treatment. The available options include consolidation chemotherapy, autologous stem cell transplantation, and allogeneic stem cell transplantation. Although the approval of novel drugs has significantly improved the survival of AML, allo-SCT is still the most curative method.

In the following paragraph of this chapter, we will focus on the allo-SCT for AML. Furthermore, considering APL is a very special subtype including treatment and prognosis, and rare cases will need allo-SCT, this chapter will not include the APL.

13.2 The Indication of Allogeneic Stem Cell Transplantation in AML

Patients receiving Intensive consolidation therapy have risk of TRM less than 5%, but relapse rate around 50–90%. The mechanism of allo-SCT includes eradication of disease by high dose of chemotherapy/irradiation, and more importantly, graft versus leukemia effect. Allo-SCT provides the strongest antileukemia effect (relapse rate 10–30% in AML-CR1), however with risk of transplant-related mortality (TRM) around 10–30% [2, 3]. Therefore, the benefit of GVL effect will be partly offset by significant higher rate of TRM. The decision of allo-SCT was based on the balance of disease risk and the TRM of HSCT. A risk-adapted treatment

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approach is generally adopted and depends on the balance of risk of relapse of the underlying disease and the risk of treatment-related mortality. Patients with an increased risk of relapse in the absence of allo-SCT are usually recommended for allo-SCT in CR1, while patients with very high risk of TRM might not benefit from allo-SCT.

13.2.1 Risk Stratification of AML

The outcome of acute myeloid leukemia is heterogeneous. Therefore, accurate prognostication of acute myeloid leukemia is important. The risk of relapse might be influenced by characteristics at diagnosis (pretreatment factors) and dynamic MRD during treatment (posttreatment factors).

13.2.1.1 Characteristics at Diagnosis (Pretreatment Factors)

Genetic analyses, including both karyotyping and screening for recurrent gene fusions and molecular mutations, provide important information about disease biology and strongly inform prognostic assessment. AML patients at diagnosis were categorized as favorable risk group, intermediate risk group, and adverse risk group according to the ELN guidelines [4]. The favorable risk group includes patients with recurrent t(8;21) or inv(16), presence of biallelic mutations of CEBPA, NPM1 mutation without FLT3 or FLT3 low; the adverse risk group includes patients with complex karyotype (defined as three or more cytogenetic abnormalities) or specific chromosomal aneuploidies (e.g., −5/−5q, −7, and −17/17p) and mutations of RUNX1, ASXL1, or TP53. The others were categorized as intermediate risk group. It is to be noted that the risk categories established by the consensus guidelines usually evolve with emerging therapies, therefore requiring continued reassessment.

Besides the variables included in ELN stratification, some other variables also have impact on the risk of relapse, such as WBC count at diagnosis, extramedullar involvements, the level of other molecular biomarker, the type of gene mutation, etc. [5]. It was reported that high aldehyde dehydrogenase activity at diagnosis predicts relapse in patients with t(8;21) acute myeloid leukemia [6]. Also, high EVI1 expression predicts poor outcomes in adult AML patients with intermediate risk receiving chemotherapy [7]. Ecotropic viral integration site 1 (EVI1) transcripts ≥1.0% at diagnosis had no effect on CR achievement, whereas it was significantly associated with lower 2-year relapse-free survival (RFS), disease-free survival (DFS), and overall survival (OS) rates (p = 0.0003, 0.0017, and 0.0009, respectively). Kong et al. reported that the initial MLL-partial tandem duplication (PTD) expression levels ≥1.0% (high-level group) at diagnosis significantly differed CR rates after the first (78.9% vs. 35.3%, p = 0.008) and second chemotherapies (84.2% vs. 47.1%, p = 0.001). The 24-month overall survival was significantly lower in the high-level group (52.6% vs. 29.4%, p = 0.043) [8].

13.2.1.2 MRD-Based Risk Stratification (Posttreatment Factors)

The depth of response is another important factor associated with relapse risk [9]. The morphologic response can be useful in predicting relapse, for example, CRi are at higher risk of relapse than CR. However, the morphologic evaluation is not sensitive enough. For those achieved CR, measurable or minimal residual disease (MRD) evaluated by multiparametric flow cytometry or quantitative PCR could predict relapse of underlying disease more sensitively [9]. Fusion genes such as RUNX1/RUNXT1, CBFB/MYH11, and NPM1 could be good candidates for MRD detection. For those without specific molecular marker, it is reported that the overexpression of WT1 is useful for MRD monitoring [10]. MFC detection of LAIP could also be used as a predictor for relapse.

It seems that dynamic MRD monitoring might be more important than the characteristics at diagnosis in some patients. For patients with CBF-AML, investigators from Peking University suggest that if patients haven't achieved major molecular remission (defined as 3 log reduction of RUNX1/RUNXT1), these patients might be at high risk of relapse (relapse rate 78.9%) [11]. It is also applicable in AML with NPM1 mutation, or AML with CBFB/MYH11. For AML patients with biallelic CEBPA mutations, patients with MRD positivity (detected by MFC) during consolidation chemotherapy had significantly higher 3-year CIR (55% vs. 36.7%; = 0.037) and decreased RFS (45% vs. 63.3%; = 0.037) than those with MRD negativity. MFC-MRD could predict relapse and was complementary to genetics for risk stratification treatment in biCEBPA AML [12]. For those with detectable MRD either in favorable risk group or intermediate risk group, patients have high risk of relapse in the absence of allo-SCT. Therefore, these patients should be reevaluated as poor risk group.

Sometimes, the MRD detected by RT-PCR and MFC might be inconsistent. Zhao et al. divided NPM1-mutated AML patients into four groups according to the results detected by real-time quantitative PCR and FCM: both negative (group 1, FCM-NPM1m-), single positive (group 2, FCM-NPM1m+; group 3, FCM + NPM1m−), or both positive (group 4, FCM + NPM1m+). There was not a significant difference in the 2-year cumulative incidence of relapse (CIR) between group 2 and group 3, while patients in groups 2 and 3 had a lower 2-year CIR than those in group 4 and a significantly higher 2-year CIR than those in group 1. These results suggested that in the MRD monitoring process of AML patients, when the results of FCM and RQ-PCR are inconsistent (especially when FCM is positive and NPM1m is negative), these single-positive results still have predictive significance for relapse [13].

13.2.2 Evaluation of Risk of Transplant-Related Mortality

The transplant-related mortality is an important factor when making the decision of allo-SCT. Patients at high risk of TRM might offset the benefit of antileukemia effect of allo-SCT. Several factors were regarded to be associated with TRM, including age, comorbidity, performance status, conditioning intensity, donor type, and other factors [14, 15]. Currently, there are several systems to evaluate the risk of TRM. Sorror et al. proposed that a prediction model "HCT-CI" has great impact on the TRM [16–18], and this model has been validated in different transplantation settings including NMA [19] and haplo [20]. Recently, the HCT-CI was augmented by the addition of age [21].

The score of the European Society for Blood and Marrow Transplantation (EBMT) includes characteristics of the patient (age), disease (status, time from transplantation), and donor (relation, donor-recipient HLA match, and sex match) [22]. Peking University proposed a haplo-EBMT model to predict the NRM after haplo-SCT [23]. Combination of the modified EBMT score model and the HCT-CI improves the stratification of high-risk patients undergoing unmanipulated haploidentical blood and marrow transplantation [24].

13.2.3 The Use of Allo-SCT in AML

AML is now the major indication for allo-HSCT from the report of CIBMTR (www.cibmtr.org), EBMT [25], and CBMTR. The long-term survival of allo-HSCT for AML ranges from 30 to 80% and depends on the disease status, the age, the donor, and other factors.

13.2.3.1 AML with Poor-Risk in CR1

Systematic review and meta-analysis have demonstrated the benefits of matched related or unrelated allo-SCT over chemotherapy for high-risk AML CR1 [26]. And for those without MSD or MUD, recent studies from Peking University also demonstrated that haplo-SCT was superior to chemotherapy for high-risk AML-CR1 [27]. It is evident that patients with poor-risk AML will benefit from allo-SCT as post-remission therapy in their first CR. Therefore, for patients with poor-risk AML, it is strongly recommended to receive allo-SCT in CR1 regardless of donor type.

13.2.3.2 AML with Intermediate Risk in CR1

Previous study conducted a donor versus no donor analysis and reported that allo-HSCT with either MSDs or MUDs was superior to chemotherapy (5-year LFS 56% vs. 28%) for int-risk AML in CR1. There is also evidence that demonstrated that myeloablative haploidentical transplantation is superior to chemotherapy for patients with intermediate-risk AML patients in CR1 [28]. However, for those with MRD negativity, there is still controversy whether these patients will benefit from allo-SCT. This might need further investigation. In summary, allo-HSCT, either from MSD, URD, or HID, is currently the preferred option for individuals with int-risk AML.

13.2.3.3 AML with Favorable Risk in CR1

It is generally considered that patients with favorable risk will not benefit from allo-SCT in CR1. However, this recommendation is based on the study performed in the era using karyotype-based stratification. The karyotype at diagnosis cannot stratify the patients perfectly. Patients with good risk still have 30–50% relapse risk. The dynamic MRD monitoring can identify a subgroup of favorable risk patients who were at high risk of relapse with chemotherapy. For patients with CBF-AML, investigators from Peking University suggest that if patients haven't achieved major molecular remission (defined as 3 log reduction of runx1/runxt1), these patients might be considered as high risk (relapse rate 78.9%), and allo-SCT could improve the LFS and OS of this group of patients [11].

In summary, the dynamic monitoring of RUNX1/RUNXT1, CBFβ/MYH11, NPM1 could identify the favorable risk groups who may benefit from allo-SCT.

13.2.3.4 Allo-HSCT in AML with CR2 or Beyond

Once relapsed after intensive chemotherapy, only a small proportion of patients could achieve a second complete remission. And most of these patients who achieved CR2 will relapse again in short duration. Therefore, for these patients, the long-term survival will be very poor in the absence of allo-SCT. The long-term survival of AML-CR2 receiving allo-SCT is around 50–80%. Therefore, patients in CR2 or beyond are generally recommended to receive allo-SCT, regardless the donor type.

13.2.3.5 Allo-HSCT in Refractory/Relapsed AML

Refractory/relapsed AML includes those with primary refractory and those at relapsed status. About 10–40% of newly diagnosed AML cannot achieve CR1. With current induction therapy, they were grouped as primary refractory AML. R/R AML has very limited chance to long-term survival via salvage chemotherapy. Allo-SCT might be a salvage option, although the long-term survival is not satisfactory (around 30–40%).

Therefore, although the prognosis of R/R AML after allo-SCT is very poor, allo-SCT is still a feasible salvage option.

13.2.4 Summary of Indication

Generally, for patients with AML in >=CR2 and R/R status, allo-SCT should be considered regardless of the risk stratification at diagnosis, the donor type. However, for patients in

CR1, the decision of allo-SCT as post-remission therapy should be based on the stratification at diagnosis and the MRD monitoring. For favorable risk group, decision of allo-SCT should be based on the dynamic monitoring of RUNX1/RUNXT1, CBFβ/MYH11, and NPM1. For intermediate risk group and poor risk group, allo-SCT was recommended in CR1.

13.3 Transplant-Related Strategies

13.3.1 Donor Selection

The donor pool includes matched sibling donor (MSD), unrelated donor (URD), haploidentical donor (HID), and cord blood (CB). Generally, MSD was the first choice and followed by well-matched URD. Unfortunately, there is less chance to find MSD and URD. The great advances in the field of alternative donor have led to great changes in donor pool, especially transplantation from HID. The great improvement of haploidentical transplantation from either Beijing Protocol or Baltimore protocol demonstrated comparable result to MSD and URD [29–33]. Several studies demonstrated that HID might have stronger GVL effect than MSD [34]. Investigators from Peking University demonstrated that for those with positive MRD before transplantation, transplantation from HID has lower relapsed incidence and higher incidence of LFS [35, 36]. Also, investigators from Peking University demonstrated that for old patients, younger HID might be better than old MSD [33]. Therefore, in some specific situation, HID might be as frontline choice. However, this needs further validation.

13.3.2 Conditioning Regimen

Conditioning regimens play important role in allo-SCT. The objectives of conditioning regimen consist of eradication of disease and suppression of host immune system. It is clear that myeloablative conditioning regimens offer better disease control, but with higher TRM. The selection of conditioning regimen depends on age of patient, disease risk, performance status, and remission status at the time of transplantation. Myeloablative conditioning regimens such as Bu/Cy or TBI/Cy were the most commonly used. However, for patients with old age or with comorbidity, the TRM was unacceptably high. MAC is the preferred choice for younger and fit patients, while RIC might be more suitable for older patients or unfit patients. However, there is still a question whether to choose MAC or RIC.

13.3.3 Graft Source

The graft source could be bone marrow or peripheral blood stem cell. Both stem cell sources are acceptable options in AML patients. It is said that PBSC are superior in engraftment, however with increased risk of GVHD. Actually, considering the convenience, the PBSC is now more popular than PB according to National Marrow Donor Program data (http://www.marrow.org). However, whether PBSC are preferable to BM harvesting as the stem cell source for patients with AML is still an open question.

13.4 Prevention of Relapse

Relapse is still the major cause of death after allo-SCT. Once relapse after transplantation, the prognosis is very poor. Several methods could help preventing posttransplant relapse. These will be discussed in detail in following parts.

13.4.1 Pretransplantation Strategies

It has been demonstrated that disease burden at transplant was associated with the risk of relapse after transplantation. Liu et al. reported the significance of peri-transplantation minimal residual disease assessed by multiparameter flow cytometry on outcomes for adult AML patients receiving allo-SCT. Therefore, it might be beneficial to achieve deep response (MRD negativity) before allo-SCT [37]. The intensified conditioning might be effective to prevent relapse.

The disease stage at the time of transplant also has impact on transplant outcomes. It is evident that patient transplant in CR1/CR2 has better outcomes than patient transplant at >CR2. Therefore, transplant at early stage (for example, transplant in CR1) in patients with high relapse risk might be helpful.

13.4.2 Posttransplant Strategies

13.4.2.1 Maintenance Therapy (Targeted Drug, HMAs)

The purpose of maintenance therapy to prevent posttransplant relapse might include, first, augment the antileukemia effect via drugs or other intervention; second, augment the graft-versus-leukemia effect. Administration of FLT3 inhibitor after transplantation has been proved effective in preventing relapse in patients with FLT3+ AML. FLT3 inhibitor could not only have antileukemia effect, but also could aug-

ment the GVL effect. In a multicenter prospective study, sorafenib was demonstrated to be associated with improved LFS (81% vs. 54%) compared with placebo [38]. Another phase 2 randomized study also found that sorafenib could significantly improve the LFS (85% vs. 53%) [39].

Administration of HMA is also effective. Several prospective studies have tested the efficacy of HMAs as maintenance therapy. Recently, a Chinese group performed a randomized controlled study. They demonstrated that use of a combination of rh-G-CSF and decetabine (100 mg/m^2 of rhG-CSF on days 0–5 and 5 mg/m^2 of Dec on days 1–5, every 6–8 weeks for up to six courses) could significantly decrease the 2-year cumulative incidence of relapse (15.0% vs. 38.3%, $p < 0.01$) compared to the nonintervention group in patients with high-risk AML after allo-SCT [40].

13.4.2.2 MRD Guided Preemptive Therapy

Lots of studies have demonstrated that post-MDR detection could identify a subgroup of patients who are more likely to relapse after allo-HSCT and could predict hematological relapse in advance to some extent (range from weeks to months). Disease-specific fusion genes (such as RUNX1/RUNXT1, NPM1, etc.) were good candidate markers for relapse prediction. Wang et al. reported that RUNX1/RUNX1T1-based MRD monitoring in high-risk t(8;21)(q22;q22) AML patients after allo-HSCT identified relapse patients (RUNX1/RUNX1T1 > 3 log reduction vs. ≤3 log reduction at 30, 60 and 90 days after HSCT, $p < 0.05$, $p < 0.001$, $p = 0.0001$, respectively). It is also demonstrated that high-risk adult AML patients with inv(16) who did not achieve major MMR within the first 3 months or lost MMR after the first 3 months following transplantation were at higher risk of relapse [41, 42]. Other nonspecific markers (such as WT1 overexpression) were detected by RT-PCR and LAIPs detected by MFC and were also used as candidate marker of MRD.

MRD-guided preemptive therapy was defined as preemptive intervention to prevent relapse based on post-HSCT MRD monitoring. DLI and interferon can significantly decrease the risk of relapse and improve the survival. Yan et al. reported that the MRD-positive patients who had mDLI had comparable relapse and survival rates when compared with those MRD-negative patients. MRD-directed mDLI significantly reduced the relapse risk (HR = 0.269, $p < 0.001$) and improved DFS (HR = 0.436; $p = 0.006$) [43, 44].

IFN-a exerts a relatively strong immunomodulatory effect and can kill AML cells through the regulation of T-cell and NK cell functions. In a prospective clinical study, MRD-positive AL patients who could receive DLI were assigned to the DLI group, whereas those who could not or did not agree to receive DLI were assigned to the IFN-a group. The 1-year relapse rate after intervention was 27.3% and 35.6%, respectively ($p = 0.514$), and the 1-year probabilities of DFS after intervention were 68.2% and 60.0% for patients in the IFN-a and DLI groups, respectively ($p = 0.517$) [45–47].

13.5 Conclusion and Perspectives

Significant progress has been made in allogeneic transplantation for AML in the last 60 years, mainly including more precise risk stratification, the better supportive treatment (such as infection, GVHD, etc.), the expansion of donor pool (especially haploidentical donor), and the improvement of conditioning regimen (such as reduced intensity conditioning regimen). These advances have led to significant improvement of long-term survival of AML after allo-SCT. However, there are also several aspects needed to be further explored in future. First, AML developed at a median age of around 70 years and a more safer transplantation regimen is needed to cover these patients who are less considered as transplant candidate before. Second, more precise risk stratification is needed to improve the outcome of risk-adapted transplant strategy. Due to the false-positive results of current MRD-detection technique, some patients may receive unnecessary treatments and these patients would experience the toxicity or mortality of the overtreatment. Thus, more precise methods for MRD monitoring should be further identified and interventions with lower toxicities may be preferred upon the initial detection of MRD. Third, relapse is still the major barrier for long-term survival. The better understanding of relapse mechanism and the development of more novel options will help improve the long-term outcomes of AML after allo-SCT.

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14 Maintenance Therapy Following Allogeneic Hematopoietic Stem Cell Transplantation in Acute Myeloid Leukemia

Yong-Xian Hu and Hong-Hu Zhu

Abstract

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) has significantly improved the outcome of patients with acute myeloid leukemia (AML). However, the relapse rate is still high, accounting for about half of treatment failures. Administration of maintenance therapy after allo-HSCT is therefore needed to prevent relapse. Different strategies and cellular therapies after allo-HSCT have been initiated as maintenance therapy to enhance graft-versus-leukemia effects, reduce the relapse rate, and eventually improve OS. In this chapter, we will review the current maintenance therapy following allo-HSCT.

Keywords

Acute myeloid leukemia · Older patients · Maintenance therapy · Hematopoietic stem cell transplantation

14.1 Introduction

Acute myeloid leukemia (AML) is a clonal, malignant hematopoietic stem cell disease with molecular and clinical heterogeneity that may be cured with allogeneic hematopoietic stem cell transplantation (allo-HSCT). Although allo-HSCT for AML has resulted in 3 ~ 5-year overall survival (OS) rates ranging 20 ~ 90%, [1] the relapse rate is still high, accounting for ~40% of treatment failures [2]. Administration of maintenance therapy after allo-HSCT is therefore needed to prevent relapse. Different strategies and cellular therapies after allo-HSCT have been initiated as maintenance therapy to enhance graft-versus-leukemia (GVL) effects, reduce the relapse rate, and eventually improve OS. Recently, with the increasing understanding of the immune biology and molecular landscape of AML, targeted and biologically directed therapies have emerged, including epigenetic modifications (e.g., hypomethylating agents (HMAs)), tyrosine kinase inhibitors (TKIs) (e.g., FMS-like tyrosine kinase 3 (FLT3, CD135) inhibitors), and immunomodulation (e.g., donor lymphocyte infusion (DLI)) [3] (Table 14.1).

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Table 14.1 The drugs used during maintenance therapy following allo-HSCT in AML

Therapy	Clinical trial	Sample size	Result		
Targeted drugs					
	FLT3 inhibitors				
		Sorafenib	SORMAIN	79	24-month RFS: 85.0% vs. 53.3% (sorafenib vs. placebo, log-rank $p = 0.002$)
					24-month OS: 90.5% vs. 66.2% (sorafenib vs. placebo, log-rank $p = 0.007$)
			NCT02474290	201	1-year cumulative incidence of relapse: 7.0% vs. 24.5% (sorafenib vs. control, $p = 0.0010$)
					2-year cumulative incidence of relapse: 11.9% vs. 31.6% (sorafenib vs. control, $p < 0.0001$)
		Midostaurin	RADIUS	30	Estimated 18-month RFS: 89% vs. 76% (midostaurin + SOC vs. SOC alone, $p = 0.27$)
					Estimated 24-month RFS: 85% vs. 76% (midostaurin + SOC vs. SOC alone, $p = 0.4297$)
					Estimated 24-month OS: 85% vs. 76% (midostaurin + SOC vs. SOC alone, $p = 0.34$)
		Quizartinib	2689-CL-0011	13	OS ranged from approximately 13 weeks to 142 weeks, with 9 subjects (69%) surviving >50 weeks and 4 subjects (31%) surviving >2 years (104 weeks)
	Monoclonal antibodies				
		Gemtuzumab	NOPHO-AML 2004	120	5-year EFS: 55% vs. 51% (gemtuzumab ozogamicin vs. observation, nonsignificant)
		Ozogamicin			5-year OS: 74% vs. 80% (gemtuzumab ozogamicin vs. observation, nonsignificant).
			SWOG S0106	169	The DFS was not significantly better in the GO group ($p = 0.97$)
			HOVON-43	232	There were no significant differences
Epigenetic drugs					
	Hypomethylating agents				
		5-azacitidine	MDACC trial	45	Median EFS: 18.2 months.
			NCT00887068	187	Median RFS: 2.07 years vs. 1.28 years
					Median OS: 2.52 years vs. 3.56 years
		Decitabine	NCT00986804	24	2-year DFS: 48%;
					2-year OS: 56%.
Checkpoint inhibitors					
	CTLA-4 inhibitors				
	Ipilimumab	NCT01822509	28	1-year OS: 49%	
	PD-1/PD-L1 inhibitors	NCT04361058		Ongoing without published results	
		NCT02846376		Ongoing without published results	
Cellular therapy					
	Donor lymphocyte infusion	Krishnamurthy P, et al.	62	5-year EFS: 65%	
				5-year OS: 80%	
		NCT01541280	30	2-year OS: 65.5%, 3-year OS: 63%	
				2-year cumulative incidence of relapse: 27.6%	
	NK cell infusion	NCT01386619	8	Relapse occurred in 4/8 AML patients	
	γδ T cells	NCT03533816		Ongoing without published results	

14.2 Targeted Drugs

14.2.1 FLT3 Inhibitors

Mutations in FLT3 occur in 25 ~ 30% of patients with AML [4] and can be divided into two categories: internal tandem duplication (ITD) and point mutations in the activation loop of the Tyr kinase domain (TKD) [5]. While the prognostic impact of FLT3-TKD mutations is not well defined [6], FLT3-ITD is proven to have a strong relevance with poor prognosis, with an increased risk of relapse and shorter overall survival after standard intensive chemotherapy compared

with FLT3-wild-type patients [7]. Allo-HSCT could improve the survival of these patients, but leukemia relapse remains high [8]. Further studies revealed that the prognosis of FLT3-ITD AML is related to the FLT3-ITD allelic ratio, length, insertion site, and cooccurring mutations, [8] thus providing the rationale for the application of FLT3 inhibitors.

FLT3 inhibitors are classified into first- and next-generation inhibitors based on their potency and specificity for FLT3 and their associated downstream targets [9]. First-generation inhibitors, including lestaurtinib, sunitinib, sorafenib, ponatinib, and midostaurin, are relatively nonspecific for FLT3, as they possess extended activity involving other potential targets, such as the KIT, PDGFR, VEGFR, RAS/RAF, and JAK2 kinases. The off-target activities may contribute to a generally higher toxicity profile and clinical efficacy in non-FLT3-mutated AML, but decreased efficacy in mutated FLT3 with high allelic burden [10]. Next-generation inhibitors, including quizartinib, crenolanib, and gilteritinib, are more specific and potent, with a lower half maximal inhibitory concentration (IC50) and fewer toxicities associated with off-target effects [10]. The approval of midostaurin and gilteritinib for newly diagnosed FLT3 mutant AML and R/R FLT3 mutant AML, respectively, has encouraged clinical trials of FLT3 inhibitors as posttransplantation maintenance therapy.

Sorafenib

In the phase I trial, out of the 19 patients who had achieved a conventional CR1 or CR2 prior to transplant, there was only one relapse, yielding a 1-year PFS of 95% and 2-year PFS of 86% at a median follow-up of 16.7 months after allo-HSCT [11]. In the phase II trial, the 24-month RFS probability was 85.0% versus 53.3% (sorafenib vs. placebo) at a median follow-up of 41.8 months after allo-HSCT [12]. In another phase III trial, the 1-year cumulative incidence of relapse was 7.0% versus 24.5% (sorafenib vs. control), and the 2-year cumulative incidence of relapse was 11.9% versus 31.6% (sorafenib vs. control) at a median follow-up of 21.3 months after allo-HSCT [13]. The most common grade 3/4 adverse events (AEs) were acute/chronic GVHD, and other AEs included skin toxicity, GI toxicity, and hematological abnormalities. Adverse effects can be managed with dose reductions, and moderate dose reductions did not seem to abolish sorafenib efficacy [12, 13]. The maximum tolerated dose (MTD) was 400 mg b.i.d. [11], but most patients receiving this dose in the trials mentioned above had to undergo dose reduction/interruption because of side effects [11–13]. The European Society for Blood and Marrow Transplantation guidelines recommend a dose of sorafenib for maintenance posttransplantation of 200 mg b.i.d. for patients without minimal residual disease (MRD) and 400 mg b.i.d. for patients with MRD [14]. With regard to the optimal time to initiate posttransplantation sorafenib maintenance therapy, there is no specific time window, and 30 ~ 120 days after allo-HSCT seem to be an acceptable range [11–13, 15].

Midostaurin

RADIUS (NCT01883362) trial is a phase II, randomized, open-label trial of standard-of care (SOC) with or without midostaurin in patients with documented FLT3-ITD-positive AML who had undergone a protocol-specified conditioning regimen before allo-HSCT in CR1 [16]. RADIUS showed that the estimated RFS at 18 months was 89% versus 76% (midostaurin + SOC vs. SOC alone, with no statistical significance), and the estimated 24-month RFS was 85% versus 76% (midostaurin + SOC vs. SOC alone, with no statistical significance) [16]. Survival outcomes also improved: the estimated 24-month overall survival (OS) was 85% versus 76% (midostaurin + SOC vs. SOC alone, with no statistical significance) [16]. Another trial, AMLSG 16–10, suggested that patients with FLT3-ITD AML who received pretransplant midostaurin and began midostaurin within 100 days posttransplant had a significantly better EFS and OS [17]. The most common midostaurin-related AEs are GI AEs, including vomiting, nausea, and diarrhea, and such GI AEs are manageable with prophylactic support treatment (e.g., antiemetics) [16, 18, 19]. Unlike sorafenib, the addition of midostaurin to SOC did not increase the rates or severity of GVHD [16]. Given the lack of experience in maintenance settings, no recommended dose is provided. For reference only, both studies mentioned above chose 50 mg b.i.d. as the initial dose (with subsequent dose reduction) [16, 17]. However, studies have suggested that midostaurin maintenance therapy should begin quickly (within 100 days, ideally <60 days after transplantation) [16, 17].

Quizartinib

In a phase I study of quizartinib as maintenance therapy in subjects with AML in remission following allo-HSCT with 13 enrolled patients, 10 subjects (77%) received treatment with quizartinib for >1 year, and 6 (46%) received treatment for close to 2 years (95 to 99 weeks). Five subjects (38%) completed quizartinib treatment. Relapse was observed in only one subject (8%) receiving quizartinib, which occurred in the first cycle. Ten subjects were still alive at the end of the study, and three had died (one disease progression, one peritoneal hemorrhage, and one unknown cause). OS ranged from approximately 13 weeks to 142 weeks, with nine subjects (69%) surviving ≥50 weeks and four subjects (31%) surviving >2 years (104 weeks). There was no significant difference in OS between treatment groups [20]. The majority of AEs reported were hematologic (e.g., neutropenia, leukopenia, and anemia); however, satisfactory overall blood counts were maintained for 11 of 13 subjects throughout the duration of the trial [20]. The 69% rate of GVHD observed following treatment with quizartinib was consistent with GVHD rates previously reported for patients with AML who underwent allo-HSCT (acute: 43–80%; chronic: 34–49%) [20, 21]. Quizartinib-associated AEs were manageable with dose adjustments and/or interruptions [20]. No MTD, nor optimal dose or optimal initiation time, was identified in the phase I trial because of the pre-

mature termination of the trial [20]. For reference, the MTD found in a phase I trial evaluating quizartinib in relapsed/refractory AML (R/R AML) was 200 mg per day, [22] and most recent trials evaluating quizartinib in R/R AML used a range of 20 ~ 60 mg per day as a therapeutic dose [23–26].

14.2.2 Monoclonal Antibodies

Gemtuzumab Ozogamicin (GO)

GO is a recombinant humanized monoclonal antibody conjugated to the antitumor antibiotic calicheamicin. The antibody component targets the $CD33^+$ cell surface marker that is expressed on cancerous cells in the majority of AML patients [27]. Although GO has been approved for treating newly diagnosed AML in adults and relapsed/refractory (R/R) AML in adults and pediatric patients, after studies using lower or fractionated doses of GO in combination with chemotherapy demonstrated efficacy with limited toxicity, [27–29] trials investigating its role in the setting of posttransplant maintenance showed poor outcomes [30].

Other Monoclonal Antibodies

Monoclonal antibodies have always drawn great attention in the treatment of AML [31]. Other than GO, unconjugated/conjugated anti-CD33 antibodies, anti-CD123 antibodies, anti-CD56 antibodies, anti-CD135 (FLT3) antibodies, radioactive conjugated antibodies, T-cell-engaging antibodies, multispecific antibodies, etc. are being widely investigated with intensive interest [31, 32]. However, few studies have focused on the maintenance setting, leaving it a field worth exploring and full of opportunities.

14.3 Epigenetic Drugs

Recent studies have demonstrated that DNA methylation is associated with gene silencing and that aberrant DNA methylation can cause abnormal gene expression, abnormal transcription, and even cancer gene mutations in tumor suppressor genes [33, 34]. Therefore, DNA methylation is a major course resulting in the occurrence of tumors and is also an important pathogenesis of AML. Hypomethylating agents (HMAs) can demethylate DNA by inhibiting DNA methyltransferases. HMAs can not only reactivate tumor suppressor genes, but also promote the expression of related tumor antigens to play a role in the antitumor response, especially in hematological malignancies [35]. Numerous studies have shown that HMAs can upregulate human leukemic antigen (HLA) and related leukemia antigens previously epigenetically silenced to enhance the GVL effect [36, 37]. HMAs seem to increase the recognition of hematopoietic cells by MAGE-specific CD8+ cytotoxic T lymphocytes and enhance CD8+ T cell responses to enhance antitumor activity [38]. Studies have shown that effective maintenance therapy may be a promising approach to treat or decrease the risk of relapse after allo-HSCT for AML.

A phase III randomized study of 5-azacitidine (NCT00887068) evaluated 187 patients with AML or myelodysplastic syndrome (MDS) after allo-HSCT. Patients were randomized to the 5-azacitidine group ($n = 93$) or the control group ($n = 94$). Although patients showed good tolerance of 5-azacitidine, there was no significant difference in median RFS or OS between the two groups [39]. Another study also analyzed the efficiency of azacytidine for patients after transplantation and found approximately the same results: administration of azacitidine ameliorated the rate of hematologic relapse and overall survival as well as nonrelapse mortality between the azacitidine group and the control group [40]. In a single-institution, open-label, prospective study (NCT00986804), the researchers examined the observed toxicity and responses to low-dose decitabine as a maintenance therapy for patients with AML and MDS after allo-HSCT and found that decitabine was also well tolerated. Owing to less hematological toxicity, 10 mg/m^2/day may be a more optimal and better tolerated dose for decitabine maintenance after allo-HSCT [41]. In addition, in a retrospective study, 21 enrolled AML patients received DAC as maintenance therapy post-allo-HSCT, intravenously infused at a dose of 20 mg/m^2/day for 5 consecutive days every 3 months for 4–6 cycles and achieved a high rate of 3-year OS (92.9% vs. 51.8%, $p = 0.003$) and 3-year LFS (94.1% vs. 55%, $p = 0.002$), with a 3-year cumulative incidence rate of relapse of 5.9% versus 45.3% ($p = 0.002$) [42].

14.4 Checkpoint Inhibitors

While hypomethylating agents, FLT3 inhibitors, and monoclonal antibodies have attracted considerable attention as maintenance therapies, checkpoint inhibitors, such as programmed death 1 (PD-1) inhibitors and cytotoxic T-lymphocyte–associated protein 4 (CTLA-4) inhibitors, will also be important as maintenance therapies.

One of the mechanisms by which tumor cells evade the surveillance of the immune system is immune checkpoint inhibition, which equally contributes to relapse after allo-HSCT in AML. PD-1 and CTLA-4, two of the hottest checkpoints, engage with their respective ligands B7-1 and B7-2 and PD-L1 and PD-L2 to inhibit effector T-cell function or mediate regulatory T cells (Tregs). Increased expression of PD-1 and CTLA-4 and their ligands is seen not only in the immune activation state, but also in the tumor microenvironment, which makes them reasonable targets for tumor immunotherapy. Tislelizumab, nivolumab, pembrolizumab, atezolizumab, avelumab, durvalumab, and cemiplimab are FDA-approved PD-1/PD-L1 inhibitors. Ipilimumab is currently the only FDA-approved CTLA-4 inhibitor.

14.4.1 CTLA-4 Inhibitors

CTLA-4 shares B7 ligands with CD28, which is expressed constitutively by T cells [43]. However, CTLA-4 has a greater affinity for ligands than CD28, proving that it is a negative regulator of T-cell activation rather than a simulator. Thus, CTLA-4 blockade results in enhancement of immune responses, which has been shown by preclinical studies with murine models [44]. A phase I/Ib study by David et al. indicated that administration of ipilimumab may be feasible in patients with relapsed hematological cancers after allo-HSCT. A total of 28 patients were enrolled, including 12 with AML. Patients received intravenous ipilimumab induction every 3 weeks for 4 cycles, followed by maintenance dosing in 12-week increments for up to 1 year. Five of 12 AML patients (42%) achieved CR, including three with leukemia cutis. Across all patients, 15 (54%) completed the full course of induction therapy and 6 (21%) received maintenance therapy [45].

14.4.2 PD-1/PD-L1 Inhibitors

PD-1 protein is another immunosuppressive receptor of the B7/CD28 superfamily expressed on B and T cells. The binding of PD-1 and its ligands, PDL-1 and PDL-2, expressed by antigen-presenting cells (APCs) or tumor cells, inhibits T-cell activation. Berthon C et al. identified PD-L1 as an immunoescape molecule in blast cells from patients with AML, suggesting a correlation between increased PD-L1 expression and AML patient relapse [46]. Albring et al. reported nivolumab treatment in three AML patients who relapsed after allo-HSCT. After 10 months on nivolumab (100 mg), the first patient, a 61-year-old male, remained in CR with no clinical signs of GVHD and with good performance status. A repeated low-dose regimen of 0.3–1 mg/kg nivolumab was applied to the second patient, a 44-year-old female. Molecular disease stabilization was observed for 6 months. The third patient, a 50-year-old man, failed to respond to nivolumab. Interestingly, all patients had an increase in CTLA-4-expressing lymphocytes [47]. An ongoing study is focusing on nivolumab for high-risk AML participants who receive HLA-matched unrelated donor HSCT or HLA-haploidentical HSCT (NCT04361058). In addition, there are several phase I or phase II clinical trials of PD-1/PD-L1 inhibitors in AML patients underway, most of which have not yet published results. Immune checkpoint inhibitors are a hot topic of research in tumor immunotherapy, among which PD-1/PD-L1 inhibitors and CTLA-4 inhibitors are the most promising. As the mechanism of the immune checkpoint in AML continues to be investigated and clinical trials of the two inhibitors in the treatment of AML continue to be conducted, more encouraging results have been obtained, indicating promise for PD-1/PD-L1 or CTLA-4 blockade as a novel tool for maintenance therapy following allo-HSCT in AML.

14.5 Cellular Therapy

14.5.1 Donor Lymphocyte Infusion (DLI)

Donor lymphocytes are donor-derived mononuclear cells (mainly CD3+ T cells) obtained via G-CSF-mobilized peripheral blood stem cells or unstimulated leukapheresis [48, 49]. According to previous studies, AML patients who achieve complete remission (CR) with chemotherapy followed by DLI tend to survive longer, as DLI can enhance GVL effects and reduce T-cell exhaustion [50]. Furthermore, Rautenberg C et al. reported that 46 high-risk AML patients were recruited and underwent DLI from day +120 after allo-HSCT. The OS achieved was 67%, compared to 31% OS for the control group after a median follow-up period of 7.2 years [49, 51]. In short, the above data supported the role of DLI as maintenance therapy for post-HSCT high-risk AML patients. Nonetheless, GVHD is a significant substantial risk associated with DLI that needs to be overcome [52]. Therefore, further investigation on the adverse events, dosing, and coadministration with other agents to maximize the GVL effect is warranted [3].

14.5.2 NK Cell Infusion

Natural killer (NK) cells, derived from CD34+ hematopoietic stem cells, are innate lymphoid cells that can recognize "non-self" cancer cells from self-MHC class I molecules. Additionally, they do not require prior antigen presentation or HLA matching to eliminate tumor cells, making them suitable candidates for adoptive cell therapy and for use in allogeneic settings. This unique antitumor effect also allows NK cells to increase GVL effects without inducing GVHD. Additionally, it is worth mentioning that the leukemic cells in AML are mostly sensitive to NK-mediated cytotoxicity. Clinically, haploidentical NK cells are adoptively transferred to cure hematological malignancies. In a previous study conducted by Miller et al. [53], 26% of AML patients with poor prognosis successfully achieved CR after receiving NK cells [54]. On the other hand, bi- and trispecific killer engager antibodies (BiKEs and TriKEs) were invented to treat relapsed AML patients following allo-HSCT. Theoretically, bispecific killer cell engagers (BiKEs) might direct NK cells to target tumor antigens, while trispecific killer cell engagers (TriKEs) are used for NK cell expansion in AML patients. Presently, the results of phase I and II clinical trials for TriKEs have yet to be determined [55]. Despite the advantages mentioned above, the significant limitations of NK cell infusions are the lack of persistence and limited cell expansion after transfusion [50]. Additionally, NK cells lack specificity and are found to have poor in vivo survival [56]. The shortcomings of NK cells should be overcome in upcoming studies to ensure NK cell infusion efficacy as maintenance therapy for post-HSCT AML patients.

14.5.3 γδ T Cells

Allogenic HSCT can cause severe transplantation-related adverse events such as GVHD. Generally, GVHD is caused by alloreactive T lymphocytes that express αβ T-cell receptors. In this process, γδ T-cell receptors are not alloreactive; instead, they exhibit antileukemic effects. Therefore, γδ T cells can induce potent GVL effects without causing GVHD in AML patients due to the absence of alloreactivity [57]. γδ T cells, with a unique role in immune surveillance, display diverse functions with resemblances to Th1, Th2, Th17, Treg, and NKT cells in various microenvironments [58]. Unlike αβ T cells, γδ T cells do not have CD4 and CD8 coreceptors [57]. Additionally, γδ T cells are not restricted to HLA matching and can be rapidly deployed for innate immune responses. A study conducted by Ross et al. [59] highlighted the importance of γδ T cells in 102 pediatric patients with acute leukemia. Event-free survival was significantly higher in patients with enhanced γδ T cells (91%) than in those with low/normal γδ T cells (55%) after allo-HSCT, with a median follow-up of 2.7 years. Moreover, γδ T cells tend to have strong GVL effects, and low GVHD occurrence guarantees their role in the context of allo-HSCT [58]. Hence, these cells are suitable for maintenance therapy for post-HSCT AML patients, but more preclinical and clinical research should be carried out to assess the efficacy and safety of γδ T cells.

14.5.4 CAR T Cells

Chimeric antigen receptor T cells (CAR T) have shown remarkable clinical results in relapsed and refractory hematological malignancies, especially in B-cell malignancies such as B-ALL non-Hodgkin's lymphoma. The current hurdle in treating AML patients with CAR T cell therapy is the lack of AML-specific antigens. Several clinical trials have been conducted using different AML target antigens, such as CD38, CD56, CD123, CD33, folate receptor β, FLT3, CLL-1, CD44v6, NKG2D, and Lewis Y [49, 60]. For instance, CD123 was recognized as one of the key target antigens for CAR T therapy in AML patients due to its overexpression in AML compared to normal stem cells [59]. This finding might help identify the role of CAR T cell therapy as maintenance therapy following allo-HSCT in AML patients. To further validate the role of CAR T cells in AML, Ritchie et al. conducted a phase I clinical trial using CAR T cells targeting the Lewis-Y antigen. The CAR T cells persisted for almost 10 months, but all patients relapsed [61]. Another newer approach was designed to transplant CD33-targeted CAR T cells altered from allogeneic donor HSPCs into AML patients and explore CAR T cell potential [62]. Nevertheless, several clinical side effects, such as cytokine release syndrome (CRS), neurologic toxicities, or tumor lysis syndromes, have been documented in various CAR T cell therapy clinical trials [55]. Despite limited data available, further CAR T studies should be performed as a novel maintenance therapy for AML patients.

14.5.5 TCR-T Cells

T cell receptor (TCR) cell therapy utilizes genetically engineered T cells with TCRab chains to treat blood cancers. Recent news reported that T cells targeting Wilms tumor antigen 1 (WT1) effectively prevent relapse in AML patients who have received allo-HSCT. In this study, Chapuis et al. isolated the high-affinity AML-relevant antigen Wilms' tumor antigen 1-specific TCR (TCRC4), and further research was carried out. HLA-A2 WT1 epitope-targeted gene-modified allogeneic T cells were infused into post-HSCT AML patients by Chapuis et al. Surprisingly, none of the 12 high-risk patients experienced disease relapse, and the infused T cells persisted for months to years without exerting toxicity [33, 37]. The relapse-free survival obtained was 100%, with a median follow-up period of 44 months following infusion. This approach is promising to prevent post-HSCT AML relapse [33]. However, in the post-HSCT setting, TCR-T cells targeting overexpressed self-antigens might have some drawbacks, such as on-target-off-tissue toxicity and TCR-mediated alloreactivity. In conclusion, the results of TCR-T cell therapy are encouraging and warrant further investigation in post-HSCT high-risk AML patients [33].

14.6 Perspectives

Given the high risk of relapse in posttransplant AML patients, the prophylactic use of certain therapies after allo-HSCT may be essential. However, for patients stratified as favorable/intermediate-risk, maintenance therapies may represent overtreatment and expose patients who are cured of their disease to long-term and late adverse effects. Generally, patients with baseline adverse biological characteristics or patients who achieve morphological CR with positive MRD are considered proper candidates for maintenance therapy. Among all the emerging therapies, sorafenib, despite the lack of approval in this setting, is the only one recommended by official guidelines with limited scenarios (FLT3-ITD-mutated patients), while other therapies require more investigation and evidence.

With the wealth of novel modalities applying various mechanisms, one has to ask the question of what should be the primary goal of maintenance therapy. Is the goal of maintenance to modulate the immune system to maintain a CR previously achieved or to eradicate MRD?

Aspects remaining regarding the development of the maintenance approach include the schedule, dose and combination of drugs/therapies and, as mentioned above, the selection of patients. The timing of initiation is challenging,

since relapse usually occurs early after allo-HSCT and intermediate intervention is favorable; on the other hand, in the early stage after allo-HSCT, patients are rather vulnerable to side effects. The proper duration of maintenance is also unclear, and the risk of late relapse after the cessation of treatment contradicts other factors, such as tolerance, drug resistance, cost-effectiveness, and patient quality of life. In terms of the stratification of patients, in addition to the standardization of the algorithm to distinguish the patient group who needs maintenance therapy from those for whom preemptive therapy would achieve similar prevention of relapse, there are questions of further classification, e.g., since FLT3 inhibitors are quite promising in FLT3-ITD AML, what is the appropriate approach for AML without FLT3-ITD mutation? Given the immunomodulatory and off-target effects shown by FLT3 inhibitors in previous studies, might they be used outside FLT3-ITD AML? More prospective, randomized trials to determine the clinical efficacy of potential therapies are needed, and the results are required to move these approaches from experimental to routine.

Conflict of Interest Statement The authors declare no conflicts of interests.

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Immunotherapeutic Targeting of AML

15

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Abstract

The use of immunotherapy has revolutionized the field of cancer management. Patients with acute myeloid leukemia (AML) have a defective bone marrow immune environment. Understanding mechanisms of immunoediting and immune escape of AML are key for development of effective immunotherapy for AML. Multiple immunotherapeutic approaches for AML have been under development over the past few years and some are advancing toward late-stage clinical testing. In this chapter we will review our latest understanding of AML immune escape mechanisms and the latest clinical results of immunotherapeutic agents for AML, with focus in monoclonal and bispecific antibodies, adoptive cellular therapy, vaccines, and checkpoint inhibitors.

Keywords

Acute myeloid leukemia (AML) · Immunotherapy Immune escape · Monoclonal antibody · Bispecific antibody · Checkpoint inhibitor

15.1 Introduction

Despite the recent advances in understanding acute myeloid leukemia (AML) pathobiology, therapeutic strategies have remained unchanged over the past four decades. These consist of intensive induction chemotherapy followed by consolidation with more cycles of chemotherapy or allogeneic hematopoietic cell transplant (allo-HCT). In the past few years, multiple new targeted therapeutic agents have been approved for the treatment of AML [1]. Nevertheless, the prognosis of AML is still poor especially in older patients and those with adverse genetic features that are usually associated with chemoresistance rapid disease relapse [2]. Thus, alternative therapeutic approaches such as immunotherapy could be the cornerstone to build upon for the future optimal management of patients with AML [3]. Allo-HCT provided a proof-of-principle for the potential effectiveness of immunotherapy for the treatment of AML [4]. In addition to the therapeutic benefit of high dose conditioning, infusion of donor alloreactive T cells mediating a graft versus leukemia (GVL) effect clearly provides an additional therapeutic advantage. The GVL effect is supported by several observations. First, reduced relapse rates were observed in patients who received an allo-HCT and especially in those patients who developed chronic graft versus host disease (GVHD) [5, 6]. Second, withdrawal of immunosuppressive therapy in those patients who relapse after allo-HCT may induce remissions [7]. Third, donor lymphocyte infusions (DLI) are effective in some cases of post-allo-HCT relapse [8, 9]. Other immunotherapy strategies have shown great success in the treatment of B cell malignancies as well as other types of solid tumors [3, 10]. The development of several forms of immunotherapy for the treatment of AML are ongoing with early studies showing some promising results. In this chapter, we will review the mechanisms of AML immune escape and discuss the recent immunotherapeutic advances for the treatment of AML including monoclonal antibodies, cellular therapies, bispecific antibodies, checkpoint inhibitors, and vaccine therapy (Fig. 15.1).

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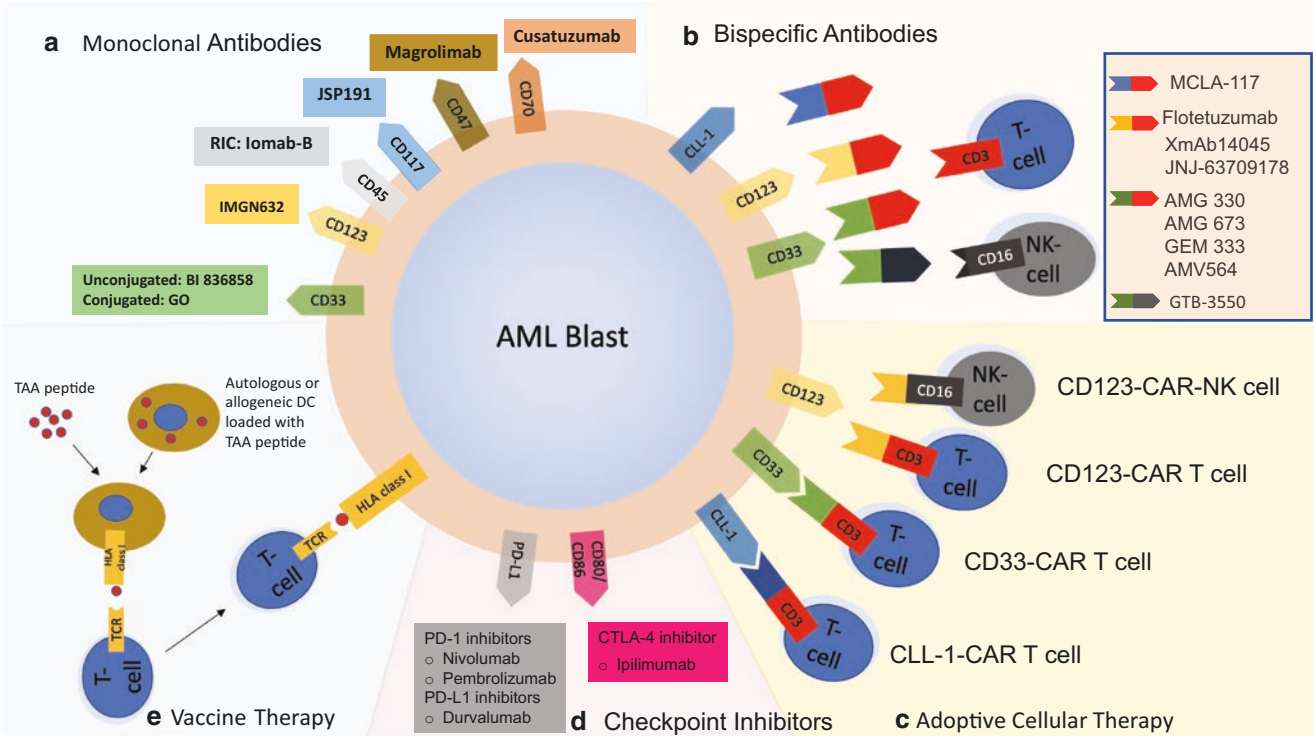

Fig. 15.1 An overview of immunotherapeutic targets for acute myeloid leukemia. (**a**) Monoclonal antibodies, (**b**) bispecific antibodies, (**c**) adoptive cellular therapy, (**d**) check point inhibitors, (**e**) vaccine therapy. *GO* gemtuzumab ozogamicin, *RIC* radioimmunoconjugate, *NK* natural killer, *CAR* chimeric antigen receptor, *CTLA-4* cytotoxic T-lymphocyte-associated protein 4, *PD1* programmed cell death protein 1, *PD-L1* programmed death-ligand 1, *TAA* tumor-associated antigen, *DC* dendritic cell

15.2 Mechanisms of Immune Escape

Multiple mechanisms contribute to AML immune escape. Some involve alterations in the innate immune system which have been linked to poor responses to chemotherapy or due to altered antigen expression or "immunoediting" of tumor cells resulting in immune evasion seen in other types of cancer [11]. In addition to altered tumor-associated antigen presentation, suppressive microenvironments may also contribute to the immune escape process in AML.

T Cell Number and Function

AML patients have higher percentage of T cells in the peripheral blood (PB) compared to healthy controls and increase percentage of T cells in the bone marrow (BM) correlated with treatment response and improved outcomes [12–14]. Studies from murine models of AML showed that AML progression was associated with increased $CD8^+$ T cells that co-expressed checkpoint inhibitory receptors (CIRs) PD-1 and TIM3, indicating T cells exhaustion, and suggested the potential positive therapeutic impact of checkpoint inhibitors [15, 16]. It was also observed that newly diagnosed AML patients have higher frequency of T cells co-expressing CIRs than healthy controls which was also observed during AML transformation indicating the role of CIRs in AML progression [17, 18]. T cells from newly diagnosed AML patients exhibit reduced levels of interferon-gamma (IFNg) indicating T cell dysfunction [19, 20]. Several studies demonstrated increased frequency and function of regulatory T cells (Tregs) in peripheral blood and bone marrow of AML patients [21–24]. Presence of Tregs was also associated with poor outcomes [23, 25]. In murine models of AML, Treg depletion was associated with improved effector T cell function and control of tumor progression [26].

Natural Killer (NK) Cells and AML

AML patients exhibit altered phenotypic and functional characteristics of NK cells which have been associated with treatment failure [27, 28]. NK cell-mediated killing is dependent on the balance between activating and inhibitory receptor signaling. Multiple studies have documented alterations in the expression of these receptors including an increase of inhibitory killer immunoglobulin-like receptors (KIRs) and a decrease in activating receptors such as NKp30 and NKp46 [29–31]. AML blasts can contribute to the defective NK cell function through the expression of inhibitory KIRs ligands and shedding of ligands for NKG2D [32–34].

Antigen Presentation in AML

Under selective immune pressure, post allo-HCT AML blasts display remarkable immunoediting resulting in altered antigen presentation and evasion of immune recognition. At least two groups showed remarkable downregulation of major histocompatibility complex (MHC) class II in AML blasts from patients who relapsed after allo-HCT and which could be restored after in vitro or in vivo exposure of IFNg [35, 36]. Similarly, complete loss of the mismatched HLA haplotype was observed in as many as one third of patients with AML relapsing after haploidentical allo-HCT [37, 38]. Others, using AML murine models, have demonstrated reduced dendritic cells frequency and function resulting in altered antigen presentation and T cell tolerance in AML [39].

AML Immunosuppressive Microenvironment

Myeloid-derived suppressor cells (MDSCs) are immature myeloid cells that exhibit immunosuppressive properties through release of indoleamine 2–3 dioxygenase (IDO), arginase (ARG1), reactive oxygen species (ROS), and nitric oxide [40]. In AML specifically, MDSCs appear to mediate T cell inhibition through V-domain Ig suppressor of T cell activation (VISTA) [41]. MDSCs can be induced by AML blasts through the release of MUC1 oncoprotein-derived extracellular vesicles, which inhibit T-cell proliferation and promote Th2 phenotype [42]. Association of MDSCs with outcome was observed in one report showing that increased numbers of MDSCs were associated with progression of myelodysplastic syndrome (MDS) to AML [43]. AML blasts can even assume features of MDSC and themselves create an immunosuppressive microenvironment through the release soluble factors such as IDO and ARG1 inhibiting T cell and NK cell proliferation and function [44, 45]. Notably, expression of IDO in AML was linked to poor outcomes [46].

15.3 Monoclonal Antibodies

Monoclonal antibody- (MoAbs) based therapies have been the center for the treatment of patients with hematological malignancies for over a decade. By targeting specific antigens, MoAbs have the ability to induce apoptosis directly in tumor cells or enhance the immune killing through antibody-dependent cellular cytotoxicity (ADCC). MoAbs can also be chemically conjugated to cytotoxic payloads which can then be internalized delivering the toxin to only those cells which express the specific receptor or surface protein to which the MoAb has been directed to. Multiple MoAbs targeting AML surface antigen have been developed for clinical application, but majority of these MoAbs did not pass the early phase clinical studies [47]. Ongoing clinical trials for MoAbs in AML are summarized in Table 15.1.

Table 15.1 Ongoing studies of immunotherapy in AML

Immunotherapy	Target	Agent	Study Design	NCT number/status
Unconjugated MoAb	CD33	BI 836858	Phase I/R/R AML and high-risk AML	NCT01690624/completed, no result
			Phase I/II, in combination with decitabine, untreated ≥65 year and R/R AML	NCT02632721/active, not recruiting
			Phase I//II, in combination with azacitidine, untreated AML ≥60 year	NCT03013998/recruiting
	CD47	Magrolimab	Phase I, as single agent for R/R AML, or with azacitidine for untreated AML or MDS and R/R AML or MDS.	NCT03248479/recruiting
			Phase Ib/II, plus venetoclax and azacitidine, R/R AML and untreated AML	NCT04435691/recruiting
	CD70	Cusatuzumab	Phase II, in combination with azacitidine, untreated AML	NCT04023526/active, not recruiting
			Phase I, in combination with azacitidine, untreated AML and high risk MDS	NCT04241549/recruiting
			Phase I, plus venetoclax and azacitidine, AML unfit for intensive chemotherapy	NCT04150887/recruiting
	CD117	JSP191	Phase I, in combination with low dose radiation and fludarabine for patient with AML or MDS undergoing Allo-HCT	NCT04429191/recruiting

(continued)

Table 15.1 (continued)

Immunotherapy	Target	Agent	Study Design	NCT number/status
Drug conjugated MoAb	CD33	Gemtuzumab ozogamicin	Phase II, AML with MRD	NCT03737955/recruiting
			Phase Ib, plus CPX-351, R/R AML	NCT03904251/recruiting
			Phase II, plus mitoxantrone and etoposide (MEGO), primary refractory AML	NCT03839446/recruiting
			Pilot study, plus Bu/CY for patients with high risk CD33+ AML or MDS undergoing Allo-HCT	NCT02221310/recruiting
			Phase I/II, plus conventional chemotherapy (cytarabine+daunorubicin) in combination with midostaurin, untreated AML with CBF or FLT3 mutation	NCT04385290/recruiting
	CD123	IMGN632	Phase I/II, CD123+ AML and other CD123+ hematologic malignancies	NCT03386513/recruiting
			Phase I/II, in combination with venetoclax and/or azacitidine for patients with CD123+ AML or monotherapy for MRD+ AML	NCT04086264/recruiting
Immunotoxin conjugated MoAb	CD123	SL-401 (tagraxofusp)	Phase I/II, as consolidation therapy for adverse risk AML in first CR, and/or +MRD	NCT02270463/completed, no result
			Phase I/II, R/R AML and BPDCN	NCT02113982/completed, no result
			Phase I, in combination with azacitidine with/without venetoclax for R/R AML, untreated AML or high risk MDS	NCT03113643/recruiting
Radioimmuno-conjugate MoAb	CD45	131I-BC8 (Iomab-B)	Phase III, in combination with low dose radiation and fludarabine for patient with R/R AML undergoing Allo-HCT	NCT02665065/recruiting
			Phase I, in combination with flu/cy/2 Gy TBI and posttransplant cy for advance AML, high risk MDS or ALL undergoing haploidentical transplant	NCT00589316/active, not recruiting
		211At-BC8	Phase I/II, in combination with low dose radiation and fludarabine for patient with high risk AML, MDS, and ALL undergoing Allo-HCT	NCT03128034/recruiting
			Phase I/II, in combination with flu/cy/2 Gy TBI and posttransplant cy for advance AML, high risk MDS or ALL undergoing haploidentical transplant	NCT03670966/recruiting
	CD33	Lintuzumab-Ac225	Phase I, plus cladribine, cytarabine, G-CSF, and mitoxantrone in R/R AML	NCT03441048/recruiting
			Phase I/II, plus venetoclax for R/R AML	NCT03867682/recruiting
CAR T cells	CD123	MB-102 (CD123. CD28.CD3ζ.EGFRt)	Phase I/II, R/R AML, BPDCN, and high risk MDS	NCT04109482/recruiting
		3rd generation CD123. CD137.CD28 signaling domains	Phase I, R/R AML	NCT04014881/recruiting
		UCART123	Phase I, R/R AML	NCT03190278/recruiting
		CD123.CD3ζ.4-1BB signaling domains	Phase I, R/R AML	NCT03766126/active, not recruiting
	CD123/ CLL1	CD123/CLL1 CAR-T cells	Phase II, R/R AML	NCT03631576/recruiting
	CLL-1	CLL-1.CD3ζ.CD28 signaling domains	Phase I, R/R AML	NCT04219163/recruiting
	CD33	CD33CART	Phase I/II, in children and adolescents/young adults with R/R AML	NCT03971799/recruiting
	CD38	CART-38	Phase I, CD38+ R/R AML	NCT04351022/recruiting
	NKG2D-ligands	NKR-2	Phase I/II, NKR-2 administration after a non-myeloablative preconditioning chemotherapy in R/R AML or MDS	NCT03466320/active, not recruiting
	FLT-3	AMG 553	Phase I, FLT3 mutated R/R AML	NCT03904069/not yet recruiting

Table 15.1 (continued)

Immunotherapy	Target	Agent	Study Design	NCT number/status
Bi-specific antibodies	CD33	AMG 330 (CD33 × CD3 bi-specific Ab)	Phase I, R/R AML	NCT02520427/recruiting
			Phase Ib, in combination with pembrolizumab, R/R AML	NCT04478695/active, not recruiting
		AMG 673 (CD33 × CD3 bi-specific Ab)	Phase I, R/R AML	NCT03224819/recruiting
		AMV 564 (CD33 × CD3 tandem Ab)	Phase I, as monotherapy or in combination with pembrolizumab for R/R AML	NCT03144245/active, not recruiting
	CD123	XmAb14045 (CD123 × CD3 bi-specific Ab)	Phase I, for CD123+ hematological malignancies	NCT02730312/recruiting
		JNJ-63709178 (CD123 × CD3 DuoBody)	Phase I, R/R AML	NCT02715011/recruiting
		Flotetuzumab (CD123 × CD3 DART)	Phase I/II, R/R AML, intermediate-2/high risk MDS	NCT02152956/recruiting
			Phase I, in children, adolescents, and young adults R/R AML	NCT04158739/recruiting
			Phase II, for relapsed AML following Allo-HCT	NCT04582864/not yet recruiting
	CLL-1	MCLA-117 (CLL-1 × CD3 bi-specific Ab)	Phase I, R/R AML and elderly untreated AML patients with high-risk cytogenetics	NCT03038230/recruiting

MoAb monoclonal antibody, *R/R* relapsed/refractory, *AML* acute myeloid leukemia, *MDS* myelodysplastic syndrome, *allo-HCT* allogeneic hematopoietic cell transplant, *Flu* fludarabine, *Bu* busulfan, *Cy* cyclophosphamide, *CBF* core binding factor, *FLT3* fms-like tyrosine kinase 3, *MRD* measurable residual disease, *BPDCN* blastic plasmacytoid dendritic cell neoplasm, *Gy* Gray, *TBI* total body irradiation, *ALL* acute lymphoblastic leukemia, *CAR T Cells* chimeric antigen receptor T cells, *Ab* antibody, *DART* dual affinity retargeting

15.3.1 Anti-CD33 Monoclonal Antibodies

One of the most attractive AML targets is CD33, which is a myeloid differentiation antigen expressed in more than 90% of AML blasts as well as normal early myeloid progenitors [48–50]. The most tested unconjugated (naked) anti-CD33 MoAb is lintuzumab (HuM195, SGN-33), which failed in two randomized trials to show any survival benefit when combined with intensive chemotherapy or low dose cytarabine in relapsed/refractory (R/R) AML, halting further clinical development [51, 52]. BI 836858 is another unconjugated anti-CD33 MoAb that has an engineered Fc domain designed to optimize interaction with the Fc receptor (CD16A) on NK cells that can exert enhanced NK cell ADCC against AML blasts through upregulation of NKG2D ligands after in vivo exposure to decitabine [53]. It is currently under clinical investigation (NCT03013998, NCT02632721), with early data from a study of biomarker-based treatment of AML (Beat AML study) showing tolerability and antileukemia activity when combined with azacitidine [54].

The most successful and best studied MoAb targeting CD33 is gemtuzumab ozogamicin (GO), a humanized CD33 antibody conjugated to the toxin calicheamicin. It was recently reapproved as a single agent or in combination with chemotherapy for treatment of newly diagnosed adult AML patients and relapsed/refractory adult and pediatric AML patients [55–57]. The survival benefit of adding GO to "7 + 3" induction chemotherapy for AML was clearly demonstrated and adopted as standard of care for the induction chemotherapy for patients with core binding factor (CBF) AMLs [58]. Further trials are ongoing to optimize the use of GO in combination with other AML therapeutic agents for R/R AML or as single agent for AML with measurable residual disease (MRD) (NCT02473146, NCT03904251, NCT02221310, NCT03737955). SGN33A (Vadastuximab Talirine), a newer antibody-drug conjugate targeting CD33, contains highly active synthetic DNA cross-linking pyrrolobenzodiazepine dimer with a more potent cytotoxic payload [59]. Early clinical testing showed promising results in combination with hypomethylating agents (HMA), which showed high rate of complete remission (CR) and CR with incomplete count recovery (CRi) of 70% in untreated AML patients [60]. However, the interim analysis from the randomized study (CASCADE trial) showed excess mortality in SGN33A arm which resulted in the closure of the study and termination of all ongoing SGN33A trials [61].

15.3.2 Anti-CD123 Monoclonal Antibodies

CD123 is low affinity α-chain subunit of the interleukin 3 (IL3) receptor that associates with the common beta signaling subunit of the GM-CSF/IL-3/IL-5 receptor. After binding to IL-3, specific signals are transmitted in hematopoietic

stem and progenitor cells resulting in cellular differentiation and proliferation [62]. CD123 is expressed in the majority of normal myeloid precursors and abnormal AML blasts. More importantly, it is overexpressed in leukemia stem cells (LSC), but not in normal hematopoietic stem cell (HSC) [63]. Multiple unconjugated antibodies targeting CD123 were developed for treatment on AML patients and showed limited activity in clinical studies. For example, CSL-362 (talacotuzumab), a fully humanized CD123 antibody with enhanced affinity for CD16A (enhancing ADCC), failed to show any significant clinical efficacy as a single agent in patients with AML or MDS and a study combining CSL-362 with decitabine versus decitabine alone was stopped early (NCT02472145) [64]. In contrast, anti-CD123 ADC (IMGN632) with an indolino-benzodiazepine payload showed early promising clinical activity as a single agent in R/R AML with objective response rates of 20% in heavily pretreated patients. These data have led to the subsequent development of IMGN632 as single agent or in combination with other agents for the treatment of AML, MDS, and other CD123+ hematologic malignancies [65]. Of note, SL-401, a recombinant IL-3 fused to a truncated diphtheria toxin payload directed to CD123 (tagraxofusp/Elzonris), was approved by the FDA for the treatment of patients with blastic plasmacytoid dendritic cell neoplasms (BPDCN) [66]. SL-401 was well tolerated with modest to moderate capillary leak syndrome, but was also associated with only modest antileukemic activity in patients with R/R AML and MDS in a phase I study [67]. A second study using SL-401 as a consolidation therapy for high-risk AML with MRD+ disease was completed recently [68]. Ongoing studies are testing the combination of SL-401 with azacitidine and venetoclax in R/R AML or untreated AML patients unfit for intensive therapy (NCT03113643).

15.3.3 Other Monoclonal Antibodies Targets

CD47, a transmembrane protein that is universally expressed in normal tissues and overexpressed in many malignant cells, acts as a "don't eat me" signal to macrophages upon interaction with its ligand signal-regulatory protein α (SIRPα) on macrophages [69]. Magrolimab is an anti-CD47 antibody that blocks the interaction with SIRPα and promotes macrophage phagocytosis. Results from a phase I study were presented at the 2020 American Society of Clinical Oncology meeting and demonstrated promising clinical activity when combined with azacitidine in patients with newly diagnosed MDS and AML (CR/CRi rate 56% in AML cohort) with high response rate (75% CR/CRi) in TP53 mutated AML patients [70]. CD27 (a TNF family receptor) and its ligand CD70 are both highly expressed in the AML blasts and leukemia stem cells and the level of soluble CD27 is associated with poor outcomes [71]. The use of anti-CD70 MoAb in murine AML xenografts led to a delay in tumor progression and improved survival. ARGX-110 (cusatuzumab) is a first-in-class high affinity humanized Fc enhanced monoclonal antibody of camelid origin targeting CD70 that was recently evaluated in a phase I study in combination with azacitidine in newly diagnosed AML patients [72, 73]. Among the 11 evaluable patients, CR/CRi was achieved in 9 patients (82%). Multiple other MoAb radioimmunoconjugates developed for treatment of AML have demonstrated promising activity in AML, especially those targeting CD33 or CD45 as reviewed by Williams et al. [74]. Iomab-B (^{131}I-BC8), an ^{131}I-radiolabeled anti-CD45 antibody that showed promising results in an early phase trial, is now being studied in a phase III trial in conjunction with a reduced intensity conditioning (RIC) regimen containing fludarabine and 2 Gy total body irradiation (TBI) prior to allo-HCT as salvage therapy for R/R AML in comparison to conventional salvage chemotherapy followed by standard allo-HCT (NCT02665065) [75]. Interim results reported in the recent 2020 American Society of Hematology annual meeting showed that 91% of patients randomized to Iomab-B arm achieved full donor chimerism at day 100 post allo-HCT despite median bone marrow blast of 26% at baseline. Furthermore, 83% of patients randomized to conventional therapy failed salvage chemotherapy and 60% of them crossed over and received Iomb-B followed by allo-HCT [76].

Anti-CD117 MoAb (JSP191, previously AMG191) is a humanized unconjugated antibody that binds human CD117 (c-Kit), a receptor tyrosine kinase expressed on the surface of HSC and progenitor cells and was able to facilitate donor engraftment (in four out of six evaluable patients) when used as the sole conditioning agent during phase I first in human study for patients with severe combined immunodeficiency undergoing allo-HCT [77]. Currently, a phase I study is ongoing to evaluate safety of JSP191 in conjunction with low dose TBI and fludarabine for patients with AML and MDS undergoing allo-HCT (NCT04429191). Recently, anti-CD45-Amanitin and anti-CD117-Amanitin ADCs have shown promising antileukemia activity in AML xenograft murine models [78]. Anti-CD45-saporin (CD45-SAP) and anti-CD117-saporin (CD117-SAP) are ADCs conjugated to saporin, a plant toxin belonging to the ribosome-inactivating protein family, and have also shown to enable donor chimerism in murine allogeneic mismatched transplant [79].

15.4 Adoptive Cellular Therapy

15.4.1 Chimeric Antigen Receptor T Cells (CAR T Cells)

The recent approval of the three CD19-directed CAR T cell products for B cell malignancies has encouraged others to

expand CAR T cells into the myeloid malignancies [80]. Multiple CAR T cell products have been tested in AML with limited success so far. Most have targeted CD33, CD123, or CLL-1. One of the first CAR T cell products tested in AML demonstrating some activity with acceptable toxicities is CAR T-directed against Lewis Y antigen, an antigen that is widely expressed in multiple malignancies with limited expression on normal tissues [81]. Multiple groups investigated CD33-directed CAR T cells and showed robust preclinical activity; however, very few progressed into clinical testing and only one study targeting CD33 alone is currently active and recruiting patients (NCT03971799) [82–86]. So far, there is only one case report of an AML patient receiving CD33-CAR T cell who showed transient reduction in marrow blasts after infusion with significant cytokine release syndrome (CRS) before progression of his disease [87].

CD123 is another attractive target for CAR T cells in AML with multiple in vitro and preclinical studies demonstrating anti-AML cytotoxicity [88, 89]. A promising result was recently reported for CD123-directed CAR T cell (MB-102) with a CR seen in one patient who received the higher dose (2×10^8) without dose limiting toxicity. The expansion phase study is still ongoing and this therapy received orphan drug designation for both AML and BPDCN from the United State FDA [90]. Multiple ongoing trials for CAR T cell targeting CD123 are currently open and recruiting AML patients, summarized in Table 15.1.

CLL-1 is another target for CAR T cell due to the high expression level in AML blasts and the lack of expression in normal human hematopoietic stem cells. CLL-1 is also co-expressed in majority of CD33 positive blasts [91]. For that reason, a "compound or bispecific CAR T cell" targeting both CD33 and CLL-1 was developed in which tandem CAR constructs were linked via a 2AP linker permitting the expression of two CARs (one directed to CLL-1 and the other directed to CD33) in the same T cell. The initial study suggested promising clinical activity reported where seven of nine patients with AML went into MRD negative CR 28 days after treatment [92]. Early phase studies testing NKG2D as a CAR T cell target in AML also demonstrated some modest antileukemia activity with acceptable safety and tolerability [93–95].

Other approaches include the use of alternative effector cells such a natural killer (NK) cell line targeting CD33 (CD33-CAR-NK cell) in R/R AML patients [96]. While early results from CAR T cell therapy in AML are encouraging, it is clear that lack of AML-specific targets makes the application of this therapy difficult to extend beyond the early phase testing due to a large "sink" of the target in non-AML cells such as monocytes, tissue macrophages, dendritic cells and granulocytes (CD123, CD33 and CLL-1) and endothelial cells (CD123), prolonged cytopenias due to the expression of many of these targets on hematopoietic stem and progenitor cells, and cytokine release syndrome (CRS) secondary to direct targeting of myeloid cells such as monocytes, the primary producers of IL-6, and many other inflammatory cytokines [97]. Another challenging aspect is the ability of AML cells to provide an immunosuppressive environment for not only CAR T cell therapy, but for other forms of immunotherapy. Many groups are focused on overcoming these barriers in targeting AML with immunotherapy, reviewed recently by Mardiana and Gill [98].

15.4.2 Antigen-Specific Cytotoxic T Cells

Another form of cellular therapy holding promise for AML is by development of T cells transduced with T cell receptor (TCR) constructs specifically recognizing tumor-associated antigens (TAA) presented in the context of a specific HLA class I and class II haplotypes. The advantage of such approach is the ability to recognize intercellular antigens that are presented by HLA molecules [99]. One of the promising targets is Wilm's Tumor 1 (WT1) antigen that was successfully targeted with both lack of toxicity and potent antileukemia effects in both preclinical and in early phase clinical studies [100–103]. Recently, Chapuis et al. published a novel strategy to prevent relapse post allo-HCT where WT1-specific TCR transduced CD8+ T cells from healthy donors were infused prophylactically into 12 high risk AML patients post allo-HCT. After a median follow-up of 44 months, none have relapsed with estimated relapse-free survival of 100% versus 54% for matched controlled group [104]. Another technique to generate T cells that recognized AML targets is through the ex vivo expansion of TAA-specific cytotoxic T cells using a peptide mixture from multiple TAA aiming to broaden cytotoxic activity of T cells in an effort to eliminate leukemic blasts. Preliminary results suggested some clinical activity of this approach when cytotoxic T cells that are expanded ex vivo to recognized TAA (WT1, PRAME, and Survivin) were infused into 11 patients with hematological malignancies relapsing after allo-HCT. Overall the infusion was well tolerated with only one patient experiencing infusion-related reactions (developed GVHD) and four of the five AML patients achieved CR [105]. Studies are ongoing to explore this therapeutic approach in patients with R/R AML or AML with MRD (NCT02494167, NCT02203903).

15.4.3 Adoptive NK Cell Therapy

NK cells are $CD3^-$ and $CD56^+$ lymphocytes and characterized by their ability to exert cytotoxic function against tumor cells without the need for prior antigen recognition or clonal expansion. NK cells express multiple inhibitory and activating receptors that mediate and modulate the activation state and

killing function of NK cells. KIRs are the most important inhibitory receptors that recognize self-major histocompatibility complex (MHC) class I molecules and lead to inhibition of killing function. Multiple reports showed that NK cell-induced killing is primarily mediated through KIRs mismatched with their ligands on leukemia cells [106]. Additional insights into the derivation, activation, function, and phenotype of NK cells have accumulated over the past years of adoptive NK cell therapy in AML as reviewed by Hansrivijit et al. [107]. It is known that NK cells are less likely or unable to cause GVHD and that haploidentical donor is probably the best source of NK cells. In a landmark study reported more than a decade ago, interleukin-2 (IL-2) was administered to enhance in vivo expansion after infusion of haploidentical NK cells resulting in clinical remissions in 5 of 19 refractory AML patients [108]. Since efficacy of NK infusions may be hampered by concurrent stimulation of host regulatory T cells with IL-2, a recombinant human IL-15 was substituted for in vivo NK cell expansion resulting modestly improved rates of CR (32%) but with significant CRS [109]. Due to concerns of reduced NK cell persistence after infusion in vivo, cytokine-induced memory-like NK cells (CIML-NK) from haploidentical donors have shown promise where ex vivo incubation with IL-2, IL-15, and IL-18 for 12–18 h resulted in remarkable alteration of NK cell function resulting in enhanced expansion, persistence, and antileukemia activity in vivo, with four of nine patients with relapsed AML achieving a CR [110].

Highly functional NK cells were also generated by ex vivo incubation with feeder cells expressing membrane-bound interleukin 21. These expanded donor NK cells were infused before and after haploidentical HCT for high risk myeloid malignancies [111]. In this phase I study, 13 patients were infused with no dose-limiting toxicities observed and only one patient relapsed. Similarly, NK cells that are primed with leukemia cell line lysate CNDO-109 produced remarkable cytotoxic function. This approach was recently tested in a phase I study using CNDO-109 activated NK cells from haploidentical donors infused after lymphodepleting chemotherapy in 12 high risk AML patients in first CR; no dose-limiting toxicities were seen and durable remissions observed in three patients [112]. Overall, NK cell therapy holds promise for the treatment of relapsed or high risk AML patients due to low toxicity and clearly established efficacy. Thus far, only small early phase studies have been reported, although many studies are currently ongoing (NCT04347616, NCT04220684, NCT04209712, NCT04166929, NCT03068819, NCT02782546, NCT03955848).

15.5 Bispecific Antibodies

Bispecific antibodies are genetically engineered constructs that contain at least two antigen binding sites with one site targeting a TAA on a cancer cell and the other a surface marker on an effector cell such as a T cell. The target on T cells is usually the T cell receptor (TCR/CD3ε) resulting in T cell proliferation and activation in close proximity to target cell resulting in target cell killing via perforin/granzyme-induced apoptosis [113]. The antitumor activity of bispecific antibodies is HLA-independent and therefore the use of bispecific antibodies overcomes the immune escape mechanisms resulting from MHC downregulation by tumor cells [114]. After the success of bispecific antibodies in the treatment of B-cell malignancies, similar therapeutic approaches are being developed in AML. Bispecific T cell engagers (BiTEs) were the first bispecifics that were developed and used in clinical trials. BiTE consists of two single-chain Fv fragments (ScFv) of different binding specificities (CD3ε and an AML target antigen such as CD33 for example) aligned as a single polypeptide chain by a flexible linker between them. BiTEs are relatively small molecules with a molecular weight of approximately 55 KD. This molecular weight is below the renal clearance threshold, thus requiring continuous infusion for maintenance of appropriate plasma levels for clinical responses. Dual affinity retargeting (DART) molecules consist of two polypeptide chains, each bearing a heavy chain (VH) specific for one target and a light chain (VL) specific for the other target. For example, in case of CD3xCD123 DART, one polypeptide chain contains VL of anti-CD3 and VH of anti-CD123 and the second polypeptide chain contains VL of anti-CD123 and VH of anti-CD3 (Fig. 15.2b). The two polypeptide chains are covalently linked via a disulfide bond resulting in a higher degree of stability and slightly longer in vivo half-life. In spite of the slight increased stability and in vivo-half-life, DARTs still need to be infused IV continuously. A "size-enhanced" CD3xCD19 DART (covalently linked to albumin to increase its size and to reduce its renal clearance) was found to induce increased B cell killing and T cell activation compared with a standard CD3xCD19 BiTE in a murine model [115]. Other forms of bispecific antibodies such as full length antibodies and trivalent and tetravalent antibodies have been also developed (Fig. 15.2) [116–119]. These have much longer in vivo half-lives and can be given by single IV or SC injections weekly, every 2 weeks or monthly.

15.5.1 CD33 Targeted Bispecific Antibodies

CD33 is expressed on myeloid progenitor cells and AML blasts but not on normal HSC, which makes it an attractive target for bispecific antibodies [48, 49, 120]. AMG 330 is a human BiTE with N-terminal and C-terminal ScFvs specific for CD33 and CD3ε respectively. Ex vivo incubation of AMG 330 with human AML samples resulted in expansion of residual autologous T cells and killing of AML blasts even

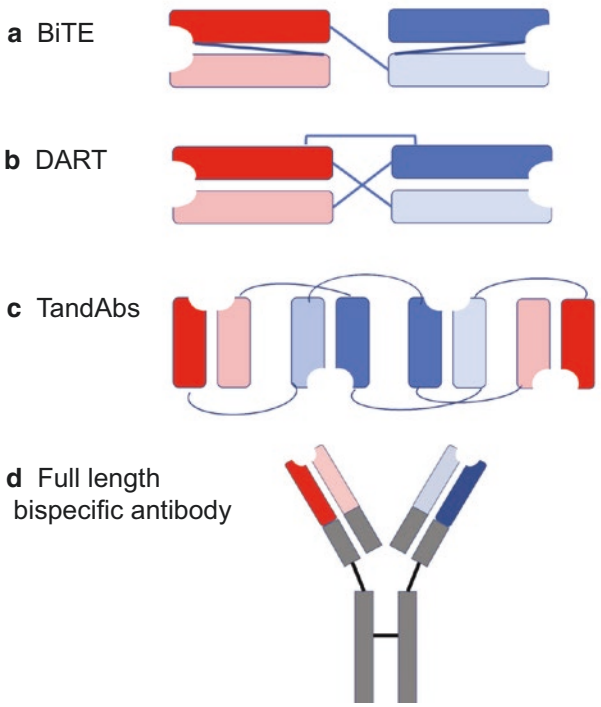

Fig. 15.2 Examples of bispecific antibody platforms. *BiTE* bispecific T cell engagers (**a**), *DART* dual affinity retargeting agents (**b**), *TandAbs* (**c**) and full length bispecific antibody (**d**)

at low effector to target (E:T) ratios [121]. In a murine model, AMG 330 prolonged the survival of NOD/SCID mice injected with human AML cell line and activated human T cells from a healthy donor [122]. The safety of AMG 330 was tested in a first in human phase 1 study which recruited 55 patients with R/R AML at the latest update [123]. The majority of included patients were heavily pretreated and about 40% of patients received prior allo-HCT. The drug was administered as a continuous 14–28 days intravenous infusion with a plan to give up to 5 cycles. CRS was the most common adverse effect and developed in two thirds of patients and was severe in 13% of patients. The CRS frequency and severity correlated with the dose level and baseline leukemic burden. Pretreatment with a single dose of corticosteroid and step up dosing strategy were applied to mitigate CRS. Doses up to 720 μg/day were associated with an acceptable safety profile and the minimal effective dose (MED) was 120 μg/day. Overall responses (ORR) were observed in 8/42 patients (19%) including 3 CR and 4 CRi. These preliminary results in heavily pretreated patients are encouraging and support conducting additional clinical trials. AMG 330 requires a 14–28 day continuous intravenous infusion because of its short half-life. One approach to prolong the half-life of CD3xCD33 BiTEs is to add an Fc fragment resulting in extended half-life BiTE (EHL BiTE) molecule and allow more extended dosing schedule. AMG 673 is an EHL BiTE in which CD3xCD33 BiTE is genetically fused to the N-terminus of a single-chain IgG Fc region. AMG 673 was tested in a phase 1 study in patients with relapsed or refractory AML [116]. The drug was administered as two short intermittent intravenous infusions during a 14-day cycle. The study showed that AMG 673 is associated with acceptable safety profile and antileukemia activity.

GEM 333 is another CD3xCD33 BiTE molecule in which the commonly used glycine serine linker was replaced with a novel linker domain to avoid unexpected recombination in the antibody construct. Preclinical studies showed antileukemia activity both in vitro and in a NSG mouse model and is currently being evaluated in a phase 1 study [124]. Tandem tetravalent bispecific antibody (TandAbs) is another bispecific antibody platform with an extended in vivo half-life. These high-avidity TandAbs provide two binding sites for each antigen with a molecular weight of approximately 106 kDa which exceeds the renal clearance threshold, and therefore, have a longer half-life compared to smaller BiTEs. In preclinical studies, TandAbs induced potent antileukemia effect regardless of disease stage (newly diagnosed or relapsed/refractory), cytogenetic or molecular profile, or the level of CD33 expression [125]. Preliminary data from phase 1 study showed that AMV564, a novel CD33 TandAb, is well-tolerated and demonstrates antileukemic activity [117]. No grade 3 or higher CRS was observed with the use of a lead-in dose escalation schedule. Immune escape through the activation of PD-1/PD-L1 axis is one of the resistance mechanisms that limits the antileukemia activity of BiTE, DART, and TandAb molecules. PD-L1 is expressed at a low intensity in only 16% of AML patients. Treatment of primary AML cells with AMG 330 induced a significant upregulation of PDL1 on AML cells and, to a lesser extent, an upregulation of PD-1 on T cells [126, 127]. A novel bifunctional checkpoint inhibitory T-cell–engaging (CiTE) antibody was developed by fusing the extracellular domain of PD-1 to a CD3 × CD33 bispecific T-cell engager [128]. The PD-1 in this construct binds PD-L1 with low affinity and is not expected to interact with PD-L1 on CD33 negative cells. This feature restricts the binding of CiTE antibody to CD33 + PD-1+ cells and the systemic side effects of PD-1/PD-L1 inhibition as one sees with checkpoint inhibitors given systemically. Preliminary preclinical studies with this novel tri-specific agent were promising.

15.5.2 CD123 Targeted Bispecific Antibodies

CD123 is expressed in 60–80% of patients with AML and high expression is associated with poor outcomes [129]. One of the advantages of CD123 as a target for bispecific antibody therapy is that it is expressed on the LSC but to a lesser extent on the normal HSC [130]. Another advantage is that high expression of CD123 is enriched in refractory AML patients [131]. XmAb14045 and JNJ-63709178 are

CD3xCD123 bispecific antibodies that contain modified Fc domains (functional Fc domain in XmAb14045 and silenced Fc domain in JNJ-63709178). Both agents are currently being evaluated in clinical trials [48, 117]. Severe CRS was observed with both agents resulting in temporary suspension of the studies by FDA. More mature data from phase I/II clinical trial are available for flotetuzumab (MGD006), which is a CD3xCD123 DART molecule that induced T-cell activation and proliferation with potent killing of CD123+ AML blasts in vitro and in vivo preclinical studies [132]. Flotetozumab was tested in phase I clinical trial with an expanded cohort at the recommended phase 2 dose (RP2D) that recruited 88 patients with relapsed/refractory AML, 46 of them received the RP2D (500 ng/kg/day) [133]. Complete remission was achieved in 18% of patients who received the phase 2 dose and one-year survival was 50% in these patients. Of note is that of those primary refractory AML patients and those with TP53 mutations, a significantly higher CR/CRi rates of 30–40% were observed validating the observations that those same patients had an "inflamed" microenvironmental RNA signature using nanostring arrays [134, 135]. As expected, CRS was observed in majority of patients and was severe (grade 3) in only 8%. No grade 4 CRS or neurotoxicity was observed. Interestingly, flotetuzomab was not associated with significant prolonged myelosuppression consistent with the fact that CD123 is not expressed on normal HSC.

15.5.3 Other Bispecific Antibody Approaches

CD3xCLL1 bispecific antibody is another form of targeted therapy and still under clinical development. CLL-1 belongs to C-type lectin-like receptor family and expressed on AML cells but not on normal HSCs. MCLA-117 is a human full length IgG1 CD3xCLL1 bispecific antibody. MCLA-117 contains a genetically modified Fc region to provide an extended in vivo half-life. Preclinical studies showed that MCLA-117 was highly active against CLL-1–expressing AML tumor cell lines, despite low effective effector-to-target (E:T) ratios [118]. Phase I clinical study (NCT03038230) of MCLA-117 to evaluate the safety, tolerability, and preliminary efficacy in adult AML patients is ongoing.

Bispecific (BiKE) and trispecific (TriKE) killer cell engagers utilizing NK cells were developed as a new potential therapeutic strategy for the treatment of AML. CD16/CD33 BiKE, also known as 1633, was shown to induce NK activation after in vitro incubation with AML cell line [136]. It also induced killing of target AML cells. A major disadvantage of 1633 is the inability to induce robust NK cell proliferation. Therefore, CD16/CD33/IL-15 TriKE (161533) was developed through insertion of a modified IL-15 crosslinking agent between CD16 and CD33 scFvs [137]. Addition of IL-15 crosslinker to CD16/CD33 BiKE promotes further NK cell activation and proliferation. GTB-3550 (CD16/CD33/IL-15 TriKE) is currently being investigated in a phase I/II clinical study (NCT03214666) for the treatment of CD33-expressing high risk myelodysplastic syndromes, R/R acute myeloid leukemia, and advanced systemic mastocytosis.

In summary, these studies of bispecific antibodies show promising activity in advanced AML. CRS is the main toxicity and can be mitigated and managed by step-wise dose escalation and the early use of steroid and tocilizumab therapy. The role of bispecific antibodies in upfront therapy as a part of induction regimen has not been studied yet. In B-ALL, blinatumomab (CD3xCD19 BiTE) was able in induce MRD negative status in patients with MRD positive CR after induction therapy [138]. Additionally, blinatumomab combined with dasatinib in the absence of any chemotherapy during induction or consolidation/intensificaiton therapy in previously untreated Philadelphia positive B cell ALL patients was associated with excellent outcomes [139]. Similar approaches to utilize bispecific antibodies in AML are warranted in future studies. Ongoing clinical trials for bispecific antibodies in AML are summarized in Table 15.1.

15.6 Checkpoint Inhibitors

Checkpoint inhibitors (CPIs) are antibodies directed against T-cell inhibitory signals to enhance the immune response against tumor cells. Increased expression of immune checkpoints (ICs) like PD-1 on CD8+ T cells or PD-L1 on AML blast has been observed in BM samples from patients with relapse or refractory AML compared with newly diagnosed AML patients [140]. This finding suggests the role of ICs in the resistance mechanism against AML therapies. Higher expression of ICs is associated with poor outcome in AML patients [141].

Given the success of CPIs in the treatment of solid malignancies, several CPIs have been tested in AML. Among several immune regulating interactions, PD-1/PD-L1 and CTLA-4/(CD80/CD86) axes are the more commonly used targets for CPIs in clinical development. Examples of CPIs include PD-1 inhibitors (nivolumab, pembrolizumab, and pidilizumab), PD-L1 inhibitors (atezolizumab, avelumab, and durvalumab), and CTLA-4 inhibitors (ipilimumab and tremelimumab). CPIs have limited clinical activity in relapsed or refractory AML if given as single agent therapy [142]. In posttransplant relapse setting, ipilimumab resulted in a CR in four patients with extramedullary AML in a small study [143]. In this study, ipilimumab was associated with severe life-treating immune-related adverse events and GVHD resulted in treatment discontinuation in some patients.

Given the limited activity of monotherapy, CPIs are being evaluated in other approaches like in combination with che-

motherapy or hypomethylating agents as upfront therapy, in relapse setting, as maintenance therapy in high risk AML patients, or for treatment of MRD positive disease. Correlative studies showed that treatment with hypomethylating agents upregulates PD-L1, PD-L2, PD-1, and CTLA4 expression on AML and MDS patients [144]. This provided a rationale for hypomethylating agent and CPIs in combination in clinical trials. In a single arm study, the overall response rate (ORR) of azacytidine and nivolumab combination in relapsed AML and high risk MDS was 33% and complete remission (CR + CRi) was achieved in 15% of patients [14]. Another study randomized previously untreated AML and MDS patients who were unfit for aggressive therapy to receive azacytidine alone or in combination with durvalumab. No statistically significant difference in ORR between treatment arms was observed [145]. The feasibility and efficacy of adding a CPI to induction chemotherapy as upfront therapy was evaluated in a phase 2 single arm study [146]. In this study, nivolumab was combined with idarubacin and cytarabine in 44 previously untreated AML patients. The ORR was observed in 34 of 44 (78%) patients, including 28 (64%) complete responses and 18 patients (53%) who achieved MRD negative status after induction. The median duration of response was 18.5 months. Interestingly, 26% of patients who proceeded with allo-HCT after induction therapy developed grade 3–4 acute GVHD. Similar findings were observed in patients with Hodgkin lymphoma who received SCT after CPI therapy. This complication can be prevented by delaying SCT and adding ATG to the conditioning regimen. While the results of some of these studies are encouraging, the use of CPIs cannot be recommended as a standard therapy for AML at present time. Ongoing studies in various settings should

Table 15.2 Ongoing studies of checkpoint inhibitors in AML

Checkpoint inhibitors	Treatment combination	Study design	NCT number/status
Nivolumab (anti-PD1)	Monotherapy	Phase II, randomized (nivolumab vs. observation) in AML patients in first CR/CRi after induction and consolidation chemotherapy; except young (<60 years) AML patients in European LeukemiaNet favorable group	NCT02275533/active, not recruiting
		Phase II, AML in first CR and at high risk of relapse	NCT02532231/recruiting
		Phase I, high-risk patients with MDS and AML after Allo-HCT using posttransplantation cyclophosphamide	NCT04361058/recruiting
	Azacitidine with or without ipilimumab	Phase II, R/R AML or untreated AML	NCT02397720/recruiting
	Azacitidine	Phase I/II, pediatric R/R AML	NCT03825367/recruiting
	Ipilimumab	Phase I, post Allo-HCT for patient with high risk or R/R AML and MDS	NCT03600155/recruiting
Pembrolizumab (anti-PD1)	Monotherapy	Phase II, for AML patients in CR and age > 60 years	NCT02708641/active, not recruiting
		Phase I, AML, MDS, or mature B cell lymphomas that have relapsed following Allo-HCT	NCT02981914/recruiting
		Phase Ib, AML, MDS, or ALL that have relapsed following Allo-HCT	NCT03286114/recruiting
	Azacitidine	Phase II, R/R AML or untreated AML age ≥ 65 years	NCT02845297/active, not recruiting
		Phase II, patients with NPM1 mutated AML and + MRD	NCT03769532/recruiting
	Decitabine	Phase I, untreated or R/R AML and MDS	NCT03969446/recruiting
	High dose cytarabine	Phase II, R/R AML	NCT02768792/active, not recruiting
Ipilimumab (anti-CTLA4)	Decitabine	Phase I, R/R AML or MDS	NCT02890329/recruiting
	CD25hi Treg depleted DLI	Phase I, AML, MDS, or MPN that have relapsed following Allo-HCT	NCT03912064/recruiting
Atezolizumab (anti-PD-L1)	Guadecitabine	Phase Ib, untreated or R/R AML unfit for intensive therapy	NCT02892318/completed, no result
	Gilteritinib	Phase I/II, R/R FLT3 mutated AML	NCT03730012/recruiting
Avelumab (anti-PD-L1)	Multiple arms combination: Azacitidine, venetoclax, GO, anti-OX40 antibody	Phase Ib/II, R/R AML	NCT03390296/recruiting

PD1 programmed cell death protein 1, *R/R* relapsed/refractory, *AML* acute myeloid leukemia, *MDS* myelodysplastic syndrome, *allo-HCT* allogeneic hematopoietic cell transplant, *CR* complete response, *CRi* complete response with incomplete count recovery, *NPM1* nucleophosmin 1, *CTLA-4* cytotoxic T-lymphocyte-associated protein 4, *DLI* donor lymphocyte infusion, *MPN* myeloproliferative neoplasm, *PD-L1* programmed death-ligand 1, *GO* gemtuzumab Ozogamicin, *FLT3* fms-like tyrosine kinase 3, *MRD* measurable residual disease, *ALL* acute lymphoblastic leukemia

define the role of CPI in the treatment of AML, summarized in Table 15.2.

15.7 Vaccine Therapy for AML

Two forms of vaccine therapy have been used in several studies, TAA peptide-based and cellular/dendritic cell- (DC) based vaccine therapies (Fig. 15.1e). The majority of these studies included only a small number of patients and demonstrated a measurable immune responses without a clear meaningful clinical benefit [147]. Both forms of vaccine therapy are safe and local skin reactions were the main associated toxicity. In peptide-based vaccine therapy, a leukemia-specific TAA peptide is administered subcutaneously. Antigen presenting cells process and present the peptide on its surface through the HLA molecules and activate T lymphocytes. WT1 and PR3 are the most widely used TAAs in peptide-based vaccine therapy in AML. Both WT1 and PR3 are HLA-A(*)0201-restricted peptides and can only be used in HLA-A(*)0201 individuals.

In cellular/DC-based vaccine therapy, autologous or allogenic DCs are pulsed with HLA-A*2402-restricted modified WT1 peptides. Early stage clinical trials showed that subcutaneous administration of WT1 peptide pulsed DCs resulted in activation of WT1-specific cytotoxic T lymphocytes. This therapy is not effective in setting of advanced disease with high leukemia burden. Multiple single arm studies of DCs vaccines as a consolidation therapy after standard chemotherapy-induced remission in high risk AML patients showed promising results [148–150]. In a study that included 17 AML patients who received an AML-DC fusion vaccine in first CR, an exceptionally high PFS rate (71% after a median follow-up of 57 months) was observed [150]. In another study, a DC vaccine as post-remission therapy-induced molecular response as measured by *WT1* transcript level in some patients translated into very durable remissions [148]. Although longer than usual CRs were reported in DCs vaccine studies in post-remission setting, the non-randomized nature of these studies precludes drawing a firm conclusion on the true efficacy with respect to relapse prevention.

15.8 Conclusion

A number of targeted therapies have been approved for the treatment of AML over the last several years. In spite of this, effective treatments of both de novo and relapsed AML represent a significant unmet medical need. The recent advances in our understanding of the immunology and biology of AML have resulted in the development of novel immunotherapies and cell-based therapies not only for AML but for other hematologic malignancies as well. The hope is that with a deeper understanding of immunoediting and mechanisms of AML immune evasion, the current therapies can be optimized for consistent robust clinical benefit especially in patients with high risk and relapsed AML for which allogeneic stem cell transplant may provide the only chance of long-term disease-free survival, but with the risk of life altering or life ending toxicities.

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In the Pipeline: Emerging Therapy for Acute Myeloid Leukaemia

16

Harinder Gill and Amber Yip

Abstract

Acute myeloid leukaemia (AML) is an aggressive, heterogenous, and age-related haematological malignancy with dismal prognosis. Conventional therapy for AML consists of frontline induction therapy with cytarabine infusion for 7 days and administration of anthracyclines, most commonly daunorubicin, for 3 days (7 + 3), followed by subsequent consolidation with chemotherapy or allogeneic haematopoietic stem cell transplant (HSCT) for high-risk disease. However, the age-related nature of AML implies that a significant portion of patients are unfit for such intensive regimens and can only be put on palliative treatment. Increasing emphasis is being put on maximizing specificities and potencies of novel agents while minimizing treatment-related toxicities, entailing a future of personalized-therapy in AML. This chapter reviews recently approved agents and agents still in the pipeline for the treatment of AML both in the frontline and the relapsed/refractory setting.

Keywords

Acute myeloid leukaemia · Novel agents · Targeted therapy · Personalized therapy

16.1 Introduction

Acute myeloid leukaemia (AML) is an aggressive, heterogenous, and age-related haematological malignancy with dismal prognosis. Conventional therapy for AML consists of frontline induction therapy with cytarabine infusion for 7 days and administration of anthracyclines, most commonly daunorubicin, for 3 days (7 + 3), followed by subsequent consolidation with chemotherapy or allogeneic haematopoietic stem cell transplant (HSCT) for high-risk disease. However, the age-related nature of AML implies that a significant portion of patients are unfit for such intensive regimens and can only be put on palliative treatment. Although treatment options for AML have remained stagnant for a long time, exciting progress has been made during recent years, with the U.S. Food and Drug Administration (FDA) approving nine novel agents indicated for this disease (Table 16.1). Increasing emphasis is being put on maximizing specificities and potencies of novel agents while minimizing treatment-related toxicities, entailing a future of personalized-therapy in AML.

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Table 16.1 Summary of recently approved agents in acute myeloid leukaemia (AML)

Agent	Class	Date of approval	Indication	Registration trial
Midostaurin	FLT3 inhibitor	1/4/2017	In combination with 7 + 3 in newly diagnosed FLT3-mutant patients ≥60 years old	CALGB-RATIFY
Enasidenib	Mutant IDH2 inhibitor	1/8/2017	r/r IDH2 mutant AML	NCT01915498
CPX-351	Liposomal daunorubicin and cytarabine	3/8/2017	AML with MRC or t-AML	NCT01696084
Gemtuzumab ozogamicin	Anti-CD33 ADJ	1/9/2017	CD33+ newly diagnosed or r/r AML	MyloFrance 1
Ivosidenib	Mutant IDH1 inhibitor	1/7/2018	r/r IDH1 mutant AML	NCT02074839
Gilteritinib	FLT3 inhibitor	28/11/2018	r/r FLT3 mutant AML	ADMIRAL
Glasdegib	Smo inhibitor	21/11/2018	In combination with LDAC in newly diagnosed AM patients ≥75 years old	NCT01546038
Oral azacitidine	HMA	1/9/2020	Maintenance therapy in adult patients achieving CR or CRi	NCT01757535
Venetoclax	Bcl-2 inhibitor	16/10/2020	In combination with azacitidine, Decitabine, or LDAC for newly diagnosed AML in patients ≥75 years old or unfit for intensive induction chemotherapy	VIALE-A VIALE-C

ADJ antibody-drug conjugate, *AML* acute myeloid leukaemia, *Bcl-2* B-cell lymphoma 2, *CR* complete remission, *CRi* complete remission with incomplete hematologic recovery, *FLT3* Fms-like tyrosine kinase 3, *HMA* hypomethylating agent, *IDH1* isocitrate dehydrogenase 1, *IDH2* isocitrate dehydrogenase 2, *LDAC* low-dose cytarabine, *r/r* relapsed or refractory, *Smo* smoothened

16.2 Novel Chemotherapeutic Formulations

16.2.1 CPX-351

CPX-351 (Vyxeos) is an FDA-approved liposomal formulation of daunorubicin and cytarabine in a 5:1 molar ratio. While the combination of cytarabine and anthracyclines (7 + 3) has long been the conventional treatment for AML, their administration in the form of a liposomal capsule significantly prolongs their half-life and efficacy [1].

After encouraging results in a phase I study, subsequent phase II and III trials were carried out [2]. In a phase II study comparing CPX-351 with 7 + 3 in newly diagnosed AML patients, remarkable clinical benefit of CPX-351 was demonstrated, especially among patients with secondary AML, which is associated with poor prognosis [3]. In another phase II trial among relapsed or refractory (r/r) patients, CPX-351 induced superior responses when compared to standard salvage chemotherapy [4]. In a phase III trial, CPX-351 showed significantly prolonged survival compared to 7 + 3 induction [5]. These promising results were consistently replicated in subsequent trials [6, 7]. Side effects of CPX-351 are generally similar to those of 7 + 3, including myelosuppression, cardiotoxicity, with the exception of slower recoveries of neutrophil and platelet counts [3–5]. Combination of vyxeos with gemtuzumab ozogamicin (GO) and FLT3 inhibitors (quizartinib, midostaurin) also demonstrated clinical and preclinical efficacy respectively [8, 9].

Trials of CPX-351 as monotherapy or in combination with Ivosidenib, enasidenib, venetoclax, gilteritinib, midostaurin, quizartinib, palbociclib, glasdegib, GO, or fludarabine are underway (NCT04230239, NCT03988205, NCT03629171, NCT04668885, NCT04269213, NCT03555955, NCT04049539, NCT04493164, NCT03825796, NCT04075747, NCT04209725, NCT04038437, NCT03826992, NCT04293562, NCT04128748, NCT03844997, NCT04231851, NCT03878927, NCT03904251, NCT03672539, NCT02272478, NCT04425655). Studies comparing CPX-351 with other intensive chemotherapy regimens are also ongoing (NCT03897127, NCT04061239, NCT04293562, NCT04195945, NCT04802161).

16.3 Targeting Tyrosine Kinases

Tyrosine kinases regulate a wide range of cellular pathways and are crucial to signal transduction. Their aberrant activities can contribute to leukaemogenesis via promoting proliferation, impeding differentiation, and inhibiting apoptosis. Therefore, various agents have been developed against these kinases for the treatment of AML (Figs. 16.1, 16.2, and 16.3).

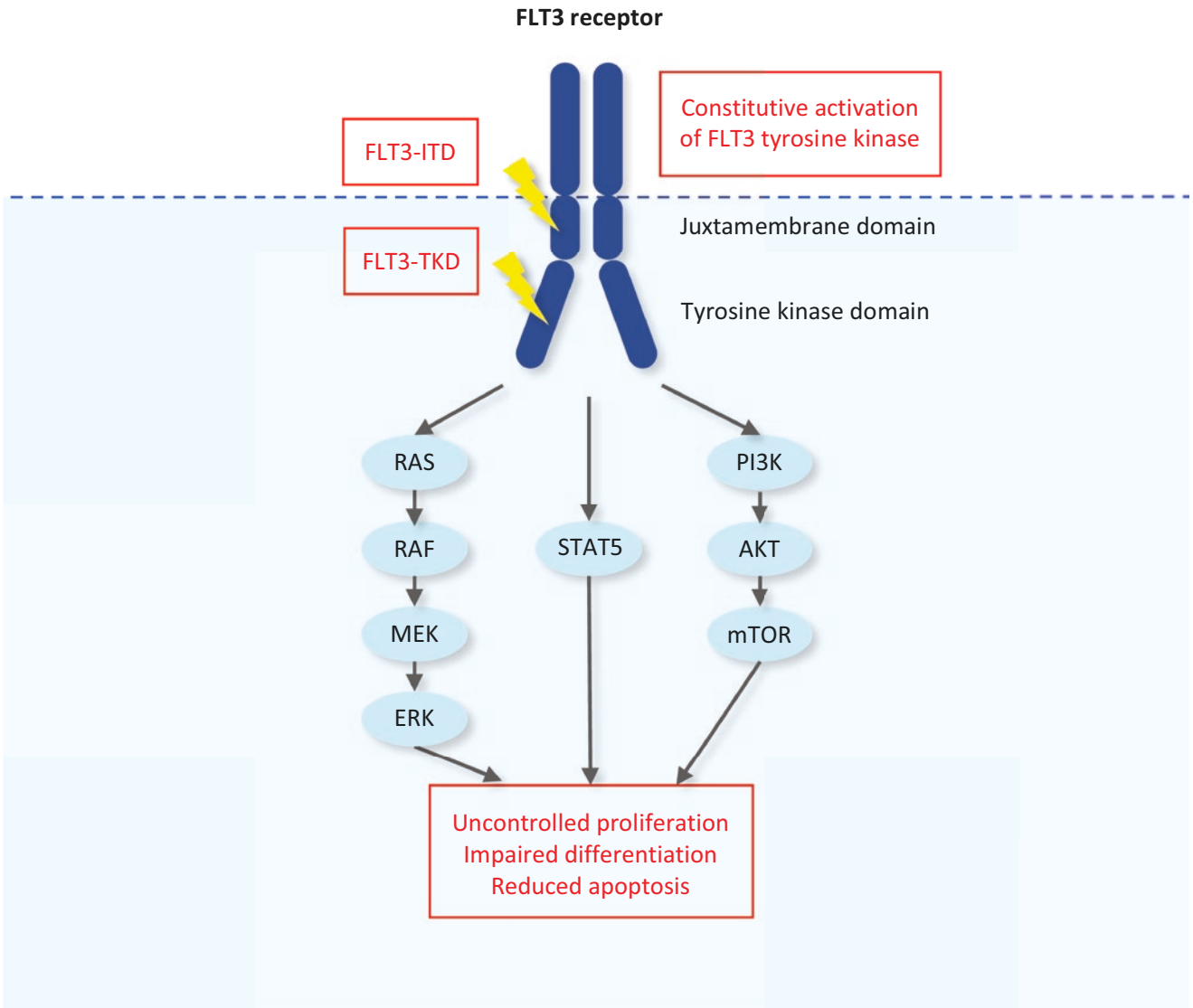
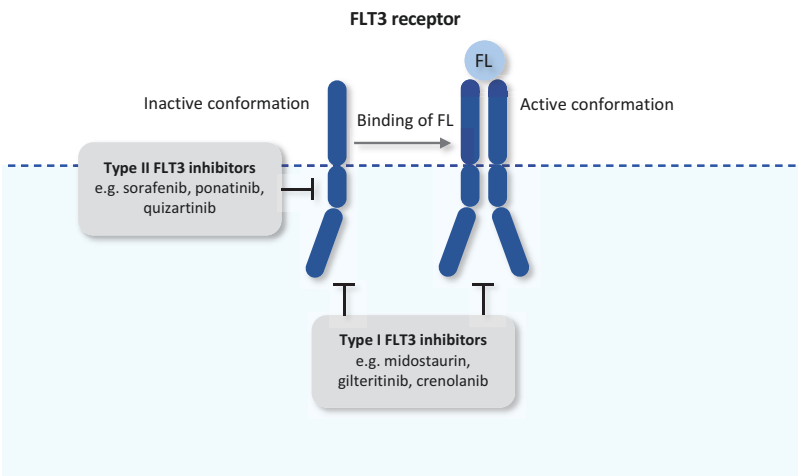


Fig. 16.1 Downstream events of aberrant FLT3 signalling. *AKT* protein kinase B, *ERK* extracellular-signal-regulated kinase, *FLT3-ITD* FLT3 internal tandem duplication, *FLT3-TKD* FLT3 tyrosine kinase domain mutations, *FLT3* Fms-like tyrosine kinase 3, *MEK* mitogen-activated protein kinase kinase, *mTOR* mammalian target of rapamycin complex, *PI3K* phosphoinositide 3-kinase, *RAF* rapidly accelerated fibrosarcoma, *Ras* rat sarcoma viral oncogene homolog, *STAT5* signal transducer and activator of transcription 5

Fig. 16.2 Actions of type I and type II FLT3 inhibitors. *FL* FLT3 ligand, *FLT3* Fms-like tyrosine kinase 3



Abbreviations: FL, FLT3 ligand; FLT3, Fms-like tyrosine kinase 3

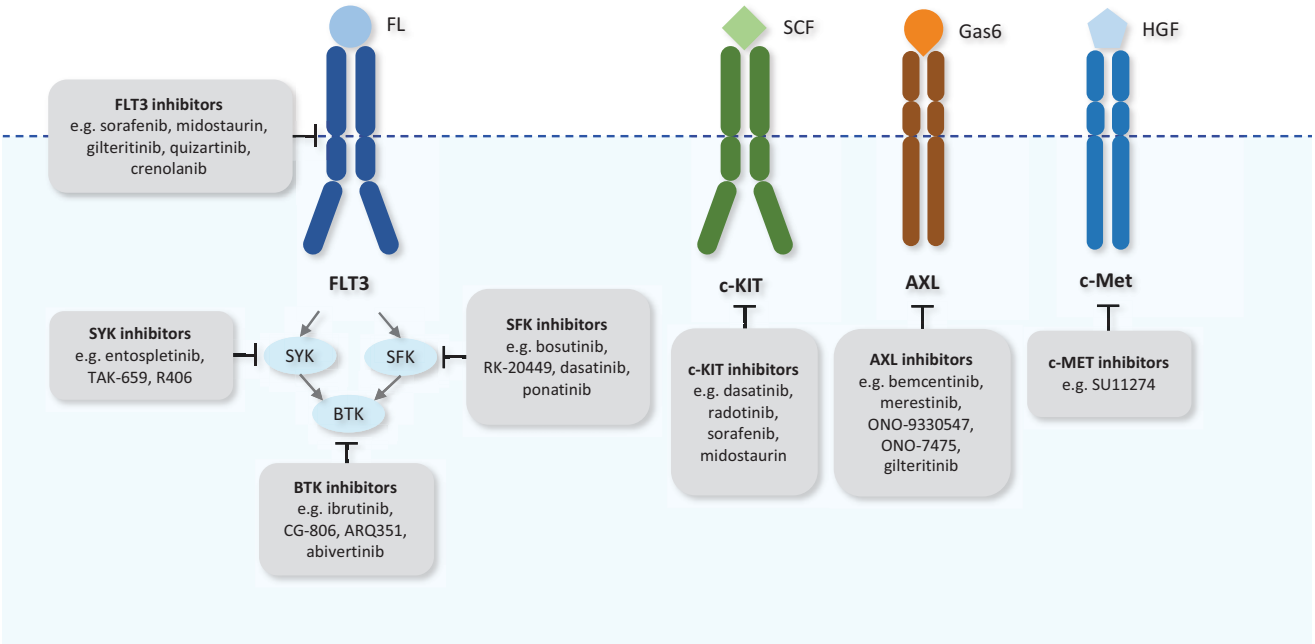


Fig. 16.3 Agents targeting tyrosine kinases. *AXL* anexelekto, *BTK* Bruton tyrosine kinase, *c-KIT* cluster of differentiation 117, *c-MET* mesenchymal-epithelial transition factor, *FL* FLT3 ligand, *FLT3* Fms-like tyrosine kinase 3, *HGF* hepatocyte growth factor, *SCF* stem cell factor, *SFK* Src family kinases, *SYK* spleen-associated tyrosine kinase

Table 16.2 Summary of the characteristics of major novel FLT3 inhibitors [10, 11]

Agent	Developmental status	Generation	Type	Off-target activity	Side effects
Sorafenib	Phase III	First	II	RAF, PDGFR, VEGFR, c-KIT, RET	Dermatological reactions (e.g. hand-foot-skin reaction, skin rash, mucositis), bleeding, cardiac events, febrile neutropenia, GI disturbance
Midostaurin	FDA-approved	First	I	PKC, SYK, SRC, c-KIT, VEGFR, PDGFR, AKT	Pulmonary toxicity (e.g. drug-induced pneumonitis), febrile neutropenia, QT prolongation, edema, bruising, GI disturbance
Sunitinib	Phase II	First	I	VEGFR, PDGFR, c-KIT	Dermatological reactions (e.g. hand-foot-skin reactions, erythema multiforme), myelosuppression, GI disturbances
Ponatinib	Phase II	First	II	RET, c-KIT, FGFR, PDGFR, BCR-ABL	Cardiovascular ischemic events, myelosuppression, febrile neutropenia, hepatotoxicity, skin rash, GI disturbances
Gilteritinib	FDA-approved	Second	I	AXL, ALK, LTK	Febrile neutropenia, liver toxicity, GI disturbance, fatigue
Quizartinib	Phase III	Second	II	c-KIT, RET, PDGFR, CSF1	Nausea, febrile neutropenia, sepsis or septic shock, QT prolongation
Crenolanib	Phase II	Second	I	PDGFR, c-KIT	Skin rash, GI disturbance, febrile neutropenia, elevation of transaminases

AKT protein kinase B, *ALK* anaplastic lymphoma kinase, *AXL* AXL receptor tyrosine kinase, *BCR-ABL1* breakpoint cluster region-Abelson murine leukaemia viral oncogene homolog 1, *c-KIT* tyrosine-protein kinase KIT, *CSF1* colony-stimulating factor 1, *FDA* U.S. Food and Drug Administration, *FGFR* fibroblast growth factor receptor, *FLT3* Fms-like tyrosine kinase 3, *GI* gastrointestinal, *LTK* leucocyte tyrosine kinase receptor, *PDGFR* platelet-derived growth factor receptor, *PKC* protein kinase C, *RAF* rapidly accelerated fibrosarcoma, *RET* rearranged during transfection, *SRC* proto-oncogene tyrosine-protein kinase SRC, *SYK* tyrosine-protein kinase SYK, *VEGFR* vascular endothelial growth factor receptor

16.3.1 FLT3 Inhibitors

Figures 16.1, 16.2, and 16.3 and Table 16.2 summarize the role of FLT3 inhibitors and the major FLT3 inhibitors in development. Readers should refer to Chap. 12 of this title for further discussion.

16.3.2 c-KIT Inhibitors

c-KIT, also known as CD117, is an RTK expressed in haematopoietic cells for their normal development. Upon binding of stem cell factor (SCF), c-KIT dimerizes and undergoes autophosphorylation, which activates downstream PI3K/

AKT/mTOR, JAK-STAT, and Ras/RAF/MAPK pathways, as well as Src family kinases (SFKs) [12, 13]. The expression of c-KIT is found in 60–80% of AML and its mutation is especially prevalent in core binding factor (CBF) AML [14]. Mutations in c-KIT mainly occur in exon 8 and exon 17, with the latter being associated with a more inferior clinical outcome [12]. Aberrant activation of c-KIT results in increased proliferation, reduced apoptosis, and subsequent leukaemogenesis [12].

Dasatinib and radotinib are multi-kinase inhibitors with potent activity against c-KIT. These agents induced apoptosis in c-KIT-positive AML cell lines and showed activity in downregulating other leukaemogenic pathways in various preclinical studies [15]. Dasatinib also showed synergistic efficacy with navitoclax against AML cells with NUP98-NSD1 and FLT3-ITD [16]. Addition of dasatinib to standard chemotherapy and its use as single agent maintenance therapy in patients with CBF AML showed favourable outcomes and a tolerable safety profile [17–19]. A phase III randomized controlled trial of chemotherapy with or without dasatinib in CBF AML patients is underway (NCT02013648). Other c-KIT inhibitors which are not actively evaluated for use in AML include imatinib, SU5416, and SU6668 [20, 21].

16.3.3 AXL Inhibitors

Anexelekto (AXL) is a member of the TYRO3, AXL, and MER (TAM) RTK family [22]. It is expressed on a multitude of cells and tissues and is crucial for the normal function of various haematopoietic cell types [22, 23]. Binding of Gas6 to AXL induces its dimerization and subsequent activation of PI3K, Ras, Src, and JAK/STAT pathways, resulting in cellular proliferation and migration [22]. In AML, AXL may be activated via mechanisms independent of Gas6 [22]. Aberrant signalling of AXL also acts as a key mediator of resistance against FLT3 inhibitors [23].

Similar to FLT3 inhibitors, AXL inhibitors are divided into two types. Type I AXL inhibitors bind to the ATP-binding site of the active AXL receptor [22]. Bemcentinib (BGB324) is a highly specific, potent, and safe small molecule type I inhibitor of AXL which showed efficacy against both FLT3-WT and FLT3-mutant AML cell lines [24, 25]. Due to promising results of its combination with LDAC in recent trials, bemcentinib has received fast track designation from the FDA [26, 27]. A phase II study regarding the use of bemcentinib in AML is currently underway (NCT03824080). Other type I inhibitors include gilteritinib and sunitinib. Type II inhibitors bind to the AXL receptor in its inactive form [22]. Among them, merestinib (LY2801653) is a potent and orally available inhibitor of AXL, FLT3, MNK, MET/RON, and other oncoproteins [28, 29]. It was proven to be safe in r/r AML patients in a phase I clinical trial [30]. Other novel AXL inhibitors with impressive preclinical efficacies against AML cell lines include the AXL/Mer dual inhibitors ONO-9330547 and ONO-7475, with ONO-7475 currently in a phase I/II trial as monotherapy or in combination with venetoclax (NCT03176277) [31–33].

16.3.4 c-MET Inhibitors

The MET RTK family consists of two members, c-Met and RON. Upon binding of their respective ligands (HGF for c-Met, MSP for RON), their tyrosine kinase domain activates and initiates signal transduction via PI3K, AKT, B-catenin, Ras/MAPK, and JAK/STAT pathways [34]. Evidence of their expression in AML blasts led to studies evaluating their potential roles as therapeutic targets [34].

SU11274 is a c-Met inhibitor which demonstrated anti-leukaemic efficacy in preclinical studies [34–36]. Crizotinib also exhibited activity against AML cells, but seemed to induce resistance via a compensatory increase in HGF expression [35].

16.3.5 SYK Inhibitors

Spleen-associated tyrosine kinase (SYK) is a cytoplasmic tyrosine kinase with diverse biological activities, including roles in adaptive immune receptors signalling [37]. In AML, its increased expression is shown to be associated with inferior clinical outcomes [38]. Upon activation by FLT3-ITD or other upstream pathways, SYK undergoes phosphorylation and initiates a series of downstream signalling pathways, ultimately contributing to leukaemogenesis [39].

Preclinical studies with R406, an active metabolite of the SYK inhibitor fostamatinib, showed efficacy against AML cell lines by inducing differentiation and inhibiting proliferation [39]. Entospletinib (GS-9973) showed efficacy as monotherapy as well as in combination with chemotherapy in two early phase trials [40, 41]. TAK-659, a dual inhibitor of SYK and FLT3, also exhibited anti-leukemic activity in murine models and showed promising efficacy and safety profile in a phase Ib/II study in r/r AML patients [42, 43].

16.3.6 BTK Inhibitors

Bruton tyrosine kinase (BTK) is a member of the Tec family kinases [44]. This family of non-receptor, cytoplasmic kinases are mainly expressed on the surfaces of haematopoietic stem cells (HSCs) and other haematopoietic cells [44]. BTK, in particular, also plays a critical role in the develop-

ment of B lymphocytes and is considered to be a key mediator in B-cell neoplasms [45, 46]. In AML, aberrant signalling of SFK, SYK, and PI3K leads to BTK activation and downstream activation of NFKB and other kinase pathways, resulting in leukaemogenesis. Emerging evidence of high BTK expression and constitutive activation in AML cells has led to interests on its potential role as a therapeutic target [47, 48]. In addition, FLT3-ITD may act as one of the upstream events leading to BTK autophosphorylation, implying the potential of BTK inhibitors for treating FLT3-ITD-positive AML [47].

Ibrutinib (CI-32765) is an irreversible inhibitor of BTK. In preclinical studies, it showed efficacy against AML cell lines by inhibiting downstream NFKB signalling, SDF1/CXCR4-mediated migration, and SDF1-induced activation of the AKT/MAPK pathway [48, 49]. Mutations in *FLT3*, *NPM1*, *and DNMT3A* were shown to be associated with increased sensitivity to ibrutinib [50]. In leukaemic blasts obtained from c-KIT-positive AML patients, ibrutinib also inhibited activation of BTK by c-KIT and their adhesion to bone marrow stromal cells [51]. Furthermore, specific inhibition of FLT3-ITD by ibrutinib in leukaemic cell lines has been reported, supporting the hypothesis that BTK inhibition may be efficacious against FLT3-ITD-positive AML [52]. Its combination with the recently approved Bcl-2 inhibitor, venetoclax, also showed promising results in preclinical studies [53]. However, a phase II clinical trial with ibrutinib monotherapy or in combination of azacitidine or cytarabine showed limited efficacy [54].

CG-806 is a dual FLT3/BTK inhibitor with remarkable activity and safety against AML cell lines and murine models [55]. This agent is currently evaluated in a phase I clinical trial (NCT04477291). Other novel BTK inhibitors with promising preclinical results include ARQ351 and abivertinib (AC0010) [56–58].

16.3.7 SFK Inhibitors

The non-receptor Src family of kinases include LYN, HCK, BLK, FGR, FYN, LCK, SRC, and YES [59]. In AML cells, FYN, LYN, HCK, and FGR are commonly expressed. Aberrant upstream signalling of FLT3, c-KIT, and other RTKs result in their activation, subsequently causing STAT5, Ras, and PI3K induction [59].

Bosutinib is an SFK inhibitor primarily used in the treatment of chronic myeloid leukaemia (CML). Recently, studies showed that its combination with all-trans retinoic acid (ATRA) enhances sensitivity of AML cell lines to ATRA, thus promoting differentiation of AML blasts [60]. It will be evaluated in a subsequent phase Ib trial in combination with glasdegib (NCT04655391).

RK-20449 is a selective HCK inhibitor which showed efficacy against chemotherapy-resistant AML cells in murine models [61]. An FGR inhibitor, TL02–59, also showed anti-leukaemic activity in a preclinical study [62]. Other SFK inhibitors with impressive preclinical evidences include PP2, dasatinib, ponatinib, PD180970, and SKI-606 [59, 63]. Although SAR103168 showed efficacy against AML cell lines in preclinical studies, results from a subsequent phase I trial were disappointing [64, 65].

16.4 Targeting the Hedgehog Pathway

The hedgehog (Hh) pathway is an essential mediator of embryonic development. In the canonical Hh pathway, Hh ligand binds to the transmembrane protein Patched (PTCH) to alleviate its inhibition on Smoothened (Smo), another transmembrane protein (Fig. 16.4). Smo then activates downstream glioma transcription factors (GLI) to stimulate gene transcription and proliferation [66]. In the non-canonical Hh pathway, activation of GLI is induced by other upstream pathways instead of Smo activation, such as PI3K/AKT/mTOR, RAS/RAF/MEK/ERK, protein kinase C (PKC), and many others [67]. In AML, the Hh pathway and oncogenic GLI activity may be constitutively activated, which is associated with radio- and chemo-resistance as well as poor prognosis [68–70]. Notably, crosstalk between the Hh pathway and FLT3-ITD has been discovered, prompting contemplations on its therapeutic role in FLT3-mutant AML [67].

16.4.1 Smo Inhibitors

Inhibition of Smo is the most widely studied among all potential therapeutic targets in the Hh pathway. Glasdegib (PF-04449913) is an FDA-approved selective Smo inhibitor. After encouraging results from preclinical studies, glasdegib was further studied in phase I clinical trials, where it was proven to be effective and tolerable in AML patients [71–73]. Subsequent trials investigating combinations of glasdegib with LDAC, decitabine, or standard chemotherapy all demonstrated clinical effectiveness [74–77]. Notably, addition of glasdegib to LDAC prolonged survival by nearly twofold compared to single agent LDAC, but did not increase toxicity in a multi-centre randomized phase II trial [76, 77]. Major side effects of glasdegib include febrile neutropenia, anaemia, and gastrointestinal disturbances [72–77]. Future trials include combination therapies with chemotherapy, LDAC, CPX-351, decitabine, azacitidine, GO, gilteritinib, ivosidenib, enasidenib, venetoclax, bosutinib, avelumab, and OX40 (NCT0341617, NCT02038777, NCT04231851, NCT04051996, NCT02367456, NCT04093505, NCT04655391, NCT03390296).

Sonidegib (LDE225) is another Smo inhibitor which demonstrated efficacy against doxorubicin-resistant AML

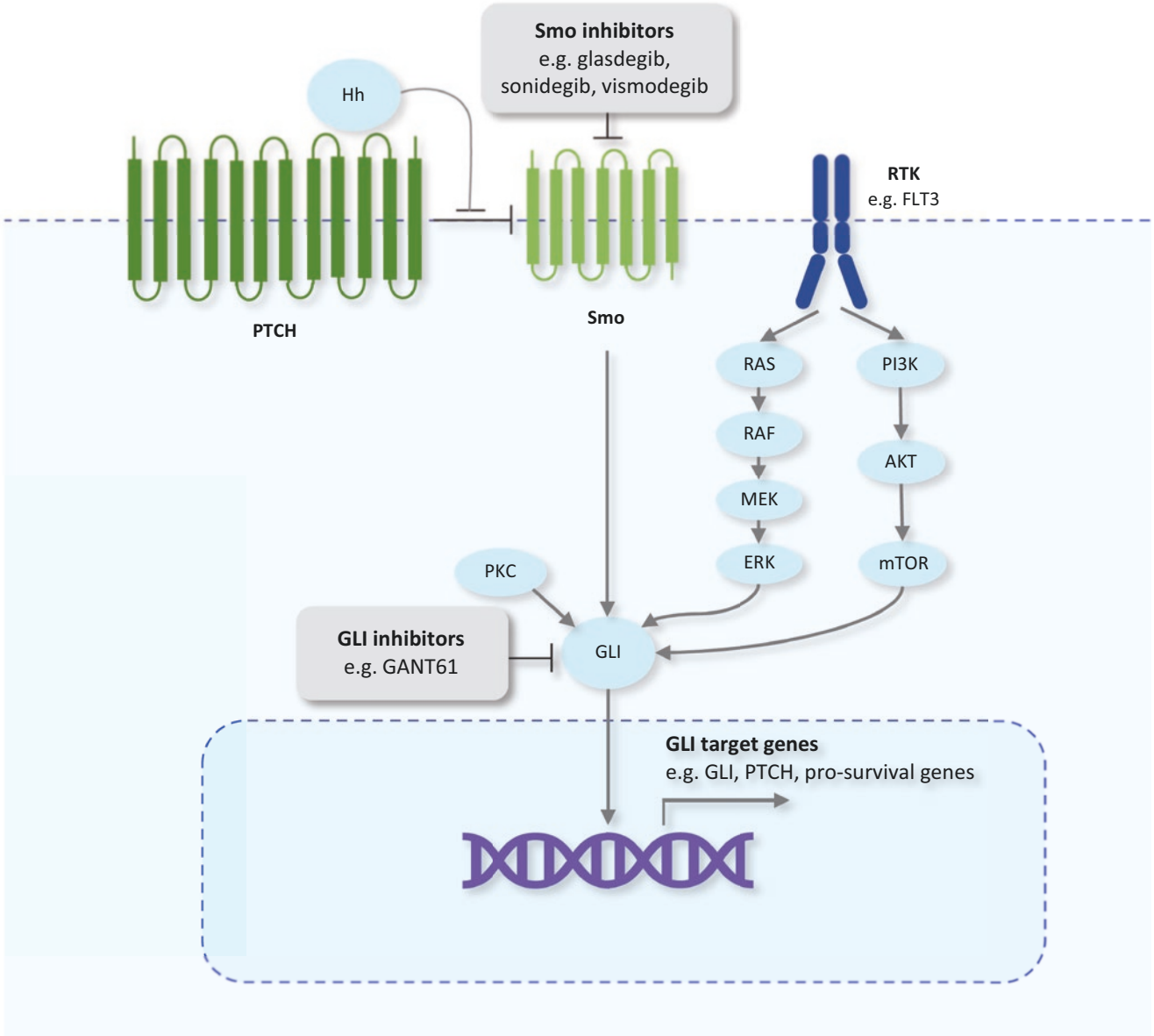


Fig. 16.4 Agents targeting the hedgehog pathway. *AKT* protein kinase B, *ERK* mitogen-activated protein kinase, *GLI* glioma transcription factors, *Hh* hedgehog, *MEK* mitogen-activated protein kinase kinase, *mTOR* mammalian target of rapamycin, *PI3K* phosphoinositide 3-kinase, *PTCH* patched, *RAF* rapidly accelerated fibrosarcoma, *Ras* rat sarcoma, *Smo* smoothened

cell lines and exhibited synergism with azacitidine in preclinical studies [78]. Its single agent therapy and combination with azacitidine or decitabine have been studied in phase I and phase II trials (NCT02129101, NCT01826214) [79].

Vismodegib (GDC-0449) also showed anti-leukaemic activity in preclinical studies, but had limited efficacy as monotherapy in a subsequent trial [80, 81]. Similarly, another trial of its use in combination with cytarabine was terminated due to the minimal responses observed among patients (NCT01880437).

16.4.2 GLI Inhibitors

Given that GLI activation can occur independently of Smo, direct inhibition of GLI is an attractive strategy against resistance to Smo inhibitors [67]. GANT61 is a GLI inhibitor which inhibited proliferation and induced apoptosis of AML in preclinical studies [82]. Its combination with sunitinib also prolonged survival of FLT3-mutant mice [83]. These optimistic results warrant clinical studies for GLI inhibitors in AML patients.

16.5 Targeting Apoptotic Pathways

16.5.1 BCL-2 Family Inhibitors

The anti-apoptotic B-cell lymphoma 2 (BCL-2) family prevents cellular apoptosis via the inhibition of proapoptotic proteins, such as BAX and BAK. Examples of members include Bcl-2 (B-cell lymphoma 2), myeloid cell leukaemia sequence 1 (MCL-1), and B-cell lymphoma-extra-large (Bcl-xL) [84]. Their actions are counteracted by the pro-apoptotic subfamily of BCL-2 (Fig. 16.5). In AML, their overexpression has been identified in multiple studies, which implies their influence on impairing apoptosis and promoting survival of leukemic cells [84, 85].

16.5.2 Bcl-2 Inhibitors

Bcl-2 inhibitors exert anti-leukaemic activity by mimicking the BH3 domain of the pro-apoptotic BCL-2 proteins and freeing them from the anti-apoptotic BCL-2 protein, which induces apoptosis [85].

Despite unsatisfactory results in early trials with oblimersen and obatoclax, efforts on investigation of Bcl-2 inhibition were persistent, which led to the development of venetoclax [85]. Venetoclax (ABT-199) is an FDA-approved, potent, and selective Bcl-2 inhibitor. Venetoclax was proven to be effective and tolerable in preclinical and clinical studies, both as monotherapy and in combination with HMAs (azacitidine, decitabine) or cytarabine, both in newly diag-

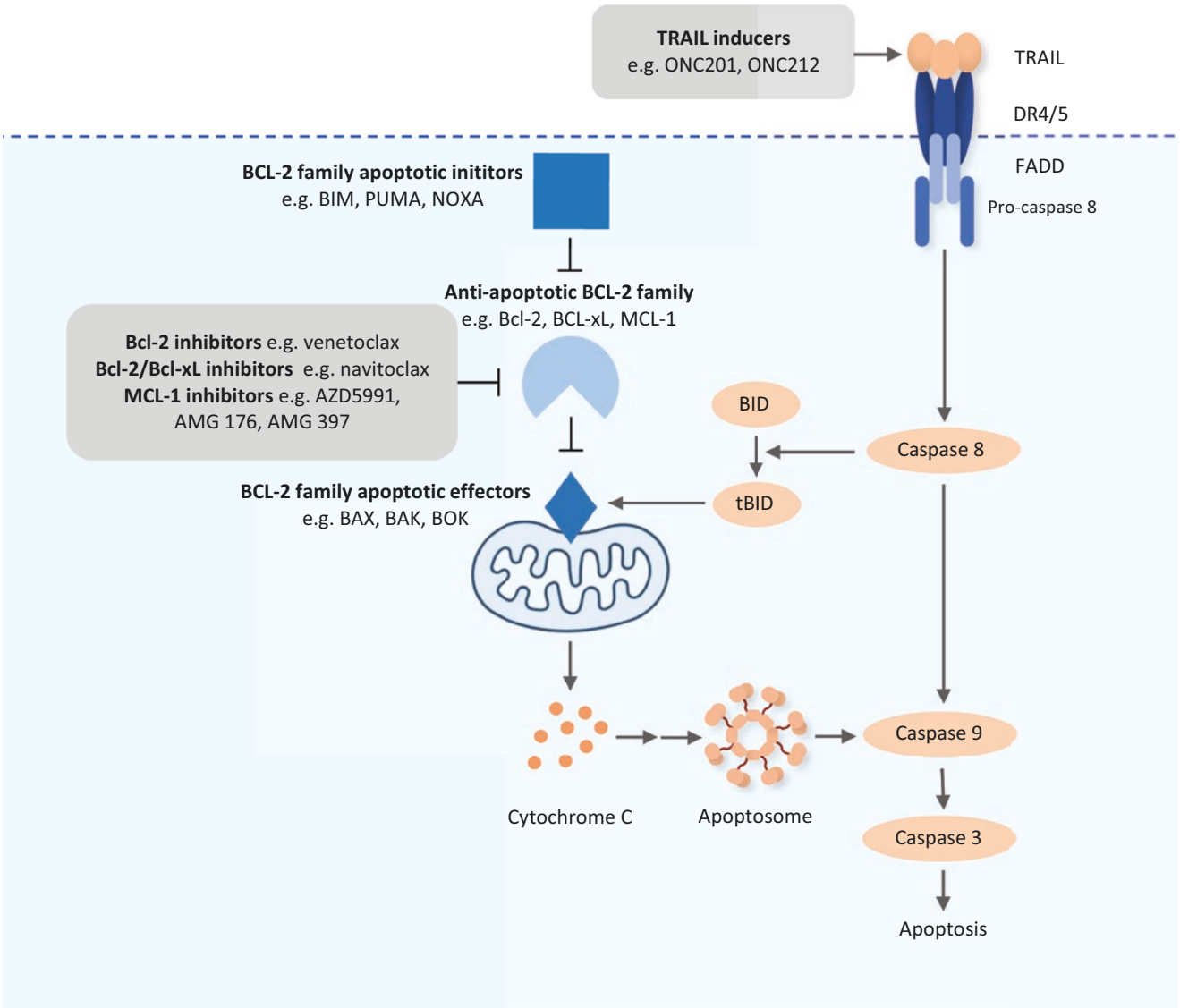


Fig. 16.5 Agents targeting apoptotic pathways. *BAK* Bcl-2-antagonist/killer 1, *BAX* apoptosis regulator BAX, *Bcl-2*, B-cell lymphoma 2, *Bcl-xL* B-cell lymphoma extra-large, *BIM* Bcl-2-like protein 11, *BOK* Bcl-2-related ovarian killer, *DR4/5* death receptor 4 or 5, *MCL-1* myeloid cell leukaemia sequence 1, *NOXA* Phorbol-12-myristate-13-acetate-induced protein 1, *PUMA* p53 upregulated modulator of apoptosis, *tBID* truncated BID, *TRAIL* TNF-related apoptosis-inducing ligand

nosed and r/r patients [86–95]. Notably, combination of venetoclax with HMAs induced remarkable responses in a wide range of patients, including those with high-risk cytogenetic features and mutant-*TP53* [95, 96]. Preclinical studies also elucidated their efficacies in targeting LSCs via inhibition of complex 2 of the ETC [97].

Full approval of venetoclax by the FDA was prompted by the phase III randomized placebo-controlled VIALE-A and VIALE-C trials, which evaluated the use of venetoclax in combination with azacitidine and LDAC, respectively. Both trials illustrated improvements of survival outcomes and remission rates upon the addition of venetoclax, along with tolerable increases in haematological toxicities [98, 99]. However, it should be noted that these benefits did not reach statistical significance in the VIALE-C study. Major side effects of venetoclax include febrile neutropenia and thrombocytopenia [98, 99].

Finally, multiple novel combinations with venetoclax are also being studied to overcome resistance. Among them, agents downregulating activity of MCL-1 are intensively evaluated owing to associations between MCL-1 upregulation and venetoclax resistance [85]. Multiple trials of venetoclax as monotherapy or in combination with other agents are ongoing (Table 16.3).

Table 16.3 Venetoclax in future clinical studies as monotherapy or in combination with other agents

Class	Combination	Developmental status	Ongoing/future trials
Bcl-2 inhibitor	Venetoclax monotherapy	Phase 2	NCT04253314, NCT04613622
Venetoclax with azacitidine			
HMA	Azacitidine	FDA-approved	NCT03466294, NCT04267081, NCT0416188, NCT04589728, NCT0299352, NCT04102020, NCT03941964, NCT03573024, NCT02203773, NCT04454580, NCT04062266, NCT04128501, NCT03236857
	Azacitidine vs. induction chemotherapy	Phase 2	NCT04801797
Venetoclax with azacitidine and other agents			
RIT	Lintuzumab-Ac225	Phase 1/2	NCT03932318
Anti-CD123	Tagraxofusp (SL-401)	Phase 1	NCT03113643
Anti-CD47	Magrolimab	Phase 3	NCT04435691, NCT04778397
Chemotherapy	Cytarabine, mitoxantrone	Phase 1/2	NCT04330820
	LDAC, cladribine	Phase 2	NCT03586609
FLT3 inhibitor	Gilteritinib	Phase 1/2	NCT04140487
TIM3 inhibitor	MGB453	Phase 2	NCT04150029
NAE inhibitor	Pevonedistat (NEDD8-activating enzyme (NAE) inhibitor)	Phase 2	NCT04172844, NCT04266795, NCT03862157
Anti-PD-1	Pembrolizumab	Phase 2	NCT04284787
MEK inhibitor	Trametinib	Phase 2	NCT04487106
LSD1 inhibitor	CC-90011	Phase 1/2	NCT04748848
Multiple	OX40, glasdegib, gemtuzumab ozogamicin	Phase 1/2	NCT03390296
Venetoclax with decitabine			
HMA	Decitabine	PDA-approved	NCT04476199, NCT04589728, NCT03941964, NCT04763928, NCT03844815, NCT02203773, NCT04454580, NCT03404193
Venetoclax with decitabine and other agents			
Grb2 anti-sense oligodeoxynucleotide	BP1001	Phase 2	NCT02781883
STAT inhibitor	OPB-111077	Phase 1	NCT03063944
FLT3 inhibitor	Quizartinib	Phase 1/2	NCT03661307
FLT3 inhibitor	Ponatinib	Phase 2	NCT04188405
Venetoclax with other agents			

(continued)

Table 16.3 (continued)

Chemotherapy	Intensive multi-agent chemotherapy	Phase 2	NCT03709758, NCT03194932, NCT04628026, NCT03214562, NCT04797767, NCT03455504, NCT02115295, NCT03613532, NCT03214562, NCT02250937
	CPX-351	Phase 2	NCT04038437, NCT03629171, NCT03826992
	Cytarabine/LDAC	FDA-approved	NCT04509622, NCT02287233
	Sapacitabine	Phase 1/2	NCT01211457
	Pegcrisantaspase	Phase 1	NCT04666649
HMA, IDH inhibitor	Oral decitabine/cedazuridine (ASTX727) ± Ivosidenib/Enasidenib	Phase 2	NCT04657081, NCT04746235, NCT04774393
	Ivosidenib ± azacitidine	Phase 1/2	NCT03471260
	Enasidenib ± azacitidine	Phase 1/2	NCT04092179
FLT3 inhibitor	Gilteritinib	Phase 1	NCT03625505
	Quizartinib ± azacitidine/LDAC	Phase 1/2	NCT03735875, NCT04687761
JAK inhibitor	Ruxolitinib	Phase 1	NCT03874052
Anti-CD33	Gemtuzumab ozogamicin	Phase 1	NCT04070768
Anti-CD47	ALX148	Phase 1/2	NCT04755244
Anti-CD123	IMGN632 ± azacitidine	Phase 1/2	NCT04086264
RIT	Lintuzumab-Ac225	Phase 1/2	NCT03867682
CDK inhibitor	CYC065	Phase 1	NCT04017546
	Dinaciclib (MK7965)	Phase 1	NCT03484520
	Alvocidib	Phase 2	NCT03969420
MDM2 inhibitor	HDM201	Phase 1	NCT03940352
	Milademetan tosylate + LDAC	Phase 1/2	NCT03634228
MCL-1 inhibitor	S64315	Phase 1	NCT03672695
	AMG 176	Phase 1	NCT03797261
	AZD5991	Phase 1/2	NCT03218683
XPO1 inhibitor	Selinexor	Phase 1	NCT03955783
AURKB inhibitor	Barasertib ± azacitidine	Phase 1/2	NCT03217838
Statin	Pitavastatin	Phase 1	NCT04512105
Salicylate	Salsalate + azacitidine/decitabine	Phase 2	NCT04146038

AURKB aurora kinase B, *Bcl-2* B-cell lymphoma 2, *CD123* cluster of differentiation 123, *CD33* cluster of differentiation 33, *CD47* cluster of differentiation 47, *CDK* cyclin-dependent kinase, *Grb2* growth factor receptor-bound protein 2, *HMA* hypomethylating agent, *IDH* isocitrate dehydrogenase, *JAK* janus kinase, *LDAC* low-dose cytarabine, *LSD1* lysine specific demethylase 1, *MCL-1* myeloid cell leukaemia 1, *MDM2* mouse double minute 2, *MEK* mitogen-activated protein kinase kinase, *NAE* neural precursor cell expressed, developmentally downregulated 8 (NEDD8)-activating enzyme (NAE); *PD-1* programmed death 1, *RIT* radioimmunotherapy, *STAT* signal transducer and activator of transcription, *TIM3* T cell immunoglobulin and mucin domain-containing protein 3, *XPO1* exportin 1

Other Bcl-2 inhibitors currently engaged in clinical trials include VOB560, S 055746 (BCL201), S6548, and APG2575 (NCT04702425, NCT02920541, NCT03755154, NCT04501120).

16.5.2.1 Bcl-2/Bcl-xL Dual Inhibitors

ABT-737 demonstrated promising efficacy against AML cell lines in preclinical studies, but its clinical development has been limited by an unfavourable pharmacokinetic profile [85, 100]. A derivative of this agent, navitoclax (ABT-263), possesses superior pharmacokinetic properties, though its clinical investigation is still not of interest due to the major adverse effect of thrombocytopenia [85].

16.5.2.2 MCL-1 Inhibitors

MCL-1 is another attractive therapeutic target in AML due to its overexpression in AML and association with venetoclax-resistance. AZD5991 is an MCL-1 inhibitor which demonstrated synergistic actions with bortezomib against AML xenograft in a murine study and is currently evaluated in combination with venetoclax in r/r AML patients in a phase I/Ib/IIa trial (NCT03218683) [101]. AMG 176 and AM-8621 both showed single agent efficacy and synergistic activity with venetoclax, though only AMG 176 is selected for further clinical investigations as monotherapy and in combination with azacitidine or venetoclax given its superior pharmacokinetic profile (NCT02675452, NCT03797261)

[102, 103]. Another agent, AMG 397, also showed favourable preclinical results and will be evaluated in r/r AML patients in a phase I trial (NCT03465540) [104]. In addition, S63845 demonstrated excellent anti-leukaemic efficacy as single agent and in combination with venetoclax, daunorubicin, or S55746 (Bcl-2 inhibitor) in preclinical studies [105–107]. A related agent, S64315, has been evaluated in AML patients in a phase I trial and will undergo further testing in combination with azacitidine, venetoclax, or VOB560 (NCT02979366, NCT04629443, NCT03672695, NCT04702425). Other MCL-1 inhibitors with preclinical efficacies against AML include Compound 42, VU661013, MIMI, and Cardone compound 9 [108–111].

16.5.3 TRAIL Inducers

TNF-related apoptosis-inducing ligand (TRAIL) induces p53-independent apoptosis upon binding to its cell surface receptors, namely death receptors (DR) 4 and 5 [112]. Imipridone compounds have been found to promote TRAIL transcription and expression, subsequently inducing apoptosis. Among them, ONC201 demonstrated potent anti-leukaemic effect against AML cells and LSCs, both as monotherapy and in combination with cytarabine or azacitidine [113–115]. Interestingly, its therapeutic activity relies on both the induction of TRAIL activity and stimulation of an integrated stress response (ISR) [113–115]. It is currently evaluated as monotherapy or in combination with LDAC, and as single agent post-HSCT maintenance in AML patients in phase I/II trials (NCT02392572, NCT03932643). ONC212, a more potent derivative of ONC201, exhibited single agent activity and synergism with venetoclax against AML cell lines and murine models [116, 117].

16.6 Targeting the *TP53* Pathway

TP53 encodes the tumour suppressor p53 and is among the most commonly mutated genes in all human malignancies [118]. WT p53 promotes cell cycle arrest, inhibits proliferation, and induces cellular apoptosis upon cellular stress [119]. Its activity is counteracted by mouse double minute 2 (MDM2), an E3 ligase which induces proteasomal degradation of p53 with the aid of MDM4 (Fig. 16.6). In AML, *TP53* mutations are associated with resistance to chemotherapeutic agents and dismal prognosis, which warrants the development of novel targeted therapies against this entity [120].

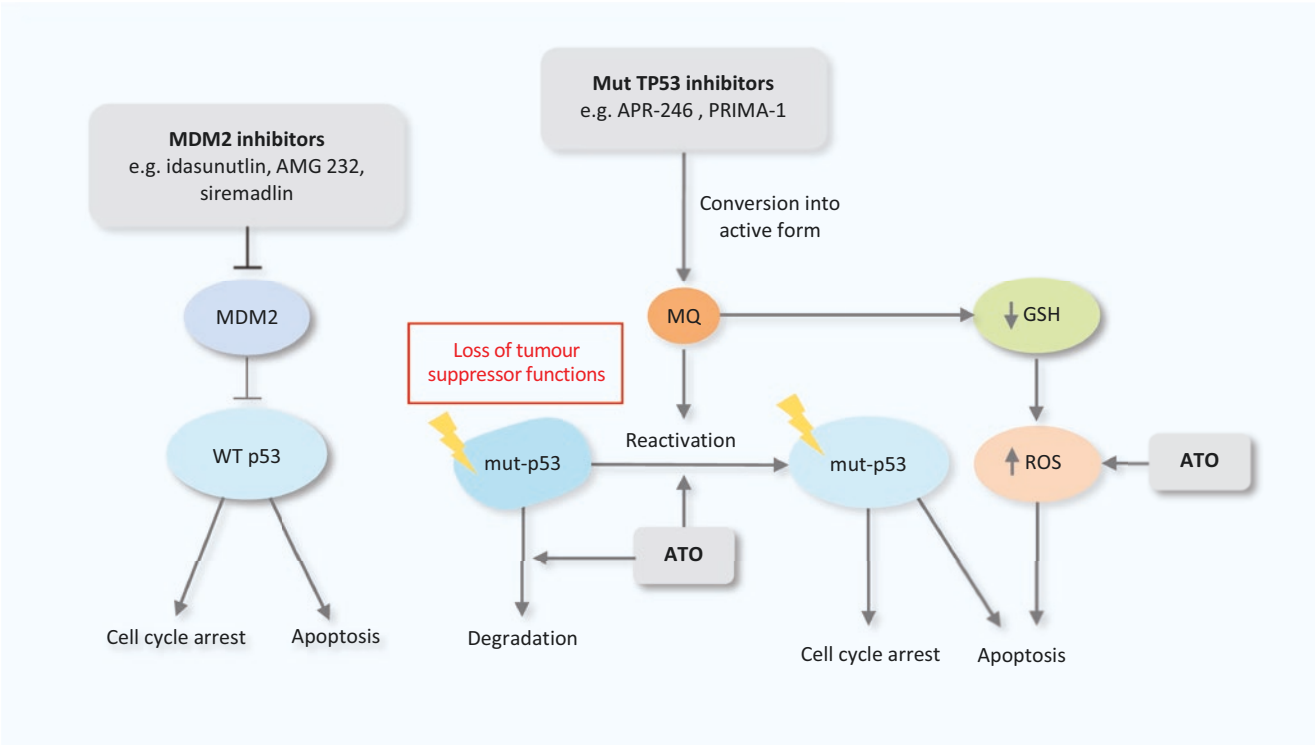


Fig. 16.6 Agents targeting the *TP53* pathway. *ATO* arsenic trioxide, *GSH* glutathione, *MDM2* mouse double minute 2, *MQ* methylene quinuclidinone, *mut-p53* mutant p53, *ROS* reactive oxygen species, *WT* wild type

16.6.1 Mutant TP53 Inhibitors

APR-246, a methylated analogue of p53 reactivation and induction of massive apoptosis (PRIMA-1), is a pro-drug of methylene quinuclidinone. Upon conversion into its active form, APR-246 restores the active conformation of p53 and its ability to induce apoptosis and cell cycle arrest in leukemic cells [120, 121]. APR0246 can also exert anti-tumour effect in a p53-independent manner via depletion of antioxidants and induction of oxidative stress [122]. Synergism with azacitidine in inducing G0/G1 cell cycle arrest, apoptosis, and downregulation of FLT3 signalling was also reported [123]. This agent demonstrated remarkable clinical efficacy in combination with azacitidine in *TP53*-mutant AML patients in an ongoing phase 1b/2 study [124] and is being further investigated in other trials (NCT03072043, NCT03931291).

Arsenic trioxide (ATO), an agent primarily used for the treatment of acute promyelocytic leukaemia, also demonstrated ability to induce proteasomal degradation of mutant p53 and restore normal function of WT p53 [125, 126]. Its combination with ascorbic acid selectively induced oxidative stress and apoptosis in *TP53*-mutant leukemic cells in a recent study [127]. In addition, this agent exhibited activity against *NPM1*-mutant AML cells by inducing mutant protein degradation in multiple studies [128–130]. The use of ATO as single agent and in combination with decitabine or all-trans-retinoic-acid (ATRA) is currently explored in patients with *TP53* or *NPM1* mutations in a number of clinical studies (NCT04689815, NCT03855371, NCT03031249).

16.6.2 MDM2 Inhibitors

Increased activity of MDM2 is associated with reduced p53 activity [118]. Therefore, inhibition of binding between MDM2 and p53 prevents degradation of p53 and restores its tumour suppressor functions [131]. Nutlins are the earliest selective inhibitors of MDM2 to be discovered, with nutlin 3 being widely used in preclinical studies investigating effects of MDM2 inhibition [131]. A small molecule MDM2 inhibitor, RG7112, demonstrated anti-leukaemic efficacy as monotherapy and in combination with cytarabine in AML patients [132, 133]. Another agent, idasanutlin (RG7388), is a potent, selective, and orally available second generation MDM2 inhibitor. Clinical studies of this agent as monotherapy and in combination with cytarabine had impressive responses. This agent was generally tolerable with gastrointestinal toxicity as a significant side effect [134]. In addition, idasanutlin exhibited synergistic activity with venetoclax in a preclinical study, which led to the initiation of a phase 1/1b trial with favourable results [135, 136]. Combination of idasanutlin with venetoclax or chemotherapy will be further evaluated in a phase 1/2 clinical trial (NCT04029688). Synergism between idasanutlin and XPO inhibitors (selinexor, eltanexor) was also discovered in a preclinical study [137].

Disappointingly, RO6839921, the pegylated prodrug of idasanutlin, showed inferior effectiveness compared to idasanutlin in a recent study and will not undergo further clinical development [138].

Another MDM2 inhibitor, AMG 232 (KRT232), showed modest clinical activity in combination with trametinib, a MEK inhibitor. This combination regimen was tolerable and common adverse effects include nausea, gastrointestinal disturbances, and poor appetite [139]. This agent will be tested in combination with cytarabine and venetoclax; cytarabine; decitabine; or with TL-895 (TKI) in subsequent trials (NCT04190550, NCT04113616, NCT04669067).

Siremadlin (HDM201) showed promising activity in a phase I trial with cytopenias and tumour lysis syndrome as the most significant side effects. It will undergo evaluation with midostaurin in r/r patients with TP53 and FLT3 mutations, as well as with MBG453 (TIM3 inhibitor) or venetoclax in AML patients (NCT04496999, NCT03940352) [140]. Another MDM2 inhibitor, Milademetan (DS-3032b), has been evaluated as monotherapy in a phase 1 trial and is currently evaluated in combination with azacitidine, or LDAC with or without venetoclax (NCT03671564, NCT02319369, NCT03634228). Finally, APG-115 is currently evaluated with azacitidine or cytarabine in a phase 1 trial (NCT04275518).

16.7 Targeting the PI3K/AKT/mTOR Pathway

The phosphoinositide 3-kinase (PI3K)-Protein kinase B (AKT)-mammalian target of rapamycin (mTOR) pathway is crucial to cellular metabolism and can be activated by a myriad of upstream pathways [141]. In AML, upregulation of this pathway supports leukaemic cell activities and can occur as a result of aberrant upstream tyrosine kinases signalling or constitutive activation [141]. Unfortunately, increased activity of this pathway seems to be associated with decreased survival [141]. Thus, pharmacological inhibition of this pathway is a logical and attractive novel strategy in AML (Fig. 16.7).

Although PI3K/AKT/mTOR inhibition demonstrated anti-leukaemic efficacies in preclinical studies, these results did not translate into meaningful clinical benefits [141]. mTORC1 inhibitors, including sirolimus, everolimus (RAD001), deferolimus (AP23573, MK-8669), and temsirolimus (CCI-779), have been tested in multiple clinical trials as monotherapies or in combination with chemotherapy regi-

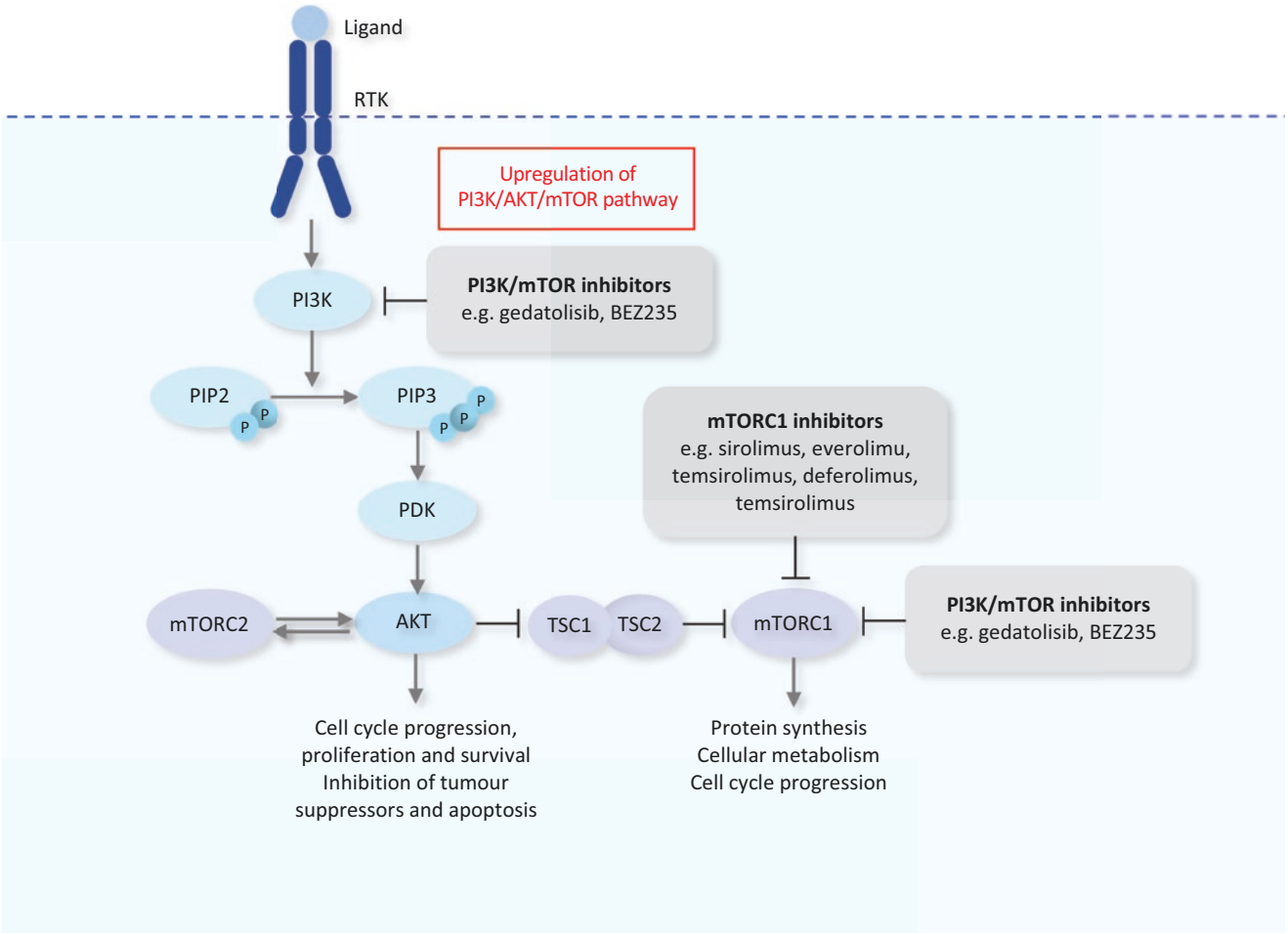


Fig. 16.7 Agents targeting the PI3K/AKT/mTOR pathway. *AKT* protein kinase B, *mTOR* mammalian target of rapamycin, *PDK* 3-phosphoinositide-dependent protein kinase-1, *PI3K* phosphoinositide 3-kinase, *TSC1* tuberous sclerosis complex 1, *TSC2* tuberous sclerosis complex 2

mens among AML patients with mostly limited success [142–147]. Although dual inhibition of PI3K and mTORC1 was proposed as a mechanism against resistance to mTORC1 inhibitors [148], two dual PI3K/mTOR inhibitors, gedatolisib (PF-05212384) and BEZ235, did not improve patient survival as single-agent and as an adjunct to chemotherapy, respectively [149, 150]. Other strategies to overcome resistance, such as dual mTORC1/mTORC2 inhibition, are being explored for the treatment of AML [148].

16.8 Targeting Metabolic Pathways

Mitochondrial activity is fundamental to supporting cellular metabolisms of almost all types of body cells. This carries paramount significance for the treatment of AML due to the presence of mitochondrial abnormalities, which can be exploited for selective AML cells targeting [151] (Fig. 16.8). In addition, other aberrant metabolic pathways discovered in LSCs are also being explored as targets for LSC eradication [151].

16.8.1 IDH1/2 Inhibitors

This role of IDH1/2 inhibition and development of IDH1/2 inhibitors are further discussed in Chap. 11.

16.8.2 Oxidative Phosphorylation Inhibitors

Leukaemic stem cells (LSCs) reply on oxidative phosphorylation (OXPHOS) for their metabolism rather than anaerobic glycolysis, which is the predominant metabolic pathway in normal HSCs [152]. Since integrity of the mitochondrial electron transport chain (ETC) is essential for OXPHOS, its

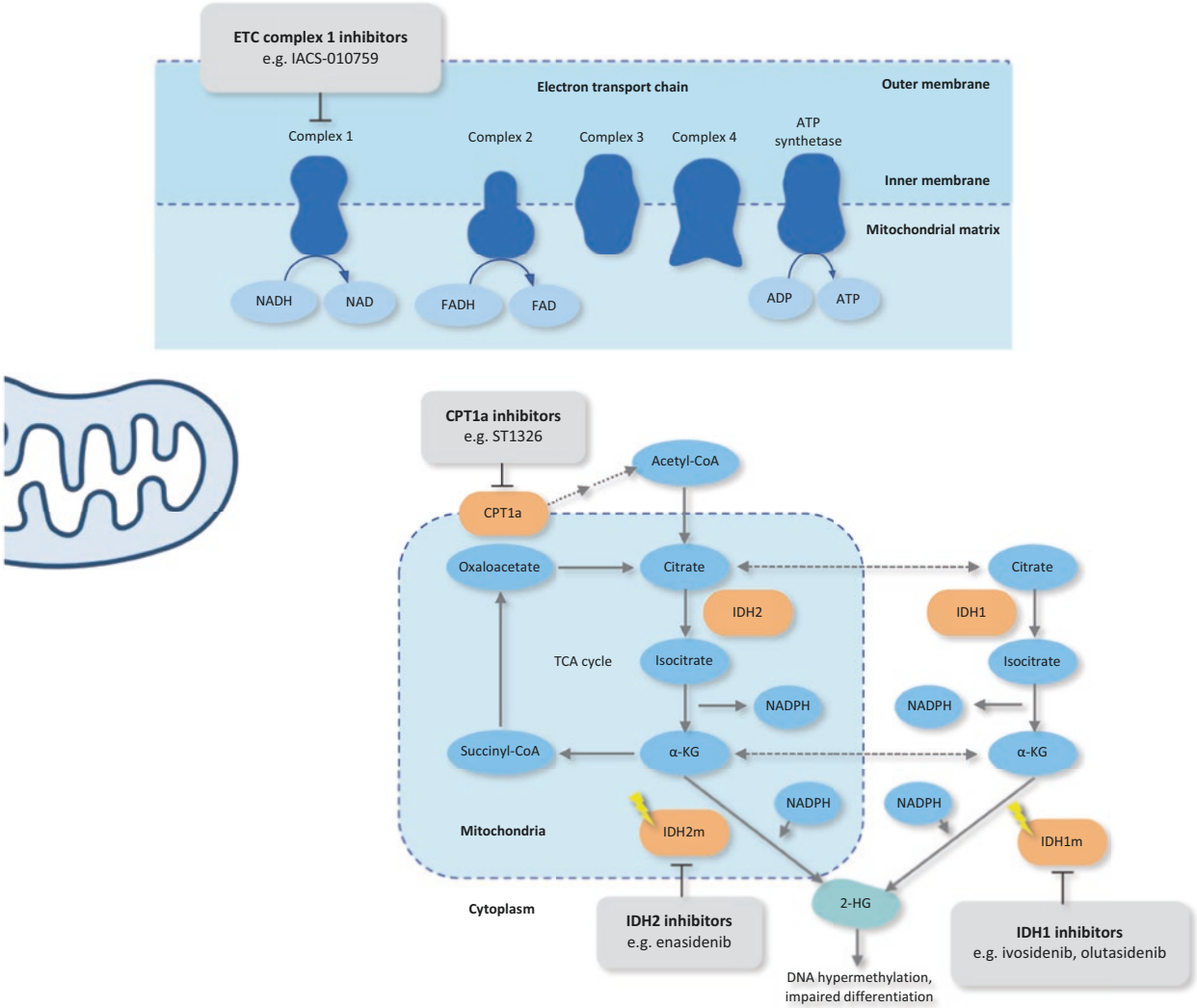


Fig. 16.8 Agents targeting metabolic pathways. *2-HG* 2-hydroxyglutarate, *Acetyl-CoA* acetyl-coenzyme A, *ADP* adenosine diphosphate, *ATP* adenosine triphosphate, *CPT1α* carnitine palmitoyl transferase 1a, *FAD* flavin adenine dinucleotide, *FADH* flavin adenine dinucleotide hydrogen, *IDH1* isocitrate dehydrogenase 1, *IDH1m* mutant IDH1, *IDH2* isocitrate dehydrogenase 2, *IDH2m* mutant IDH2, *NAD* nicotinamide adenine dinucleotide, *NADH* nicotinamide adenine dinucleotide hydrogen, *NADPH* nicotinamide adenine dinucleotide phosphate hydrogen, *α-KG* α-ketoglutarate

inhibition can disrupt metabolic activities of LSCs. IACS-010759, an inhibitor of complex 1 of the ETC, demonstrated selective anti-leukemic activity as monotherapy and synergism with venetoclax and vinorelbine, a microtubule destabilizer, against AML cells and xenograft models while sparing normal haematopoietic cells [153–155]. Compared to its predecessor BAY 87–2243, IACS-010759 also has a superior safety profile [152]. It is currently being studied in r/r AML patients in a phase I trial (NCT02882321). Another ETC complex 1 inhibitor, mubritinib (TAK-165), also exhibited activity against AML cells in a preclinical study [156].

16.8.3 Fatty Acid Oxidation Inhibitors

Fatty acid oxidation (FAO) generates acetyl coenzyme A (Acetyl-CoA) for the TCA cycle, and ultimately, OXPHOS [152]. The rate limiting step in FAO is catalysed by carnitine palmitoyl transferase 1a (CPT1a), thus, inhibition of this enzyme selectively impedes metabolism of leukaemic stem cells [152]. ST1326 is a CPT1a inhibitor which induced growth arrest, mitochondrial disruption, and apoptosis in various leukaemic cell lines, with the highest activity towards AML cells [157].

16.9 Targeting the Proteasome

The proteasome is a multimeric protein complex which mediates degradation of ubiquitinated proteins (Fig. 16.9). It controls a wide range of cellular activities, including cell cycle progression and survival [158]. Aberrant activities of the proteasome contribute to leukaemogenesis through various mechanisms, such as the activation of NF-κB signalling via degradation of its regulatory protein IκBα. Inhibition of the proteasome attenuates these pathways and induces autophagy of abnormal proteins, such as FLT3-ITD [158].

16.9.1 Proteasome Inhibitors

Bortezomib inhibits the 26S subunit of proteasome complex 2 [159]. This agent has been shown to exert anti-tumour activity via stabilization of p53, p27, IκBα, pro-apoptotic proteins BID and BAX, and other signalling proteins [159]. After demonstrating anti-leukaemic activity in preclinical studies, it was tested in AML patients in a number of clinical trials as monotherapy and in combination with other agents, including chemotherapy, hypomethylating agents, and HDAC inhibitors [158, 160]. Although it was minimally effective as a single agent, its combination regimens successfully induced remissions in varying portions of patients, with the highest response rates when added to intensive chemotherapy. Although bortezomib was generally tolerable, the risks of bortezomib-related peripheral neuropathy and potentially, pulmonary toxicity, are concerning [158, 160]. Other side effects of this agent include febrile neutropenia, nausea, and gastrointestinal disturbances [158, 160]. A phase 2 trial evaluating its role as a chemo-sensitizing agent is underway (NCT04173585).

16.9.2 NAE Inhibitors

Neural precursor cell expressed, developmentally downregulated 8 (NEDD8)-activating enzyme (NAE) promotes conju-

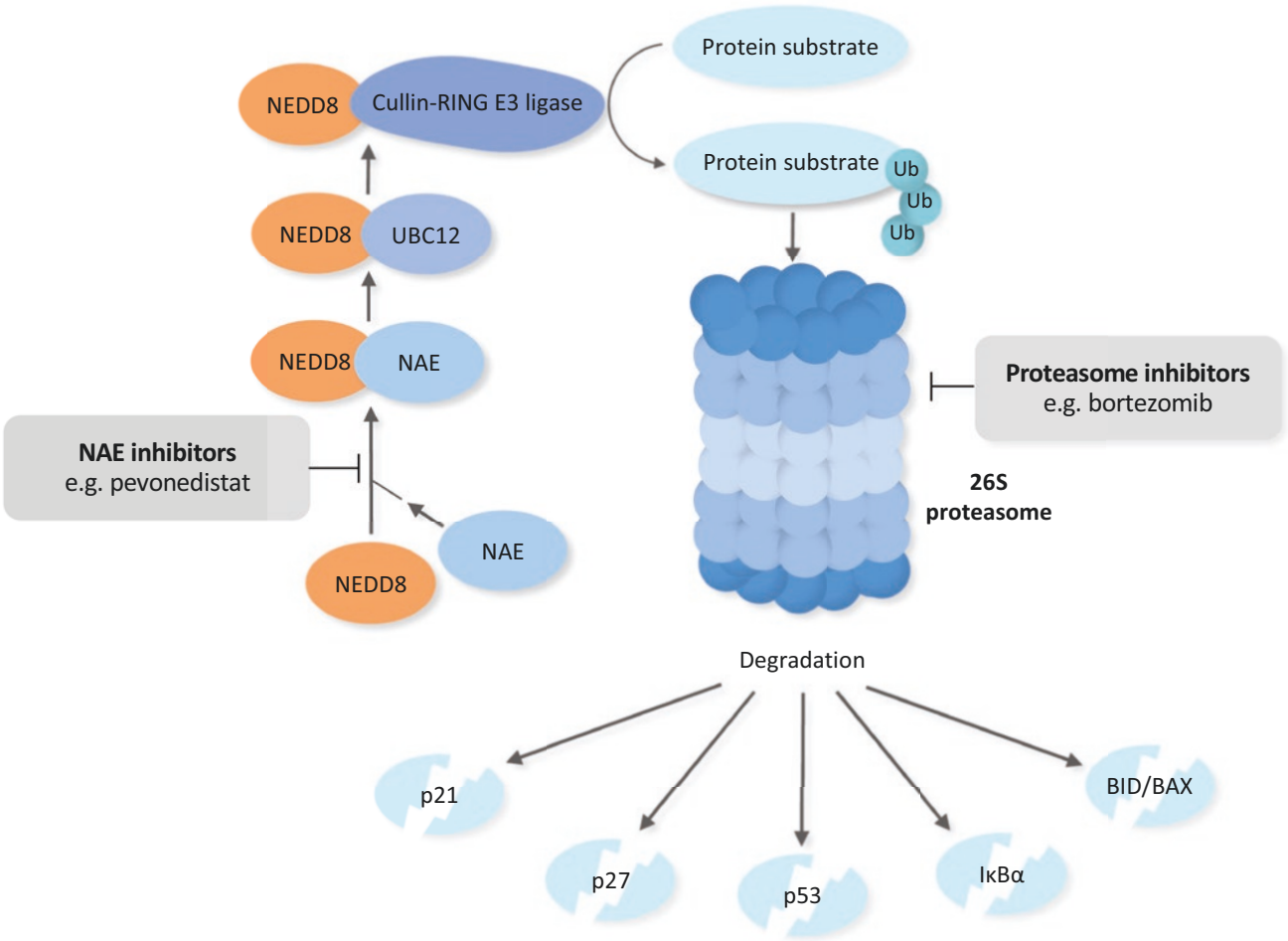


Fig. 16.9 Agents targeting the proteasome. *BAX* apoptosis regulator BAX, *BID* BH3 interacting-domain death agonist, *IκB α* inhibitor of NF-κB alpha, *NAE* neural precursor cell expressed, developmentally downregulated 8 activating enzyme, *NEDD8* neural precursor cell expressed, developmentally downregulated 8, *Ub* ubiquitin, *UBC12* ubiquitin-conjugating enzyme 12

gation of NEDD8 to proteins, which results in their ubiquitination by Cullin-RING E3 ubiquitin ligase (CRL) and subsequent proteasomal degradation [161, 162].

Pevonedistat (MLN4924) is a first-in-class small molecule inhibitor of NAE. In preclinical studies, it downregulated NF-κB signalling, triggered oxidative stress, and caused apoptosis in AML cells [161, 162]. In view of its synergistic action with belinostat in inducing DNA SSBs and apoptosis in AML cells [163], this combination regimen will be tested in a phase I study in r/r AML patients (NCT03772925). The combination of pevonedistat and venetoclax also showed synergism in a preclinical model and yielded promising preliminary results in a phase I/II study [164, 165], prompting other phase I to III trials regarding this regimen (NCT04172844, NCT04266795, NCT03862157). Synergism between pevonedistat and LSD1 inhibitors was also demonstrated in another murine study [166]. These optimistic results paved way to phase I and randomized phase II trials evaluating the combination of pevonedistat and azacitidine, where it was effective and provided superior survival over azacitidine monotherapy along with a favourable safety profile [167, 168]. Common side effects of this agent include fever, peripheral edema, dyspnea, febrile neutropenia, nausea, gastrointestinal disturbances, and transaminitis. Pevonedistat will be evaluated in combination with LDAC (NCT03459859), cytarabine, and idarubicin (NCT03330821), HMAs (NCT04712942, NCT04090736, NCT03009240).

16.10 Targeting Nuclear Transport

16.10.1 XPO1 Inhibitors

Exportin 1 (XPO1), or chromosome maintenance protein 1 (CRM1), is a nuclear exporter responsible for the export of substances from the nucleus [169]. Aberrant activity of XPO1 contributes to the pathogenesis of AML via shuttling tumour suppressors, such as NPM1 and p53, into the cytoplasm, which perturbs their functions [169] (Fig. 16.10). Upregulation of XPO1 is also associated with FLT3 mutations and confers inferior prognosis in AML [169].

Small molecule inhibitors of XPO1, known as selective inhibitors of nuclear export (SINE) or KPT-SINE, have diverse anti-leukaemic functions. These orally available agents irreversibly bind to the cysteine^{528} residue of XPO1 and alter its conformation, preventing export of tumour suppressors. They also induce differentiation via upregulation of the myeloid differentiation marker CD11b and downregulate WT and mutant FLT3 as well as c-KIT [169]. In addition, their strong activity against *NPM1*-mutant blasts is highlighted by a lower IC50 compared to NPM1-WT blasts [169]. An early KPT-SINE, KPT-185, demonstrated downregulation of FLT3 and induction of apoptosis in AML cell lines, while its analogue, KPT-276, prolonged survival in murine models [170].

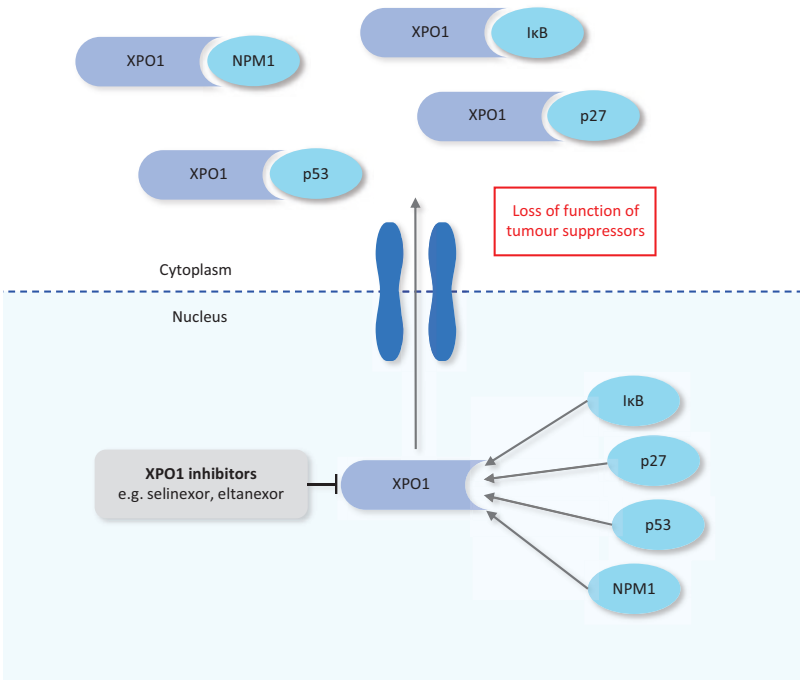


Fig. 16.10 Mechanism of actions of XPO1 inhibitors. *IκB* inhibitor of NF-κB, *NPM1* nucleophosmin 1, *p27* tumour protein 27, *XPO1* exportin 1

Selinexor (KPT-330), a first generation SINE, demonstrated preclinical synergism with topoisomerase inhibitors (idarubicin, daunorubicin, mitoxantrone, etoposide), cytarabine, and sorafenib [171–173]. As monotherapy, Selinexor produced modest responses among patients in a phase I trial, but the subsequent randomized phase II Selinexor in Older Patients with Relapsed/Refractory AML (SOPRA) trial was terminated due to a failure of meeting the expected survival endpoint [174, 175]. Selinexor has been tested with multiple agents, including 7 + 3 induction (daunorubicin/idarubicin and cytarabine), fludarabine and cytarabine, cladribine, cytarabine, G-CSF (CLAG), high-dose cytarabine (HDAC) and mitoxantrone, and decitabine, where it induced excellent responses among patients [172, 176–184]. In combination with sorafenib, it also exhibited anti-leukemic efficacy in FLT3-mutant AML patients [185]. The use of selinexor as post-HSCT maintenance therapy has been explored with optimistic results in a phase I trial [186]. However, due to the CNS-penetrating properties of selinexor, its therapy is associated with dose-limiting toxicities such as cerebellar toxicity, anorexia, weight loss, and nausea [169, 174, 175]. Other major side effects include gastrointestinal disturbances, myelosuppression, and asymptomatic hyponatraemia [172, 174–185]. Preclinical studies also suggested that it may exert undesirable activity against normal haematopoietic cells [172]. Nevertheless, selinexor is currently studied as monotherapy in r/r paediatric AML, in combination with standard chemotherapy or with venetoclax in adult patients, and as post-transplant maintenance therapy (NCT02091245, NCT02403310, NCT02835222, NCT03955783, NCT02485535).

Eltanexor (KPT-8602) is a second-generation SINE with similar potency as selinexor. It is suggested to have an improved safety profile due to a lower degree of CNS penetration and reduced effect on normal haematopoiesis [187]. It exhibited potent single-agent anti-leukaemic effect and synergism with venetoclax in preclinical studies [187–190].

16.11 Targeting Epigenetic Pathways

Epigenetic regulators, such as DNMTs and HDACs, regulate transcription via controlling DNA methylation and acetylation [191] (Fig. 16.11). Aberrant activities of these pathways result in transcription of oncoproteins and/or transcriptional silencing of tumour suppressors, resulting in leukaemogenesis [191].

16.11.1 Hypomethylating Agents

Hypomethylating agents exert anti-leukemic activities by inhibition of DNA methyltransferases (DNMT), causing demethylation and reactivation of tumour suppressor genes

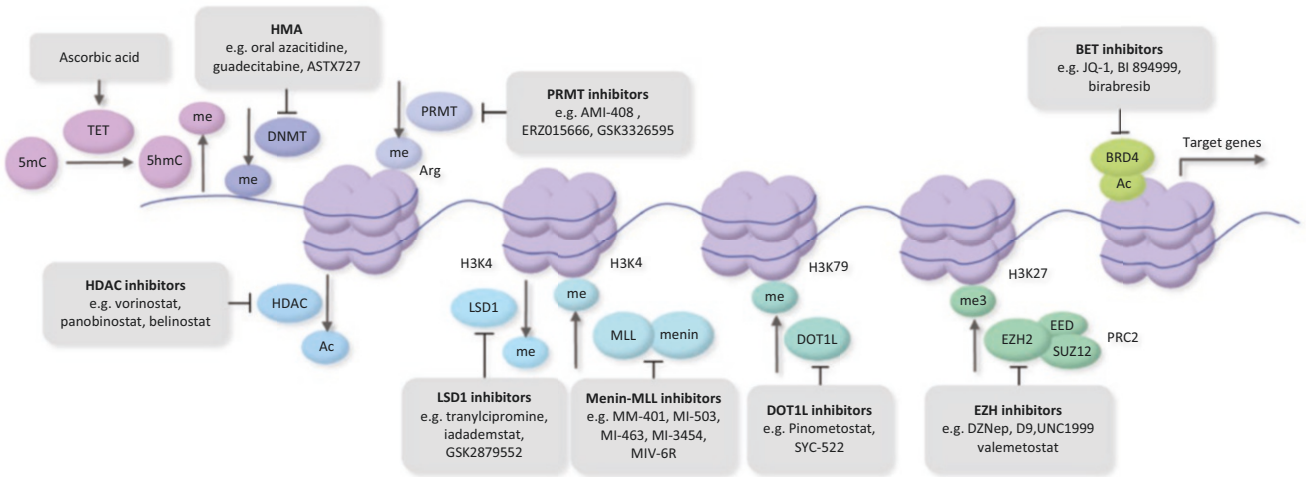


Fig. 16.11 Agents targeting epigenetic pathways. *5hmC* 5-hydroxymethylcytosine, *5mC* 5-methylcytosine, *Ac* acetyl group, *Arg* arginine residue, *BET* bromodomain and extra-terminal domain, *BRD* bromodomain-containing protein 4, *DNMT* DNA methyltransferase, *DOT1L* disruptor of telomeric silencing 1-like, *EED* embryonic ectoderm development, *EZH* drosophila enhancer of zeste homolog, *HDAC* histone deacetylase, *HMA* hypomethylating agents, *LSD1* lysine specific demethylase 1, *Me* methyl group, *Me3* trimethyl group, *MLL* mixed-lineage leukemia, *PRC2* polycomb repressive complex 2, *PRMT* protein arginine methyltransferase, *SUZ12* suppressor of Zeste 12, *TET* ten-eleven-translocation

[192]. Azacitidine and decitabine have been extensively studied and are widely used in AML patients. To enhance the ease of administration, an oral formulation of azacitidine (Onureg, CC-486) was developed and has recently been FDA-approved for the treatment of AML. In phase I trials, oral azacitidine demonstrated efficacy in DNA demethylation with a prolonged duration compared to subcutaneous azacitidine and a favourable safety profile, with common side effects being myelosuppression and gastrointestinal disturbances [193, 194]. In a subsequent randomized placebo-controlled phase III trial evaluating its use as maintenance therapy, oral azacitidine was significantly more effective at providing survival benefits [195, 196]. More randomized studies of oral azacitidine compared with placebos as maintenance therapies are ongoing (NCT04173533, NCT01757535).

Guadecitabine (SGI-110) is a deoxyguanosine analogue of decitabine with resistance to cytidine deaminase (CDA), thus prolonging its activity. Several trials of this agent in AML patients showed remarkable responses with tolerable toxicities, such as myelosuppression and infections [197–199]. However, subsequent phase III trials had disappointing results [200]. It is currently undergoing evaluation with talazoparib in r/r AML patients and with donor lymphocyte infusion (DLI) in post-HSCT patients (NCT02878785, NCT03454984, NCT02684162).

ASTX727, an oral formulation of decitabine with a cytidine deaminase inhibitor, cedazuridine, is currently compared with intravenous decitabine in a phase III randomized trial (NCT03306264). Its combinations with venetoclax, ivosidenib, enasidenib, and ASTX 660, a dual antagonist of cellular inhibitor of apoptosis protein (cIAP) 1 and X-linked inhibitor of apoptosis protein (XIAP), are also undergoing evaluation in clinical trials (NCT04657081, NCT04746235, NCT04774393, NCT04155580).

16.11.2 HDAC Inhibitors

Histone deacetylase (HDAC) and histone acetyltransferase (HATs) mediate deacetylation and acetylation of both histone and non-histone proteins. They are integral to the regulation of numerous cellular activities, such as gene transcription [201]. In AML, aberrant activation of HDAC by oncoproteins impairs the tumour suppressor function of p53, inhibits cellular differentiation, mediates aberrant signaling pathways (e.g. c-MYC), and induces abnormal proliferation [201]. Thus, the efficacies of multiple HDAC inhibitors have been studied in AML (Table 16.4) [201]. HDAC inhibitors can be classified into hydroxamines, benzamides, cyclic peptides, aliphatic acids, and electrophilic ketones according to their spectrum of activities and molecular structures [201].

Table 16.4 HDAC inhibitors and their developments

Agent	Phase	Observations	Ongoing/future trials	References
Hydroxamines				
Vorinostat (SAHA)	II	Preclinical	Combination with azacitidine (NCT00392353, NCT03843528)	[202–218]
		1. Synergism in combination with tozasertib (AURKi, MK-0457), NPI-0052 (proteasome inhibitor), cytarabine, etoposide, obatoclax (GX15–070), adavosertib, BPRK-341 (FLT3 inhibitor).	Combination with decitabine, cytarabine, G-CSF, fludarabine (NCT03263936)	
		Clinical	Combination with fludarabine, clofarabine, busulfan (NCT02083250)	
		1. Minimal activity as monotherapy.		
		2. Synergism with idarubicin; idarubicin, and cytarabine; GO and azacitidine; decitabine; decitabine, and cytarabine; sorafenib and bortezomib.		
		3. No additional survival benefit when added to azacitidine		
		4. Minimal efficacy in combination with alvocidib		

Table 16.4 (continued)

Agent	Phase	Observations	Ongoing/future trials	References
Panobinostat (LBH589)	III	Preclinical	Single agent post-HSCT maintenance therapy (NCT04326764)	[219–236]
		1. Greater potency than vorinostat		
		2. Synergism in combination with decitabine, azacitidine, venetoclax, adavosertib, BC2059 (β-catenin inhibitor), SP2509 (LSD1 inhibitor), JQ1, quizartinib, bortezomib, CXCR4 antagonists, doxorubicin, DZNep		
		Clinical		
		1. Minimal activity as monotherapy		
		2. Safe and effective in combination with idarubicin and cytarabine; daunorubicin, and cytarabine		
		3. No additional survival benefit when added to azacitidine; cytarabine, and mitoxantrone		
Belinostat (PXD101)	II	1. Synergism with bortezomib; pevonedistat	Combination with pevonedistat (NCT03772925)	[163, 237–240]
		2. Limited activity as monotherapy		
		3. Anti-leukemic efficacy in combination with bortezomib		
Pracinostat (SB939)	III	1. Modest clinical activity as monotherapy	Combination with GO (NCT03848754)	[241, 242]
		2. Combination with azacitidine effective in phase I trial, but phase III trial discontinued due lack of efficacy		
Givinostat (ITF2357)	Preclinical	1. Anti-leukemic efficacy in AML cell lines and murine models		[243, 244]
Tefinostat (CHR-2845)	Preclinical	1. Anti-leukemic efficacy, especially in monocytoid AML cell lines		[245]
Abexinostat (PCI-24781)	I	1. Phase I trial discontinued due to lack of efficacy		[246]
Benzamides				
Chidamide	I/Ib	1. Anti-leukemic efficacy against AML cell lines as single agent	Monotherapy (NCT03031262)	[247–258]
		2. Synergism in combination with decitabine; cytarabine ± sorafenib; anthracyclines; daunorubicin, idarubicin, cytarabine; venetoclax; MI-3 (menin-MLL inhibitor) and betulinic acid in preclinical studies		
		3. Clinically safe and effective in combination with decitabine, cytarabine, aclarubicin, and G-CSF		
Entinostat (MS-275)	II	1. Anti-leukemic efficacy in AML cell lines and murine models	Combination with azacitidine (NCT01305499)	[259–266]
		2. Evidence of activity against FLT3-mutant AML		
		3. Synergism with AZD6244 (MEK/ERK inhibitor); RAD001 (mTOR inhibitor); decitabine in preclinical studies		
		4. Limited clinical activity as monotherapy		
		5. Mixed clinical results in combination with azacitidine		
Mocetinostat (MGCD0103)	I	1. Anti-leukemic efficacy against AML cell lines		[267, 268]
		2. Effective and safe in phase I trial		
Cyclic peptides				
Romidepsin (FK228)	II	1. Effective against chemo-resistant AML murine models		[269–273]
		2. Synergism in combination with decitabine; azacitidine in preclinical studies		
		3. Limited clinical activity as monotherapy		
		4. Clinically safe and effective in combination with azacitidine		
Trapoxin A	Preclinical	Anti-leukemic efficacy against AML cell lines		[274, 275]

(continued)

Table 16.4 (continued)

Agent	Phase	Observations	Ongoing/future trials	References
Aliphatic acids				
Valproic acid		1. Anti-leukemic synergism in combination with ATRA; cytarabine; GO; bortezomib; dasatinib; nutlin-3; proteasome inhibitors (NPI-0052, PR-171) and curcumin in preclinical studies	Post-HSCT maintenance in combination with azacitidine (NCT02124174)	[265–272, 274–298]
		2. Unfavourable pharmacokinetic profile		
		3. Clinically effective in combination with HU; 6-MP; azacitidine; decitabine		
		4. Mixed clinical results in combination with cytarabine		
		5. Monotherapy and combination with ATRA ineffective in multiple trials		
		6. Risk of neurological toxicity		

6-MP 6-mercaptopurine, *AML* acute myeloid leukaemia, *ATRA* all-trans retinoic acid, *AURKi* aurora kinase inhibitor, *CXCR4* C-X-C chemokine receptor 4, *ERK* extracellular-signal-regulated kinase, *G-CSF* granulocyte colony-stimulating factor (G-CSF), *GO* gemtuzumab ozogamicin, *HU* hydroxyurea, *MEK* mitogen-activated protein kinase kinase, *MLL* mixed-lineage leukaemia

Among them, vorinostat, panobinostat, and belinostat appear to be the most clinically promising. These agents are generally safe with only mild side effects, such as fatigue, nausea, and gastrointestinal disturbances.

16.11.3 LSD1 Inhibitors

Lysine specific demethylase 1 (LSD1) controls demethylation of H3K4 and can function both as a transcription activator and repressor [299]. Inhibition of LSD1 was shown to promote differentiation of AML cells [299]. Multiple agents targeting this enzyme have been studied as potential therapies for AML.

Tranylcypromine (TCP) is a selective LSD1 inhibitor which induced differentiation of AML cell lines and demonstrated synergistic effect with ATRA [300]. In a subsequent phase I/II trial, this combination was proven to be effective in AML patients [301]. This agent was tolerable, with hypotension, orthostatic dysregulation, vertigo, confusion, and cytopenias as its major adverse effects. Another trial regarding these two agents in AML is ongoing (NCT02717884).

Various analogues of TCP also demonstrated preclinical activities against AML cells [302–313]. Notably, iadademstat (ORY-1001) exhibited remarkable preclinical anti-leukemic efficacy and was effective and tolerable as monotherapy in AML patients in a phase I trial [314, 315]. A phase II trial regarding its combination with azacitidine is underway (EudraCT No.: 2018–000482-36). Another agent, GSK2879552, synergized with ATRA to exert anti-leukaemic efficacy in preclinical studies, but disappointing survival benefits from a phase I trial led to termination of the study (NCT02177812) [316]. Another LSD1 inhibitor, CC-90011, is also undergoing evaluation in combination with venetoclax and azacitidine (NCT0474884).

16.11.4 BET Inhibitors

Bromodomain and extra-terminal domain (BET) is a family of epigenetic readers responsible for regulating gene transcriptions [317]. Importantly, bromodomain-containing protein 4 (BRD4) is a member of this family which has been identified as a crucial mediator of various oncogenic pathways [299]. JQ-1 is a selective BRD4 inhibitor with potent preclinical anti-leukaemic efficacy as monotherapy and in combination with other agents, including cytarabine, ATRA, azacitidine, and ponatinib [318–321]. BI 894999 is another BRD inhibitor which also demonstrated marked single-agent anti-leukaemic activity and synergism with LDC000067, a CDK9 inhibitor in a preclinical study [322]. In addition, birabresib (OTX015/MK-8628) showed preclinical activity against AML cells as monotherapy and therapeutic synergy with either panobinostat or azacitidine [323]. It is now undergoing evaluation as monotherapy in a phase I/II trial (NCT02698189).

16.11.5 TET Inhibitors

Ten-eleven-translocation (TET) enzymes inhibit DNA methylation via oxidizing 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) [299]. In AML, mutant-TET causes hypermethylation of various gene loci, resulting in impaired differentiation and uncontrolled proliferation [299]. Ascorbic acid serves as a co-factor for TET2 to restore its normal activity and is frequently found to be deficient in AML patients. It showed anti-leukemic efficacy in preclinical studies and synergized with decitabine to prolong patient survival in a clinical trial [324–326]. A phase II trial of azacitidine in combination with ascorbic acid is currently underway (NCT03397173).

16.11.6 Menin-MLL Inhibitors

Mixed-lineage leukemia (MLL) is a lysine methyltransferase which methylates H3K4, while menin functions as its co-factor. MLL translocations result in generation of oncoproteins and are generally markers of poor prognosis. Small molecule inhibitors with preclinical efficacies against MLL complexes include MM-401, MI-503, MI-463, and MIV-6R [327–330]. Strikingly, these agents also exhibited potent activity against *NPM1*-mutant AML cell lines, possibly due to the reliance of mutant NPM1 on Menin-MLL1 interactions for its aberrant gene expression [331]. In particular, MI-503 and MI-3454 selectively targeted *MLL1*-rearranged and *NPM1*-mutant cells and prolonged survival in murine models [331, 332].

16.11.7 DOT1L Inhibitors

Disruptor of telomeric silencing 1-like (DOT1L) is a histone methyltransferase mediating the methylation of H3K79 [299]. Since its function is integral to the oncogenic activities of MLL fusion complexes, it can be used as a potential target against *MLL*-rearranged AML [299]. Pinometostat (EPZ5676) is a DOT1L inhibitor with remarkable preclinical efficacy against *MLL*-rearranged cell lines and showed modest single-agent clinical activity along with a favourable safety profile [333–335]. It is currently being tested in combination with standard chemotherapy in *MLL*-rearranged AML patients (NCT03724084). SYC-522 is another agent with preclinical efficacy against *MLL*-rearranged AML [336].

16.11.8 EZH Inhibitors

Drosophila enhancer of zeste homolog (EZH) is a subunit of polycomb repressive complex (PRC) 2, which regulates trimethylation of H3K27 and mediates gene transcription [299]. Interestingly, they can function both as a tumour suppressor and oncoprotein in AML [299]. The selective EZH2 inhibitor 3-Deazaneplanocin A (DZNep) and its analogue D9 both showed efficacy against MLL-rearranged AML cells [337–339]. UNC1999 is a dual inhibitor of EZH1 and 2 with preclinical efficacy against AML models with *MLL* gene rearrangement [340]. Finally, valemetostat (DS-3201) is another dual EZH1/2 inhibitor currently evaluated as monotherapy in a phase I trial (NCT03110354).

16.11.9 PRMT Inhibitors

Protein arginine methyltransferases (PRMTs) are mediators of arginine methylation of histone as well as non-histone proteins and their overexpression is frequently found in AML [299]. AMI-408 is a specific inhibitor of PRMT1 with growth suppressive effect on AML cell lines and murine models [341]. ERZ015666, an inhibitor of PRMT5, induced differentiation of AML cells and showed efficacy in murine models with *MLL* rearrangements [342]. GSK3326595 is another PRMT5 inhibitor currently undergoing evaluation in combination with azacitidine in a phase I trial (NCT03614728).

16.12 Targeting DNA Damage Response Pathways

DNA damage response (DDR) is essential for the maintenance of genomic stability via halting cell cycle progression for DNA repair [343]. In the case of substantial DNA damage beyond repair, the apoptotic cascade would be initiated [343]. Studies have shown that AML cells have defective DDR mechanisms and are thus more susceptible to combined inhibition of chemical and DDR pathways [344]. Importantly, *IDH*-mutant AML is proposed to be sensitive to further inhibition of DDR due to their intrinsic defects in homologous recombination (HR).

16.12.1 PARP Inhibitors

Poly (ADP-ribose) polymerases (PARP) are a superfamily of 18 enzymes responsible for DNA single strand breaks (SSBs) repair and survival of cells with DNA damage [345]. Some subtypes of AML, such as those with *IDH1/2* and *FLT3* mutations, are proposed to be more sensitive to the effects of PARP inhibitors [345]. PARP inhibitors are nicotinamide analogues which function via the inhibition of DNA SSB repair by PARP and induction of cytotoxic allosteric effects by trapping PARPs to damaged DNA [345, 346].

Olaparib is a potent and selective PARP inhibitor which showed excellent potency against AML cell lines and synergistic activity with two anti-CD33 antibody drug conjugates, GO and IMGN779, in preclinical studies [347, 348]. Olaparib is in a trial as monotherapy for r/r IDH-mutant AML (NCT03953898).

Other PARP inhibitors with promising preclinical activities against AML include veliparib, talazoparib (BMN-673), niraparib, rucaparib, and PJ34 [345, 349, 350]. These agents showed synergistic activity against AML cell lines in combination with IMGN632 (anti-CD123 antibody drug conjugate), MS275 (HDAC inhibitor), entinostat (MS275, HDAC inhibitor), and AZD1775 in preclinical studies [351–354]. Among them, results of veliparib as single agent or in combination with temazolomide (alkylating agent) or topotecan and carboplatin in r/r ALM patients were impressive [355, 356]. Two trials regarding the use of these two combinations

in AML patients are ongoing (NCT00588991, NCT01139970). Talazoparib also demonstrated potent efficacy against *IDH1*-mutant AML cells [357]. Trials of talazoparib as monotherapy and in combination with decitabine are currently underway (NCT03974217, NCT02878785).

16.12.2 ATR Inhibitors

Ataxia telangiectasia and Rad3-related kinase (ATR) is responsible for detecting DNA SSBs. It subsequently activates downstream repair pathways or apoptotic cascades depending on the extent of DNA damage [343]. VX-970 and AZ20 are two ATR inhibitors which demonstrated single agent efficacy against AML cell lines [358, 359]. AZ20 also synergistically induced anti-leukemic activity with cytarabine in another preclinical study [360].

16.12.3 ATM Inhibitor

The function of ataxia telangiectasia mutated kinase (ATM) resembles that of ATR except for its detection of double strand breaks (DDBs) instead of SSBs in DNA [343]. AZD0156, an ATM inhibitor, prolonged survival of MLL-rearranged mice in a preclinical study [359]. In another study, KU-59403 also induced apoptosis in AML cell lines [361].

16.12.4 CHK Inhibitors

Checkpoint kinase (CHK) 1 and 2 inhibit CDK 1 and 2 and cause cell cycle arrest upon activation by ATR and ATM [343]. Their overexpression in AML is associated with inferior prognosis [362]. Prexasertib (LY2606368), MK-8776 (SCH900776), and rabusertib (LY2603618) are CHK inhibitors which synergistically induced apoptosis in combination with CPX-351 in TP53-WT and TP53-deleted AML cells [363]. Rabusertib also exhibited synergism with venetoclax against AML cells [364]. MK-8776 demonstrated activity at overcoming chemotherapeutic resistance and synergized with cytarabine and vorinostat [362, 365, 366]. However, the combination of MK-8776 with cytarabine did not provide survival benefit over single agent cytarabine in r/r AML patients in a subsequent trial [367]. A phase I trial of prexasertib in combination with cytarabine and fludarabine is underway (NCT02649764).

16.12.5 WEE1 Inhibitors

Wee1-like protein kinase (WEE1) is activated by CHK and induces cell cycle arrest by inhibition of CDK1 and 2 [343]. Adavosertib (AZD1775, MK-1775) exhibited synergism with panobinostat and olaparib, respectively, in AML cell lines [224, 354]. It also synergistically overcame cytarabine-resistance when combined with cytarabine in leukemic cells [368]. Unfortunately, a trial of adavosertib as monotherapy was terminated due to safety concerns and another trial of its combination with belinostat was terminated for unspecified reasons (NCT03718143, NCT02381548).

16.13 Targeting the Cell Cycle

The cell cycle is a 4-phased process and progression through each phase is under strict regulation by several mediators, including cyclin-dependent kinases (CDKs) and cell cycle checkpoints. Aberrant progression of the cell cycle results in uncontrolled proliferation and leukaemogenesis [151].

16.13.1 CDK Inhibitors

Cyclin-dependent kinases (CDKs) are regulators of cell cycle progression which are activated upon binding of cyclins. Among them, transcriptional CDKs (CDK7, 8, 9) are mainly responsible for regulating transcription (Fig. 16.12). Inhibition of CDKs can halt the cell cycle and inhibit aberrant gene expression, giving rise to anti-leukemic effects. Information regarding various CDK inhibitors is summarized in Table 16.5. Among them, palbociblib and alvocidib are the most widely studied in AML. These agents have an excellent safety profile with myelosuppression as a significant side effect.

16.13.2 Aurora Kinase Inhibitors

The aurora kinase (AURK) family consists of three members, AURK A, B, and C. These enzymes are responsible for entry into the M phase and normal progression of mitosis [393]. In AML, their overexpression has been observed and is associated with poor-risk cytogenetics. Alisertib (MLN8237) is an AURKA inhibitor with oral bioavailability. Investigational use of this agent in a phase II study in combination with induction chemotherapy among poor-risk AML patients illustrated its clinical effectiveness and safety [394]. Barasertib (AZD1152) is an AURKB inhibitor which demonstrated anti-leukaemic efficacy along with a less desirable safety profile in a phase I/II study in AML patients, with major side effects being febrile neutropenia and oral mucositis [395]. In another trial, it was tested in combination with LDAC and showed favourable outcomes and tolerability [396]. A trial of barasertib as monotherapy or in combination with venetoclax and/or azacitidine is underway (NCT03217838).

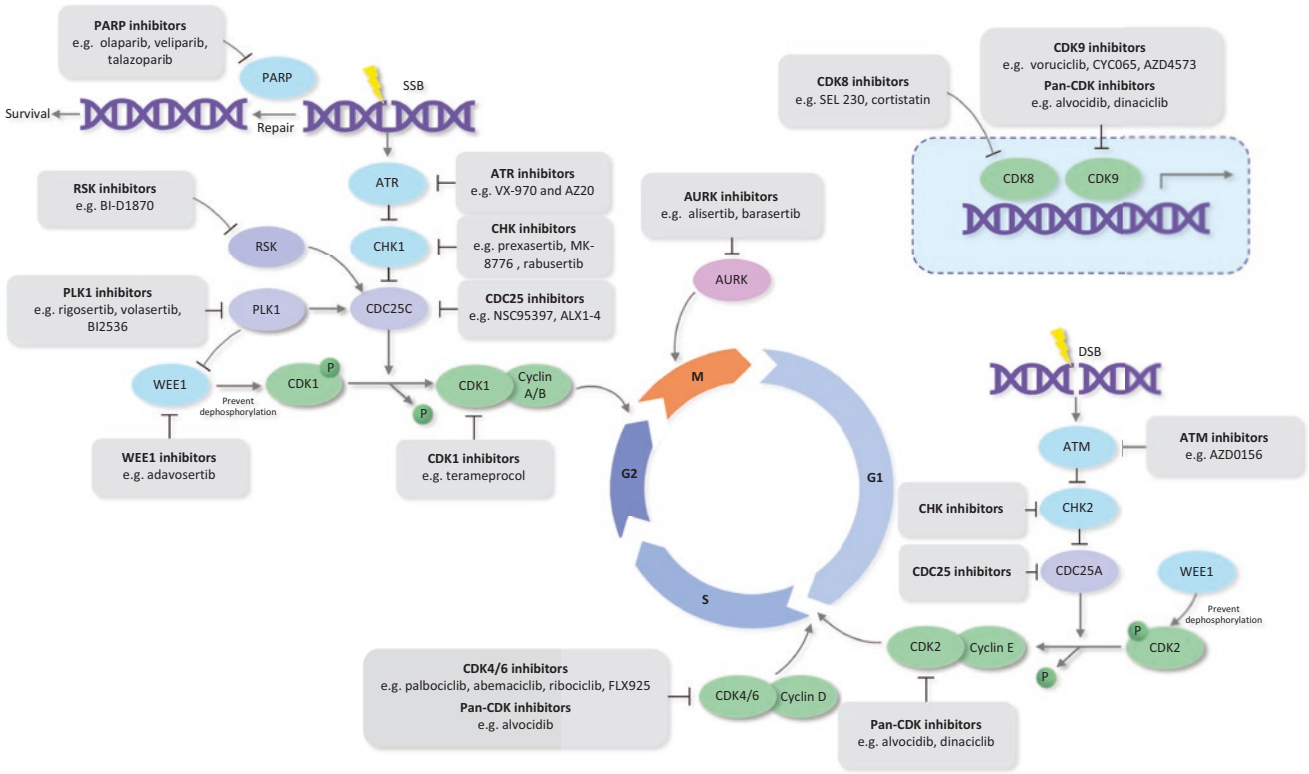


Fig. 16.12 Agents targeting DNA damage responses and cell cycle. *ATM* ataxia telangiectasia mutated kinase, *ATR* ataxia telangiectasia and Rad3-related kinase, *AURK* aurora kinase, *CDC25A* cell division cycle 25 A, *CDC25C* cell division cycle 25 C, *CDK1* cyclin-dependent kinase 1, *CDK2* cyclin-dependent kinase 2, *CDK4/6* cyclin-dependent kinase 4/6, *CHK1* checkpoint kinase 1, *CHK2* checkpoint kinase 2, *DSB* double strand breaks, *P* phosphate group, *PARP* poly (ADP-ribose) polymerases, *PLK1* polo-like kinases 1, *RSK* p90 ribosomal S6 kinase, *SSB* single strand breaks, *WEE1* Wee1-like protein kinase

16.13.3 PLK Inhibitors

Polo-like kinases (PLKs) promote cell cycle progression via inducing degradation of WEE1 and activating CDK1 [397, 398]. It also inhibits apoptosis by activating Bcl-xL [398]. In AML, its overexpression is frequently observed [399].

Rigosertib (ON01910) is a dual inhibitor of PLK1 and PI3K. In addition to exhibiting preclinical anti-leukemic efficacy, it was effective and tolerable as monotherapy and in combination with azacitidine in clinical trials [400–402]. Major adverse events were gastrointestinal disturbances, myelosuppression, and pneumonia. A phase II study of oral rigosertib in combination with azacitidine is underway (NCT01926587).

Volasertib (BI6727) is a selective PLK1/2/3 inhibitor. Encouraging results from preclinical studies in AML models paved way for subsequent clinical trials in AML patients [403]. In summary, volasertib was safe and effective as monotherapy and demonstrated synergism in combination with LDAC and decitabine, respectively, among AML patients in phase I and II trials [404–407]. However, responses of its combination with LDAC did not meet expectations in the randomized phase III POLO-AML-2 trial [408]. Significant side effects of volasertib include myelosuppression and fatigue. It is currently undergoing evaluation as monotherapy or in combination with cytarabine in several trials (NCT00804856, NCT01721876). Another oral PLK1 inhibitor, onvansertib (NMS-1286937), demonstrated impressive efficacy and safety in combination with decitabine, but limited activity with LDAC in a phase Ib study [409].

BI2536 also exhibited anti-leukemic effect in a preclinical study and had modest single agent activity in AML as reported in a phase I/Ib trial [410–412]. Other PLK1 inhibitors with preclinical efficacies against AML include TAK-960 and NMS-P937 [413, 414].

Table 16.5 Developments of cyclin-dependent kinase (CDK) inhibitors

Agent	Target	Phase	Observations	Ongoing/future trials	References
Selective CDK inhibitors					
Terameprocol	CDK1	I	1. Limited single agent anti-leukemic efficacy in phase 1 trial		[369]
Palbociclib (Ibrance, PD0332991)	CDK4/6	II	Preclinical	Monotherapy in MLL-rearranged acute leukaemia (NCT02310243)	[370–376]
			1. Inhibition of leukemic colony formation, induction of G1 cell cycle arrest in AML cells	Combination with CPX-351 (NCT03844997)	
			2. Synergism with cytarabine, danusertib, tozasertib, CCT137690 (AURKi), everolimus, MK-2206 2HCl (AKT inhibitor)	Combination with sorafenib, decitabine, or dexamethasone (NCT03132454)	
			3. Activity against FLT3-ITD and FLT3-D835Y-positive AML cells		
			4. Re-sensitization of FLT3 inhibitor and multi-drug-resistant AML cells		
			Clinical		
			1. Safe and effective in MLL-rearranged AML patients		
			2. Limited single agent activity in non-MLL-rearranged AML patients		
Abemaciclib	CDK4/6	I	1. Re-sensitization of multi-drug-resistant AML cells to mitoxantrone		[376]
			2. Phase I trial in r/r AML terminated after dose-escalation completion (NCT02335814)		
Ribociclib	CDK4/6	Preclinical	1. Re-sensitization of multi-drug-resistant AML cells to mitoxantrone		[376]
FLX925 (AMG925)	CDK4/6, FLT3	Preclinical	1. Re-sensitization of FLT3 inhibitor-resistant AML cells		[373]
SEL120	CDK8	Ib	1. Efficacy against AML cells and murine models via inducing apoptosis and differentiation	Monotherapy in AML (NCT04021368)	[377]
Cortistatin	CDK8	Preclinical	1. Efficacy against AML cells and murine models		[378]
CYC065	CDK9	I	1. Efficacy as monotherapy in AML cell lines	Combination with venetoclax (NCT04017546)	[379]
			2. Synergism with venetoclax; azacitidine; cytarabine		
AZD4573	CDK9	I	1. Anti-leukemic efficacy as monotherapy in AML cells and murine models	Monotherapy (NCT03263637)	[380]
Voruciclib	CDK9	I	1. Synergism with venetoclax in AML cells and models	Monotherapy (NCT03547115)	[381]
CDKi-73	CDK9	Preclinical	1. Efficacy against MLL-rearranged AML models		[382]
A-1592668	CDK9	Preclinical	1. Efficacy as monotherapy		[383]
			2. Synergism with venetoclax		
Pan-CDK inhibitors					
Alvocidib (Flavopiridol)	CDK1, 2, 4, 6, 7, 9[a]	II	Preclinical	In combination with venetoclax (NCT03969420)	[384–390]
			1. Single agent anti-leukemic activity		
			2. Synergism with cytarabine and venetoclax		
			Clinical		
			1. Safe and effective in combination with cytarabine in multiple trials		
			2. Combination with cytarabine and mitoxantrone (FLAM) provided no significant benefit over 7 + 3 induction in interim results of phase II trial, but showed benefit in subsequent analysis		
Dinaciclib (MK7965/ Scheme 727965)	CDK1, 2, 5, 9[a]	I	1. Efficacy against MLL-rearranged AML cells	In combination with venetoclax (NCT03484520)	[391, 392]
			2. Limited clinical efficacy as monotherapy		

AML acute myeloid leukaemia, *AURKi* aurora kinase inhibitor, *CDK* cyclin-dependent kinase, *FLT3* Fms-like tyrosine kinase 3, *FLT3-ITD* FLT3 internal tandem duplication, *MLL* mixed-lineage leukaemia, *r/r* relapsed or refractory

[a]Both alvocidib and dinaciclib are most potent against CDK9 [151]

16.13.4 CDC25 Inhibitors

Cell division cycle 25 (CDC25) is a protein phosphatase which modulates cell cycle progression via dephosphorylation of CDKs [415]. In a preclinical study, several CDC25 inhibitors, namely NSC95397, ALX1, ALX2, ALX3, and ALX4, inhibited proliferation of AML cells, but did not demonstrate cytotoxic effects [415].

16.13.5 RSK Inhibitor

p90 Ribosomal S6 Kinase (RSK) is a downstream mediator of the Ras/MAPK/ERK pathway and controls a wide range of cellular pathways, including the promotion of cell cycle progression via activation of CDC25 and CDK1 [416]. In AML, upregulation of RSK has been discovered in patient samples and is indicative of poor prognosis. BI-D1870 is an RSK inhibitor which exerts potent anti-leukemic activity via S phase cell cycle arrest, impeding mitotic exit, and induction of DNA damage [416, 417]. It was effective as monotherapy and showed synergism with vincristine in AML cell lines [416, 417].

16.14 Targeting the Bone Marrow Microenvironment

The bone marrow microenvironment (BMM) plays crucial roles for the normal development of HSCs and other haematopoietic cells. In AML, the complex interactions between leukaemic cells and the BMM are integral to their development and disease progression [418]. With overwhelming evidence suggesting the substantial abnormalities in the BMM of AML patients, multiple therapeutic strategies to target these aberrant pathways are being explored (Fig. 16.13) [418].

16.14.1 SDF1/CXCR4 Inhibitors

C-X-C chemokine receptor (CXCR) type 4 is a HSC surface G-protein-coupled chemokine receptor for stromal-derived factor 1 (SDF1), also known as CXCR12, which is produced by mesenchymal stromal cells. Their interactions promote survival, quiescence, and marrow homing of HSCs. Leukaemic cells exploit this mechanism by upregulating their expressions of CXCR4, which grants them chemoresistance due to protection by marrow stromal cells.

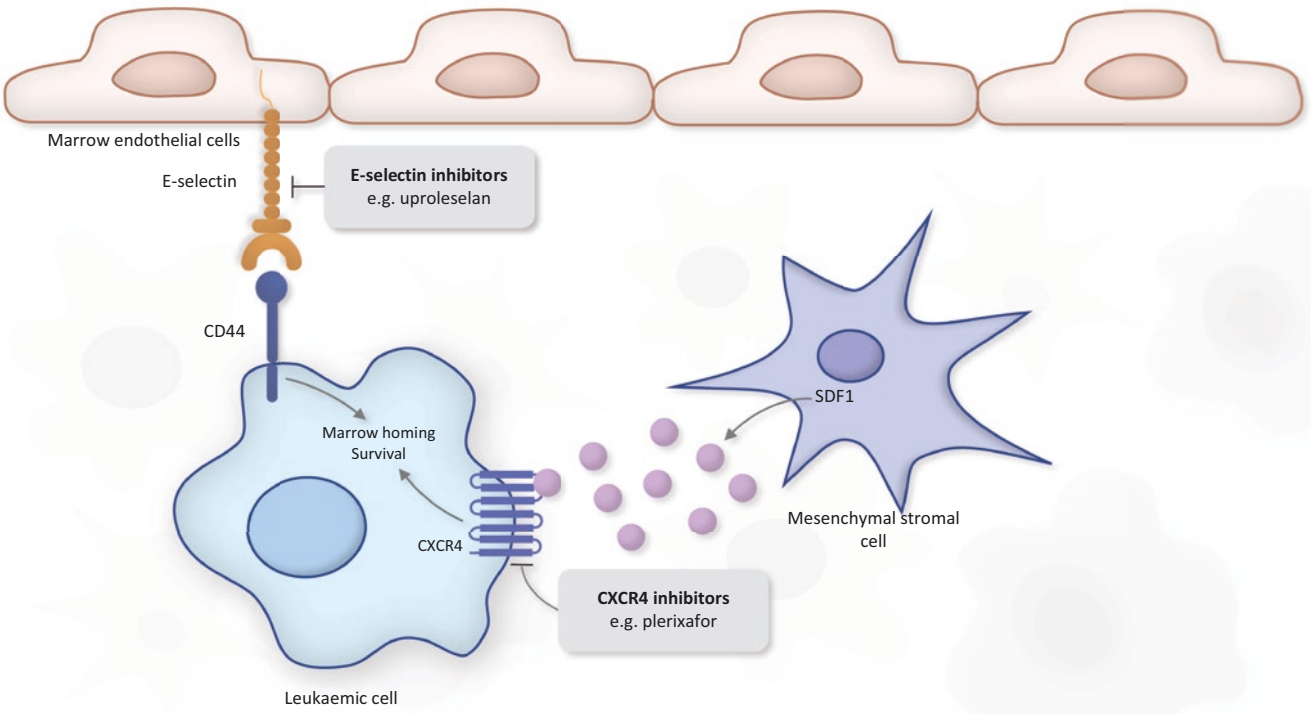


Fig. 16.13 Agents targeting the bone marrow microenvironment. *CD44* cluster of differentiation 44, *CXCR4* C-X-C chemokine receptor type 4, *SDF1* stromal-derived factor 1

Plerixafor (AMD3100) is a small molecule inhibitor of CXCR4 commonly used as an off-label stem cell mobilizing agent. Promising results from preclinical studies prompted several trials of plerixafor in combination with chemotherapies [419, 420], decitabine [421], as well as with G-CSF with or without sorafenib [422]. These studies all showed impressive survival outcomes and demonstrated the remarkable potential of plerixafor as a chemo-sensitizing and AML blast mobilizing agent. It will be tested as a chemo-sensitizing agent prior to pre-transplant conditioning in a phase II trial (NCT02605460).

16.14.2 E-Selectin Inhibitors

E-selectins are molecules expressed by vascular endothelial cells which mediate cellular adhesion. Leukaemic cells express CD44, the ligand for E-selectins, to promote their engraftment in the bone marrow. Uproleselan (GMI-1271) is an inhibitor of e-selectin which showed preclinical efficacy in overcoming chemoresistance and synergism with chemotherapeutic agents [423]. Its use in several clinical trials in combination with chemotherapy yielded profound response rates, excellent tolerability, and even reduction in risks of mucositis [424–426]. Phase III trials evaluating comparing responses to chemotherapy with or without uproleselan are underway (NCT03616470, NCT03701308).

16.15 Immunotherapy

Immunotherapy represents a new era of therapies in AML and has been intensively studied in recent years. Broadly, these strategies can be classified into antibody-based or T/NK-cell-based depending on their mechanism of actions. The former involves targeting cell surface antigens of leukemic cells, while the latter relies on activation of immune responses against leukaemic cells. Compared to conventional chemotherapy, they are generally more tolerable due to reduced toxicity on normal cells.

16.15.1 Antibody-Based Immunotherapies

16.15.1.1 Antibody-Drug Conjugates

Antibody-drug conjugates (ADJs) are synthesized via the conjugation of cytotoxic agents to antibodies against various cell surface antigens of AML cells or LSCs. Upon cell surface receptor binding, they are endocytosed and release their cytotoxic moieties to induce leukaemic cell death (Fig. 16.14).

Anti-CD33 ADJs

CD3 is expressed primarily on LSCs and not in normal haematopoietic cells [152]. Thus, targeting this cell surface antigen allows selective eradiation of LSC while sparing normal haematopoietic cells [152]. Gemtuzumab ozogamicin (GO; Mylotarg) is an FDA-approved anti-CD33 ADJ with the cytotoxic agent calicheamicin as a conjugate. GO was first FDA-approved for the treatment of AML in 2000, but was withdrawn in 2010 in view of its non-superior survival benefit compared to standard 7 + 3 induction and high risks of toxicities, such as veno-occlusive diseases (VODs), and hepatotoxicity [427]. Despite these discouraging events, GO was continually studied at fractionated and lower doses with optimistic results. Notably, the phase III randomized ALFA-0701 study showed that the addition of GO to standard induction chemotherapy provided marked survival benefits with only a slight increase in risks of VODs [428, 429]. Another randomized phase III trial (AML-19) also reported that GO improved patient survival to a larger extent than best supportive care [430]. Following these encouraging results, GO was re-approved by the FDA for newly diagnosed and relapsed AML patients. However, it should be noted that subsequent controlled trials still reported higher risks of VODs and early mortality with GO therapy than in control groups [431]. Apart from VODs, other toxicities of GO include haemorrhage, infections, gastrointestinal disturbances, febrile neutropenia, and myelosuppression.

Further trials of GO include its use as monotherapy (NCT03737955) or in combination with pracinostat (NCT03848754) and venetoclax (NCT04070768); talazoparib (NCT04207190); OX40, venetoclax, avelumab, glasdegib, and azacitidine (NCT03390296); mitoxantrone and etoposide (NCT03839446), CPX-351 (NCT03904251, NCT03878927, NCT03672539), midostaurin and standard induction therapy (NCT03900949, NCT04385290), CLAG (NCT04050280), CLAG, and mitoxantrone (CLAG-M) (NCT03531918); fludrabine, cytarabine, G-CSF, idarubicin (NCT00801489); cytarabine, daunorubicin, erwinase, and etoposide (NCT04326439). It will also be tested as induction therapy followed by glasdegib (NCT04093505) or non-engraftment donor leukocyte infusion (NCT03374332),

Vadastuximab talirine (SGN33A) is another anti-CD33 ADJ linked to a pyrrolobenzodiazepine dimer. In preclinical studies, it exhibited remarkable anti-leukaemic activity in a diverse panel of cell lines, including those with *TP53*-mutant and multi-drug-resistant phenotypes [432]. It induced remarkable responses as monotherapy in AML patients in a number of trials, with some achieving MRD negativity [433, 434]. Although it also showed potent efficacy and induced MRD-negativity in combination with azacitidine in a phase I trial [435, 436], the subsequent randomized-phase III

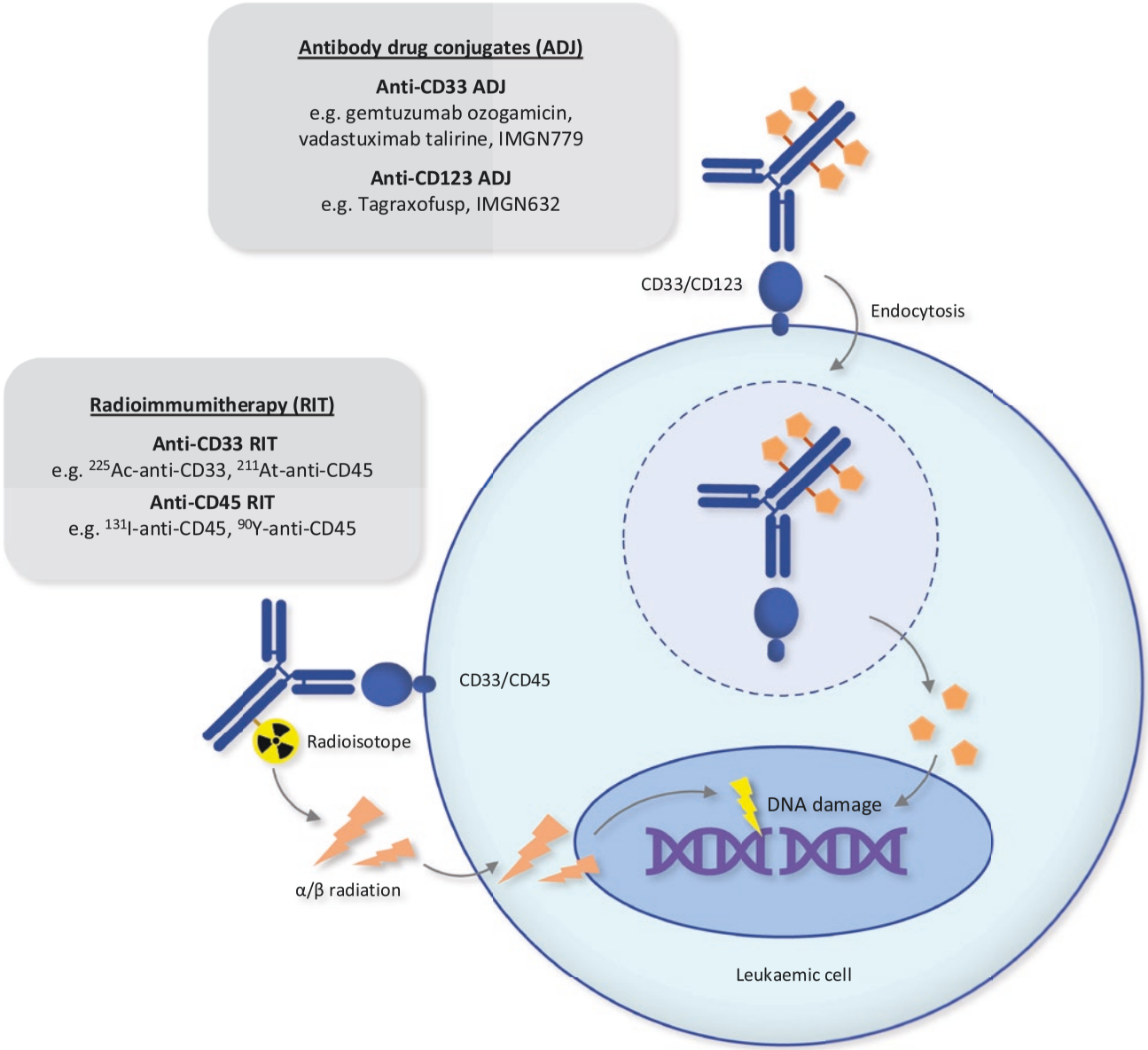


Fig. 16.14 Mechanism of actions of antibody-based immunotherapies. ^{131}I Iodine-131, ^{225}Ac Actinium-225, ^{90}Yt Yttrium-90, *CD123* cluster of differentiation 123, *CD33* cluster of differentiation 33, *CD45* cluster of differentiation 45, *DNA* deoxyribonucleic acid

CASCADE trial was discontinued due to increased mortality in the experimental arm [437]. Treatment-related deaths were attributed to severe infections rather than VODs [437].

IMGN779 also targets CD33 and is conjugated to DGN462, an alkylating agent. It showed anti-leukaemic activity against multiple AML cell lines and murine models, with the highest activity in cells harbouring FLT3-ITD [438, 439]. Its use in a phase I trial yielded impressive response rates and tolerability [440].

Anti-CD123 ADJs

CD123 functions as an interleukin (IL)-3 receptor and mediates downstream proliferation induced by IL-3 [152]. It was also found to be highly expressed on LSCs and is an attractive target for eliminating leukaemic colony forming activities [152].

Tagraxofusp (SL-401) consists of an anti-CD33 antibody conjugated to part of the diphteria toxin [152]. It will be evaluated as monotherapy or in combination with venetoclax with or without azacitidine in AML patients (NCT04342962, NCT03113643).

IMGN632 is conjugated to DNA mono-alkylating portion of the indolinobenzodiazepine pseudodimer. It showed encouraging activity and favourable safety profile in a phase I trial [441]. Since it demonstrated synergism with azacitidine and venetoclax in a preclinical study [442], its combination with venetoclax, azacitidine, or both agents will be tested in a phase I/II trial (NCT04086264). Its use as monotherapy in AML patients will also be tested (NCT03386513).

16.15.1.2 Radioimmunotherapy

Radioimmunotherapy (RIT) involves the use of monoclonal antibodies linked with radionuclides, which then bind to leukaemic cell surface antigens and continually release ionizing radiation, resulting in selective anti-leukaemic effects (Fig. 16.14) [443]. In AML, RITs mainly utilize Iodine(I)-131 and Yttrium(Yt)-90 and are usually studied as pre-transplant conditioning regimens.

^{131}I-anti-CD45 RIT markedly improve post-transplant outcomes in various clinical trials in combination with various conditioning regimens, including total body irradiation (TBI), busulfan and cyclophosphamide, as well as fludarabine and low-dose TBI [444–447]. The use of ^{90}Y-anti-CD45 RIT also resulted in remarkable survival outcomes and prolonged donor engraftment [448, 449].

While the above two agents emit β-radiation, ^{225}Actinium(Ac)-lintuzumab (^{225}Ac-anti-CD33) emits short-ranged α-radiation. Although it induced blast reduction in patients, no remissions were seen in a phase I trial combining this agent with LDAC in newly diagnosed patients [450]. ^{225}Ac-lintuzumab and ^{211}Astatine(At)-anti-CD45 will undergo further testing either as therapy for r/r patients or as part of conditioning regimens in multiple clinical trials (NCT03867682, NCT03441048, NCT03670966, NCT03128034).

16.15.2 T-Cell-Based Immunotherapies

T cells are integral to the normal functioning of the adaptive immune system. In particular, cytotoxic T cells are responsible for the elimination of cells carrying abnormal antigens, including leukaemic cells. However, these activities often impaired in AML, giving rise to immune evasion of leukaemic blasts. Thus, intensifying the anti-tumour responses of T cells is an attractive strategy against AML.

16.15.2.1 Immune-Related Adverse Events

Although immune-cell-based immunotherapies are generally considered to be more tolerable than conventional therapies, their resulting alterations in immune responses cause a distinct group of side effects termed "immune-related adverse events". They can present in a multitude of ways, including as skin rash, pneumonitis, and colitis, among others [451]. Fortunately, the majority of these events are tolerable and not lethal. In addition, cytokine release syndrome (CRS) is especially common with the use of multivalent antibodies and CAR-T, with presentations ranging from mild flu-like symptoms to severe multi-organ failures and encephalopathy [452]. Cautious monitoring and proper management of CRS are keys to preventing significant morbidities and mortality.

16.15.2.2 Immune Checkpoint Inhibitors

Immune checkpoints (ICs) inhibit aberrant T-cell responses against normal body cells and are paramount to self-tolerance. However, leukemic cells can also express checkpoint ligands, which cause anergy of T-cells upon their binding, resulting in immune evasion and uncontrolled proliferation. Therefore, inhibition of these pathways allows reactivation of immune responses against leukemic cells (Fig. 16.15).

PD-1/PD-L1 Inhibitors

Programmed death 1 (PD-1) is expressed on the surface of T cells while its ligand, Programmed death ligand 1 (PD-L1), is expressed on leukaemic cells [453]. Nivolumab, an anti-PD-1 antibody, induced impressive responses in combination with azacitidine in older r/r AML patients in a phase II trial [454]. Addition of ipilimumab to this regimen further improved survival outcomes at the cost of increased toxicities and immune-related adverse events [455]. The above study is still currently ongoing (NCT02397720). Another trial of nivolumab with cytarabine or idarubicin showed remarkable remission rates with measurable residual disease (MRD) negativity in more than half of the cohort [451].

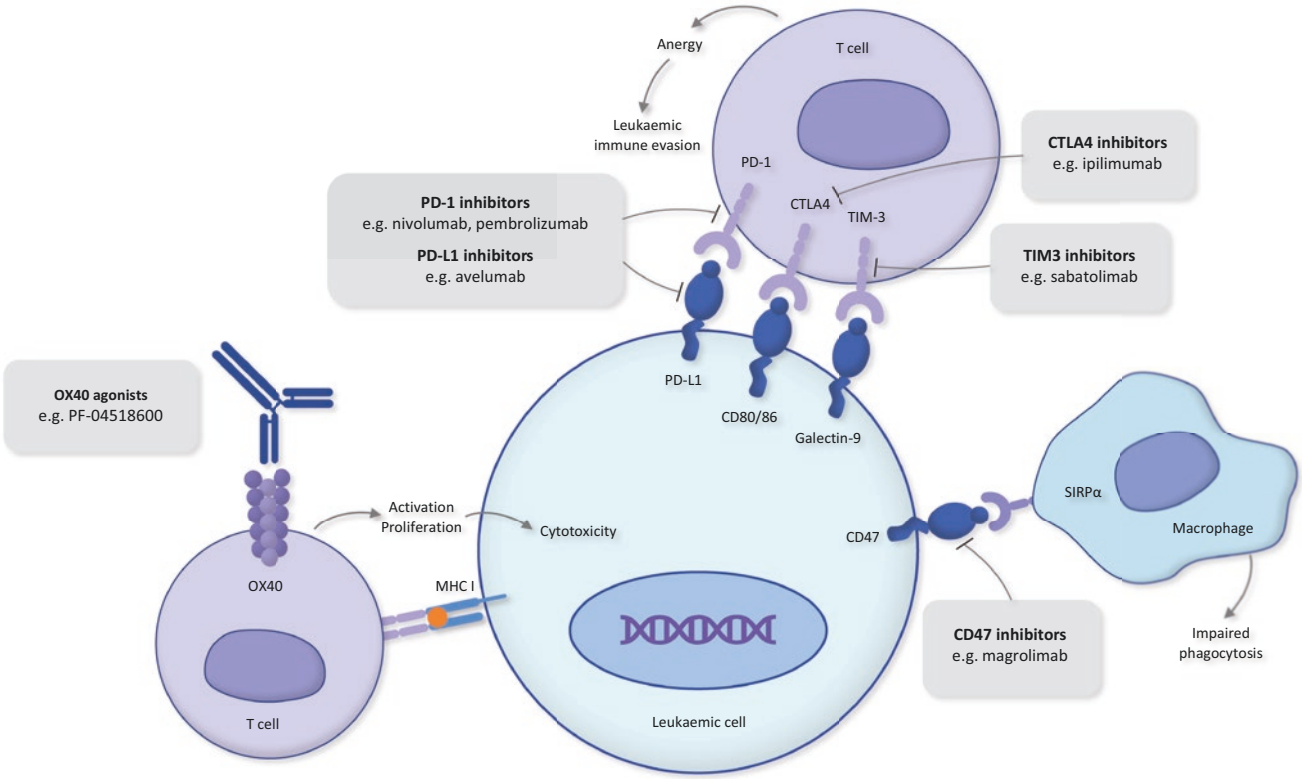


Fig. 16.15 Mechanisms of actions of immune checkpoint inhibitors and OX40 agonists. *CD47* cluster of differentiation 47, *CD80/86* cluster of differentiation 80 or 86, *CTLA4* cytotoxic T-lymphocyte-associated protein 4, *MHC I* major histocompatibility complex class I, *OX40* tumor necrosis factor receptor superfamily, member 4, *PD-1* programmed death 1, *PD-L1* programmed death ligand 1, *SIRPα* signal regulatory protein alpha, *TIM3* T cell immunoglobulin and mucin domain-containing protein 3

Nivolumab is generally tolerable with mostly immune-related adverse events, such as skin rash, transaminitis, and nephritis. However, another trial of nivolumab as post-transplant therapy demonstrated minimal efficacy and unacceptable adverse effects [456]. In view of these encouraging results, it will be further evaluated in multiple trials, including combination with azacitidine in r/r paediatric AML patients, as monotherapy in post-transplant relapsed patients, and as post-chemotherapy or post-HSCT maintenance as monotherapy or in combination with ipilimumab (NCT03825367, NCT01822509, NCT02275533, NCT02532231, NCT03600155, NCT02846376). Its combination with NY-ESO-1 vaccination and decitabine will also be tested in a clinical trial (NCT03358719).

Pembrolizumab is another anti-PD-1 antibody which has been tested in combination with azacitidine, decitabine, and following HDAC in AML patients; their respective trials all showed optimistic outcomes and tolerable adverse effects which were mostly immune-related [457–459]. Further trials will evaluate this agent in combination with decitabine, galinpepimut-S, azacitidine, venetoclax, with intensive chemotherapy as frontline therapy, with azacitidine in *NPM1*-mutant AML patients with molecular relapse, and as monotherapy in patients with post-HSCT relapse (NCT03969446, NCT03761914, NCT04284787, NCT04284787, NCT03769532, NCT02981914, NCT03286114).

Avelumab, an anti-PD-L1 mAb, showed anti-leukaemic efficacy and tolerability in combination with decitabine in a phase I trial and is currently involved in a phase I/II trial with venetoclax, PF-04518600, glasdegib, GO, and azacitidine (NCT03390296) [460].

However, durvalumab (MEDI-4736), another anti-PD-L1 antibody, did not provide additional survival benefits when added to azacitidine in a randomized phase II study [461].

CTLA-4 Inhibitors

Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) is expressed on the surface of T cells. It competitively binds to CD80 or CD86 expressed by leukaemic cells with a higher affinity than CD28 [453], inducing T cell anergy. Ipilimumab is an anti-CTLA-4 antibody which induced responses in AML patients who experienced relapse after HSCT, with graft-versus-host disease (GVHD) as the dose limiting toxic-

ity in some patients [462]. It is undergoing evaluation in combination with decitabine, as monotherapy in patients with post-transplant relapse, as post-HSCT maintenance either as monotherapy, in combination with nivolumab, or in combination with donor lymphocyte infusion (NCT02890329, NCT01822509, NCT03600155, NCT02846376, NCT03912064).

TIM-3 Inhibitors

T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3) receptors on T cells are activated by the binding of galectin-9 on leukemic cell surface [453]. Sabatolimab (MBG453) is an anti-TIM3 antibody. In a phase Ib trial, its combination with either azacitidine or decitabine showed promising anti-leukaemic activity [463]. This agent was tolerable, with myelosuppression and immune-related adverse effects being major side effects. Trials will further explore its combination with azacitidine and venetoclax, HDM201, and decitabine (NCT04150029, NCT03940352, NCT03066648). Its use in MRD-positive post-transplant patients will also be tested (NCT04623216).

CD47 Inhibitors

CD47 functions as an immune checkpoint by binding to Signal regulatory protein alpha (SIRPα) receptors on macrophage and preventing phagocytosis of CD47-positive cells [464]. The anti-CD47 mAb magrolimab is currently undergoing clinical evaluation in combination with azacitidine with optimistic preliminary results from a phase Ib trial [465]. It has been granted fast track designation by the FDA and will be tested in combination with azacitidine and venetoclax in a phase I/II trial (NCT04435691). A phase III trial comparing magrolimab combined with azacitidine against standard therapy is also underway (NCT04778397).

16.15.2.3 Targeting Co-Stimulatory Pathways

OX40 Agonists

OX40 is a cell surface receptor predominantly expressed by activated T cells, while its ligand, OX40L, is widely expressed by activated antigen presenting cells. The binding of OX40L to OX40 provides a co-stimulatory signal necessary for further T cell activation, clonal expansion, and anti-leukaemic immune responses [466] (Fig. 16.15). (PF-8600) is an anti-OX40 agonist monoclonal antibody currently investigated in combination with venetoclax, avelumab, glasdegib, GO, and azacitidine in a phase I/II trial (NCT03390296).

16.15.2.4 Multivalent Antibody Therapies

Multivalent antibody therapies facilitate interactions between immune cells and leukaemic cells. These recombinant antibodies are constructed by the combination of antibodies targeting these two types of cells and thus carry specificity against multiple antigens. This allows them to bring immune cells to the proximity of leukaemic cells for exerting anti-tumour effects (Fig. 16.16). Broadly, they can be divided into non-IgG-like and IgG-like, where only IgG-like multivalent antibodies retain the Fc region to promote additional immune pathways, such as antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) [467]. Currently, bivalent antibodies are the most widely studied in AML.

Non-IgG like Multivalent Antibodies

BiTE

Bispecific T-cell engagers (BiTEs) contain both the heavy and light chain variable domains (VH and VL) of the single chain variable fragments (scFv) from two antibodies targeting T cells (e.g. CD3) and leukaemic cells (e.g. CD33, CD123), respectively.

AMG330 is an anti-CD33 x anti-CD3 BiTE with encouraging preclinical efficacy and is now currently evaluated in a trial with r/r or MRD-positive AML patients (NCT02520427) [468, 469]. AMG673 is another anti-CD33 x anti-CD3 BiTE. Preliminary results from an ongoing first-in-human phase I study revealed anti-leukaemic efficacy (NCT03224819) [470]. Manageable adverse effects, such as CRS and myelosuppression, were observed. AMV564 has the same antigen specificity as the above BiTEs, but is tetravalent, i.e. it contains two VH and VL chains from each types of antibody, which further promotes target binding. Favourable preclinical data promoted a phase I trial, which showed promising preliminary results (NCT03144245) [471, 472].

Dart

Dual-affinity retargeting (DART) antibodies are similar in principle to BiTEs, except the VH and VL chains from the two antibodies are cross-linked to further increase efficiency [467]. Flotetuzumab (MGD-006) is anti-CD3 x anti-CD123 DART which induced T-cell activation against CD123-positive leukaemic blasts in preclinical studies. Preliminary results from the subsequent in-human trial showed potent anti-leukaemic activity and manageable side effects, which mainly included CRS [473].

IgG-Like Multivalent Antibodies

XmAb14045 is an anti-CD123 x anti-CD3 multivalent antibody. Its long serum half-life of 6.2 days can be attributed to the binding of its bispecific Fc domain to neonatal Fc receptor (FcRn), which prevents its degradation [452, 467]. This agent demonstrated preclinical anti-leukaemic efficacy and induced T-cell activation. Although a phase 1 study in AML patients was suspended following occurrences of patient mortalities and major toxicities, including CRS and pulmo-

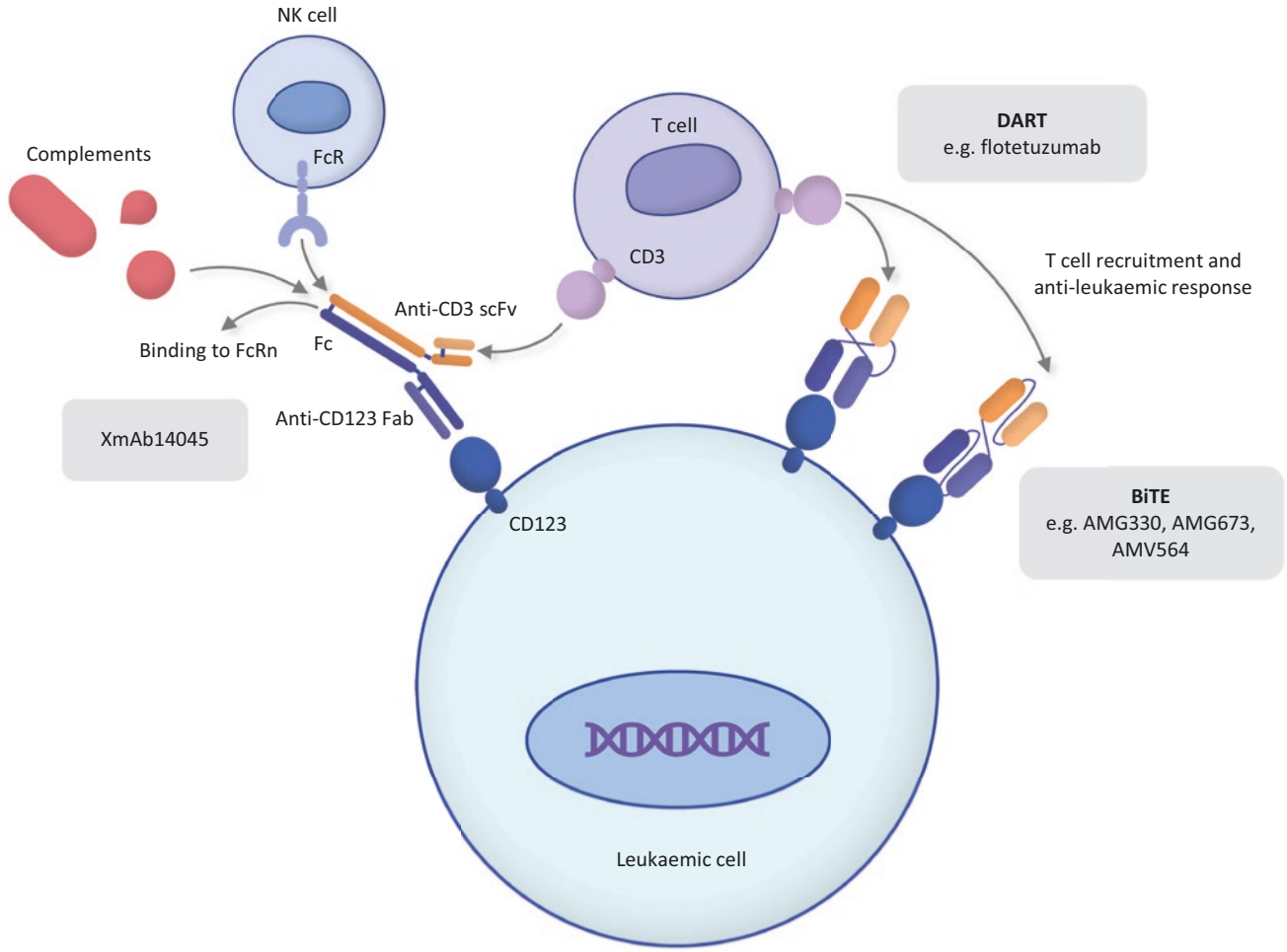


Fig. 16.16 Mechanism of actions of multivalent antibodies. *BiTE* bispecific T cell engager, *CD3* cluster of differentiation 3, *CD123* cluster of differentiation 123, *DART* dual-affinity retargeting antibodies, *Fab* antigen-binding fragment, *Fc* fragment crystallizable, *FcRn* neonatal Fc receptor, *NK* natural killer, *scFv* single chain variable fragment

nary edema, the partial suspension was shortly lifted by the FDA [474, 475]. A subsequent phase I study reported encouraging efficacy with manageable adverse events, such as CRS (NCT02730312) [476, 477]. The clinical activity and tolerability of XmAb14045 will be further elucidated in another trial (NCT02730312).

16.15.2.5 Chimeric Antigen Receptor T Cells Therapy

Chimeric antigen receptor T (CAR-T) cells therapy involves the use of genetically engineered T cells expressing chimeric antigen receptors (CAR) against leukaemic cell surface antigens. After infusion of CAR-T cells, binding of CAR to leukaemic cells triggers cytotoxic responses and leukaemic cell death (Fig. 16.17) [427].

CYAD-01 is a CAR-T cell product expressing the natural killer group 2D (NKG2D) fused to a CD3ζ signalling domain. NKG2D is normally expressed by natural killer cells, CD8+ T cells and NK-T cells. It is activated upon binding to NKG2D ligand (NKG2D-L) expressed by leukaemic cells, while a co-stimulatory signal is provided by DNAX-activating protein 10 (DAP10) [478]. Astonishingly, CYAD-01 is capable of inducing a co-stimulatory signal via DAP10-independent pathways. In a phase I trial, it was determined to possess anti-leukaemic activity. However, frequent adverse effects, including CRS and pneumonitis, were observed [479]. Another phase I trial with the use of NKG2D CAR-Tx cells in AML patients is underway (NCT04658004).

CAR-T cell products can also be engineered to target more than one leukaemic antigens to further improve potency. Notably, a compound CAR-T (cCAR-T) product constructed to target C-type lectin-like molecule-1 (CLL1) and CD33 was evaluated in a phase I study with remarkable results, with seven out of nine patients achieving remission and MRD-negativity [480, 481].

Other CAR-T products with preclinical successes in AML include c-Kit-targeting and FLT3 scFv-targeting CAR-Ts [482, 483]. CAR-T is undergoing intensive studies in numer-

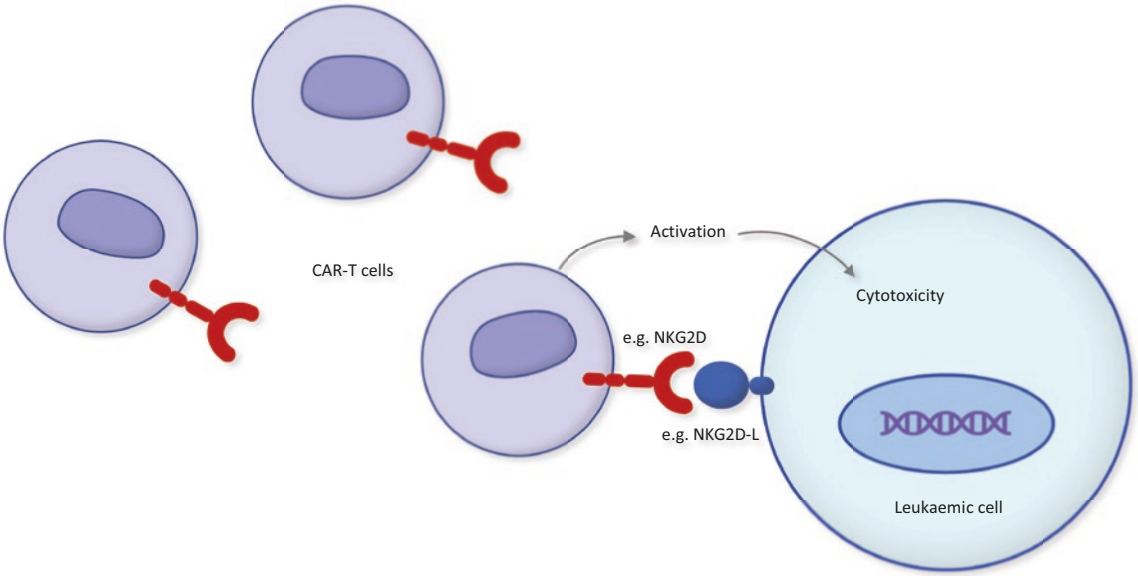


Fig. 16.17 Mechanism of action of chimeric antigen receptor (CAR) T cells. *NKG2D* natural killer group 2D, *NKG2D-L* NKG2D ligand

ous clinical trials, including novel CAR-T strategies such as donor-derived CAR-T (NCT04766840), CD123/CLL1 CAR-T (NCT03631576), CD38-targeted CAR-T (NCT04351022), and IL3 CAR-T (NCT04599543), among many others.

16.15.3 NK Cell-Based Immunotherapies

NK cells are paramount effectors of anti-tumour immune responses. After binding of an antibody to a cell surface antigen, the Fc receptors of NK cells bind to the Fc region of the antibody, resulting in activation of NK cells and release of cytotoxic materials for target cell killing. This process is known as antigen-dependent cellular cytotoxicity (ADCC). NK cell-based immunotherapies aim at harnessing the cytotoxic activity of intrinsic or foreign NK cells against leukaemic cells (Fig. 16.18).

16.15.3.1 Unconjugated Antibodies

Unlike ADJs, unconjugated antibodies exert cytotoxicity by stimulating ADCC as well as CDC activated by their Fc domains. Daratumumab is an anti-CD38 mAb which demonstrated anti-leukaemic activity against AML cell lines via induction of ADCC and CDC [484]. Interestingly, it also targets leukaemic blasts via perturbing cellular metabolism [485]. It is currently studied as monotherapy and in combination with FT538 (NCT04714372, NCT03067571). A study of daratumumab in combination with DLI for patients who relapsed post-HSCT is also underway (NCT03537599).

Isatuximab is another anti-CD38 mAb with potent anti-leukaemic activity in a preclinical study [486]. It will undergo further evaluation in combination with chemotherapy in r/r paediatric AML patients (NCT03860844).

Talacotuzumab (CSL362) is a CD123 which demonstrated high potency against CD123 in preclinical studies [487]. However, its uses as monotherapy or in combination with decitabine were only minimally effective in multiple clinical trials and caused high incidences of treatment termination [488, 489].

16.15.3.2 CAR-NK Cells Therapy

CAR-NK cells are engineered to express receptors which enhance ADCC and are administered in conjunction to unconjugated antibodies [490]. FT538 is a CAR-NK cell product expressing an IL-5 receptor alpha fusion protein and high affinity non-cleavable CD16 [490]. It demonstrated promising preclinical efficacy against multiple myeloma cells and will undergo further testing in a phase I trial with daratumumab in r/r AML patients (NCT03067571) [491].

16.15.4 Vaccination

Vaccination of tumour-associated antigens is a strategy created to induce antigen presentation of dendritic cells to T cells, which then generate anti-leukaemic immune responses and prolonged immunological memory against leukaemic cells (Fig. 16.19) [427]. They can potentially prevent future relapses by inducing eradication of all remaining abnormal blasts in the haematopoietic system. Given the dismal prognosis of r/r AML, their development carries substantial significance for patients.

Wilm's tumour 1 (WT-1) antigens are attractive targets for peptide vaccination due to their high expression in leu-

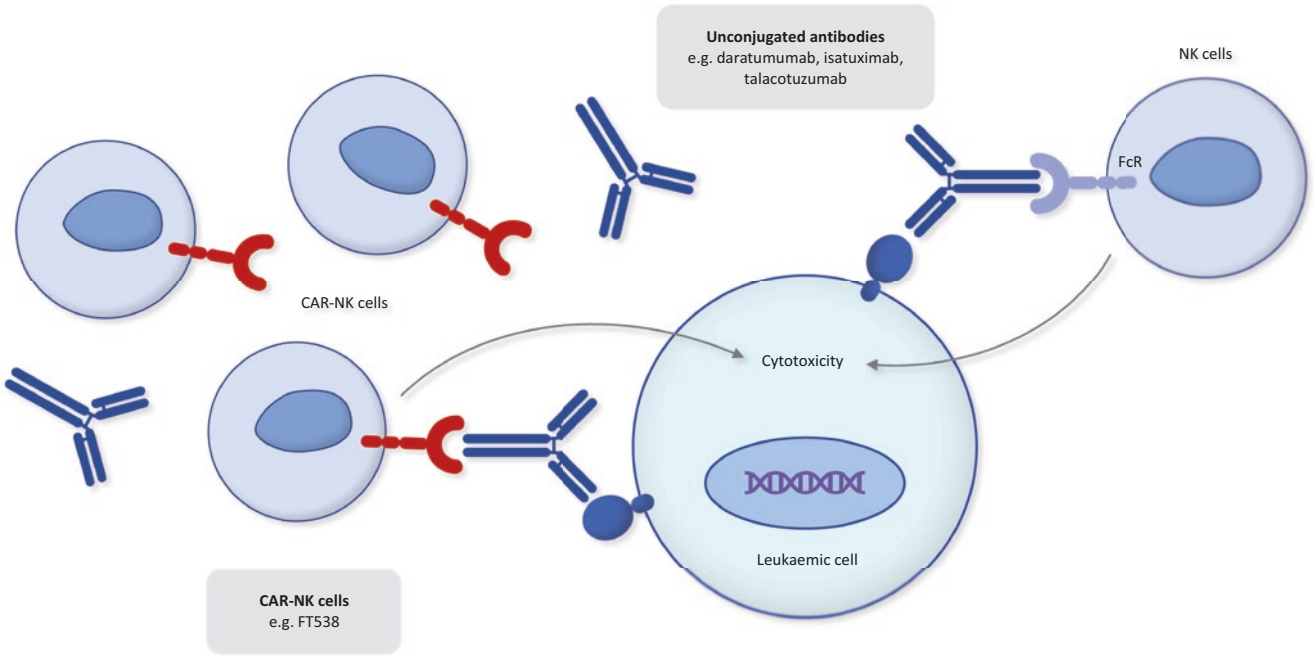


Fig. 16.18 Summary of NK cell-based immunotherapies. *CAR* chimeric antigen receptor, *NK* natural killer

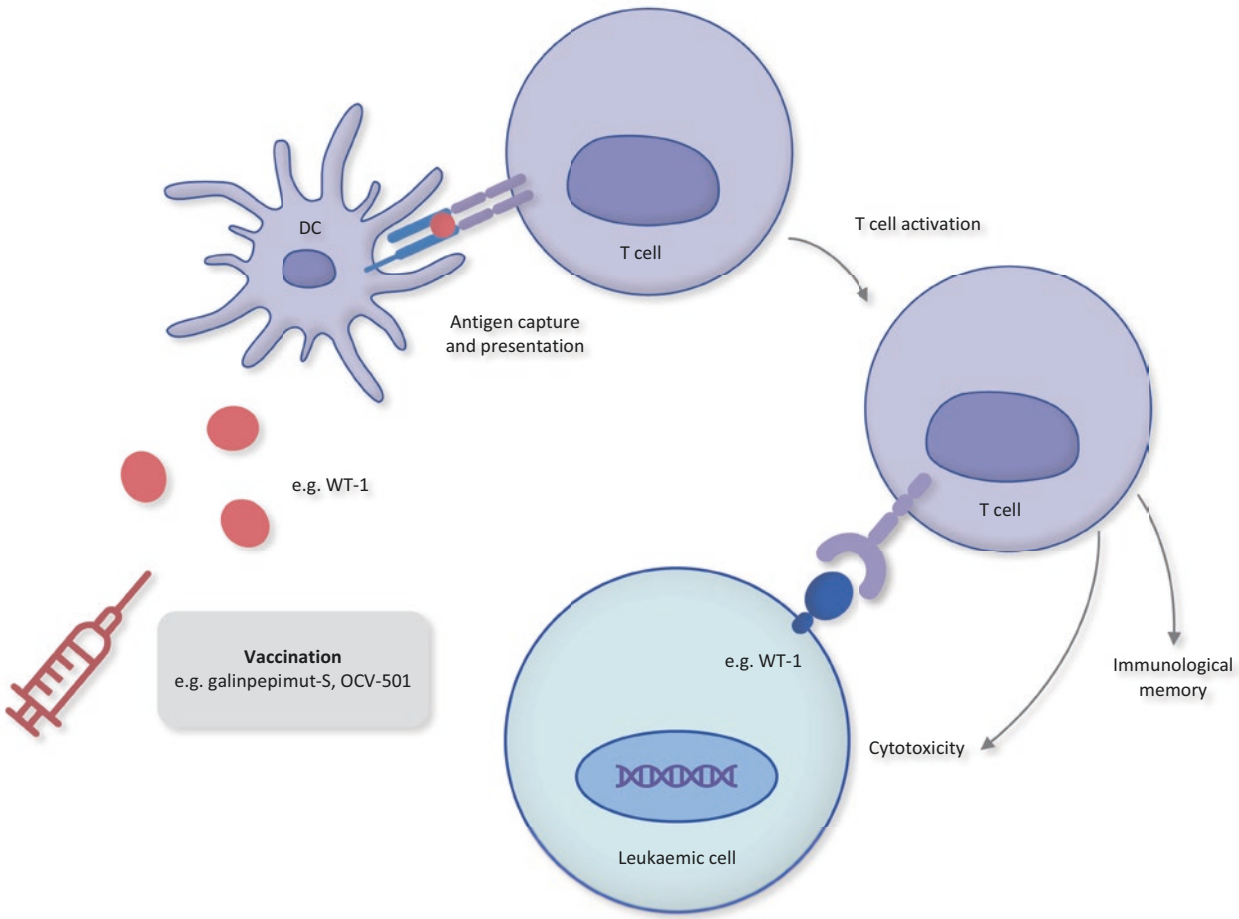


Fig. 16.19 Mechanism of action of vaccination for the treatment of AML. *DC* dendritic cell, *WT-1* Wilm's tumour 1

kaemic cells [427]. Galinpepimut-S and OCV-501 are examples of WT-1 vaccines which showed potency at inducing immunological responses and improving survival in AML patients in CR1/2 in phase I or II trials [492–494]. The combination of galinpepimut-S with pembrolizumab is currently under evaluation (NCT03761914). Ombipepimut-S (DSP-7888) is another WT-1 peptide vaccine evaluated in AML (NCT04747002).

Owing to evidence suggesting that allogeneic dendritic cells (DCs) induce stronger immune responses compared to autologous ones, an allogeneic DC vaccine, DCP-001, was manufactured and examined in a phase I trial with optimistic outcomes and tolerability [495]. This vaccine is under clinical investigation in a phase II trial among AML patients in remission (NCT03697707).

In addition to the above agents, NY-SEO-1 vaccination is currently studied in a phase I trial in combination with decitabine and nivolumab (NCT03358719). This vaccine formulation comprises three components: (1) a mAb against DEC-2015 (CD205), a dendritic cell surface receptor which promotes antigen presentation; (2) NY-SEO-1, a leukaemic cell surface antigen; and (3) polyinosinic-polycytidylic acid complexed with poly-L-lysine and carboxymethylcellulose (Poly-ICLC), a double-stranded mRNA complex which serves as an immune stimulant [496, 497]. No preliminary results are available at the moment.

16.16 Conclusion

Given the plethora of aberrant pathways in AML, the aforementioned novel strategies only provide a glimpse of the endless therapeutic options against this aggressive haematological malignancy. Although the current prognosis of AML remains suboptimal, intensive efforts on the development of novel agents may soon bring about unprecedented pharmacological breakthroughs.

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Frontline Management of Acute Promyelocytic Leukemia

17

Harinder Gill

Abstract

In the frontline management of acute promyelocytic leukemia (APL), prevention of early deaths and the selection of the optimal frontline induction strategies incorporating arsenic trioxide (ATO) and all-trans retinoic acid (ATRA) are the more important factors in determining the outcome of APL. In this chapter, we discuss the problem of early deaths and principles in circumventing this important issue. Second, we discuss the optimal frontline induction regimens incorporating ATO and ATRA.

Keywords

Acute promyelocytic leukemia · Arsenic trioxide · Frontline induction

17.1 Introduction

Acute promyelocytic leukemia (APL) is characterized by t(15;17)(q24;21) and the fusion gene *PML*::*RARA* [1]. With optimal supportive care and frontline use of all-*trans* retinoic acid (ATRA) and chemotherapy, first complete remission (CR1) rates of more than 90% and long-term survivals of more than 80% can be achieved [2–5]. Intravenous arsenic trioxide (I.V.-ATO) is highly efficacious for APL in first relapse (R1), inducing second complete remission (CR2) in more than 90% of patients [6, 7]. Frontline treatment of APL has evolved rapidly. An emerging theme is incorporation of ATO early in the treatment algorithm, starting from induction to consolidation [8–14]. Oral formulation of ATO (oral-ATO) has also been developed and shown to induce CR2 in more than 90% of R1 patients [15, 16]. Furthermore, oral-ATO has been evaluated in the frontline induction and maintenance of CR1. This strategy has led to favorable overall-survival (OS) and leukemia-free-survival (LFS) [17], implying that prolonged oral-ATO treatment may prevent relapses. Frontline treatment of APL has evolved rapidly.

17.2 Early Deaths in Newly Diagnosed APL: The Major Predictor of Outcome in APL

Despite treatment advances, early deaths (EDs) constitute the major challenge in APL. In multicenter clinical trials of patients treated with ATRA, ATO, and anthracyclines, ED rates of only 3–10% were reported [9–11, 18, 19] (Table 17.1). However, population-based and single institution studies in unselected patients reported ED rates ranging from 9.6% to 61.5% depending on the populations studied [23, 25–29, 32–39]. Life-threatening complications at presentation, which often preclude enrolment into clinical trials, are one of the major factors contributing to differences in early death rates. Nearly half of the patients who were considered ineligible for the Spanish PETHEMA trials (LPA96 and LPA99) had life-threatening hemorrhage complications [40]. A significant proportion as high as 29% of patients were excluded from clinical trials in France, as a result of disease severity at initial presentation, which might lead to underestimation of early mortality in the real-world setting [41]. These patients had statistically higher leukocyte count ($\geq 50 \times 10^9$/L), lower platelet count ($<40 \times 10^9$/L), more frequent microgranular variant APL, and a nonsignificant increase in admission to intensive care unit during induction. Together, these factors translated into the significantly higher early death rate observed in patients not enrolled into clinical trials, and hence the discrepancy in early morality between clinical trials and unselected populations.

Efforts in the development of international recommendations have led to a gradual improvement in ED rates over the last three decades from 28% in the 1990s to approximately 15% over the last two decades [27, 42, 43]. Major risk factors for ED include advanced age, high-risk disease, poor

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Table 17.1 Early deaths in the clinical trials and real-world settings in newly diagnosed acute promyelocytic leukemia

Studies	Period	Patients (no.)	Median age (range)	Men (%)	High risk (%)	Early deaths(%)
Clinical trials						
C9710 [9]	1999–2005	481	N/A (15–79)	51.3	23.4	7.7
LPA99 [20]	1996–2002	561	40 (2–83)	48	25	8.9
LPA2005 [21]	2005–2009	402	42 (3–83)	52	29	7.5
APML4 [10]	2004–2009	124	44 (3–78)	50	20	3.2
APL0406 [11]	2007–2010	156	N/A (18–70)	48.7	0	2.6
Single centre studies						
Rome, Italy [22]	1993–2008	105	N/A	50.5	29.5	4.8
California, United States [23]	1997–2009	70	50 (19–93)	37.1	18.6	26
Auckland, New Zealand [24]	2000–2017	70	43 (3–89)	45.7	27	5.8
Real-world population-based studies						
France [25]	2006–2011	399	51 (16–87)	51.6	27	9.6
United States [26]	1992–2007	1400	44	47.0	N/A	17.3
United States [27]	2000–2014	2962	48 (20–96)	51.4	N/A	20.5
Canada [28]	1993–2007	399	N/A	50.8	N/A	21.8
Canada [28]	1999–2010	131	50 (7–85)	45.8	20.6	14.6
Sweden [29]	1997–2006	105	54 (18–86)	38	40.9	29
Zhejiang, China [30]	2015–2019	1233	44 (1–91)	52.9	23.4	8.2
Hong Kong [31]	2007–2020	358	47 (1–97)	48.3	36.0	16

performance status, and coexisting infection [44]. High white blood cell count, high lactate dehydrogenase levels, low fibrinogen, prolonged prothrombin time and activated partial thromboplastin time, and the presence of differentiation syndrome are associated with fatal bleeding [45–48]. Intracranial hemorrhage (ICH) in the presence of hypofibrinogenemia is often fatal [49]. In addition, large datasets have shown that delays in ATRA administration are a major factor contributing to early death [50].

Fatal hemorrhage is the leading cause of EDs [45–49, 51]. In the PETHEMA trials, the most common lethal bleeding were intracranial and pulmonary [40]. In addition, up to 69% of these fatal hemorrhages ran a fulminant course, resulting in deaths within 24 h from the onset of bleeding. Analyses from the Swedish registry reported that majority (77%) of early deaths occurred within the first week of diagnosis [29]. The most significant factors predictive of fatal bleeding were the presenting leukocyte count [31, 52] and hypofibrinogenemia [31]. The underlying pathophysiological basis is related to hyperfibrinolysis caused by abnormal promyelocytes through expression of annexin II, which is cell-surface receptor with high affinity for plasminogen and tissue-type plasminogen activator (tPA) [53, 54].

APL differentiation syndrome (APL-DS) is the other leading cause of early mortality in APL, especially in patients above the age of 50. The incidence of APL-DS varied from 2% to 48% in the published literature due to variations in diagnostic criteria and differences in induction regimens and prophylaxis [55–58]. Leucocyte count $\geq 5 \times 10^9$/L was shown to be a major predictive factor for severe DS [59, 60]. Severity of DS was associated with increase in mortality [59]. Prompt treatment with steroids could reduce the mortality related to DS and hence should be initiated when the diagnosis is first suspected [61]. Concurrent uses of cytoreductive therapy such as hydroxyurea, correction of haemostatic abnormalities, close monitoring of fluid balance, and avoidance of interruption in ATRA treatment were also advocated to reduce mortality due to DS [31]. Treatment with ATRA and/or ATO corrects coagulopathy through the down-regulation of tissue facture and annexin II expression on abnormal promyelocytes [62, 63]. It has been shown that ATRA and ATO act synergistically [64–67].

17.3 Principles of Initial Management and Supportive Care for APL

1. All cases of suspected APL should be managed as a medical emergency.
2. ATRA +/− ATO must be started urgently at the earliest suspicion of the diagnosis based on clinical and hematological features together with close monitoring and aggressive correction of coagulopathy and thrombocytopenia.
3. High white blood cell (WBC) count at presentation is NOT a contraindication to ATRA +/− ATO initiation.
4. Maintain a platelet count of $>50 \times 10^9$/L ($>100 \times 10^9$/L if there is any bleeding in life-threatening sites) and a fibrinogen >1.5 g/L. In the correction of hypofibrinogenemia, options include the use of cryoprecipitate and

fibrinogen concentrates. Fresh frozen plasma (FFP) for the correction of hypofibrinogenemia should only be administered if cryoprecipitate or fibrinogen concentrates are not available. FFP may be beneficial in patients with established DIC with prolongation of both prothrombin time (PT) and activated partial thromboplastim time (aPTT).

5. Monitoring of blood counts, biochemistry, clotting profile, and fibrinogenemia levels must be done every 12 hourly until the patient is out of disseminated intravascular coagulopathy, hyperfibrinolysis, or APL-DS.
6. Cytoreduction with hydroxyurea or anthracyclines (daunorubicin or idarubicin) should be started as soon as practicable in patients with presenting WBC > 10×10^9/L.
7. Corticosteroids (e.g., intravenous dexamethasone 10 mg every 12 h) must be initiated if there is any suspicion of APL-DS or if there are any symptoms or signs suggestive of APL-DS.
8. Prophylactic corticosteroids may be used at the discretion of the managing physician in patients at very risks of developing APL-DS.
9. Avoid excessive intravenous fluids, as fluid overload may aggravate pulmonary complications APL-DS. The use of xanthine oxidase inhibitors such as febuxostat is adequate to prevent tumor lysis syndrome in most situations without the need for excessive intravenous fluids.
10. Avoid the insertion of central venous catheters or any unnecessary invasive procedures with the exception of bone marrow aspiration.
11. Administration growth colony-stimulating factor G-CSF is contraindicated in newly diagnosed APL.
12. All patients with APL must be referred to and managed at tertiary centers with the experience of managing APL if feasible.

17.4 Arsenic Trioxide (Intravenous or Oral) Plus All-Trans Retinoic Acid: The Preferred Frontline Induction Regimen for All Patients with Newly Diagnosed APL

The benefits of frontline ATO-ATRA regimens have been shown by several groups (Table 17.2). The combination of ATO, ATRA, and gemtuzumab ozogamacin was studied by investigators from the M.D. Anderson Cancer Center [68, 69]. A CR rate of 90% and the 3-year OS of 85% were achieved in 82 patients studied over 6 years. Survivals were comparable to the LPA99 and C9710 trials [9, 18]. The APLM4 trial combined ATO, ATRA, and idarubicin during induction followed by consolidation with As_2O_3-ATRA and 2 years of maintenance with ATRA, 6-mercaptopurine, and methotrexate [10]. A CR rate of 95% was achieved together with a 2-year disease-free survival of 97.5% and a 5-year OS of 94%. The 5-year OS was significantly better than that in the APML3 trial [70]. The APL0406 trial was a randomized phase 3 multicenter study of using ATO-ATRA induction and consolidation without maintenance in low to intermediate-risk APL [13]. An impressive 100% CR rate and a 2-year OS of 99% were achieved, non-inferior to the AIDA-2000 protocol [4]. The AML17 trial randomized newly diagnosed APL patients to either the AIDA induction and consolidation protocol or ATO-ATRA combinations [12]. Gemtuzumab ozogamacin was given to high-risk patients. A CR rate of 94% was achieved in the As_2O_3-ATRA cohort compared to 89% in the AIDA cohort. The ATO ATRA cohort had a significantly better event-free survival (91% vs. 70%, $p = 0.002$) and cumulative incidence of relapse (1% vs. 18%, $p = 0.0007$). In most of these trials, high-risk APL had worse outcomes (Table 17.2).

Oral tetra-arsenic tetra-sulfide (As_4S_4) in combination with ATRA during induction was shown to be non-inferior to intravenous As_2O_3 and ATRA with a CR rate of 99.1% and a 3-year OS of 99.1% [71].

The combination of oral ATO, ATRA, and ascorbic acid (AAA) developed by Hong Kong was given for induction for newly diagnosed APL [72, 73]. The regimen resulted in a universal molecular remission and excellent leukemia-free survival (LFS) and OS in all risk and age categories of APL. Twenty-two patients in our cohort belonged to the conventional high-risk group. With a median follow-up of more than 3 years, neither relapse nor disease-related death occurred. Comparison with a concurrent cohort treated with ATRA-chemotherapy showed a lower risk of relapse and a better 5-year LFS. The presence of *FLT3*-ITD did not appear to impact on response rates or survival of patients treated with frontline AAA. *FLT3*-ITD mutations occur in up to 38% of patients with APL portend worse LFS and OS in patients treated with ATRA-chemotherapy regimens without ATO [74, 75]. The use of ATO-ATRA combinations in the frontline setting appears to overcome the deleterious effect of *FLT3*-ITD [10, 13].

ATO induces dose-dependent apoptosis and differentiation of abnormal promyelocytes via the effect on the PML moiety of the PML-RARA fusion protein. Arsenic binds to the cysteine residues of the B2 domain of the PML protein inducing oxidation and disulfide bond formation [76, 77]. That leads to reformation of the PML-containing nuclear bodies (NBs) followed by sumoylation of the PML-RARA protein and recruitment of the SUMO-dependent ubiquitin ligase, RNF4, and polyubiquitination [78, 79]. Finally, the PML moiety is degraded by the proteasome together with the partner RARA. Arsenic also induces degradation of PML-RARA via the production of reactive oxygen species (ROS). The combination of ATO and ATRA, via the distinct interactions on PML and RARA, results in the synergistic

Table 17.2 Frontline arsenic trioxide-based regimens for newly diagnosed acute promyelocytic leukemia (APL)

Study	Median FU (y)	N (risk)	CR (risk)	ED	5-year LFS	5-year EFS	5-year OS
APL0406: ATRA-ATO	3.4	127 (S)	100% (all)	0%	NR	97.3%	99.2%
UK AML17: ATRA-ATO +/− GO	2.5	86 (S)	94% (all)	4%	97%	92%	95%
		30 (H)					
MD Anderson: ATRA/ATO +/− GO	1.9	56 (S)	95% (S)	3.6% (S)	NR	89% (S)	89% (S)
		26 (H)	81% (H)	19% (H)		65% (H)	75% (H)
APML4: ATRA-ATO-Ida	4.2	189 (S)	96% (S)	2% (S)	96% (S)	92% (S)	96% (S)
		23 (H)	88% (H)	8.7% (H)	95% (H)	83% (H)	87% (H)
Hong Kong: ATO-ATRA-ascorbic acid +/− Daunorubicin	2.2	40 (S)	100% (all)	0%	100%	100%	100%
		22 (H)					

ATRA all-trans retinoic acid, *ATO* arsenic trioxide, *GO* gemtuzumab ozogamacin, *Ida* idarubicin, *FU* follow-up, *y* years, *N* number of patients, *CR* complete remission, *ED* early deaths, *LFS* leukemia-free survival, *EFS* event-free survival, *OS* overall survival, *S* standard-risk APL, *H* high-risk APL

degradation of the PML-RARA fusion protein and leukemia-initiating cells (LICs) eradication [66, 80]. Concurrent use of anthracyclines stimulates the production of ROS and potentiate PML-RARA degradation [81]. The addition of ascorbic acid in the AAA regimen was based on the synergism observed in vitro and in vivo, albeit in non-APL cells [82–84]. Ascorbic acid enhances the formation of ROS in ATO-treated cells in vitro [85]. Ascorbic acid also depletes intracellular glutathione in vitro and subsequent oxidation-induced cell death [84].

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18 Management of Relapsed Acute Promyelocytic Leukemia and the Role of Hematopoietic Stem Cell Transplantation

Harinder Gill

Abstract

This chapter addresses one of the most controversial issues in the management of acute promyelocytic leukemia (APL) which is the optimal management of relapsed APL following second remission and role of hematopoietic stem cell transplantation in APL. Historical data on the outcomes with HSCT in the preoral arsenic trioxide trioxide (ATO) era will be discussed. The role of consolidation and maintenance with oral ATO-based regimens will be discussed.

Keywords

Acute promyelocytic leukemia · Relapse · Arsenic trioxide · Hematopoietic stem cell transplantation

18.1 Introduction

Arsenic trioxide (ATO) is highly efficacious for APL in first relapse (R1), inducing second complete remission (CR2) in more than 90% of patients [1–3]. Similar to other acute myeloid leukemias, consolidation of CR2 with autologous or allogeneic hematopoietic stem cell transplantation (HSCT) is then recommended [4]. For R1 patients in CR2 after As_2O_3 re-induction, the optimal post-remission strategy has not been defined. Prevailing recommendations are based on retrospective analyses of highly selected patients [4–6]. Previous studies had shown that CR2 patients had poor long-term relapse-free survivals of only 22–37% [1, 7, 8]. HSCT was conventionally regarded as a consolidation strategy.

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However, prospective data validating HSCT or other approaches as the optimal post-remission therapy for APL in CR2 are not available. Since the introduction of ATRA, extramedullary relapse of APL has increasingly been reported, [9–12] with the central nervous system (CNS) the most common site involved. CNS relapse of APL has a dismal prognosis [13]. Therefore, prevention and optimal treatment of CNS relapse is an important facet in the management of APL. An oral preparation of ATO (oral-ATO) is equally efficacious for APL in R1, inducing CR2 in more than 90% of patients [14, 15].

18.2 HSCT for APL in the Pre-ATO and Post-ATO Era

In the pre-ATO era, autologous HSCT in CR2 resulted in relapse rates of 18–54%, leukemia-free survival (LFS) of 31–69%, and overall survival (OS) of 40–76% (Table 18.1) [16–23]. Allogeneic HSCT appeared worse, with therapy-related mortality (TRM) of 17–40%, relapse rates of 8–64%, LFS of 22–59%, and OS of 46–52% (Table 18.1) [16, 20–22]. A registry study showed that autologous was superior to allogeneic HSCT for OS, owing to a lower TRM [24]. In a report comparing chemotherapy, allogeneic, and autologous HSCT, there were no differences in 7-year OS (40–86%) [25]. Another retrospective study showed that autologous HSCT, allogeneic HSCT, and other regimens resulted in comparable 5-year OS and relapse rates (51–58%) [26].

In the post-ATO era, few studies addressed the optimal post-CR2 strategy. In 35 cases with ATO-induced CR2, 23 patients received an autologous HSCT. The 5-year failure-free-survival and OS were 59% and 77% for transplanted patients, respectively, but were significantly worse on an intention-to-treat basis [27]. Another study evaluated allogeneic HSCT in 15 APL patients in CR2 (6 induced by ATO) [28]. The 4-year OS and relapse rate were 62% and 32%, respectively. Another registry study evaluated 140 patients in CR2 undergoing autologous HSCT, most of whom were

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Table 18.1 Results of hematopoietic stem cell transplantation in patients with acute promyelocytic leukemia (APL)

Study (Ref)	Year	Status	No.	TRM (%)	RR (%)	DFS/EFS/LFS	OS (%)
Autologous HSCT							
Mandelli et al. [16]	1979–1992	CR2	58	23	54	LFS: 31%	–
Meloni et al. [17]	1984–1996	CR2	15	0	53	–	40
Thomas et al. [18]	1994–1998	CR2	22	–	–	3-year DFS: 77%	–
Capria et al. [19]	–	CR2	16	–	44	10-year DFS: 56%	68
de Botton et al. [20]	1991–1998	CR2	50	6	18	7-year EFS: 61%	60
Sanz et al. [21]	1993–2003	CR2	195	16	37	LFS: 51%	–
Kohno et al. [22]	1999–2004	CR2	15	–	20	4-year DFS: 69%	76
Linker et al. [23]	1996–2000	CR2	12	–	–	5-year DFS: 62%	–
Holter Chakrabarty et al. [24]	1995–2006	CR2	62	2	30	5-year DFS: 63%	75
Pemmmaraju et al. [25]	1980–2010	CR2	7	20	30	7-year EFS: 68.6%	85.7
Fujita et al. [26]	1998–2005	CR2	6	0	67	5-year EFS: 41.7%	83.3
Yanada et al. [27]	2005–2009	CR2	23	0	13	5-year EFS: 65%	77
Ganzel et al. [29]	–	CR2	140	–	–	-	78
Allogeneic HSCT							
Mandelli et al. [16]	1979–1992	CR2	33	40	64	LFS: 22%	–
de Botton et al. [20]	1991–1998	CR2	23	39	4	7-year EFS: 53%	52
Sanz et al. [21]	1993–2003	CR2	137	17	24	LFS: 59%	–
Kohno et al. [22]	1999–2004	CR2	13	–	8	4-year DFS: 46%	46
Holter Chakrabarty et al. [24]	1995–2006	CR2	232	30	18	5-year DFS: 50%	54
Pemmaraju et al. [25]	1980–2010	CR2	8	47	18	7-year EFS: 40.6%	49.4
Fujita et al. [26]	1998–2005	CR2	21	19	9.5	5-year EFS: 71.7%	76.2
Ramadan et al. [28]	2000–2010	CR2	15	19.6	41.9	DFS: 50%	45

Ref Reference number, *Year* period of recruitment, *Status* leukemia status at transplantation, *No* Number of patients studied, *TRM* treatment-related mortality, *DFS* disease-free survival, *EFS* event-free survival, *LFS* leukemia-free survival, *OS* overall survival, *CR2* second complete remission

treated before ATO was available. The 5-year OS was 78%, but the survival curve was not plateauing [29].

18.3 Oral Arsenic Trioxide-based Consolidation and Remission of CR2 Instead of HSCT

Oral is equally efficacious for APL in R1, inducing CR2 in >90% of patients [14, 15, 30]. Furthermore, in an effort to prevent relapse, oral-ATO has been studied in the maintenance of CR1. This strategy results in favorable overall-survival (OS) and leukemia-free-survival (LFS), [31, 32] implying that prolonged treatment with oral-ATO may prevent relapses.

Current protocols have incorporated I.V.-ATO in the treatment of newly diagnosed APL [33–39]. Patients may receive frontline I.V.-ATO for induction and/or consolidation.

Patients with APL in R1 in Hong Kong were treated with protocols that incorporate oral-ATO re-induction followed by ATO maintenance without HSCT at CR2 [30]. The use of an oral-ATO-based re-induction regimen resulted in CR2 uniformly in R1 patients, irrespective of previous exposure to oral-ATO as CR1 maintenance [30]. In patients who further developed second relapse (R2), all of whom had received oral-ATO as CR2 maintenance, only 2 cases (6.7%) were refractory to ATO re-induction [30]. These results show that arsenic-resistance during re-induction is not a concern for patients in R1 or R2, despite previous ATO exposure. However, in the third relapse or beyond, ATO-refractoriness during re-induction developed in 25% and 50% of cases. Hence, arsenic-resistance will only develop upon repeated and multiple administration of ATO [30].

In R1 patients treated with oral-ATO, the LFS plateaued after 4 years at 56.8%, implying that these CR2 patients were potentially cured [30]. Because oral-ATO remained effective in R2 and salvage was still feasible, the 5-year and 10-year OS were 79.5% and 67.3%, respectively. Notably, about 40% of patients who developed R2 were long-term survivors. Hence, oral-ATO maintenance regimen is an effective post-remission strategy for CR2, with outcome at least comparable with that of HSCT, which would have involved highly selected good-risk patients. The short-term risk (TRM) and long-term sequelae (sterility, menopause, secondary cancers) of HSCT are obviated by the oral-ATO-based non-HSCT strategy. This may be one of the best approaches for transplant-ineligible patients.

For patients who relapsed more than 24 months after CR1, the 5-year LFS was close to 70% and the 5-year OS above 90% in patients treated with oral-ATO-based regimens at R1.

Conventional prognostic indicators and risk scores did not impact on relapsed APL treated with oral-ATO-based re-induction [30].

In the post-ATRA era [40], CNS relapse has become an important problem, occurring in 1.1–3.2% of cases [13, 41]. In a more recent registry study, the incidence of isolated CNS relapse was 5% [42]. Risk factors predisposing to isolated CNS involvement at R1 included CNS hemorrhage, presentation leucocyte count >10 × 10^9/L, APL differentiation syndrome, microgranular variant, and the short isoform of PML::RARA [43–46].

Currently, not every newly diagnosed APL patient will be exposed to ATO. Hence, for R1 patients not previously exposed to ATO, a significant proportion will remain in durable CR2 after re-induction and maintenance with ATO without HSCT [30]. For R1 patients previously exposed to ATO, either as upfront induction, consolidation, or maintenance [33, 35, 36, 47, 48], ATO re-induction is highly effective. Whether HSCT in patients previously exposed to ATO improves the outcome will have to be better defined. The use of alternative strategies, including gemtuzumab Ozogamicin [49, 50] or targeted therapy, will also have to be explored.

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19 Genomic Landscape of Acute Lymphoblastic Leukemia (ALL): Insights to Leukemogenesis, Prognostications, and Treatment

Sin Chun-fung

Abstract

Acute lymphoblastic leukemia is a heterogenous disease under the current WHO 2017 classification. The prognosis in certain subgroups of patients is particularly poor with limited treatment options available. Moreover, risk stratification was difficult in some patients due to lack of recurrent genetic aberrations identified. With the advance of research in genomics, we identified some novel genetic subgroups of acute lymphoblastic leukemia with unique biological features and this information is important for disease prognostication. Moreover, targeted therapies are emerging for acute lymphoblastic leukemia with targetable genetic lesions. The findings of novel genomic signatures in acute lymphoblastic leukemia also facilitate deep mechanistic study to reveal leukemogenesis and development of novel therapeutic strategies. The advance of research in genomics further improves the prognosis of acute lymphoblastic leukemia.

Keywords

Genomics · Acute lymphoblastic leukemia · Genetics

19.1 Introduction

Acute lymphoblastic leukemia (ALL) is a heterogenous disease which is subclassified by various genetic aberrations. Those genetic aberrations lead to differentiation block and promote malignant proliferations of leukemic cells. In the past, conventional cytogenetics was the only tool to characterize ALL genetically and risk-stratified patients for treatment. With the advance in genomics, more and more recurrent genetic aberrations are discovered. Those discoveries not only help us to understand the disease biology of individual subtypes of ALL, it also revolutionized the management of ALL by refining the risk-stratification scheme of patients, possibilities of targetable genetic lesions, and targets for minimal residual disease (MRD) monitoring.

The prognosis of childhood ALL is excellent with overall survival rate approaching 90% [1]. Although ALL is much less common in adolescence and adult, their prognosis is much less favorable and the overall survival is only around 30–40% [2]. Recent discoveries in genomics revealed the difference in genomic landscape of patients with ALL among pediatric and adolescent/adult population, which accounts for the difference in disease biology and thus the prognosis of patients.

19.2 World Health Organization (WHO) Classification of ALL

Various recurrent genetic aberrations had been described in WHO 2017 classification (Table 19.1) [3]. Those genetic aberrations are subclassified by conventional cytogenetics and their prevalence varies among different age groups of patients. Broadly speaking, those recurrent genetic aberrations described by WHO classification can be subclassified as copy number changes (aneuploidy) and genetic fusion.

19.2.1 Aneuploidy

Hyperdiploidy is defined as gain of at least 5 chromosomes randomly and this subgroup constitutes around 25% of pediatric ALL cases. However, it is much less common in adolescence and adult patients which constitute only <5%. Genetic aberrations involving Ras pathway, including KARS, NRAS, and PTPN11 mutations, are the most common genetic aberrations [4]. Aberrations in chromatin-modifying genes had also been reported, e.g., CREBBP, NSD2, SUV420H1, SETD2, and EZH2. Other genetic abnormalities that had been described include NF1, CDKN2A/B, IKZF3, PAG1, and the

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Table 19.1 Classification of B-ALL and recurrent genetic aberrations described in 2017 WHO classification [3]

B lymphoblastic leukemia/lymphoma	B lymphoblastic leukemia/lymphoma, NOS	B-lymphoblastic leukemia/lymphoma with t(9;22)(q34.1;q11.2);*BCR-ABL1*
	B-lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities	B-lymphoblastic leukemia/lymphoma with t(v;11q23.3);*KMT2A*-rearranged
		B-lymphoblastic leukemia/lymphoma with t(12;21)(p13.2;q22.1); *ETV6-RUNX1*
		B-lymphoblastic leukemia/lymphoma with hyperdiploidy
		B-lymphoblastic leukemia/lymphoma with hypodiploidy
		B-lymphoblastic leukemia/lymphoma with t(5;14)(q31.1;q32.3) *IL3-IGH*
		B-lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3);*TCF3-PBX1*
		B-lymphoblastic leukemia/lymphoma, BCR-ABL1–like
		B-lymphoblastic leukemia/lymphoma with iAMP21

6p22 histone gene cluster aberrations [5]. Hypodiploidy is defined as chromosome number less than 44 and it is further subclassified into two subtypes: low-hypodiploidy (31–39 chromosomes) and near-halploid (24–30 chromosomes). Low hypodiploidy commonly acquired IKZF2 deletions or TP53 mutations. This subgroup of ALL accounts for less than 1% in childhood ALL, but it becomes more common in adolescence and young adult, approaching 5–10% of ALL cases. Ras-activating mutations and IKZF3 mutations are commonly reported in near-halploid subtype and it constitutes around 2% of childhood ALL and less than 1% of ALL cases in adolescence and young adults. The prognosis is very poor for ALL with hypodiploidy [6].

19.2.2 Intrachromosomal Amplification of Chromosome 21 (iAMP21)

Intrachromosomal amplification of chromosome 21 was first reported by research groups in 2003 [7, 8]. The utilization of fluorescence in situ hybridization (FISH) to detect ETV6-RUNX1 fusion that made the discoveries of this entity of B-ALL and the FISH test utilizing RUNX1 probe is not recognized as one of the reliable methods to detect iAMP21 [9]. We defined the intrachromosomal amplification of chromosome 21 by the presence of 3 or more copies of RUNX1 in a single chromosome 21 (i.e., 5 or more RUNX1 copies per cell) [10].

Despite the fact that RNUX1 had been amplified in the B-ALL cases with iAMP21, there are no evidence of aberrations involving the gene RUNX1 and also the expression level of RUNX1 gene in those cases of B-ALL with iAMP21 has no significant difference from those B-ALL cases with other numerical and structural abnormalities of chromosome 21 [11–13].

The common region of amplification on chromosome 21 was refined to a 5.1-mb region that included RUNX1, miR-802, and genes mapping to the Down syndrome critical region. Recurrent abnormalities affecting genes in key pathways were identified: IKZF1 (22%), CDKN2A/B (17%), PAX5 (8%), ETV6 (19%), and RB1 (37%). Investigation of clonal architecture provided evidence that these abnormalities, and P2RY8-CRLF2, were secondary to chromosome 21 rearrangements [14].

B-ALL with iAMP21 constitute around 2% of pediatric ALL cases and the median age of diagnosis is around 9 years old [15]. It is associated with a poor outcome. Heerema et al. showed that iAMP21 was more commonly associated with NCI high-risk category, positive MRD post-induction and those patients with iAMP21 with MRD positivity after induction had the worst outcome. The outcome of patients was inferior when treated with standard-risk protocol and thus a high-risk-intensified regimen is needed for those patients [16].

19.2.3 Gene Translocation

ETV6-RUNX1 fusion is caused by t(12;21)(p13;q22) and it is prevalent in childhood ALL (25%). It confers favorable prognosis. The fusion is much less common in adolescence and young adults. WHSC1 mutations and PAX5 deletional mutations are commonly found in patients with ETV6-RUNX1 fusion ALL [17, 18]. TCF3-PBX1 fusion is characterized by t(1;19)(q23;p13) and it accounts for 5% of childhood ALL. The fusion is rare in adult patients with ALL. Although this genetic aberration was considered as high-risk genetic lesion in the past, the prognosis was much improved by treating with modern contemporary therapy [19, 20].

BCR-ABL1 fusion is characterized by t(9;22)(q34;q11) and it is prevalent in adult patients (25% of ALL), while it constitutes 2–5% of ALL cases in childhood ALL. The prognosis was unfavorable in the past with overall survival of less

than 25% [21]. However, the prognosis is much improved after introducing combined conventional chemotherapy and tyrosine kinase inhibitors (TKIs) therapy. Now the data show a much-improved overall survival in both pediatric and adult patients [22–24].

ALL with KMNT2A rearrangement is associated with poor prognosis and it is prevalent in infantile ALL (less than 1 year old of age) and adult patients (around 15%). Those cases of ALL with KMNT2A rearrangement are usually presented as sole mutation and seldom associated with secondary mutations which suggested that it is a strong driver mutation in ALL [25, 26]. Introduction of KMMT2A-AF4 fusion into hemopoietic stem cell was sufficient to induce pro-B-ALL without the introduction of secondary mutations [27]. The protein complex of KMNT2A is responsible for binding to promoter region of genes and caused subsequent histone modification. Thus, the disruption of KMNT2A due to genetic rearrangement promotes leukemogenesis via modulating gene transcription by histone modification [28]. Therefore, targeting histone modification and epigenetic mechanism is one of the approaches of novel treatment for KMNT2A-rearranged leukemia. Treatment with histone deacetylase inhibitor promoted differentiation of leukemic cells via epigenetic modifications in preclinical study [29]. In addition, histone modification via KMNT2A rearrangement activated BCL2 and thus this type of leukemia is sensitive to the treatment of BCL2 inhibitors [30]. Another preclinical study also showed excellent sensitivity of KMNT2A-arranged ALL towards venetoclax [31].

19.3 Ph-Like ALL

Ph-like ALL is a well-defined subtype of B-ALL under WHO 2017 classification. Upon genomic profiling study, study groups identified that Ph-like ALL showed similar gene expression signature with those cases of Philadelphia chromosome-positive (Ph-positive) ALL, but without BCR-ABL1 fusion [32]. Ph-like ALL account for 15% of pediatric B-ALL and around 20% of adult B-ALL [33–37]. The incidence of Ph-like ALL is the highest in patients of adolescence and young adult with the incidence approaching 20–27%. The prognosis of Ph-like ALL is poor [38]. Diverse genomic aberrations are described in this subtype of B-ALL; however, it consistently shows a high frequency of IKZF1 deletion and mutations of genes involving transcription factors of lymphoid progenitors. Although the genomic composition of Ph-like ALL is complex, we can simply classify them into five subgroups: (1) CRLF2 rearrangement, (2) Rearrangement of ABL-class gene, (3) Rearrangement of JAK2 and EPOR, (4) Aberrations leading to activation of JAK-STAT or MAPK pathway, and (5) Other rare kinase alterations [39]. The incidence of these five genetic subgroups varies with age and the distribution among pediatric ALL, adolescence, and young adult ALL and adult ALL is shown in Fig. 19.1.

19.3.1 Role of Lymphoid Transcription Factor IKAROS in Ph-Like ALL

IKAROS is a transcription factor belonging to zinc finger protein and some recent studies proposed it was an epigenetic regulator which controls critical process of hemopoiesis and lymphopoiesis [40–42]. IKAROS also serves an important function in B-cell maturation and thus it is critical in the leukemogenesis of B-ALL [43].

The aberrations of IKAROS are caused by deletion of IKZF1 gene which occur in 60–80% of Ph-positive ALL [44]. The presence of IKZF1 deletions is also very common in Ph-like ALL which account for 80% of Ph-like ALL cases [45]. Studies show that the functional loss of IKAROS activity caused differentiation block in B-cell and IKZF1 deletion was associated with resistance to chemotherapy in leukemic cells [36, 43].

Large deletions, small Indel, or SNVs are also common types of genetic aberrations reported in IKZF1 mutations [46]. Various genes are commonly associated with IKZF1 mutations and IL-7R mutations are exclusively found in IKZF1 mutation cases [47]. Studies show that IL7R was overexpressed due to IL7R mutations and combining with the effect of loss of inhibitory effect of IKAROS due to IKZF1 loss-of-function mutations, these mutations drive the development of ALL synergistically via activation of JAK/STAT pathway as well as PI3K/Akt/mTOR pathway [48]. IKZF1 is also reported to be associated with CDK2A/B mutations, PAX5, and PAR1 deletions and it confers worse prognosis when compared with the presence of IKZF1 mutations alone in childhood ALL [6].`

A study from Chinese group on pediatric and adolescence patients found that IKZF1 mutations were associated with higher white cell count and higher rate of minimal residual disease- (MRD) positive after day 15 of induction therapy. Moreover, the presence of IKZF1 mutations, together with IL7R mutations, confers glucocorticoid resistance and thus contributes to treatment failure. In that study, IKZF1 mutations were found to be associated with genetic aberrations in various signaling pathway and transcription factor, e.g., NRAS, SETD2, FLT3, and CREBBP [46].

19.3.2 CRLF2 Rearrangement

CRLF2 overexpression resulting from CRLF2 rearrangements is the most common type of genetic aberrations in

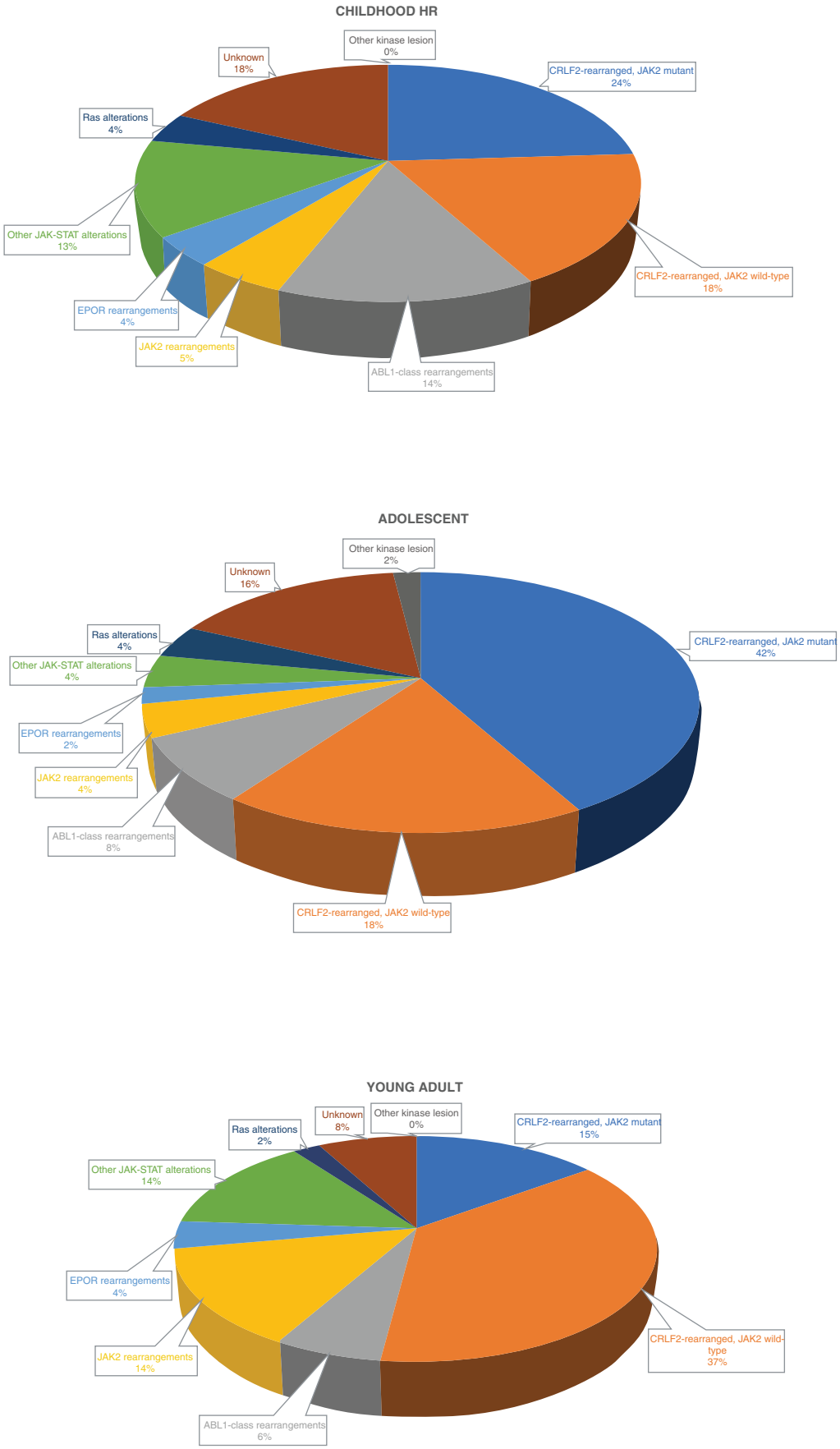

Fig. 19.1 Distribution of genetic aberrations in Ph-like ALL among pediatric, adolescence, and young adult patients and it showed difference in prevalence of various genetic aberrations

Ph-like ALL which constitutes 47% of Ph-like ALL cases [38]. The gene of CRLF2 is located at locus Xp22.3/Yp11.3 which encodes the thymic stromal lymphopoietin receptor. It works as heterodimer with IL7R α-chain, and upon binding of ligand, subsequent downstream signaling will trigger and control the process of lymphopoiesis [49]. The main mechanisms of CRLF2 overexpression are as follows: (1) translocation of CRLF2 to the immunoglobulin heavy-chain transcriptional enhancer (14q32.3; IGH@-CRLF2); (2) fusion of CRLF2 to a G-protein purinergic receptor P2RY8 gene (P2RY8-CRLF2) resulting from small interstitial deletion of the pseudo-autosomal region of the sex chromosomes (Xp22.23 or Yp11.32) centromeric to CRLF2; (3). Activating mutation of CRFL2 F232C [50–52]. Those cases with P2RY8-CRLF2 rearrangements are more commonly found in younger patients, while IGH@-CRLF2 is commonly found in adolescence and young adult [52]. Around 50% of Ph-like ALL with CRLF2 rearrangement harbor concomitant mutations in JAK of which the point mutation of R683G in JAK is the most common [53, 54].

19.3.3 ABL Gene Rearrangement

This subgroup accounts for 13% of Ph-like ALL cases and it involves rearrangement of ABL-class genes including ABL1, ABL2, PDGFRB, and CSF1R. Multiple fusion partners are identified, but they uniformly present as aberrant kinase activity [38, 55].

19.3.4 Rearrangement of JAK2 and EPOR

This genetic subgroup accounts for about 11% of Ph-like ALL and the prevalence of JAK2 fusion is increased in young adult when compared with pediatric and adolescence patients (15% vs 5%) [38, 50]. Around 4% of Ph-like ALL cases harbor EPOR rearrangement. There are four types of EPOR rearrangements reported and all these rearrangements involve the fusion of juxtaposition of the EPOR gene to the enhancer regions of immunoglobulin gene and cause subsequent aberrant expression of EPOR protein [56].

19.3.5 Other Kinase Fusion

Around 13% of Ph-like ALL have JAK-STAT pathway hyperactivation resulting from fusions of other kinase genes, sequence mutations, and focal deletions of genes [38]. Examples of genetic aberrations in this subgroup include mutations of IL7R, FLT3, IL2RB, activating mutations of JAK1 & JAK3, and deletion of genes that encode negative regulators of the JAK-STAT pathway (SH2B3). Aberrations in RAS-MAPK pathway account for 4% of Ph-like ALL which include mutations in NRAS, KRAS, and PTP11 as well as NF1. Other rare kinase fusions reported include NTRK3 and DGKH-fusion and they account for 0.9% of Ph-like ALL cases. There are still around 5% of Ph-like ALL that have no identifiable kinase-activating lesions, but the unique signature of these cases was revealed by gene expression profile [39].

19.3.6 Diagnostic Approach of Ph-Like ALL

The gold standard of diagnosing Ph-like ALL is gene expression profile. However, it is difficult to implement in routine diagnostic laboratory due to difficulties in standardization. Cytogenetic study is a routine diagnostic tool for ALL cases and it allows a global assessment of genetic defects in terms of numerical and structural chromosomal abnormalities. However, most genetic aberrations of Ph-like ALL are cryptic and conventional cytogenetics most often missed those lesions, e.g., interstitial deletion of CRLF2. Fluorescent in situ hybridization (FISH) is also a good diagnostic tool for detecting Ph-like ALL genetic lesions. The rearrangements involving ABL1, ABL2, PDGFRB, JAK2, CRLF2, and P2RY8 can be readily picked up by breakapart probes which are available commercially. The findings of FISH study provide valuable information of establishing the diagnosis of Ph-like ALL, although the positive result of FISH study needs to be confirmed by addition of fusion probes ideally. However, FISH study fails to detect some of the Ph-like ALL genetic aberrations, for example intrachromosomal inversions (e.g., inv.(9) resulting in PAX5-JAK2 fusion) and intrachromosomal deletions (e.g., del(X)(p22p22)/del(Y)(p11p11) resulting in P2RY8-CRLF2 fusion) [57]. NGS techniques with targeted sequencing are increasingly available in diagnostic laboratory and it is an excellent tool in diagnosing Ph-like ALL lesions [57, 58]. Table 19.2 shows comparison and contrast of each diagnostic techniques in diagnosing Ph-like ALL.

The monoclonal antibody against CRLF2 is available for detection of CRLF2 rearrangement by flow cytometry. The result of CRLF2 detection by flow cytometry was found to be correlated reasonably well with CRLF2 rearrangement by genomic testing [59].

The detection of Ph-like ALL is ultimately important not only because of its prognostic significance, but also because most of the genetic alterations of Ph-like ALL are targetable kinase lesions, which could be treated by tailored kinase inhibitor therapy (Table 19.3) [39]. Preclinical studies show that ruxolitinib was effective in targeting Ph-like ALL with JAK2 hyperactivation [60].

Table 19.2 The methods used to diagnose Ph-like ALL. The clinical utility, advantages, and disadvantages are listed in the table [57]

Technology	Detection method	Advantages	Disadvantages
Flow cytometry	Identifying antigen expressing pattern of blasts	1. Widely available in diagnostic laboratory 2. Rapid turnaround-time	1. No unique antigenic expression pattern for Ph-like ALL
	Detecting surface CRLF2 expression	1. Widely available in diagnostic laboratory 2. Rapid turnaround-time 3. Good correlation with JAK mutation status	1. Negative result does not exclude the diagnosis of Ph-like ALL 2. Results of JAK mutation status are ultimately needed for planning of targeted therapy
Cytogenetics	Conventional karyotyping	1. Widely used technique in diagnostic laboratory 2. Global assessment of genetic aberrations	1. Slow turnaround time 2. Most Ph-like genetic aberrations are cryptic
Fluorescence in situ hybridization (FISH)	Fusion probe or breakapart probe to detect common non-Ph-like ALL fusions	1. Widely used in routine diagnostic laboratory 2. Positive results can rule out Ph-like ALL	1. Negative result does not give extra information of the diagnosis of Ph-like ALL
Reverse-transcription PCR (RT-PCR)	RT-PCR for BCR-ABL1, *TCF3-PBX1, ETV6-RUNX1*	1. Widely available in diagnostic laboratory 2. Rapid turnaround time 3. Positive results made the diagnosis of Ph-like ALL unlikely	
	Multiplex RT-PCR targeting Ph-like fusions, e.g., *ABL1, ABL2, CRLF2, EPOR, JAK2, PDGFRβ*	1. Widely available in diagnostic laboratory 2. Rapid turnaround time 3. Positive results are diagnostic of Ph-like ALL and provide information on targeted therapy	1. Negative results do not exclude the diagnosis of Ph-like ALL
Sequencing-based	Hybrid-capture-based Targeted Next-Generation Sequencing	1. Clinically available in diagnostic laboratory 2. Identifying rearrangements/ mutations/insertions/deletions on a single platform	1. Expensive 2. Slow turnaround-time 3. Able to detect known fusion partners only
	Anchored Multiplex PCR-based Targeted Next-Generation Sequencing	1. Clinically available in diagnostic laboratory 2. Identifying rearrangements/ mutations/insertions/deletions on a single platform 3. Useful diagnostic test to confirm the diagnosis of Ph-like ALL 4. Able to detect known and novel fusion partners	1. Expensive 2. Slow turnaround-time 3. Fail to detect fusion of both partners are novel
	Whole exome sequencing	1. Able to detect established and novel mutations/insertions/deletions in coding region. 2. Detect translocations if occurring in coding regions	1. Cost not realistic to diagnostic laboratory 2. Difficult to standardize the test for use of diagnostic purpose 3. Slow turnaround time
	Whole transcriptome sequencing	1. Independent confirmation of *BCR-ABL1*-like B-ALL diagnosis 2. Able to detect established and novel gene fusions	
	Whole genome sequencing	1. Independent confirmation of *BCR-ABL1*-like B-ALL diagnosis on a single platform 2. Able to detect novel mutations/ insertions/deletions/gene rearrangements	
Gene expression analysis	Large scale microarray probe-set classifiers (110- or 257-gene)	1. Gold standard for diagnosis of Ph-like ALL	1. Not widely available 2. Difficulties in standardization
	Low-density microarray classifiers (8- or 15-gene)	1. Good indicator of *BCR-ABL1*-like phenotype 2. Probes for kinase mutations are included for planning of targeted therapy	

Table 19.3 Targetable kinase lesions in Ph-like ALL [38]

Genetic aberrations involved	Targeted therapy
ABL1	Dasatinib
ABL2	Dasatinib
CSF1R	Dasatinib
PDGFRB	Dasatinib
CRLF2	JAK2 inhibitors
EPOR	JAK2 inhibitors
IL2RB	JAK2 inhibitors or JAK3 inhibitors or both
NTRK3	Crizotinib
TSLP	JAK2 inhibitors
TYK2	JAK2 inhibitors

19.4 Other New Subtypes of B-ALL

19.4.1 ETV6-RUNX1-Like ALL

ETV6-RUNX1-like ALL is a subtype of B-ALL that has gene expression profile resembling those ALL with ETV6-RUNX1 fusion. Their immunophenotype is also similar to that of ALL harboring ETV6-RUNX1 (CD27-positive, CD44-negative to dim). This subtype of ALL is exclusively identified in childhood ALL. Various genetic aberrations are identified including gene fusion and copy number changes involving lymphocyte development and transcription factors, e.g., ETV6, IKZF1, and TCF3. The prognostic significance is unclear in the meantime for this subtype of ALL [6].

19.4.2 DUX4-Rearranged ALL

A special subtype of B-ALL with distinct immunophenotype (CD2-positive) was identified recently. This subtype is characterized by genetic aberrations in DUX4 gene and ETS transcription factor (ERG). DUX4 is expressed in germinal tissue. However, the role of DUX4 in gonadal development is uncertain. The translocation of DUX4 to IGH, resulting into overexpression in DUX4 in B-cell precursors, is an early event of leukemogenesis. Subsequently, the aberrantly expressed DUX4 binds to ERG and produced an abnormal ERG protein. It leads to loss-of-function of wild-type ERG protein and promotes leukemic growth in mice model. This subtype of B-ALL constitutes around 5–10% of ALL cases and the incidence is increased in adolescence and young adult. It confers an excellent prognosis even in the presence of poor-risk genetic aberrations such as IKZF1 deletions. DUX4-rearrangement ALL commonly has concomitant IKZF1 deletions which account for 40% of those cases [60].

19.4.3 Alterations in Transcription Factors

Gu et al. identified a recurrent fusion involving MEF2D (monocyte enhancer factor 2D) in a subset of B-ALL, which comprised around 5.3% of pediatric and adult ALL cases. Several fusion partners were identified including BCL9, CSF1R, DAZAP1, HNRNPUL1, SS18, and FOXJ2. The most common fusion was MEF2D-BCL9. IKZF1 mutations were identified as co-occurrence with MEF2D fusion. They also found that NRAS was also commonly co-occurred in this subgroup of ALL, as a subclone or secondary events of leukemogenesis. Those genetic aberrations enhance the transcriptional activity of MEF2D and thus the leukemic cells had unique genetic profile as a result of MEF2D dysregulation. A distinct immunophenotype was identified in patients with MEF2D rearrangement and they showed an aberrant immunophenotype of CD10-negative and CD38-positive. However, leukemic cells with MEF2D-CFS1R fusion displayed a Ph-like ALL phenotype. Nevertheless, the rearrangement of MEF2D disrupted MEF2D as normal function for B-cell development and overexpression of histone deacetylase 9. Therefore, this subtype of ALL is theoretically sensitive to the treatment with HDAC inhibitors [6, 61, 62]. Zhang et al. studied the molecular pathogenesis of MEF2D-HNRNUPL1 in B-ALL. They introduced knock-in mutation of MEF2D-HNRNUPL1 in mice. The mice showed disrupted B-cell development at pre-pro-B-stage and an aggressive leukemic phenotype was developed after introduction of $NRAS^{G12D}$mutation. The mice with MEF2D-HNRNUPL1 fusion showed an increase of chromatin-binding ability through MEF2D-responsive element (MRE) motifs in target genes. The study found that by modulating the MEF2D-HNRNUPL1 and DNA interaction, the transcription of aberrant target genes was alleviated. Moreover, the mice with MEF2D-HNRNUPL1 were sensitive to the treatment of histone deacetylase inhibitor [63]. The prognosis of patients who carry MEF2D fusion is more inferior than wild-type subgroup [61].

ALL with ZNF384 fusion is another gene fusion identified in B-ALL with the advance in genomic techniques. The gene zinc-finger protein 384 (ZNF384) is responsible to encode a transcription factor and this transcription factor is responsible to regulate the promoter of another gene, extracellular matrix genes 8. The fusion between ZNF384 and genes in TET family, e.g., Ewing sarcoma breakpoint region 1 gene (EWSR1), TATA box-binding protein-associated factor (TAF15), and transcription factor 3 (TCF3), was found to be involved in leukemogenesis of ALL. The immunophenotype of ALL with ZNF384 rearrangement was also distinct with CD10-negative and aberrant expression of one or more myeloid antigens. Activating mutations in Ras pathway genes (NRAS, KRAS, PTPN11) were the most com-

mon co-occurring mutations. Other genes such as EZH2, MLL2, and ASH1L were also reported to be co-occurred in patients with ZNF384 fusion. Gene expression profile of patients with TCF3-ZNF384 showed enrichment of genes hemopoietic stem cell features [64]. Indeed, a study showed that ZNF384 rearrangement also constitutes a significant proportion of B/myeloid mixed phenotype leukemia, indicating that this genetic aberration leads to leukemic transformation at the level of hemopoietic progenitor with multilineage potential [65].

The clinical behavior is highly variable, depending on the fusion partner of ZNF384. S. Hirabayashi et al. found that patients with TCF-ZNF384 fusion often had a higher white cell count, poorer response to steroid, and younger age at presentation. They also showed a higher frequency of relapse and all these clinical behaviors were totally different from those with EP300-ZNF384 fusion [64].

Both subtypes of ALL (MEF2D rearrangement and ZNF384 rearrangement) constitute around 5% in childhood ALL and around 7–10% of ALL in adolescence and young adults [6].

19.4.4 TCF-HLF Fusion

Next-generation sequencing revealed a distinct subtype of B-ALL involving rearrangement of TCF3 which disrupted the B-cell differentiation and fused with partner gene HLF. The fusion of TCF3-HLF constitutes a rare subtype of ALL (<1% in pediatric ALL) and is associated with relapse/refractory disease. It is typically associated with relapse disease and death within 2 years of diagnosis. This fusion was found exclusively in leukemic precursor B-cells, but not in stem cells or cells of pluripotent progenitor stage, indicating that the leukemic fusion occurs in lymphoid-committed stage. Moreover, Fischer et al. found that those cooperative mutations mainly involve genes regulating the maturation of B-cell, for example, PAX5, BTG1, and VPREB1. Other co-occurring mutations detected include JAK2, CDKN2A/B, and genes of transcriptional regulators, e.g., ERG, NCOR1, TOX, BACH2, BCL7C, MLLT3, SMARCA2, and MAFK. Moreover, the presence of TCF3-HLF fusion was strongly associated with gene mutation in Ras pathway including NRAS, KRAS, and PTPN11. Genomic profiling showed enrichment of genes of stem cells and myeloid signature. The study group found that this subtype of ALL was extremely sensitive to BCL2 inhibitor, venetoclax [66].

19.4.5 B-Other ALL

Despite the advancement of genomic technology, a couple of ALL cases lack a well-defined recurrent genetic aberrations and hence proper risk stratification cannot be performed on those cases. Some study groups perform large-scale genomic studies in B-ALL cases and successfully identified a few novel recurrent genetic aberrations in B-ALL.

19.4.5.1 IGH Rearrangement

The prevalence of IGH rearrangement involving various partners, e.g., CRLF2, CEBP family members (CCAAT/enhance binding protein), and ID4, is increasing with age and approaching around 10% of ALL cases in adolescence and young adult. Moreover, a novel subtype was identified by some study groups with unique transcriptional signature. They were characterized by rearrangement of IGH to BCL2, MYC2, and/or BCL6, resembling those "double-hit" lymphoma. This subtype was identified in adult with median age of 48.5 years old. The B-ALL with IGH rearrangement confers a very poor prognosis [67, 68].

19.4.5.2 NUTM1 Rearrangement

This subtype of ALL involves rearrangement of nuclear protein in testis midline carcinoma family 1 (NUTM1) with various partner genes including ACIN1, BRD9, CUX1, IKZF1, SLC12A6, and ZNF618. This subtype constitutes 1% of childhood ALL with a median age of 3 years old [6]. The BRD4-NUTM1 fusion was also found in NUT midline carcinoma, a highly fatal and aggressive pediatric cancer [69]. No other known co-occurring driver mutations were identified and thus it indicated that the NUTM1 rearrangement itself is a primary event of leukemogenesis. Gene expression profile showed upregulation of proto-oncogene including HOXA and BMI1. This subtype of ALL carries favorable prognosis [70].

19.4.5.3 PAX5-Driven Subtypes

PAX5 acts as a haploinsufficient tumor suppressor gene in ALL and heterozygous deletions or loss-of-function mutations present in around 33% of ALL cases. Mice had heterozygous loss-of-function PAX5 allele that showed B-cell maturation arrest and shorten the latency of development of leukemic phenotype. Genomic profiling showed frequent co-occurrence of mutations involving genes of JAK-STAT pathway, Ras pathway [71]. The introduction of concurrent PAX5 heterozygous loss and JAK-STAT pathway mutation in mouse model promoted development of B-ALL [71]. Besides genes in JAK-STAT pathway, genes responsible for cell cycle regulation (CDKN2A, RB1, and BTG1 deletions), B cell development (IKZF1, VPREB1, and BTLA deletions), transcriptional regulation (e.g., ZFP36L2, ETV6, and LEF1), and epigenetic modification (e.g., KDM6A, KMT2A, ATRX) were also reported to be co-occurred with PAX5 alterations. This subtype of PAX5-driven B-ALL constitutes around 10% of cases in childhood ALL as well as adolescence and young adult, while it constitutes around 7% in adult ALL. Rearrangement involving PAX5 genes was reported to constitute around 2–3% of ALL cases and the most common

rearrangement was PAX5-JAK2. PAX5-ZCCHC7 was also another type of genetic fusion of PAX5 reported [72].

Another subgroup of PAX5-driven B-ALL is characterized by PAX5 P80R mutation. Mutations in signaling pathway (Ras, JAK/STAT, FLT3, BRAF, and PIK3CA) were the most common co-occurring mutations (95% of PAX5 P80R cases), which suggested the cooperative role of PAX5 aberrations and activating kinase mutations in promoting leukemic phenotype. The Ras pathway mutations were the most common co-occurring mutation in signaling pathway including NRAS, KRAS, PTPN11, and NF1 aberrations (75% of PAX5 P80R cases). Gene expression profile not only showed enrichment of genes in cytokine signaling pathway, but also downregulation of B-cell-associated genes, indicating the impairment of B-cell maturation by PAX5 P80R mutation. Particularly, MEGF10 gene was overexpressed in B-ALL with PAX5 P80R mutation [72].

19.5 Genomic Landscape of T Lymphoblastic Leukemia (T-ALL)

T-ALL comprises 10–15% of childhood ALL and 20–25% of adult ALL cases in most parts of the world [26, 73]. The prognosis of T-ALL was inferior to that of B-ALL in general with around 10% of long-term remission rate. With the introduction of intensified chemotherapeutic regimen, the long-term remission rate approaches 60% and it is comparable with that of B-ALL [74].

In the past decades, genetic aberrations causing dysregulation of cell cycle regulator CKD2A/CDK2B, NOTCH1-activating mutations are well-established oncogenic mutations in T-ALL. With the advance of genomic studies, more and more genetic aberrations were identified, and the findings gave us insight on the molecular pathogenesis of T-ALL. From those findings, we can identify a unique genetic profile for different subtypes of T-ALL, together with genetic aberrations causing ectopic expression of one or more transcription factor genes. Figure 19.2 showed the spectrum of mutations across different subtypes of T-ALL [75]. Early precursor T-ALL (ETP-ALL) was characterized by lowest prevalence of NOCTH1-activating mutations and loss-of-function mutations of CDK2A/2B, but more enriched with mutations in signaling pathway (FLT3, RAS), transcription factors involving thymocyte development (RUNX1, GATA3, ETV6), and epigenetic regulators (e.g., DMNT3A, EZH2). Cortical T-ALL has high frequency of NOTCH1-activating mutations and loss-of-function mutations of CDKN2A/2B and high prevalence of activating mutations of TLX1 and TLX3. The presence of rearrangement of

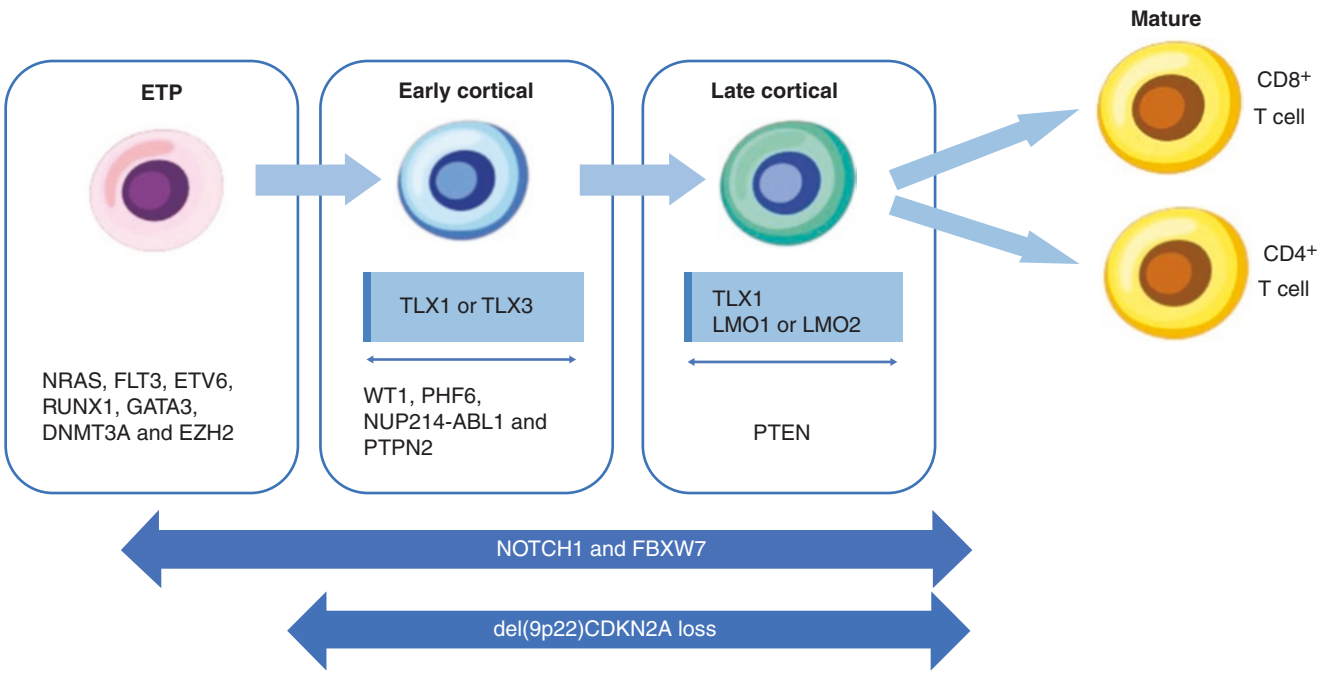

Fig. 19.2 Gene expression signature in different subgroups of T-ALL which defined their cellular origin. ETP-ALL showed lower frequency of aberrations in NOTCH1 and CDKN2A but more prevalent in activating mutations of signaling pathway such as NRAS and FLT3 and aberration involving key developmental transcription factors such as RUNX1 and GATA3. Aberrations in epigenetic regulators (DNMT3A, EZH2) are also common. Early cortical stage of thymocyte commonly has overexpression of various oncogenes of TLX1 and TLX3 and they encode transcription factors. Moreover, a high frequency of NOTCH1 and CDKN2A aberrations is noted. Some aberrations are unique to this stage of thymocytes including NUP214-AB1 rearrangement, aberrations in PFH6, WT1, and PTPN2. Late cortical thymocyte stage showed overexpression of TAL1 and it encodes LMO1 and LMO2. NOTCH1 and CDKN2A aberrations are very common. They also show high frequency of loss-of-function aberrations of PTEN

NUP214-ABL1, mutation of PHF6 and WT1, is also characterized in cortical T-ALL. T-ALL at late cortical thymocyte stage is characterized by high prevalence of mutation in TAL1 causing its aberrant expression, as well as mutations of LMO1 and LMO2. This subtype of T-ALL has high prevalence of PTEN loss-of-function mutations which is unique to T-ALL at late cortical thymocyte stage [76]. These genetic events cooperate together to promote leukemogenesis. On average, a T-ALL case comprises more than 10 genetic aberrations and these genetic aberrations promote malignant transformation of normal T-cell to leukemic phenotype via impairment of differentiation, enhancement of survival of leukemic cells, increased proliferation, and altered cellular metabolism [75]. The prevalence of different genetic aberrations is different between pediatric and adult T-ALL. Table 19.4 summarized different categories of genetic aberrations and their prevalence in pediatric and adult T-ALL.

Table 19.4 Genes involved in leukemogenesis in T-ALL and nature of genetic aberrations. Their frequencies of occurring in pediatric and adult T-ALL are shown [75]

Types of gene	Gene involved	Mechanism of aberrations
NOTCH1 signaling pathway	*NOTCH1*	*Activating mutations/chromosomal rearrangement*
	FBXW7	*Inactivating mutations*
Cell cycle regulators	CDKN2A	*Deletions*
	CDKN2B	
	RB1	
Transcription factors	BCL11B	*Inactivating mutations/deletions*
	ETV6	
	GATA3	
	HOXA	*Chromosomal rearrangements/gene inversion/overexpression*
	NKX2.1/NKX2.2	*Chromosomal rearrangements/overexpression*
	TLX3	*Chromosomal rearrangements/overexpression*
	MYB	
	LMO2	
	RUNX1	*Inactivating mutations/deletions*
	TAL1	*Chromosomal rearrangements/mutations in 5′ super-enhancer/gene deletions/overexpression*
	TLX1	*Chromosomal rearrangements/deletions/overexpression*
	WT1	*Inactivating mutation/deletion*
	LEF1	
Signaling pathway	AKT	Activating mutations
	FLT3	
	JAK1	
	JAK3	
	IL7R	
	KRAS	
	NRAS	
	PI3KCA	
	STAT5B	
	NUP214-ABL1 fusion/ ABL1 *gain*	Chromosomal rearrangement/gene amplification
	PTEN	Inactivating mutations
	PTPN2	
	DNM2	
	NF1	*Deletions*
Epigenetics	*DNMT3A*	Inactivating mutations
	EED	
	EZH2	
	KDM6A/UTX	
	PHF6	
	SUZ12	
Protein translation and RNA stability	CNOT3	*Missense mutations*
	RPL10	
	RPL5	*Inactivating mutations*
	RPL22	
	mTOR	*Activating mutations*

19.5.1 Oncogenic NOTCH1 Signaling Pathway

NOTCH1 is a transmembrane glycoprotein and it acts as a ligand-activated transcription factor which promotes direct transduction of signals from extracellular stimuli to cell membrane, resulting in transcription of various target genes and those genes often play an important role in thymocyte development [77, 78]. Activating mutations in NOTCH1 domain cause disruption of initiation and termination of signaling [79]. In addition, mutations in F-Box and WD Repeat Domain Containing 7 (FBXW7) were identified in 8–30% of T-ALL. FBXW7 is the negative regulator of NOTCH1 and its mutation causes failure of degradation of activated NOTCH1 and around 9–12% of T-ALL harbor these mutations [80, 81]. Upon introduction of NOTCH1-activating mutation in hemopoietic stem cell in mice resulting into generation of constitutively active intracellular form of NOTCH1, leukemic phenotype compatible with human T-ALL could be developed [82]. Some NOTCH1 mutations have weaker oncogenic properties, e.g., mutations in NOTCH1 heterodimerization domain (HD) and mutations in NOTCH1 PEST domain. Those mutations require additional oncogenic mutations such as $KRAS^{G12D}$ to drive the development of T-ALL [83]. Dysregulation of paracrine and cell-intrinsic regulatory mechanisms of NOTCH1 can also lead to development of hyperactivation of NOTCH1 signaling and thus drive the leukemogenesis. Mutations in NOTCH1 ligand, delta-like 4 (DLL4), and loss-of-function mutations of other negative regulators of NOTCH1 including RNA-binding proteins ZFP36L1 and ZFP36L2 were shown to drive T-ALL in mice model [83–85].

The hyperactivation of NOTCH1 signaling resulting into enhanced transcription of genes that promote leukemogenesis and MYC is one of the direct targets of NOTCH1 signaling [86–88]. Hairy and enhancer of split-1 (HES1) is also a downstream target of NOTCH1 signaling and it is responsible to activate PI3K-Akt and nuclear factor kappa b (NF-κB) pathway [89, 90].

Gamma secretase is responsible for cleavage of NOTCH1 upon binding of ligand, resulting into release of intracellular domain of NOTCH1 (ICN1) from plasma membrane. After that, ICN translocates to nucleus and mediates the transcription of various gene. Gamma secretase inhibitors, e.g., nelarabine, inhibit the cleavage of NOTCH1 and thus prevent the release of intracellular domain of NOTCH1. Therefore, the cellular effect of NOTCH1 signaling is halted [76].

19.5.2 Cell Cycle Regulator Mutations

The cell cycle inhibitors, $p16^{INK4A}$ (inhibitor of G1 to S phase of cell cycle) and $p14^{ARF}$ (inhibitor of p53-negative regulator, MDM2), are encoded by the gene CDKN2A which is located in short arm of chromosome 9. More than 70% of T-ALL harbor mutations causing loss-of-function of these cell cycle inhibitors [90]. Also, around 15% of T-ALL have deletion of gene involving retinoblastoma 1 (RB1) locus, resulting from deletion of chromosome 13q14.2. RB1 is a tumor suppressor and the phosphorylated form of RB1 inhibits E2 promoter-binding–protein-dimerization partner (E2F-DP) dimers and thus prevents the cell cycle progression from G1 to S phase [17, 91].

Deletion of CDKN1B gene results from chromosomal deletion of 12p13.2 and this genetic aberration accounts for 12% of T-ALL. The gene CDKN1B is responsible to encode cell cycle inhibitor, p27KIP1, and it is responsible for inactivating the cyclin E-CDK2 complex, resulting into cell cycle arrest at G1 phase [92].

Around 3% of T-ALL harbor t(12;14)(p13;q11) and t(7;12)(q34;p13) translocations and these chromosomal translocations lead to increased expression of cyclin D2 (CCND2) and thus promoting the cell cycle progression from G1 phase to S phase [93].

T-ALL with dysregulation of cell cycle pathway may be targetable by various cyclin-dependent kinase (CDK) inhibitors available in the market. Preclinical study showed that CDK4/6 inhibitor, ribociclib, was active in T-ALL. Moreover, combination of ribociclib and corticosteroid or mTOR inhibitor showed synergistic effect [94]. Another preclinical study showed that dinaciclib, a multi-CDK inhibitor targeting CDK2, CKD5, and CDK9, was active against T-ALL cell lines [95]. More extensive preclinical studies and clinical trials are needed to ascertain the use of CDK inhibitors in treating T-ALL with cell cycle dysregulation.

19.5.3 Aberrations in Transcription Factor Genes

Distinct genetic aberrations are identified in each subtype of T-ALL and each subgroup of T-ALL is characterized by 1 or more defects in transcription factors. The mechanisms of aberrations of transcription factor genes are as follows: (1) chromosomal translocation involving T-cell receptor (TCR) gene and other regulatory genes. (2) Gene amplification involving transcription factor. (3) Function enhancement by small insertions or mutations of gene. Table 19.5 shows examples of genetic aberrations involving transcription factor and their mechanisms.

19.5.3.1 bHLH and LMO Transcription Factors

Genetic aberrations of bHLH transcription factor including TAL1, TAL2, LYL1, and BHLHB1 as well as LMO1 and LMO2 comprise 60% of T-ALL cases [76]. Besides structural rearrangement of chromosomes involving TCR and SCL/TAL1 interrupting locus (STIL), somatic muta-

Table 19.5 Examples of chromosomal rearrangement and mechanisms of leukemogenesis [75]

Gene involved	Chromosomal rearrangement	Mechanism of leukemogenesis
TAL1	del(1)(p32p32)	Overexpression of TAL1 by STIL promoter
	t(1;14)(p32;q11)	Overexpression of TAL1 by TCR enhancer
	t(1;14)(p32;q11)	
TLX1	t(10;14)(q24;q11)	Overexpression of TLX1 by TCR enchancer
	t(7;10)(q34;q24)	
	t(10;14)(q24;q11)	
TLX3	t(5;14)(q35;q11)	Overexpression of TLX3 by TCR enhancer
	t(5;14)(q35;q32)	Overexpression of TLX3 by BCL11B
HOXA9/HOXA10	inv(7)(p15;q34)	Overexpression of HOXA genes by TCR enhancer
	t(10;11)(p13;q14)	Formation of PICALM-MLLT10 fusion transcript and results into upregulation of HOXA genes
	del(9)(q34;q34)	Formation of SET-NUP214 fusion transcript and results into upregulation of HOXA genes
NKX2–1	inv(14)(q11;q13)	Overexpression of NKX2-1 by TCR enhancer
NKX2–2	t(14;20)(q11;p11)	Overexpression of NKX2-2 by TCR enhancer
LMO1	t(11;14)(p15;q11)	Overexpression of LMO1. The LYL1 or TAL1 also overexpressed.
LMO2	t(11;14)(p13;q11)	Overexpression of LMO1. The LYL1 or TAL1 also overexpressed.
MEF2C	del(5)(q14;q14)	Overexpression of MEF2C
LYL1	t(7;19)(q34;p13)	Overexpression of LYL1 by TCR enhancer
MYB	dup(6)(q23;q23)	Overexpression of MYB
	t(6;7)(q23;q34)	Overexpression of MYB by TCR enhancer

tions in the upstream region of TAL1 gene are also identified [95, 96].

TAL1 serves as autoregulatory loop of GATA3 and RUNX1 and positively activates MYB to promote leukemogenesis [97]. Overexpression of TAL1 in T-cells promotes leukemic phenotype in mice with activation of genes including tribbles pseudokinase 2 (TRIB2)65, NKX3-1 [98].

Around 10% of T-ALL cases have overexpression of LMO1 and LMO2, resulting from structural rearrangement of chromosome. The leukemogenic ability of transgenic mice with LMO1 and LMO2 overexpression was much increased after introduction of TAL1, indicating a cooperative role of TAL1 and LMO1/LMO2 in promoting T-ALL development [99, 100]. It is not surprising since the proteins, LMO1 and LMO2, form complex with TAL1 and other transcription factors of bHLH family which may indicate that they are interrelated in functional aspect [76].

19.5.3.2 HOX Transcription Factor

Around 3% of T-ALL cases show genetic aberration of HOX9 and HOX10 gene. These genetic aberrations are common in ETP-ALL (See section of ETP-ALL) and ALL with chromosomal rearrangements such as KMNT2A rearrangement and NUP214 rearrangement.

TLX genes such as TLX1 and TLX3 are some of the HOX oncogenic factors and genetic aberrations of TLX1 and TLX3 are usually caused by chromosomal arrangement. One of the example is TLX3 aberrations caused by t(5;14)(q35;q32) and this chromosomal translocation exists in 20–25% of pediatric T-ALL and around 5% of adult T-ALL [76]. NKX2-1 and NKX2-2 are also HOX-related genes. T-ALL with NKX2-1 and NKX2-2 rearrangement show similar transcription signature with those T-ALL cases with TLX1 aberrations [101].

19.5.3.3 Other Transcription Factors Aberrations

ETV6 is a tumor suppressor gene which is important for differentiation of hemopoietic stem cell. RUNX1 is another tumor suppressor which regulates hemopoietic stem cell development. GATA3 is a regulator responsible for early T-cell development. These mutations in transcription factor suppressors are prevalent in ETP-ALL (See the section of ETP-ALL). BCL11B inactivating mutations occur in around 10% of T-ALL cases and inactivation of BCL11B caused maturation arrest of early T-cell in mice model. Deletion and loss-of-function mutation of WT1 gene occur in 10% of T-ALL and it frequently co-occurs with aberrations of TLX1, TLX3, and HOXA gene [76].

19.5.4 Aberrations in Epigenetic Regulators

19.5.4.1 PHF6

Genetic aberrations of PHF6 include deletion and inactivating mutations and it occurs in around 16% of pediatric T-ALL and 38% of T-ALL [102]. Börjeson–Forssman–Lehmann syndrome is characterized by germline mutation of PHF6 and thus development of T-ALL has been reported in those patients. PHF6 gene is located in X chromosome

(Xq26) and thus T-ALL with PHF6 aberrations are found predominantly [76]. PHF6 had been implicated to have a role in ribosome biogenesis and splicing, as well as chromatin remodeling [103–105].

19.5.4.2 Other Epigenetic Regulators

Another H3K27me3 histone demethylase called KDM6A is a tumor suppressor gene. Loss-of-function mutations of KDM6A comprise 5–15% of T-ALL cases [106].

EZH2, EED, and SUZ12 are collectively called Polycomb repressive complex 2 (PRC2) and it involves transcriptional regulation via epigenetic mechanisms. The aberrations in PRC2 are prevalent in ETP-ALL (see the section of ETP-ALL) [76].

19.5.5 Aberrations in Oncogenic Signaling Pathway

PI3K-Akt Pathway

Thymocytes depend on PI3Kγ and PI3Kδ signaling for their growth. Loss-of-function mutations and deletions of PTEN, a negative regulator of PI3K-Akt pathway, comprise 10–15% of T-ALL and it is the most common cause of hyperactivation of PI3K-Akt pathway [107, 108]. Transgenic mice with heterozygous Pten knockout showed T-ALL phenotype [109]. Other mechanisms of PI3K-Akt pathway hyperactivation include AKT1-activating mutations, PI3K mutations in catalytic and regulatory subunit, as well as chromosomal rearrangement resulting into overexpression of insulin receptor substrate 4 (IRS4) [110, 111].

RAS-MAPK Pathway

Activating mutations in MPAK pathway including NRAS, KRAS, and PTP11 mutations account for 5–10% of T-ALL and these mutations are more prevalent in ETP-ALL (See the section of ETP-ALL). Moreover, deletions or loss-of-function mutations of neurofibromatosis type 1 (NF1) gene account for 3% of T-ALL and NF1 gene serves as a negative regulator of RAS-MAPK signaling pathway [112].

19.5.6 Mutations of Genes of Ribosomal Proteins

The increased rate of protein synthesis is crucial in survival of leukemic cells. Aberrations in NOTCH1 and PI3K-Akt signaling as well as MYC aberrations increased the rate of protein synthesis [113]. Mutations in ribosomal protein genes including RPL5, RPL10, and PRL11 as well as CCR4–NOT transcription complex subunit 3 (CNOT3) were identified in certain subset of T-ALL. For example, RPL10 mutations comprise 6% of childhood T-ALL [114].

19.5.7 Genomic Landscape of ETP-ALL

Early precursor T-cell acute lymphoblastic leukemia (ETP-ALL) is an aggressive subtype of T-ALL described by Coustan-Smith et al. in 2009 due to its unique immunophenotype and genomic expression profile [115]. ETP-ALL account for 5%–17% of childhood ALL and 7.4% of adult ALL cases [116]. This subtype of T-ALL was originated from earliest thymic progenitors (ETPs) derived from multipotent progenitor cells of bone marrow [117, 118]. Due to its cellular origin, the genomic profile of ETP-ALL is unique when compared with other subtypes of T-ALL whose genomic expression profile resembles hematopoietic stem cells of myeloid leukemia [76]. Table 19.6 summarized various genetic aberrations that occur in ETP-ALL. Those genetic aberrations affect the development and differentiation of early thymocytes, aberrant kinase signaling causing increased proliferation and enhancement of survival, as well as epigenetic regulators. Those common mutations found in other subtypes of T-ALL such as NOTCH1 mutation and CDKN2A/2B aberrations are much less common in ETP-ALL, but the presence of mutations occurred in myeloid malignancies is much more common in ETP-ALL such as FLT3 mutations and DMNT3A mutations [115]. In addition, some other aberrations which seldom reported in other subtypes of T-ALL were identified, for example genetic aberrations in IL7R, JAK3, RUNX1, EZH2, and EED [76]. The genetic aberrations of epigenetic regulators including EZH2, PHF6, and SUZ12 are more frequent in ETP-ALL [119, 120]. Gene rearrangements such as MEF2C rearrangement and KMT2A rearrangement were also identified in ETP-ALL [119, 121]. The discoveries of those genetic aberrations had stimulated some of the mechanistic studies to elucidate the molecular mechanisms of leukemogenesis of ETP-ALL. Those findings are relevant in development of novel therapeutic strategies for preclinical and clinical studies.

19.5.8 Genetic Aberrations in Transcription Factors in ETP-ALL

LMO2 expression in thymocytes promotes the transcription of hemopoietic stem cell-related genes and suppression of those genes responsible for T-cell maturation. LYL1 also plays a role to induce abnormal self-renewal and impairment of differentiation of thymocytes. The genes LMO2 and LYL1 often overexpress together in ETP-ALL cases [122, 123]. Around 36.8% of ETP-ALL cases show overexpression of LMO2 and LYL1 [119]. Overexpression of BCL11B induces T-cell maturation and development into CD4 and CD8 double positive T-cell stage and genetic aberrations causing its underexpression were reported in ETP-ALL [119, 124]. RUNX1 is responsible for thymocyte maturation and matu-

Table 19.6 Examples of genetic aberrations found in ETP-ALL and mechanisms of aberrations [146]

Types of genes	Genes involved	Mechanisms of aberrations
Transcription factors	ETV6	Inactivating mutations/deletions
	GATA3	
	RUNX1	
	WT1	
	HOXA	Chromosomal rearrangements/inversions/overexpression
	LMO2	Chromosomal rearrangement/deletions/overexpression
Signaling pathway	FLT3	Activating mutations/internal-tandem repeat
	JAK1	Activating mutations
	JAK3	
	IL7R	
	KRAS	
	NRAS	
Epigenetics aberrations	DNMT3A	Inactivating mutations
	EED	Inactivating mutations/deletions
	EZH2	
	PHF6	
	SUZ12	
Others	STIL-TAL1 fusion	Rearrangement of Genes
	MEF2C-rearrangement	
	KMT2A-rearrangement	
	NUP98-rearrangement	

ration arrest will occur if there are presence of loss-of-function mutation or deletion of RUNX1 [125]. GATA3 plays an important role in differentiation of ETPs into mature T-cells and around 33% of adult ETP-ALL show underexpression of GATA3 due to hypermethylation [126, 127].

19.5.9 Molecular Mechanism of Gene Arrangement in Leukemogenesis of ETP-ALL

Around 50% of ETP-ALL cases harbor MEF2C rearrangement which involves MEF2C-related cofactors [128]. MEF2C overexpression occurs due to MEF2C rearrangement and subsequently leads to overexpression of LMO2 and LYL1, thus causing differentiation block of early thymocytes. The addition of other genetic events such as RAS and MYC aberrations promotes development of leukemic phenotype [129]. KMNT2A rearrangement is identified in ETP-ALL and it leads to overexpression of HOXA-associated genes which causes impairment of maturation of progenitors [76, 130, 131]. The overexpression of HOXA in ETP-ALL is associated with increased risk of relapse and hence poor survival [132].

19.5.10 Activating Mutations in IL7R in ETP-ALL

Around 45% of ETP-ALL cases harbor IL7R-activating mutations and these are associated with poor response to chemotherapeutic agents [133, 134]. PRC2 mutations are also commonly associated with IL7R mutations in T-ALL [135].

The gene IL7R encodes IL7 receptor alpha chain and this is crucial in maturation of early lymphoid progenitors [136]. The mutations in IL7R induce its activation with autonomous homodimerization and lead to STAT5 phosphorylation. The subsequent events of STAT5 phosphorylation include BCL2 expression, downregulation of cyclin-dependent inhibitor p27 kip1, and hyperactivation of the PI3K/Akt/mTOR and JAK/STAT pathways [134, 137–141]. Moreover, IL7R can cause overexpression of LMO2 and downregulation of BCL11 which subsequently lead to differentiation block in early thymocyte. These findings were supported by upregulation of myeloid-associated genes in thymocytes with IL7R mutations [137]. Therefore, IL7R mutations promote development of leukemia through a variety of mechanisms, from differentiation block of early thymocytes to dysregulation in pathway affecting cellular growth and survival (Fig. 19.3).

19.5.11 Mutations in Epigenetic Regulators and Leukemogenesis of ETP-ALL

Polycomb repression complex 2 (PRC2) is a protein complex that is responsible to participate in histone modifications and thus control the genetic expression of development-related genes. The PRC2 complex comprises EZH2 and EZH2 and involves in methylation of histone 3 tail at lysine 27 residual via its SET domain. The process of histone modification by EZH2 recruits PRC1 and leads to

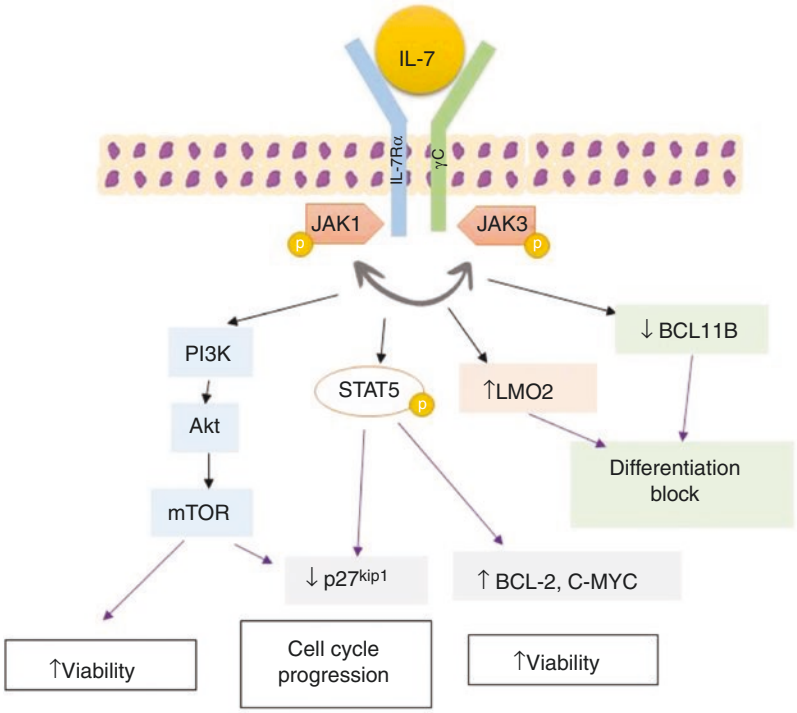

Fig. 19.3 Mechanism of leukemogenesis of IL7R aberrations in ETP-ALL

condensation of chromosome which causes suppression of gene transcription (Fig. 19.4) [142]. Around 48% of ETP-ALL cases involve mutations in PRC2 complex and loss-of-function mutations. EZH2 is the most common type of mutation reported. Mutations of other genes in PRC2 complex including SUZ12 and EED were also reported [133].

Transgenic mice with CDKN2A −/−/NRAS Q61K/EZH2 Δ/Δ mutated lineage-negative, SCA-positive, and c-Kit-positive (LSK) cells promoted the development of phenotype resembling ETP-ALL. Differentiation block of ETP with upregulation of genes relating to stem cell and early progenitor cell programming was associated with homozygous loss of EZH2 and it was also associated with upregulation of HOXA and hyperactivation of JAK/STAT pathway [143] (Fig. 19.4). Another experiment using transgenic mice with EZH2 Δ/Δ RUNX1 Δ/Δ mutated lymphoid progenitor cells showed that an additional mutation of signaling pathway such as FLT3-ITD was required to develop ETP-ALL phenotype. The addition of FLT3-ITD mutations in EZH2 and RUNX1 double knockout mice was associated with upregulation of genes in RAS pathway and Il7R signaling [144]. Figure 19.5 summarizes the current concepts of leukemogenesis causes by mutations in epigenetic regulators.

19.5.12 Challenges and Future Perspectives

The incorporation of recurrent genetic aberrations into risk stratification of patients is a major breakthrough in management of patients with ALL. With the advance of technology of deep sequencing, the discoveries of many new recurrent genetic aberrations aid the risk stratification of patients, especially those patients with their genetic abnormalities not being described in the current risk stratification system. Moreover, the research on molecular mechanism of leukemogenesis leads to the discoveries of novel therapies. The research on histone deacetylase inhibitor in KMNT2A-rearranged ALL is an example.

However, ALL is not a simple disease which is caused by a single drive mutation. It is a complex disease process and many genetic aberrations occur together in the same patient, with clonal diversities. Therefore, a detail dissection of molecular pathogenesis of individual genetic aberration and their interactions with other mutated genes is needed to uncover the key leukemic pathway through mechanistic study. Moreover, the entity of ETP-ALL is a separate subtype of T-ALL with its unique cellular origin. The exact cellular origin and the molecular pathogenesis were not clear. Recent mechanistic studies had proven the cellular origin of ETP-ALL and delineated the molecular pathogenesis.

Novel research platform should be developed in order to study multiple genetic aberrations and their interactions in ALL. With the development of such detailed mechanistic studies, a more effective novel therapeutic approach can be developed for treating ALL of various genetic subtypes. The utilization of genomic technology not only provides mechanistic insight of disease and important tools for risks stratification, but also serves as a tool for monitoring of disease after treatment. Recent study had showed that MRD measurement by high-sensitivity NGS platform could be more

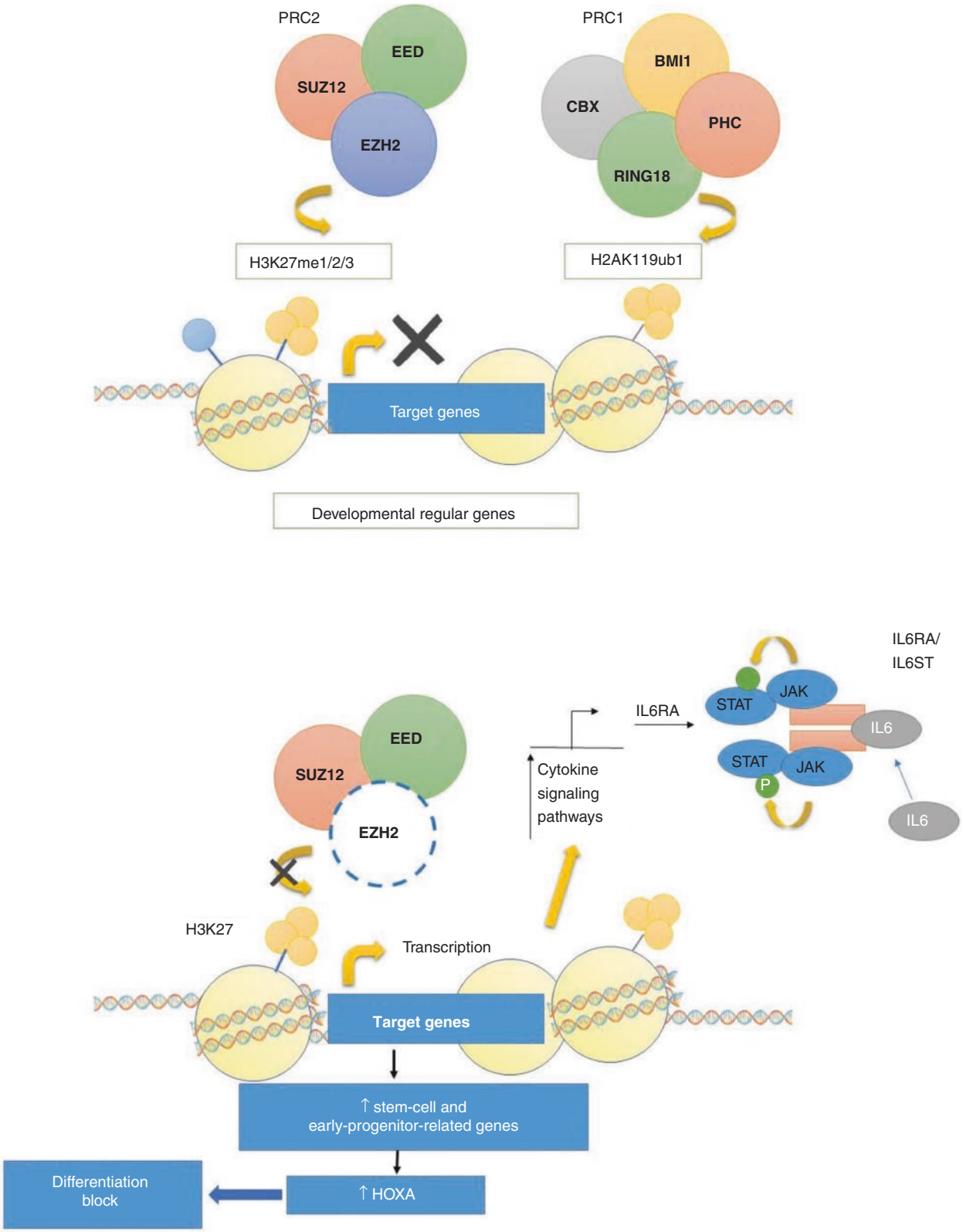

Fig. 19.4 Mechanism of leukemogenesis involving aberrations in epigenetic regulators in ETP-ALL. The diagram represents the function of PRC2 complex in normal state of hemopoiesis. The methylation of lysine 27 residue of histone 3 tail (H3K27me3) is mediated by EZH2. After the histone modification, the K2K27me3 recruits PRC1. Afterward, the lysine 119 residual of histone 2A (H2AK119ub) is mono-ubiquitinated by CBX1 in PRC1. The gene transcription of developmental regulatory genes is suppressed after this histone modification. The loss-of-function mutations in EZH2 will increase the transcription of genes involving stem-cell and early-progenitor programming. Therefore, differentiation block of thymocytes will occur. The IL6RA gene will increase in transcription as well and lead to STAT3 hyperactivation

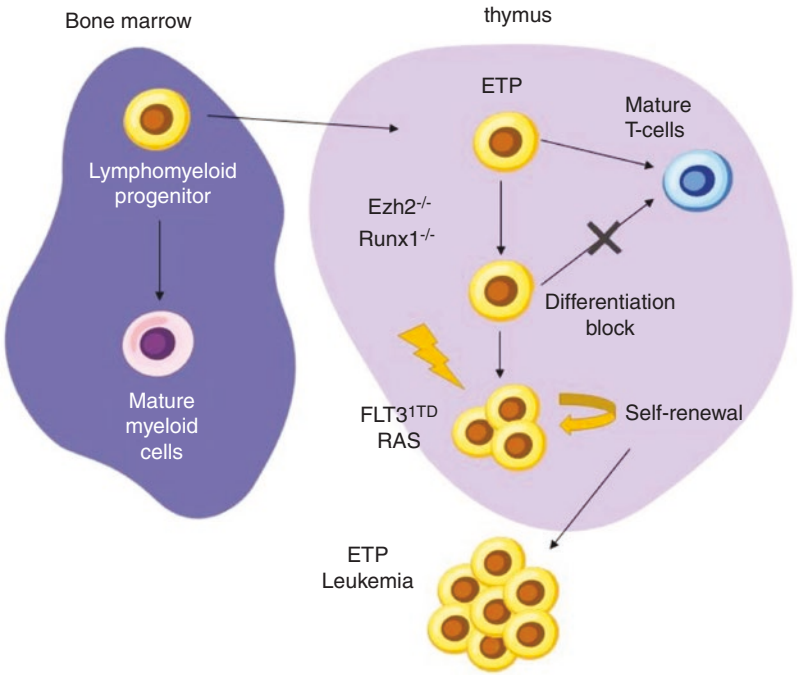

Fig. 19.5 Schematic diagram showing the process of leukemogenesis in ETP-ALL involving aberrations in EZH2, RUNX1, RAS, and FLT3-ITD genes

informative of patient's prognosis comparing with MRD detection by flow cytometry [145]. In the future, despite all those challenges mentioned, the prognosis of ALL will further improve with all advancements made in discovery of novel genomic data and robust research work.

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Management of Adolescent and Young Adults with Acute Lymphoblastic Leukaemia

20

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Abstract

Acute Lymphoblastic Leukaemia (ALL) is the commonest malignancy in children and the treatment outcome improved significantly in the past few decades, the 5-year survival increased from 10% to now 90%. The good results of paediatric ALL treatment protocols are also observed in older children in large multicenter clinical trials. Recently, the application of paediatric-inspired treatment protocols extends to adolescents and young adults (AYA) up to 30–40 years of age. The treatment outcome in AYA also demonstrated improved survival outcome to 60–70%. The more intensive chemotherapy regimens adopted in paediatrics are associated with more side effects and complications in AYA as compared to children. Guidelines on management of intensive chemotherapy protocols will help to reduce severe complications and improve compliance to treatment, thus improves the outcome. With the success of chemotherapy treatment, allogeneic haematopoietic stem cell transplant (HSCT) may not be required for most of the AYA with ALL, but reserved for very high-risk ALL or relapsed ALL.

Keywords

ALL · AYA · Chemotherapy · Clinical trials · Compliance · HSCT

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20.1 Introduction

Acute Lymphoblastic Leukaemia (ALL) happens in all age groups, but is most common in children with peak at 2–6 years. About 53% of patients with ALL are diagnosed before age of 20 years, the median age at diagnosis for ALL is 17 years [1]. Adolescents and young adults (AYA) accounted for 10–20% of all ALL cases, and definition of AYA varies among different centres or study groups. The upper age limit of young adults may be from 30 to 40 years of age, while adolescents usually refer to those children 15 years of age or older. Adolescents below age of 18 years are managed in paediatric haematology units in most countries and they are managed typically with paediatric ALL protocols. The recent results of paediatric ALL protocols improve remarkably with 5-year overall survival of 90% in younger children and adolescents also have survival in the range of 70–80% [2]. However, adults were treated with less intensive chemotherapy protocols, the long term survival outcome was inferior at around 40%, and allogeneic haematopoietic stem cell transplantation (HSCT) has been included as standard treatment in many centres.

In recent years, paediatric-inspired chemotherapy approach has been studied in young adults and the results are encouraging. The treatment outcome of young adults is similar to that of adolescents treated under paediatric centres, and HSCT may not be required as first line treatment. There are, however, more complications encountered in AYA when managed with more intensive chemotherapy. With better experience in managing the complications, AYA patients can be managed successfully with paediatric-inspired chemotherapy protocols.

20.2 Genetic Subtypes of ALL in AYA

Genetic mutations in patients with ALL carry prognostic significance. The management of AYA with ALL follows the genetic-guided approach on stratification of treatment and

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possible target therapy. The good prognostic genetic markers in children, *ETV6-RUNX1* and high hyperdiploidy (51–65 chromosomes), constituted about 50% of genetic changes in children, but are much less common in AYA with only around 15%. But the Philadelphia chromosome (*BCR-ABL1)*-positive and Ph-like ALL are getting more prevalent in AYA, and up to 15–30% [3]. The version 1.2022 of National Comprehensive Cancer Network (NCCN) Clinical Practice guideline for ALL recommends comprehensive testing by next-generation sequencing (NGS) for other gene fusions and pathogenic mutations associated with Ph-like ALL [4]. The high-risk genetic subtype of *KMT2A*-rearranged is also more common in AYA at 5–10%. Intrachromosomal amplification of chromosome 21 (iAMP21) is associated with a higher relapse rate and the incidence of this abnormality was also higher in AYA and was 12% [5]. Thus, most of the AYA with ALL will be stratified in the intermediate- or high-risk groups according to childhood ALL stratification criteria. AYA with ALL will be treated with more intensive chemotherapy which usually includes intensified asparaginase treatment and high-dose methotrexate. Tyrosine kinase inhibitors are effective in achieving haematological and molecular remission when used in combination with standard induction chemotherapy. Continuous TKI with chemotherapy may achieve long-term disease remission and some AYA with ALL may not require allogenenic HSCT as frontline treatment. Ph-like ALL with ABL fusions are studied with TKI combined with standard chemotherapy recently. The treatment results are still preliminary and need longer follow-up to confirm the efficacy.

20.3 Immunophenotyping of ALL in AYA

ALL is classified into precursor B-ALL, mature B-ALL, and T-ALL according to immunophenotyping. In children, B-precursor ALL constitutes 85–90%, while T-ALL only 10–15%, and mature B-ALL is less than 2%. In adults, T-ALL is more prevalent and is up to 25%, and mature B-ALL constitutes about 5% ALL. Early T-cell precursor ALL (ETP-ALL) is a distinct subtype of T-cell lineage ALL and is more common in AYA. A study showed that 17% of T-ALL in AYA was ETP-ALL and associated with lower complete remission (CR) rate as compared with non-ETP-ALL patients (73% vs 91%; $P = 0.03$) [6]. The median overall survival was also significantly inferior in patients with ETP-ALL.

20.4 Risk Stratification in ALL

Paediatric ALL is stratified into different risk groups based on the biological and genetic factors. Minimal residual disease (MRD) is shown to be a strong prognostic marker after initial chemotherapy. The NCI criteria are commonly adopted in many protocols; age 1 to less than 10 years with presenting WBC $<50 \times 10^9$/L in B-lineage ALL is classified as standard risk ALL. Whereas B-lineage ALL with age <1 year of age or WBC $>50 \times 10^9$/L or T-cell ALL are classified as high risk. Failure to achieve remission after induction chemotherapy has high risk of relapse even after subsequent remission achieved with salvage therapy. MRD by flow cytometry or quantitative PCR methods is performed in many centres for monitoring of treatment response. Positive MRD of $>10^{-3}$ to 10^{-4} after induction or early intensification is associated with high risk of relapse and intensive therapy including allogeneic HSCT is indicated. *ETV6-RUNX1* and high hyperdiploidy are favourable genetic markers and are included as criteria for standard risk group, while *BCR-ABL1* is an unfavourable genetic marker which requires target therapy. For AYA managed at adult oncology centres, there is different criteria for stratification; B-lineage with WBC $>30 \times 10^9$/L and T-lineage with WBC $>100 \times 10^9$/L are considered as high-risk features in adult with ALL. However, the recent studies would put more emphasis on genetic factors and MRD response for stratification. Recent studies demonstrated NGS MRD may be more accurate in predicting relapse [7].

20.5 Reports of Traditional Adult ALL Protocols and Paediatric-Inspired ALL Protocols

Hyper-CVAD developed at MD Anderson Cancer Center for ALL has been widely adopted for treatment of AYA with ALL for the past two decades in many adult centres [8]. The induction and consolidation therapy consisted of combination chemotherapy of high-dose cyclophosphamide, vincristine, doxorubicin and dexamethasone, and alternating with high-dose cytarabine and high-dose methotrexate for total of 8 courses. Maintenance chemotherapy is then continued as daily mercaptopurine, weekly oral methotrexate, and monthly pulses of vincristine and steroid up to 2.5 years from diagnosis. Central nervous system (CNS)-directed treatment included intrathecal chemotherapy or cranial irradiation prophylaxis. Complete remission rate was 92% and death during remission induction was 5%. With a median follow-up of 63 months, the 5-year survival rate was 38%. There are some variations of treatment protocols based on Hyper-CVAD; some centres add on rituximab but asparaginase is seldom included [9]. Overall, the adult protocols based on Hyper-CVAD have much higher doses of several agents: cyclophosphamide 3 times higher, cytarabine 40 times higher, and methotrexate 2 times higher as compared to paediatric protocols.

In large paediatric multicenter clinical trials, adolescents with ALL are treated with paediatric protocols as in younger

Table 20.1 Treatment outcomes of AYA with chemotherapy protocols

Protocols	Patients	Survival outcome	Death in induction or remission
Adult studies			
1. Hyper-CVAD	204 (MDACC)	5-year OS 39%	Induction death 6%
2. Modified Hyper-CVAD and rituximab	280 patients (MDACC)	3-year OS 50% 3-year OS in rituximab arm 75%	Death in CR 8–17%
Paediatric studies			
3. AIEOP-BFM ALL 2000 Study	1094 (10–17 years)	5-year OS 83.4% 5-year EFS 74.6%	Induction death 2.2% Remission death 4%
4. COG AALL0232 Study	3154 patients (16–30 years)	5-year OS 77.4% 5-year EFS 65.4%	Remission death 5.7%
Paediatric-inspired studies			
5. CALGB 10403	295 patients (17–39 years)	3-year OS 73% 3-year EFS 59%	Treatment-related death 3%
6. UKALL 2003 study	229 patients (16–24 years)	5-year OS 76.4% 5-year EFS 72.3%	Death in remission 6.1%

children. BFM group and Children's Oncology Group reported better survival outcome in adolescents with overall survival up to 70–80% [10, 11]. Some adult studies also adopted the paediatric-inspired protocols with some modification. CALGB 10403 protocol included chemotherapy agents with doses and schedule identical to COG AALL0232. The conclusion of the study confirmed that the use of the paediatric regimen was safe; the treatment-related mortality was only 3%. Most remarkable finding is the much improved survival outcome, the 3-year EFS was 59% and the 3-year OS was 73% [12].

Paediatric ALL protocols have been extending to young adults in some study groups [13]. The Nordic Society of Pediatric Hematology and Oncology (NOPHO) ALL2008 protocol recruited newly diagnosed patients aged 1–45 years with Philadelphia chromosome negative B-precursor or T-lineage ALL [5]. Overall, 1288 patients were of age 1–17 years and 221 patients were 18–45 years. Patients were treated with same protocol with the exception of adult patients having the option of HSCT in those with translocation t(4;11)[*KMT2A/AFF1*]. Adult patients had higher incidence of T-ALL at 32% and also t(4;11) at 6.5%. Thus, only 20% of adults were stratified to standard risk arm and 31% were stratified as high risk arm. There was no difference in the risk of induction death, 3 adults and 13 children. When the outcome of EFS of standard-risk and intermediate-risk groups was compared among different age groups, adults did significantly worse than patients aged 1–9 years of age, but did not differ significantly from patients aged 10–17 years. For the high-risk group, there was no significant difference in 5-year EFS observed between the three age groups. The 5-year EFS and overall survival for adults were 74 ± 4% and 78 ± 3%, respectively. The toxicity profiles were different among the age groups; there were more thrombosis, pancreatitis, and osteonecrosis for patients 10 years and older, while allergic reaction to asparaginase was more common in children <10 years. In a Dana Faber study that included ALL patients aged 18–50 years, the patients were treated with the paediatric protocol and the 4 years EFS was 62% for Ph-negative patients [14]. Table 20.1 compares the outcomes of adult protocol and paediatric-inspired protocol.

20.6 Ph-Positive ALL

Ph-positive ALL is more common in AYA as compared to younger children, up to 25%. The prognosis of this type of ALL was poor in the past and allogeneic HSCT was considered as standard of care for AYA with Ph-positive ALL. In the pre-target therapy era, the 7-year EFS and OS was 32% and 45%, respectively, and there was no difference in outcome for those received matched-related donor or unrelated donor HSCT [15]. With the introduction of target therapy with tyrosine kinase inhibitors (TKI), Ph-positive ALL was studied in multicenter trials for the efficacy of combination of TKI and chemotherapy. The COG AALL-0031 study and EsPhALL study conducted phase II and phase III randomized studies using imatinib as the standard TKI. Imatinib given as continuous administration over 2 years was tolerable, and the 5-year EFS and OS was improved to 57% and 71.8%, respectively [16, 17]. Dasatinib combined with chemotherapy was studied in a COG phase II trial, AALL-0622, and the 5-year EFS and OS was similar to the imatinib-based study [18]. More recently, a Chinese Children Cancer Group (CCCG ALL 2015) conducted a randomized study comparing dasatinib 80 mg/m^2 versus imatinib 300 mg/m^2 with the same chemotherapy backbone. The dasatinib arm showed better 4-year EFS and OS as compared with imatinib, 71.0% versus 48.9% and 88.4% versus 69.2% (*P* 0.04), respectively [19]. Allogeneic HSCT was reserved for high-risk patients who had high MRD after induction and consolidation. The number of patients required HSCT at first remission reduced to 40% and mainly in the high MRD group. There was a higher remission death rate in the EsPhALL study at 16%, mainly due to infection and followed by complications of HSCT. The remission rate and MRD negativity rates with TKIs combined with chemotherapy are higher than the historical studies with chemotherapy only. Patients who achieved MRD negativity may be cured with chemotherapy and continuous TKI.

In the adult studies, TKI was combined with standard adult ALL chemotherapy protocols. A phase II study at MDACC evaluated combination of dasatinib with hyper-CVAD regimen. Among the 35 patients treated, 4 received HSCT at first complete remission. The 2-year EFS and OS were 57% and 64%, respectively [20]. Ponatinib combined with chemotherapy has also been tried in other studies. TKIs including imatinib or dasatinib or nilotinib had been studied with other chemotherapy regimen in Europe and Asia. Other than allogeneic HSCT, autologous HSCT had also been applied in some patients after achieving good MRD response with TKI and chemotherapy. The results of autologous HSCT and allogeneic HSCT appeared to be similar [21]. Use of TKIs in Ph-positive ALL achieved good response, but some TKI such as imatinib does not enter into CNS effectively. The CNS-directed treatment should be adequately provided to achieve complete response. The value of adding TKI after HSCT has also been tested in some studies. TKI will be started at 1–2 months after transplant with engraftment, and the duration is at least one year after HSCT. The value of TKI post-HSCT is still under investigation.

20.7 Relapsed ALL in AYA

In children, about 15–20% of patients with ALL relapsed after frontline chemotherapy. The prognosis of patients with relapse depends on the time of relapse, site of relapse, immunophenotyping, and genetic subtypes. Very early relapse (<18 months from diagnosis) and early relapse (18–36 months from diagnosis) are associated with poorer prognosis, in particular those with bone marrow relapse. The second remission rate is only around 60–70% in this group of high risk relapse and the long-term survival without HSCT is less than 10%. Allogeneic HSCT is recommended for patients with early and very early bone marrow relapse. For late bone marrow relapse, a subgroup of patients with good MRD response after re-induction have relatively good outcome with further chemotherapy. Patients had 3-year EFS and OS of 84.9 ± 4.0% and 93.8 ± 2.7% without HSCT when MRD at end of re-induction was <0.1% [22]. Late extra-medullary relapse is also having better outcome after systemic chemotherapy and local therapy with 5-year survival of 78%. T-cell ALL with bone marrow relapse is having dismal outcome with chemotherapy alone; allogeneic HSCT should be arranged early once CR2 is achieved.

For adults with relapse of ALL, the outcome is rather poor and the median OS after relapse was around 4.5–6 months, and the 5-year OS was just 7–10% [23]. Only around 20–30% of patients after relapse could achieve second remission with re-induction chemotherapy. Younger age and first CR duration more than 2 years had better outcome, but the 5-years OS was only 38% [24]. HSCT is the standard treatment for adults with relapsed ALL and survival outcome is superior to those treated with chemotherapy. MRD negativity before HSCT improved the survival outcome significantly; one study reported the 3-year EFS and OS of 62% and 69%, respectively [25].

20.8 New Treatment

In recent years, there are new treatments introduced for patients with ALL including target therapy and immunotherapy. The new treatment may improve the remission rate and MRD negativity rate, thus leading to better long-term survival or successfully bridging to HSCT. The safety profiles in general are acceptable and the side effects are manageable.

Blinatumomab is a bispecific T-cell engager (BiTE) antibody which links up the CD19 receptor on leukaemia cell surface and the CD3 receptor of the normal T cells. The early results of blinatumomab as single agent in relapsed/refractory ALL showed remission rate of 32%. Subsequent randomized studies of blinatumomab with consolidation chemotherapy versus chemotherapy alone in first relapse ALL showed improved MRD remission rate (90% vs 54%), and more patients could have HSCT (88.9% vs 70.4%). The 2-year overall survival was 71.3% for the blinatumomab group versus 58.4% for the chemotherapy group, and the serious adverse effects were also lower in Blinatumomab group [26]. Another immunotherapy was also introduced for relapsed or refractory ALL, inotuzomab ozomagacin (InO), which also showed promising results. In the phase II study, InO could achieve CR of 59%, and the MRD negativity rate was 82% among the responders [27]. The median Relapse-free survival (RFS) and OS rate were 8 and 11 months, respectively. One of the major complications of InO was sinusoidal obstructive syndrome (SOS) or veno-occlusive disease of liver which usually does not happen during InO treatment, but often after HSCT. The incidence of SOS may be reduced with lower dose of InO in each cycle, and also combined with Blinatumomab [28]. The interval period between InO and HSCT is not well defined in relation to the risk of SOS.

T-cell ALL did not have target immunotherapy available yet, only case reports of daratumomab (anti-CD38) showed encouraging results. Nelarabine is a nucleoside analog and had been studied in children and adolescents with relapsed or refractory T-ALL with a response rate of 55%. Similar study was conducted in adult patients and the response rate was around 41% [29]. Nelarabine is approved for T-ALL with relapse or refractory disease. The major toxicity is neurological events, both central and peripheral neuropathy.

Combination of nelarabine with cyclophosphamide and etoposide had been shown to be an effective regimen to achieve remission in refractory disease.

Chimeric antigen receptor T cell (CAR T) therapy is the most promising treatment for refractory or multiple relapsed B-lineage ALL. In the recent decade, many clinical trials using lentiviral vectors of different constructs and costimulatory proteins for transduction of autologous T cells had been conducted for relapsed or refractory ALL. The ELIANA trial based on anti-CD19 CAR T cell therapy demonstrated 81% remission rate in children and young adults. The 5-year EFS of tisagenlecleucel therapy was recently updated to be 42% [30]. Tisagenlecleucel (Kymriah) was approved by FDA in August 2017 for refractory or multiple relapsed ALL in children and young adults up to 25 years, the first genetic manipulated medicinal product approved. Another anti-CD19 CAR T product, brexucabtagene autoleucel, was conducted in adults with B-ALL in the ZUMA-3 study. The complete remission or complete remission with incomplete haematological recovery was observed in 71%. Among the responders, the MRD negativity rate was 97%. The median duration of remission was 12·8 months and median overall survival was 18·2 months. The product (Tecartus) also obtained FDA approval for adults with relapsed or refractory B-ALL in Oct 2021 [31]. Both CAR T products may be associated with complications including severe cytokine release syndrome and immune effector cell-associated neurotoxicity syndrome (ICANS), and other infectious risk. After acquiring more clinical experience with CAR T products in treatment of leukaemia and lymphoma, most patients who received CAR T products can be managed successfully with supportive treatment, or tocilizumab and sometimes steroid treatment. Consensus statement on management of complications of CAR T therapy was also released.

20.9 Management Issues in AYA Patients Receiving Paediatric-Inspired Protocols

The paediatric-inspired chemotherapy protocol is more intensive than the adult protocols. High cumulative dose of corticosteroid may be associated with more side effects in AYA, including osteonecrosis in adolescent, steroid-induced psychosis, hypertension, hyperglycaemia, and infections. The higher cumulative dose of vincristine can lead to troublesome neuropathy in some patients. Asparaginase is associated with more toxicities in AYA, including coagulopathy and thrombosis, hypertriglyceridemia, and pancreatitis. Liver impairment is also more common; patients may require dose reduction or temporary suspension of asparaginase for the hyperbilrubinaemia or raised transaminase. The recommended pediatric dosage of Peg-Asparaginase is 2500 IU/m^2, but older AYAs may need to reduce to 1500–2000 IU/m^2 or less. Sinusoidal obstructive syndrome may now be managed with supportive treatment or defibrotide. Anaphylactic reaction to asparaginase is however less commonly seen in adults. Asparaginase level monitoring is more commonly adopted as this can detect the silent inactivation in some patients; switching from E Coli preparation to Erwinia preparation will maintain the anti-leukaemic effect. Drug toxicity should be monitored closely and provide supportive treatment as indicated. Drug adherence for the rather complicated chemotherapy protocol can be challenging in adolescents and young adults. Patients may not be able to adhere to the time schedule during consolidation or intensification phases with long gaps, and the drug compliance of maintenance treatment of oral mercaptopurine and methotrexate may be low. Education and supervision of medication utilization is important to keep the compliance. Previous studies showed that patients managed under a clinical trial may have better outcome, but the recruitment rate of AYA into clinical trials is much lower than those of children.

In summary, AYA patients with newly diagnosed ALL are recommended to be managed under a clinical trial. In case of absence of clinical trial for the patients, the paediatric-inspired regimens should be considered such as COG AALL0232, CALGB 10403. After achieving remission with the multi-agent induction, patients should be monitored with MRD for subsequent stratification of treatment. For non-high-risk patients with MRD negative remission, they can be managed with consolidation and intensification treatment followed by maintenance treatment for up to 2–2.5 years. Allogeneic HSCT may not be required for the good responders. However, patients with high-risk features such as slow MRD clearance and unfavourable genetic markers may be considered for allogeneic HSCT while in first CR. For Ph-positive ALL, TKI should be incorporated with the chemotherapy protocols. For those with MRD-negative remission after chemotherapy and TKI treatment, they may be closely monitored with serial MRD without HSCT. Patients who developed relapse after achieving first remission may be stratified according to time of relapse and MRD response after initial induction treatment. Most early relapses involving bone marrow require allogeneic HSCT and the best results would be MRD-negative before HSCT. New agents such as Blinatumomab may bring the patients in MRD remission before bridging to HSCT. CAR T therapy is now available to those with multiple relapses or refractory disease, but the long-term disease free is below 50%. Innovative approach is required to further improve the outcome of AYA with ALL.

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Management of Older Patients with Acute Lymphoblastic Leukemia

21

Xiao-Xia Hu and Hong-Hu Zhu

Abstract

Although substantial progress is achieved in ALL total therapy in adolescent young adults and adults, marginal improvements in survival over time in older patients present a great challenge. Older patients often carry high-risk genetic lesions that confer resistance to conventional chemotherapy, and a higher incidences of comorbidities and impaired performance status limit their tolerance to receive the same intensity as younger patients, such as hematopoietic stem cell transplantation. In this chapter, we review the current treatment of older ALL patients with good performance.

Keywords

Acute lymphoblastic leukemia · Older patients · Targeted therapies · Tyrosine kinase inhibitors

21.1 Introduction

Approximately 21.5% of adult acute lymphoblastic leukemia (ALL) occur in patients aged over 55 years, and 17% in those older than 60 years [1]. Although substantial progress is achieved in ALL total therapy in adolescent young adults and adults, marginal improvements in survival over time in older patients are demonstrated by Dutch registry and SEER database of U.S [2, 3]. ALL in older patients presents a great challenge in the real world, as well as clinical trials, with low rates of continuous complete remission (CR), and inferior long-term survivals vary from 5% to 20% [2, 4–8]. Older patients often carry high-risk genetic lesions that confer resistance to conventional chemotherapy, and a higher incidences of comorbidities and impaired performance status limit their tolerance to receive the same intensity as younger patients, such as hematopoietic stem cell transplantation (HSCT).

In this chapter, we discuss the treatment of older ALL patients with good performance. The definition of an older patient varies but most commonly refers to ages ≥60 years or 65 years.

21.2 Philadelphia Chromosome (Ph) Negative ALL

Improvements in outcomes in younger adults have largely been attributed to the widespread adoption of pediatric-inspired protocols in Ph-negative patients. However, the tolerance of pediatric-inspired protocols is usually poor in older patients [9, 10]. The backbone of chemotherapy is similar for childhood, adult, and older patients, comprising with corticosteroids, vincristine, and anthracyclines. The CR rates for older ALL are around 80% with different combinations [11, 12]. Achieving high quality of CR is the prerequisite for long-term survival. However, application of standard chemotherapy regimens in older patients with ALL results in excessive toxicity and induction mortality. Certain drugs, such as asparaginase and vincristine, are avoided or dose reduced in older patients [13]. Early death occurred in 35% older patients in the first 60 days, and the mortality of patients who did not achieve CR reached 60% in the following month. Collectively, 51% of ALL-related deaths occur in patients ≥55 years old [1]. The quality of CR and the risk of intensive therapy should be cautiously balanced. In older patients, a higher cumulative incidence of chemotherapy-related death (23%) and early death in first CR (22%) offset the benefits of

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the lower relapse rate brought by the intensified therapy and result in a dismal long-term outcome.

Several trials, including modified DFCI [14], GRAALL-SA1 [12], and PETHEMA ALL-96 [15], produced unsatisfactory results in older patients [13]. The optimal therapy for older patients is still exploring. Application of modified pediatric-inspired ALL regimens in older adults has been attempted. Reduced-dose PEG-ASP (500 IU/m^2) was successfully introduced in patients ≤75 years old [14, 16]. The Spanish PETHEMA group compared outcomes of older adults (55–65 years old) with ALL treated either on intensive pediatric-inspired protocols (ALL-HR03 and ALL-HR11) or on a less intensive adult protocol (ALL-OLD07). Their analysis showed superiority of intensive regimens with regard to CR rate, OS, and event-free survival, with comparable induction mortality rates [16]. Regimens specifically tailored for older adults by dose adjust and combination of different drugs would be preferred.

21.3 Ph-positive ALL

Ph-positive ALL represents the largest subset of ALL in older adults. The highest incidence of 47% was found in patients 50–59 years and did not increase further with age [17]. The advent of tyrosine kinase inhibitors (TKIs) has revolutionized the treatment modalities and prognosis of older patients with Ph-positive ALL. The use of first-generation (imatinib), second-generation (dasatinib, nilotinib), and the third-generation (ponatinib) TKIs, in combination with chemotherapy, has clearly improved patient outcomes, allowing to decrease the intensity of chemotherapy and lessen toxicity [11, 18–22].

The concept of "Chemo-free modality" is sprout out first for older patients with Ph-positive ALL in the last years. Imatinib plus steroid as induction therapy and imatinib as consolidation therapy in patients >60 years were evaluated by Gruppo Italiano Malattie Ematologiche dell'Adulto (GIMEMA). The CR rate was 100% and no early death occurred. However, fourteen patients relapsed after a median time of 4 months (range, 3–28 months) and a median survival was 20 months [23]. Similar results with dasatinib were reported by European Working Group on Adult ALL (EWALL) and GIMEMA ALL Study (GIMEMA LAL 1509) [19]. Although TKIs plus with low-intensity chemotherapy could yield high CR rates, the challenge is the durability of therapeutic responses. Most recently, GIMEMA treated patients with a median age of 54 years (range, 24–82 years) with dasatinib plus glucocorticoid as induction, and followed by two cycles of blinatumomab. The CR rate was 98%. After a median follow-up of 18 months, OS was 95% and DFS was 88% [24]. The addition of novel agents would turn Ph-positive ALL into the second curable acute leukemia in the future.

21.4 Allogeneic Transplantation

Allogenic HSCT for older patients remains challenging at least in part due to graft-versus-host disease (GVHD) and higher non-relapse mortality (NRM) owing to myeloablative conditioning regimens. In recent years, with the wider use of reduced intensity conditioning (RIC) regimens, and increased application of posttransplant cyclophosphamide (PTCy) as GVHD prophylaxis, patients older than 55 years constituted 5% of all adults treated with allogenic HSCT between 2001 and 2003 that increased to 18% between 2013 and 2015 [25, 26]. RIC regimen combining fludarabine, busulfan, and melphalan has demonstrated a potential strategy to decrease NRM in older and/or unfit individuals [27]. Lower doses of melphalan (100 mg/m^2) appeared to be better tolerated in older individuals. Higher NRM in older patients was also in part due to higher incidence of grade III-IV GVHD. Hence, control of GVHD remains even a greater priority for older individuals. PTCy-based GVHD prophylaxis has been shown to induce tolerance by eliminating rapidly proliferating alloreactive T-cells with proven efficacy in haploidentical HSCT. Several efforts were made to further control GVHD with the backbone of PTCy. The addition of cyclosporine A, methotrexate, mycophenolate mofetil, and tacrolimus would enhance its effect and further reduce the risk of sever GVHD [28, 29]. Use of PTCy and antithymocyte globulin (ATG) is reported to significantly reduce NRM, decrease risk of acute and chronic GVHD, and have no increase in relapse risk in older patients [30, 31].

21.5 Targeted Therapies

The advent of monoclonal antibodies that target cell surface antigens, such as CD20, CD22, CD3-CD19 bispecific antibodies, and chimeric antigen receptor (CAR) T-cell therapy, has dramatically changed the treatment paradigm in ALL without age limitation. About 80% ALL is B-cell origin and express CD20, CD22, and CD19 antigens. Recently, immunotherapy has become the mainstream of ALL treatment. Blinatumomab is an anti-CD19-directed CD3 bispecific T-cell engager. In relapsed/refractory ALL setting, blinatumomab was shown to be effective in CR induction and minimal residual disease (MRD) clearance. In frontline setting, blinatumomab combined with hyper-CVAD in newly diagnosed B-ALL patients is evaluated [32, 33]. The combination of blinatumomab with TKI is safe and effective in patients with relapsed/refractory Ph-positive disease. The CR rate was 50%, and with a median follow-up of 8 months, the median survival was not reached, and 1-year OS rate was 73% [34]. Blinatumomab in combination with TKI is being studied as frontline therapy for newly diagnosed B-ALL with Ph chromosome.

Ongoing studies are also investigating the combination of immune checkpoint blockade with blinatumomab in an effort to enhance the T-cell and hence augment the activity of blinatumomab [35]. Inotuzumab ozogamicin is a humanized anti-CD22 antibody conjugated to calicheamicin. Kantarjian et al. [36] first reported inotuzumab ozogamicin combined with mini-Hyper-CVAD, resulting in better outcomes in patients with relapsed or refractory ALL than does standard therapy. In 48 newly diagnosed older patients, 98% of them reached CR with a median follow-up of 29 months (range, 13–48 months) and 59% for progression-free survival (PFS). It would be promising that Inotuzumab with low-intensity chemotherapy (mini-Hyper-CVAD) ± blinatumomab will benefit older patients [36].

Despite the relatively impressive response rates with immunotherapy strategies, including among older patients, responses appear short-lived and most patients will relapse. To date, CD-19-directed T cells, modified with a chimeric antigen receptor (CAR), have been among the most extensively studied and promising technologies for CD19 expressing B-ALL patients [37]. Many of the promising CAR T trials have treated younger patients with ALL, but data in older adults have been equally robust with an OS of around 70%. Unlike traditional chemotherapy, age has not been shown to be a major prognostic delineator for response to CAR T therapy. Cytokine release syndrome and neurological toxicity are needed to be better managed, especially among more vulnerable, elderly ALL patients.

21.6 Closing Remarks

Despite the remaining challenges, options for the therapy of older ALL patients are rapidly increasing. More rigorous clinical studies and novel combinations are needed to establish their utility and safety in this high-risk population.Conflict of Interest StatementThe authors declare no conflicts of interests.

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Management of Philadelphia Chromosome-positive Acute Lymphoblastic Leukaemia

22

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Abstract

The distinct Philadelphia chromosome-positive (Ph+) subtype of acute lymphoblastic leukaemia (ALL) has historically been difficult to manage and has resulted in poor long-term survival in both paediatric and adult populations. However, drastic improvements have occurred with the addition of BCR-ABL1 tyrosine kinase inhibitors (TKIs) such as imatinib, dasatinib, and ponatinib to traditional cytotoxic chemotherapy regimens. Diagnostic advances including sensitive minimal residual disease (MRD) measurement by PCR and multi-parameter flow cytometry methods as well as BCR-ABL1 mutation analysis have enabled more accurate assessment of disease response allowing the type and intensity of treatments to be tailored accordingly. This chapter covers the diagnosis and management of Ph+ ALL in both children and adults, including discussion of TKIs, their combination with chemotherapy, and the role of haematopoeitic stem cell transplantation in this disease.

Keywords

Acute Lymphoblastic Leukemia · ALL · Philadelphia Chromosome · Philadelphia Chromosome-positive · Ph+ Tyrosine kinase inhibitor · TKI · Imatinib · Dasatinib · Ponatinib · BCR-ABL · BCR-ABL1

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22.1 Introduction

22.1.1 Definition, Background, and Incidence

Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukaemia (ALL) is a distinct subtype of B-ALL. Its incidence increases with age, comprising 3–5% of paediatric ALL cases and up to 50% in adults aged over 50 years [1–4]. Historically, this ALL subtype has been associated with a poor prognosis compared to Philadelphia chromosome negative (Ph−) disease [5, 6]. However, the availability of the BCR-ABL1 tyrosine kinase inhibitors (TKIs) such as imatinib, dasatinib, and ponatinib has resulted in significant improvements in Ph+ ALL outcomes [7, 8], with long-term overall survival (OS) increasing from ~20% in the pre-TKI era to ~40–50% with contemporary treatments [9]. Ph+ ALL outcomes are predicted to further improve with the development of new-generation TKIs and the utilisation of ALL-directed monoclonal antibody therapies.

22.1.2 Molecular Biology

The Philadelphia chromosome (Ph) is derived from a reciprocal translocation between the *ABL1* gene on the long arm of chromosome 9 and the *BCR (*breakpoint cluster region) gene on the long arm of chromosome 22, denoted as t(9;22)(q34;q11) on cytogenetic reports [10]. The resultant fusion gene encodes for the oncogenic constitutively active BCR-

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ABL1 tyrosine kinase [11]. There are three isoforms of the *BCR-ABL1* oncogene: p190, p210, and p230 (with the number designating molecular weights of the respective products), as a result of fusion between exon 2 of *ABL1* and exons 1, 13/14 or 19 on *BCR*, respectively [12]. Approximately 70% of Ph+ ALL cases contain the p190 isoform while 95% of chronic myeloid leukaemia (CML) cases demonstrate p210, with the p230 variant being rare [13].

The abnormal constitutively active BCR-ABL1 contributes to leukaemogenesis via multiple molecular interactions in the cytoplasm which activate downstream signalling cascades. The result is increased phosphorylation and activation of numerous proteins, of which the most important are signal transducers and activators of transcription factors (STAT), RAS, phosphatidylinositol 3-kinase (PI3K), and focal adhesion kinase (FAK). This, in turn, causes increased cell proliferation, disruption of cell differentiation, and interference of cell adhesion leading to the leukaemic clinical presentation [14, 15].

Although *BCR-ABL1* is central to the pathogenesis of Ph+ ALL, a number of cooperating lesions have been identified [16]. Most variants affect transcription factors such as deletions of IKAROS family zinc finger 1 (*IKZF1*) gene and alterations in paired box 5 (*PAX5*) genes, as well as deletions in cyclin-dependant kinase inhibitor 2A/B (CDKN2A/B) [17, 18]. Less common mutations occurring in Ph+ ALL include those involving B-cell translocation gene 1 (*BTG1*), retinoblastoma protein 1 (*RB1*), early B-cell factor 1 (*EBF1*), and translocation-ets-leukemia virus (*ETV6*) [18]. These mutations, along with mutations in the ABL1 kinase domain, may contribute to therapy resistance and suggest that alterations in B-cell-specific transcription factors may be critical second hits in the pathogenesis of Ph+ ALL [19, 20].

With regard to immunophenotype, virtually all Ph+ ALL express one or more B-cell lineage defining marker, though additional aberrant expressions of the CD13 and CD33 myeloid antigens are common, with variable expression of CD20 [21]. This is an important consideration in the context of targeted antibody treatments such as blinatumomab, inotuzumab ozogamicin, and rituximab which require expression of CD19, CD22, and CD20, respectively.

22.1.3 Chronic Myeloid Leukaemia with Lymphoid Blast Crisis

Progression of CML to the blast crisis phase is now rare due to the efficacy of BCR-ABL1 TKIs in the treatment of this disease. However, myeloid or lymphoid blast crisis can occur, the latter of which has similar presentation to Ph+ ALL. The two may be indistinguishable except in patients with a clear antecedent history of chronic phase CML, though concomitant splenomegaly and granulocytosis may hint at the origins of the disease. Clinical management is similar.

22.2 Diagnosis, Monitoring, and Minimal Residual Disease

22.2.1 Diagnosis

The initial approach to diagnosis of Ph+ ALL is the same as for any suspected acute leukemia, including urgent morphological examination of bone marrow aspirate and trephine, immunophenotyping via flow cytometry, and cytogenetic studies.

As soon as the diagnosis of ALL is confirmed, efficient identification of the disease as either Ph+ or Ph- is vital, as this will have a significant impact on the initial therapy. This can be done using either cytogenetic or molecular methods. The former includes formal karyotype analysis as well as fluorescence in situ hybridisation (FISH). With FISH, fluorescent molecular probes (a complementary sequence of the DNA to the genes of interest, in this case, *BCR* and *ABL1*), each with a different colour fluorescent marker (typically red for one, green for the other), is applied to the sample. Each of these probes will then anneal to the complementary genomic sequence. Juxtaposition of the red signal to the green signal signifies an abnormal fusion. This technique is rapid and may yield a result overnight.

A full karyotypic examination takes longer to analyse than FISH, but may reveal additional cytogenetic abnormalities in addition to the Philadelphia chromosome, such as hyperdiploidy, monosomy 7, monosomy or trisomy 8, and derivatives of chromosomes 9 or 22. The significance of these additional cytogenetic abnormalities is not yet fully clear and may be therapy-dependent. While some studies suggested that additional cytogenetic abnormalities were associated with a worse prognosis, this finding has not been consistent [22–25].

Detection of *BCR-ABL1* transcripts via quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) has been a significant advance in the therapy of both Ph+ ALL and CML [26]. An inclusive method may be necessary to determine the type of fusion transcript (e1a2 vs e13/e14a2 vs other atypical transcripts) at diagnosis, after which the specific quantitative assay may be applied for the rest of the patient's management. This method allows highly sensitive quantification of the burden of disease at a given time point and is used to monitor response to treatment, serving as a valuable marker of minimal (or measurable) residual disease (MRD) [27]. Persistent MRD may guide the need for allogeneic stem cell transplantation, as well as indicating resis-

tance to therapy. Given the respective characteristics, all three techniques may have a role depending on local availability and practice.

22.2.2 Monitoring Response and Minimal Residual Disease

Assessment of response to therapy relies on similar techniques to diagnosis. A patient is assessed for morphological and cytogenetic remissions. FISH may also be used to evaluate the remaining proportion of cells bearing the *BCR-ABL1* fusion. These techniques have limited sensitivity of detection and are more suited to assess early treatment milestones, as negative results are not reliable enough to exclude significant residual disease.

MRD, referring to either measurable or minimal residual disease, is the detection and quantification of residual leukemia below the traditional limits of microscopy and its detection is now a standard tool used in the management of ALL. Multiparametric flow cytometry, looking for a leukemia-associated immunophenotype, and allele-specific PCR for *IgH*/*TCR* rearrangements are both accepted methodologies for monitoring MRD. Each method has its own advantages and disadvantages, but either can be applied successfully in >90% of ALL cases [28]. Consensus guidelines exist to optimise assay performance (e.g. EURO-MRD and Euro- Flow), covering technique standardisation and reporting standards to reduce inter-laboratory variability and risk of false negative results [29]. Most paediatric studies of Ph+ ALL continue to utilise these methods to monitor MRD.

The consistent presence of *BCR-ABL1* in Ph+ ALL facilitates a third method of monitoring MRD in this subtype, namely transcript gene-specific monitoring via qRT-PCR. Unlike CML where peripheral blood monitoring of *BCR-ABL1* provides accurate assessment, European consensus guidelines recommend that bone marrow should be used to quantify *BCR-ABL1* in Ph+ ALL. Transcripts may be 10–1000× lower in blood compared with bone marrow, so may underestimate disease burden. A complete molecular response is defined as undetectable *BCR-ABL1,* or *BCR-ABL1* detected below 0.0032%, which is the technical limit of sensitivity of many assays.

For most Ph+ ALL patients, *BCR-ABL1* qRT-PCR results are concordant with the results of the other MRD methodologies described above. However, occasional patients exhibit discordant results, with detectable *BCR-ABL1* and negative *IgH/TCR* [30]. Cell sorting in a small number of patients with such discordant results has demonstrated the presence of *BCR-ABL1* in non-ALL lymphoid cells and myeloid cells. This suggests that a subtype of Ph+ ALL has a more CML-like biology; however, the impact of this on outcome has not yet been determined in large cohorts.

It is important to note that MRD is a time-sensitive variable, and patients should be assessed against optimal time-dependent milestones specific for the treatment given, though in general, timepoints after induction, as well as pre and post SCT, are the most informative. Persistent positivity is associated with inferior outcomes [31].

Approximately 50% of adults who achieve morphological remission will have detectable MRD following induction, and MRD positivity at any time point is associated with inferior OS [32]. In Ph+ ALL, the achievement of a complete molecular response by 3 months is associated with superior OS and event-free survival (EFS), and in some instances, may result in the avoidance of allogeneic haematopoietic stem cell transplant (alloHCT) [33]. MRD-directed therapy has been standard in paediatric protocols for many years, with early intensification strategies used for MRD positivity. The advent of new therapies such as blinatumomab, inotuzumab, or CD19-directed Chimeric Antigen Receptor T-cells (CAR-T) may provide additional targeted strategies to ameliorate MRD positivity and improve long-term survival.

22.3 Therapy for Newly Diagnosed Disease

22.3.1 Tyrosine Kinase Inhibitor Overview

TKIs are a cornerstone in the treatment of Ph+ ALL in patients of all ages. Addition of these agents to traditional chemotherapy regimens has resulted in significant improvements in OS by enabling the achievement of higher rates of first complete remission (CR1), decreasing early relapse, and allowing more patients to progress to alloHCT [34].

As noted above, *BCR-ABL1* mediates its oncogenic effects through phosphorylation of secondary messengers that carry signals downstream promoting tumorigenesis. TKIs bind to the adenosine triphosphate (ATP) binding site on the BCR-ABL1 tyrosine kinase to inhibit secondary messenger phosphorylation. The affinity of this binding, and the particular protein interaction involved, is different for each of the TKIs, resulting in differential activity. This may also be interrupted by any changes in the protein constituents of the kinase domain, leading to therapeutic resistance [35]. Point mutations of the kinase domain are the most common reason for TKI resistance. Mutations can be characterised as *direct contact* where the ATP binding site is altered so the TKI can no longer bind, *P-loop mutations* which decrease the efficiency of TKI binding, and *activation loop mutations* which direct the equilibrium for the kinase toward existing in its active conformation [36, 37]. The differential activity of the TKIs means some agents may remain active despite a mutation conferring resistance to another. The direct contact T315I mutation resulting from isoleucine replacing threonine at position 315 of

BCR-ABL1 represents the most significant cause of therapeutic resistance to both first- and second-generation TKIs [38]. TKI structures, interactions with BCR-ABL1, binding conformations, and important tyrosine kinase domain mutations are shown in Fig. 22.1. As TKIs selectively act against *BCR-ABL1* bearing cells, they have a favourable safety prolife compared to traditional cytotoxics and can be combined with chemotherapy with little additional toxicity. In addition to their specific activity against BCR-ABL1, TKIs have an emerging role in the treatment of Ph-like ALL with *ABL*-class fusions, a new diagnostic grouping that includes cases driven by *ABL*-class fusions other than *BCR-ABL1*. These often involve constitutively active kinases such as ABL1/2, CSF1R, and PDGFRB [39].

All TKIs are administered via the oral route with gastrointestinal side effects such as nausea, vomiting, and diarrhoea being commonly reported. Oedema and skin reactions are other important side effects related to off-target activity against other tyrosine kinases such as C-KIT and platelet-derived growth factor receptor *(PDGFR)*. Metabolism of these agents occurs via cytochrome P450 enzyme CYP3A4, so other drugs causing inhibition or induction of this pathway can lead to significant drug-drug interactions [41].

Imatinib, the first TKI developed, was used originally in CML, but also soon proved to be efficacious in Ph+ ALL. This agent is still commonly used in the frontline setting. Subsequent TKIs developed include the second-generation drugs dasatinib, nilotinib, and bosutinib, of which dasatinib is the most commonly used in Ph+ ALL, either in the frontline setting, or in cases with imatinib resistance. Current clinical trials are investigating the role of the third-generation drug ponatinib upfront, although this drug is usually reserved for cases with resistance to other TKIs.

Imatinib competitively binds to the ATP binding site of BCR-ABL1 and is selective for the inactive conformation of the enzyme [42]. Of all the TKIs, the activity of imatinib is limited by the largest number of kinase domain mutations. Because imatinib is a substrate for P-glycoprotein (PGP), resistance can also occur via drug efflux mechanisms [37].

Dasatinib is a second-generation TKI that demonstrates highly avid binding to the BCR-ABL1 ATP site with 100 times the inhibitive potency of imatinib [36]. This enables

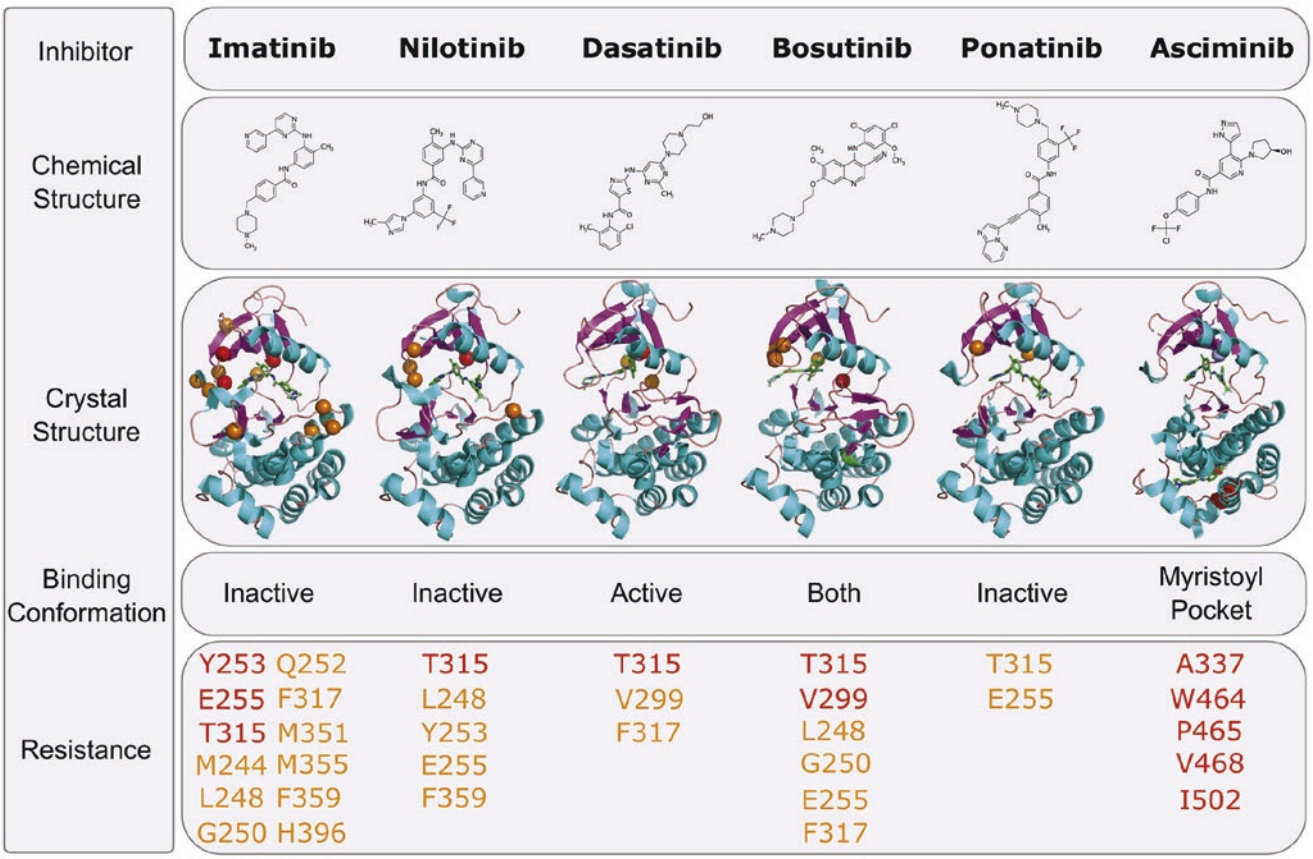


Fig. 22.1 BCR-ABL1 Tyrosine Kinase Inhibitor structures, binding, and resistance characteristics. Chemical structures and published X-ray crystallographic structures of ABL1 complexed with kinase inhibitors are shown. Residues at which mutations are associated with strong resistance to a given TKI are indicated in red, while those associated with lesser degrees of resistance are listed in orange. The structure of ABL1 complexed with asciminib shows nilotinib in the ATP-binding site for reference. T315I is indicated in purple for visual reference. Used with permission from Braun et al. 2020 [40]

the activity to be retained for many *BCR-ABL1* mutations overcoming numerous mechanisms of imatinib resistance [43]. In contrast to imatinib, dasatinib inhibits the active conformation of the enzyme [44] and can overcome resistance to imatinib caused by mutations in the activation loop resulting in equilibrium shift to the active conformation. However, dasatinib is less selective towards BCR-ABL1 and has increased off-target TKI activity against the Src kinase family, BMX, Eph family, VEGFR2, and TIE2 that may lead to a wider range of toxicities. Pleural effusions tend to be the most problematic adverse effect, occurring in 10–20% of patients due to increased pulmonary vascular endothelial permeability [45].

The highly potent third-generation TKI, ponatinib, binds to different residues in the ATP binding pocket compared to earlier generation TKIs. This was designed to overcome resistance mediated by kinase domain mutations, in particular T315I [46], which has historically been associated with poorer outcomes in both CML and Ph+ ALL [47]. Ponatinib binds to the inactive conformation of the enzyme and off-target activity against VEGFR, FGFR, SRC, and PDGFR kinase families are seen [38, 46]. Clinically important cardiac and vascular toxicities occur more frequently with ponatinib than other TKIs and are thought to be due to its wide range of TKI activity and prothrombotic effects [48]. Pancreatitis is also observed more commonly [49].

22.3.1.1 Which Tyrosine Kinase Inhibitor Is Preferred in Frontline Treatment?

Imatinib and dasatinib are currently the two TKIs indicated for the first-line treatment of Ph+ ALL, commonly combined with other traditional cytotoxic chemotherapy agents. Imatinib is more widely available worldwide, and given its longer history, has the larger body of evidence supporting its use [50–53]. In some practices, dasatinib, a second-generation TKI, is preferred because of its higher potency in vitro, and it was suggested that it may penetrate the CSF better than imatinib. There is currently limited data directly comparing the two agents in Ph+ ALL. In CML, where Phase III studies have reported outcomes in patients randomised to imatinib or dasatinib, dasatinib led to significantly faster achievement of molecular responses, as well as lower rates of disease transformation to the blastic phase, although survival and the rates of kinase domain mutation development remain similar. It is difficult to know whether this can be generalised from CML to Ph+ ALL. A recently published paediatric study demonstrated superior overall and relapse-free survival in paediatric Ph+ ALL, without an overall increase in toxicity [54]. It is unclear whether dasatinib is superior to imatinib in adults, where the relapse rate is significantly higher than those seen in children. For instance, the MD Anderson Cancer Center reported that Hyper-CVAD combination chemotherapy + imatinib resulted in a 5-year OS and disease-free survival (DFS) of 43%. Using dasatinib instead of imatinib with the same chemotherapy backbone conferred a 5-year OS and DFS of 46% and 44%, respectively [55, 56].

Aside from efficacy, there are differences in the toxicity profile between the two drugs. The most common side effects of imatinib include fluid retention/oedema, periorbital oedema, nausea, rash, and muscle cramps, whereas pleural effusion is much more commonly encountered with dasatinib [57]. The CML literature suggests that the risk of pleural effusion with dasatinib is dose-dependent, with a potential role for therapeutic drug monitoring (TDM). The risk is highest in the elderly, who are also more likely to have decreased cardiopulmonary reserve to deal with pleural effusions [58]. Interestingly, fractionated dosing increases the risk of pleural effusion, and once daily dosing is preferred [59]. Pericardial effusion and pulmonary hypertension are infrequent complications of dasatinib, which are seen less commonly with imatinib. Thus, in patients with pulmonary comorbidities (e.g. smoking, chronic obstructive pulmonary disease), imatinib is often the preferred first-line option. In other patients, either agent can be selected, with the agent chosen generally guided by local experience and cost.

Current clinical trials are investigating the third-generation TKI, ponatinib, together with chemotherapy in the frontline setting. MD Anderson Cancer Center data suggested that adding ponatinib instead of dasatinib to Hyper-CVAD yielded superior outcomes [60]. Ponatinib has also been explored with promising results in other studies, with or without intensive chemotherapy [61, 62]. A phase III study comparing imatinib against ponatinib with combined abbreviated chemotherapy is currently ongoing (NCT03589326).

22.3.1.2 Tyrosine Kinase Inhibitor Resistance, BCR-ABL1 Mutations, and Mutation Analysis

A significant contributor to relapsed and refractory disease are point mutations within the BCR-ABL1 kinase domain. Nucleotide changes in the DNA sequence that lead to amino acid changes alter the interaction between BCR-ABL1 protein and drug binding. Such alterations are detected in up to 90% of relapsed/refractory cases in some series. This is higher than the rate observed in chronic phase CML, presumably due to greater genomic instability in Ph+ ALL [34, 63–65]. Traditionally, kinase domain mutations are detected through direct (Sanger) sequencing, prompted either by rising *BCR-ABL1* or overt morphological relapse. Direct sequencing has limited sensitivity and can only detect mutations when their clone size exceeds 15%, and only if total *BCR-ABL1* is present in sufficient quality for PCR amplification (usually >0.01%) [66, 67]. There is now increasing adaptation of next-generation sequencing (NGS) methods for this purpose [68]. Greater than 90 kinase domain muta-

tions have been identified; the resistance profiles of the more common ones are known through in vitro sensitivity analysis and clinical data [69]. Imatinib is susceptible to the widest range of kinase domain mutations, while V299L and F317V/L will confer resistance to dasatinib. Of particular importance is the gate keeper mutation T315I, which confers resistance to all TKIs except for ponatinib [38]. This mutation has been associated with disease progression and reduced survival in Ph+ ALL [47]. More than one kinase domain mutation may be seen in combination. They are compound mutations when they exist on the same *BCR-ABL1* allele; this is often difficult to ascertain, though compound mutations involving T315I as a component may confer resistance even against ponatinib [65].

22.3.1.3 Central Nervous System Penetration of Tyrosine Kinase Inhibitors

Involvement of the central nervous system (CNS) in ALL is seen at diagnosis in approximately 6% of patients. However, Ph+ ALL has a higher risk for CNS involvement, estimated at 8–17%. In the absence of effective CNS-directed therapy, CNS relapse occurs in up to 30% of cases and may occur even in those without overt CNS disease at diagnosis. This can be minimised to 5–10% with incorporation of CNS-directed therapy in all patients [70–72]. The inability of many cytotoxic chemotherapy agents to achieve therapeutic concentrations within the cerebrospinal fluid (CSF) makes this a sanctuary site, where established disease can often be very difficult to treat. The administration of systemic cytotoxic chemotherapy agents that achieve adequate concentrations in this compartment combined with intrathecal administration is a vital component of ALL regimens.

Whether TKIs can effectively cross the blood brain barrier is an important consideration for Ph+ ALL treatment. Imatinib demonstrates poor CNS penetration, with CSF concentrations being only 1.5% of corresponding plasma concentrations [72, 73]. This is likely due to its properties of being highly protein-bound and a substrate of the drug efflux pump P-glycoprotein (PGP) [74]. Dasatinib has improved CNS penetration compared to imatinib, with improved activity against CNS leukemia, and may be preferred on this basis [75]. However, concentrations achieved in CSF may still only be approximately 10% of plasma concentrations and the activity of dasatinib against CNS disease has not been shown consistently [76, 77]. While clinical data for ponatinib are scarce including a discouraging case report describing CNS relapse during ponatinib therapy [78], CNS penetration in vitro and in animal studies has been more promising and further investigation is warranted [79]. Based on current data, TKIs may be a useful adjunct, but cannot be relied upon as sole agents for CNS-directed therapy.

22.3.2 Chemotherapy + Tyrosine Kinase Inhibitor Regimens

22.3.2.1 Paediatrics

In the pre-TKI era, paediatric Ph+ ALL had poor outcomes with 7-year OS reported as 36–45% and progression to alloHCT in CR1 universally recommended [80, 81]. Incorporation of TKIs into intensive chemotherapy protocols has yielded significant improvements with doubling of OS rates and alloHCT in CR1 being limited to patients deemed to be high risk. However, the optimal duration of TKI therapy and chemotherapy backbone requires further elucidation [82, 83].

The Children's Oncology Group (COG) AALL0031 trial was the first to incorporate imatinib into an intensive chemotherapy regime and included patients aged 1–21 years [82]. A key question in this study was whether concurrent administration of imatinib would exacerbate chemotherapy-induced toxicity and impair treatment delivery. After standard induction consisting of vincristine, asparaginase, and corticosteroid ± daunorubicin, imatinib was initiated as early as the beginning of the first consolidation at a dose of 340 mg/m^2/day. Five different cohorts were administered progressively dose-dense imatinib regimens, with the highest exposure cohort receiving continuous imatinib therapy from the beginning of consolidation until maintenance where a 2 weeks on - 2 weeks off regimen was adopted. Patients with matched sibling donors proceeded to alloHCT after consolidation chemotherapy. This trial demonstrated significantly improved outcomes when imatinib was used concurrently with chemotherapy and showed that continuous imatinib administration led to the best outcomes [52]. To assess the toxicity of imatinib, the Ph+ group was also compared to a high-risk Ph- ALL group given the same chemotherapy backbone without imatinib. Adverse effects seen at significantly higher rates in the imatinib group were transaminitis in the maintenance cycles, infection with ≥grade 3 neutropenia in reinduction block 2, and lower total white cell count plus hypokalaemia in consolidation block 2. Longer delays in therapy also occurred in the imatinib group in the first blocks of consolidation and reinduction. Nonetheless, imatinib was deemed to be well tolerated overall. Importantly, this study was the first to suggest that adding imatinib to dose-dense chemotherapy may obviate the need for alloHCT in some patients. While the numbers in this specific analysis were limited, 3-year EFS for those undergoing HLA-identical-related alloHCT (56.6%, n = 21) or unrelated-donor alloHCT (71.6%, n = 11) was similar to those treated with chemotherapy and intensive imatinib dosing alone (87.7%, n = 25, P = 0.14) [82]. Longer follow-up confirmed no advantage in the transplanted groups [52].

At a similar time to AALL0031, the Europeans conducted the EsPhALL study. This was an open label randomised trial

for Ph+ ALL patients aged 1–18 comparing the use of imatinib combined with a high-risk Berlin-Frankfurt-Munster (BFM) chemotherapy backbone against high-risk BFM chemotherapy alone. Patients were classified as either good risk or poor risk depending on their response to induction chemotherapy with good-risk patients randomised to receive imatinib + chemotherapy or chemotherapy alone, while all poor-risk patients were non-randomly assigned to imatinib + chemotherapy. Imatinib was administered at a dose of 300 mg/m^2/day from the start of consolidation in an alternating regimen with subsequent high-risk and reinduction blocks for a total of 126 days. Patients were eligible to proceed to alloHCT after high-risk block 3 if they were in CR and had a matched sibling- or unrelated-donor. EFS at 4 years was 75.2% in the good-risk imatinib group, compared to 55.9% in good-risk patients not receiving imatinib, with the difference almost reaching statistical significance (P = 0.06). There were no significant differences in major toxicities between the two cohorts. The total imatinib exposure of 126 days was far less than the 616 days in the COG AALL0031 study and may have led to a lesser therapeutic benefit of the imatinib and a reduced incidence of adverse effects. In the poor-risk group, 4-year EFS was 53.5% which was an encouraging improvement compared to historical data [84]. This study further strengthened the place of imatinib treatment in Ph+ ALL and indicated along with the COG AALL0031 study that the dose and time intensity of TKI therapy is likely of importance [85].

The Spanish SHOP-2005 trial also included Ph+ ALL patients aged 1–18 and imatinib was initiated at a dose of 260 mg/m^2 on day 15 of induction with the chemotherapy backbone again being an intensive multiagent paediatric regimen. A significant improvement was reported, with a 3-year EFS of 78.7% in the imatinib cohort versus 29.6% in a non-imatinib historical cohort [86].

With the highly encouraging results seen with imatinib + intensive chemotherapy combinations, attention then turned to the newer more potent second-generation TKIs, particularly dasatinib. COG AALL0622 included patients aged 1–30 years old and incorporated dasatinib 60 mg/m^2 starting at day 15 of induction either given continuously or for 2 weeks of each treatment block, with two thirds of patients receiving the discontinuous regimen. The chemotherapy backbone was the same as that used in COG AALL0031. Patients were classified as either standard risk or high risk based on flow cytometry-based MRD results after induction and consolidation treatments. AlloHCT was recommended for high-risk patients based on slow response and those with a matched family donor. The post induction CR rate was 98%, which was significantly better than the 89% reported in AALL0031. This potentially resulted from earlier TKI initiation. The 5-year EFS and OS rates for standard-risk patients were 61% and 87%, while for high-risk patients they were 67% and 89%, respectively. Interestingly, there were no significant outcome differences between the standard-risk and high-risk groups, and in contrast to other studies, the level of early response measured by MRD did not correlate with an effect on survival [87]. Rather, the presence of *IKZF1* deletions was associated with a significantly worse prognosis. An additional finding was the surprisingly high isolated and combined CNS relapse rate of 15% in patients treated without radiotherapy (compared with 6% in the AALL0031 cohort who received radiation therapy), despite dasatinib's purported better CNS penetrance. This raises the question of whether cranial irradiation can be safely omitted in Ph+ ALL patients not undergoing AlloHCT. Further information is expected from the successor study AALL1122 which only administered cranial irradiation to patients with CNS3 disease (i.e. CSF white cell count >5/μL with blasts on cytospin).

While the COG Ph+ ALL studies yielded impressive outcomes, the chemotherapy backbone was associated with considerable toxicity. Consequently, the subsequent AALL1122/BMS CA180-372 study investigated the addition of continuous dasatinib 60 mg/m^2 to the less intensive AIEOP-BFM chemotherapy backbone which had been used in the EsPhALL study. The initial analysis of this study, which enrolled children from both COG institutions in USA and Australia and EsPhALL institutions in UK and Italy, confirmed that this approach was non-inferior to the imatinib-based regimens used in AALL0031 and EsPhALL [88]. Furthermore, this result was achieved with only 14% of patients being referred for AlloHCT in CR1 compared with 81% in EsPhALL 2004 and 38% in EsPhALL 2010. Hence, it is now evident that most children with Ph+ ALL can be effectively treated without AlloHCT.

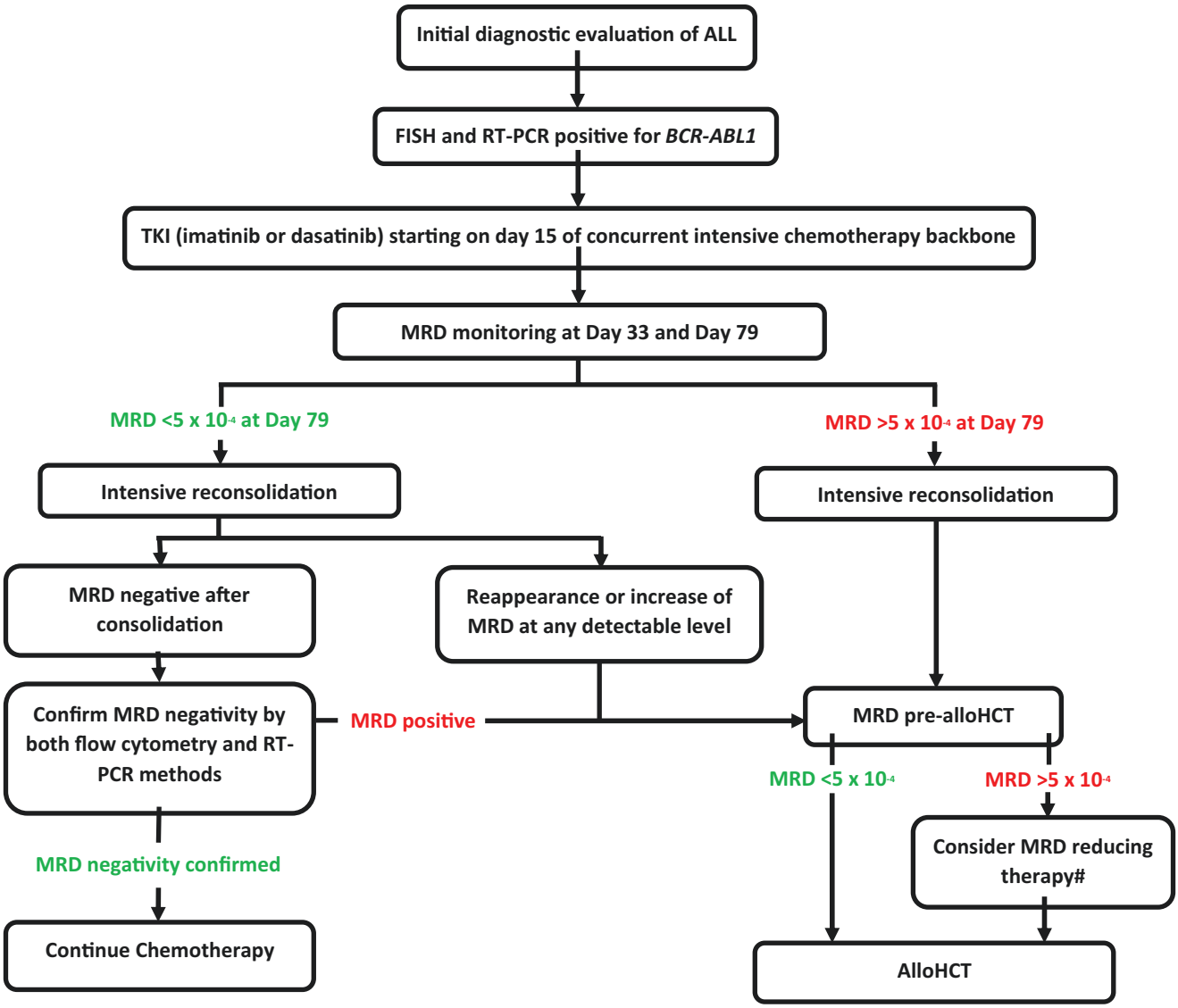
The current AALL1631 study (NCT03007147), which is being run in conjunction with the EsPhALL group, is attempting to determine whether chemotherapy can be further de-intensified in standard-risk patients to reduce treatment-related toxicities without compromising disease-free survival. Specifically, patients with IgH/TCR PCR MRD $<5 \times 10^{-4}$ at the end of the second block of chemotherapy are randomised to receive either imatinib plus the more intensive EsPhALL/AALL1122 chemotherapy backbone or imatinib plus the less intensive BFM2000-based chemotherapy regimen that is used by the COG for Ph- high-risk ALL. High-risk patients will be non-randomly assigned to EsPhALL chemotherapy before proceeding to AlloHCT in CR1. Imatinib was selected based on the fact that it was associated with similar EFS and end-of-consolidation MRD to dasatinib and was more readily available in most participating countries. This study will also explore the role of post-transplant imatinib and the prognostic impact of *IKZF1* deletions. The COG are also currently investigating the safety and efficacy of ponatinib in Ph+ ALL patients who are

relapsed/refractory, intolerant of other TKIs, or harbour the T315I mutation (NCT04501614).

A suggested approach to the treatment of Ph+ ALL in paediatrics, adolescents, and young adults is shown in Fig. 22.2. Emerging data on the prognostic role of *IKZF1* deletions and post-transplant TKI may further refine this algorithm in the coming years.

22.3.2.2 Adults

There is now clear evidence that paediatric or paediatric-inspired chemotherapy regimens confer superior outcomes in young fit adults (<40 years age) with Ph- ALL compared to historical outcomes from previous adult-based protocols [89, 90]. Studies in children and young adults with Ph+ ALL where ages ranged from 1 to 30 years old have demonstrated



A suggested approached to diagnosis and treatment of Ph+ ALL in paediatric, adolescent and young adults from the EsPhALL collaborative group, as an example management algorithm. The suggested chemotherapy back bone is an intensive paediatric protocol. MRD is measured either by PCR for IgH/TCR rearrangement, by *BCR-ABL1* qRT-PCR, or by multiparameter flow cytometry. Modified from Bleckmann and Schrappe 2016 [83}.

 # E.g. Blinatumomab, Inotuzumab Ozogamicin, alternative TKI

Fig. 22.2 Suggested Treatment Algorithm for Ph+ ALL in Paediatrics and Adolescents/Young Adults. A suggested approach to diagnosis and treatment of Ph+ ALL in paediatric, adolescent, and young adults from the EsPhALL collaborative group, as an example management algorithm. The suggested chemotherapy of backbone is an intensive paediatric protocol. MRD is measured either by PCR for IgH/TCR rearrangement, by *BCR-ABL1* qRT-PCR, or by multiparameter flow cytometry. Modified from Bleckmann and Schrappe 2016 [83]. # E.g. Blinatumomab, Inotuzumab Ozogamicin, alternative TKI

that use of imatinib or dasatinib with paediatric-based protocols significantly improves outcomes, with no advantage for alloHCT in CR1 for most patients [52, 82, 87]. Head-to-head trials directly comparing the combination of TKI therapy with paediatric-inspired protocols versus TKI therapy plus conventional adult-based protocols have not been performed. The toxicity of paediatric regimens in adults can be problematic – particularly due to the use of asparaginase, the higher cumulative doses of corticosteroids, and potentiation of these toxicities by TKIs [91]. Considering current evidence still favouring alloHCT in adult Ph+ ALL and the comparatively low incidence of Ph+ disease in young adults able to tolerate paediatric like ALL regimens, addition of a TKI to the generally less toxic adult-based protocols is often favoured.

The Hyper-CVAD regimen, pioneered by the MD Anderson Cancer Center, is widely used for remission induction for both Ph- and Ph+ ALL. This treatment regimen involves 21-day cycles containing hyper-fractionated cyclophosphamide, doxorubicin, vincristine, and dexamethasone alternating with 21-day cycles incorporating high-dose methotrexate and cytarabine. The TKI is generally administered from days 1 to 14 of the first induction cycle (1A), to avoid compounding myelosuppression in the aftermath of remission induction. TKI therapy then resumes and continues without breaks starting from day 1 of cycle 1B. Four cycles of each arm are given followed by 13 months of maintenance therapy including continuous TKI therapy, monthly vincristine, and 5-day prednisolone pulses each month.

The initial trial combining imatinib with Hyper-CVAD included Ph+ ALL patients aged 17–75 and demonstrated CR, 5-year EFS, and OS of 93%, 43%, and 43%, respectively, with significant improvements compared to CR rates of 52–66% and 5-year EFS and OS of <20% in historical control groups. Imatinib doses were 400 mg daily during induction/consolidation and 600 mg daily during maintenance. Eligible patients proceeded to alloHCT and there were no significant EFS differences in patients who underwent alloHCT compared to those who did not; however, numbers were small [55, 92].

A subsequent trial combining dasatinib at a total daily dose of 70-100 mg with Hyper-CVAD in patients aged 21–80 (median = 55) showed similar CR, 5-year EFS, and OS rates of 96%, 44%, and 46%, respectively. Eligible patients again proceeded to alloHCT in CR1, but only 17% of patients underwent transplantation likely due to the advanced age of the cohort [56]. A further study investigated the same treatment regimen in a younger cohort of patients aged 18–60 (median = 44) where 49% of patients proceeded to alloHCT in CR1. Overall, the CR rate was 88%, while 3-year EFS and OS were 55% and 69%, respectively. Importantly, 3-year EFS and OS were significantly higher in those who had undergone alloHCT [93].

Ponatinib combined with Hyper-CVAD was a logical next step in the evolution of this regimen and has shown promising results with a 100% CR rate and 3-year EFS and OS of 70% and 78% and a 5-year OS of 73% in a cohort of patients aged 21–80. Ponatinib was given initially at a dose of 45 mg with subsequent dose reductions occurring as the trial proceeded due to concerns over vascular toxicity. Patients proceeded to transplant at the physician's discretion, resulting in a transplant rate of 21%. Despite previous concerns regarding potential cardiac and vascular toxicities, ponatinib was well tolerated with no mortality during induction [62, 94, 95]. A propensity analysis of the imatinib, dasatinib, and ponatinib with Hyper-CVAD historical cohorts has subsequently been undertaken and has suggested superior outcomes with ponatinib compared with dasatinib [60]. This led the authors to suggest that initial treatment using ponatinib combined with chemotherapy may become the new standard of care for Ph+ ALL in adults. In general, hepatotoxicity, coagulopathies, and corticosteroid-related side effects are less common in Hyper-CVAD compared with paediatric-based regimens due to the omission of asparaginase and reduced cumulative corticosteroid dose, although myelosuppression and infective complications are common, with grade 3–4 infection occurring in 50–70% [92].

Similar improvements in outcomes have been demonstrated when imatinib was added to the UKALLXII/ECOG2993 study standard chemotherapy backbone, consisting of daunorubicin, vincristine, prednisolone, asparaginase, cyclophosphamide, 6-mercaptopurine, cytarabine, methotrexate, etoposide, dexamethasone, and tioguanine. This study was conducted when imatinib first became available, resulting in a pre-imatinib cohort treated between 1993 and 2003, a cohort of patients receiving imatinib as a single agent following induction at 400-600 mg once daily (late imatinib, 2003–2005), and a cohort who had imatinib incorporated into the second phase of induction (2005 onwards, early imatinib). Patients in CR with a sibling- or matched-unrelated donor (MUD) were then offered a myeloablative alloHCT. The complete remission (CR) rate was 92% in the imatinib cohort vs 82% in the pre-imatinib cohort ($P = 0.004$). The 4-year OS of imatinib-treated patients was 38% versus 22% in the pre-imatinib cohort ($P = 0.003$), with an EFS of 33% vs 18% and an RFS of 50% vs 33% in imatinib vs pre-imatinib cohorts, respectively. This allowed a higher transplant realisation rate of 46% in the imatinib-treated patients, as opposed to 31% in the pre-imatinib cohort [50].

While TKIs have undoubtedly improved disease response in adults with Ph+ ALL, toxicity from the combination with chemotherapy remained an ongoing challenge. For example, the UKALL14 study reported significant toxicity. In this study, patients were treated using a 4-drug induction consisting of pegylated asparaginase, daunorubicin, vincristine, and dexamethasone that continued from

the pre-phase, and continuous imatinib was added at 400 mg then escalating to 600 mg per day for Ph+ ALL. Patients were randomised to receive rituximab in addition to the common backbone. In their initial report of 91 B-ALL patients which included 26 Ph+ ALL (29%), there were 16 induction-related deaths, half of them accompanied by recognised pegylated asparaginase toxicities. This was disproportionately observed in the Ph+ ALL patients, suggesting that the combination of imatinib and pegylated asparaginase was associated with increased toxicity. The UKALL14 study was amended to omit pegylated asparaginase in induction for Ph+ ALL patients [96].

In addition to highlighting the potential for additional toxicity when imatinib is combined with asparaginase containing regimens in adults, the UKALL 14 study demonstrated the potential benefit of adding rituximab. Other groups had demonstrated that rituximab, a monoclonal antibody against the CD20 antigen on B-cells, has provided benefit when added to chemotherapy regimens for Ph- B-ALL expressing CD20, defined as CD20 expression at diagnosis ≥20% [97, 98]. The phase III randomised UKALL14 study included both Ph- and Ph+ precursor B-ALL patients regardless of CD20 cell surface expression and compared one group of patients receiving standard chemotherapy with four doses of rituximab in induction against another group receiving standard chemotherapy without rituximab. The initial analysis has shown a non-significant trend towards better outcomes in the rituximab group regardless of Ph or CD20 status [96]. Their 4-dose schedule during induction was a much lower rituximab exposure than in the previous Ph- studies which used 8 to 18 doses, which may indicate that a higher number of rituximab doses are required throughout all stages of treatment. Final publication of the results of the UKALL14 study is still awaited.

The clear survival benefit associated with TKIs has prompted several groups to consider reducing the intensity of chemotherapy to minimise toxicity. This is particularly important during induction remission when infective complications are common. The GRAAPH group, for instance, treated Ph+ ALL patients in France and Switzerland with imatinib and randomised them to concurrent Hyper-CVAD chemotherapy versus an abbreviated regimen (omitting cyclophosphamide and anthracycline for A cycles, while keeping B cycles the same). Patients then proceeded to an allogeneic or autologous haematopoietic cell transplant (autoHCT) depending on donor availability upon achieving remission. The GRAAPH regimen demonstrated improved CR1 rates in the less intensive arm (98% vs 91%) owing to decreased induction mortality, but with similar rates of major molecular response (MMR, BCR-ABL1 <0.1%) (66.1% in the modified arm vs 64.5% in the standard arm). Five-year EFS and OS was also improved in the less intensive arm (EFS 42.2% vs 32.1% and 48.3% vs 43% respectively). AlloHCT in first CR was associated with improved relapse-free survival and OS; however, this was only seen in patients who did not achieve a MMR after cycle 2 [51]. This study established that an abbreviated chemotherapy regimen may be used for induction if transplant is incorporated as consolidation within a comprehensive treatment plan.

A suggested treatment algorithm for Ph+ ALL in adults is shown in Fig. 22.3. Some of the commonly used TKI plus chemotherapy regimens are shown in Table 22.1.

22.3.2.3 Older Adults

Older adults diagnosed with Ph+ ALL have historically had dismal outcomes. Reasons for this include their higher comorbidity burden, increase in poor prognostic markers, and inability to tolerate the dose- and time-intensive chemotherapy required to achieve a durable remission [99]. However, the advent of TKI therapy has provided improvement in this patient group. In a study of patients >60 years old, imatinib plus corticosteroids led to a haematological response rate of 100%, although responses were not durable for the majority. Median remission duration and OS were 8 and 20 months, respectively, with a 12-month disease-free survival of 48%. However, the regimen was delivered with minimal toxicity, suggesting that in the elderly and those unfit for induction chemotherapy, TKI therapy combined with corticosteroids may be a meaningful treatment to extend and maintain quality of life [100]. The more potent TKI, ponatinib, plus corticosteroid therapy, may be even more effective, with a CR rate of 95% and 2-year OS of 62% reported [61].

Other studies adding low intensity chemotherapy to steroids and TKIs have also demonstrated high remission rates and minimal induction-related mortality. For instance, a French cohort using the DIV regimen consisting of dexamethasone, imatinib, and vincristine alone reported a CR rate of 90% [101]. Similarly, the EWALL-PH-01 study used dasatinib in place of imatinib and achieved a CR rate of 96% in a cohort of older patients (>55 years of age), with an MMR rate of 65%. Even though this protocol included consolidation with cytarabine, asparaginase, and methotrexate, responses were not durable, with the 5-year RFS and OS rates of only 28% and 36%, respectively, with most relapses mediated by T315I mutations [102]. The subsequent EWALL-PH-02 trial using nilotinib with a similar backbone led to a CR rate of 94%, with a 4-year RFS and OS of 42% and 47%, respectively [103]. Despite the high late relapse rate, such approaches continue to be attractive in the elderly and unfit, given their high CR rate, low rate of toxic deaths during induction, and the small number of patients apparently achieving long-term disease control.

Current clinical trials are investigating whether immunotherapies combined with TKIs can improve on the outcomes

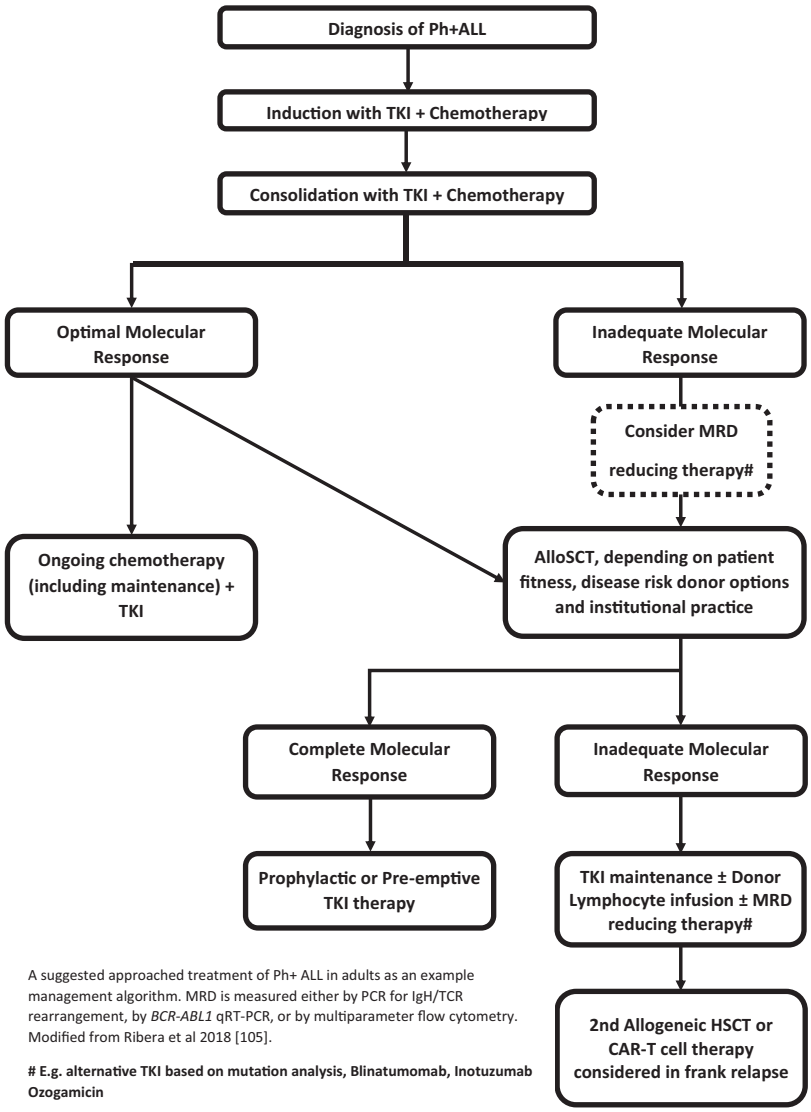


Fig. 22.3 Suggested Treatment Algorithm for Ph+ ALL in Adults. A suggested approached treatment of Ph+ ALL in adults as an example management algorithm. MRD is measured either by PCR for IgH/TCR rearrangement, by *BCR-ABL1* qRT-PCR, or by multiparameter flow cytometry. Modified from Ribera et al. 2018 [105]. # E.g. alternative TKI based on mutation analysis, Blinatumomab, Inotuzumab Ozogamicin

attained with minimal chemotherapy regimens (see Sect. 22.4.3). This may provide an effective strategy for elderly individuals with Ph+ ALL, whose outcomes continue to lag behind younger groups.

22.3.3 Stem Cell Transplantation

22.3.3.1 Who Needs Stem Cell Transplantation?

Given the adverse prognosis of Ph+ ALL, alloHCT has been historically indicated in all adult patients in CR1 who are transplant eligible. This remains the only long-term curative option for a subset of patients, even in the TKI era. A pressing challenge is to determine which patients are most likely to benefit from AlloHCT and which patients are potentially curable without the morbidity and mortality associated with transplant. As new biomarkers are identified and as new agents are incorporated into the treatment of Ph+ ALL, indications for transplant will probably continue to require re-evaluation.

In the aforementioned UKALLXII/ECOG2993 study, patients who achieved CR1 and had a sibling or MUD donor were recommended to proceed to myeloablative alloHCT. While the OS of imatinib-treated patients at 4 years was only 38%, a sub-analysis showed significantly superior 4-year OS for patients who received alloHCT in CR1 versus those who did not (50% vs 19%) with EFS of 46% vs 14%. These results, within the context of the superior CR rate in the imatinib cohort versus the pre-imatinib cohort, suggested that the higher transplant realisation rate was the significant contributor to improved survival. The same study reported dismal outcomes in patients with relapsed disease, with a 6% 5-year OS [104]. Similarly, long-term follow-up of the JALSG ALL202 cohort suggested an advantage for patients

Table 22.1 Examples of Ph+ ALL TKI + Chemotherapy Regimens (for reference only – do not prescribe or dispense against this table – consult primary source document for clinical treatment decisions)

	COG AALL1122 (1–17 years)	UKALL14 (25–65 years)	HyperCVAD (17–80 years)	GRAAPH (18–59 years)	EWALL-PH-01 (>55 years)
TKI Therapy	**Induction:** Dasatinib 60 mg/m^2 Daily from day 15 of induction phase 1a **Consolidation:** Dasatinib 60 mg/m^2 Daily continuously **Maintenance:** Dasatinib 60 mg/m^2 Daily continuously	**Induction:** Imatinib 400 mg Daily aiming to up-titrate to 600 mg Daily from day 1 of induction phase 1 **Consolidation:** Imatinib 600 mg continuously **Maintenance:** Imatinib 600 mg continuously	**Induction:** Imatinib 600 mg Daily OR Dasatanib 100 mg Daily OR Ponatinib 45 mg Daily from day 1–14 of induction 1A, then restart Imatinib 600 mg Daily OR Dasatinib 70 mg Daily OR Ponatinib 30 mg Daily on Day 1 of induction 1B **Consolidation:** Imatinib 600 mg Daily OR Dasatanib 70 mg Daily OR Ponatinib 30 mg Daily[a] all given continuously **Maintenance:** Imatinib 600 mg Daily OR Dasatanib 70 mg Daily OR Ponatinib 30 mg Daily[a] all given continuously	**Induction:** Imatinib 400 mg Twice a Day continuously **Consolidation:** Imatinib 400 mg Twice a Day from Day 1–14 of consolidation cycle 1 then restart Imatinib 300 mg BD from Day 1 of consolidation cycle 2 and continue until transplant **Maintenance:** Can consider TKI therapy post HSCT	**Induction:** Dasatinib 140 mg Daily continuously **Consolidation:** Dasatinib 100 mg daily from days 14–27 of consolidation cycles **Maintenance:** Dasatinib 100 mg daily on alternative months
Induction	**Phase 1a (33 days):** Prednisolone 60 mg/m^2 (28)[b] Vincristine 1.5 mg/m^2 (4)[c] Daunorubicin 25 mg/m^2 (4) PEG-asparaginase 2500 IU/m^2 (1) IT Methotrexate[d] IT Cytarabine[d] **Phase 1b (28 days):** Cyclophosphamide 1 g/m^2 (2) Cytarabine 75 mg/m^2 (16) 6-MP 60 mg/m^2 (28) IT Methotrexate[d]	**Phase 1 (28 days):** Daunorubicin 30 mg/m^2 (4) Vincristine 1.4 mg/m^2 (4)[c] Dexamethasone 10 mg/m^2 (12) IT Methotrexate 12.5 mg (1) **Phase 2 (28 days):** Cyclophosphamide 1 g/m^2 (2) Cytarabine 75 mg/m^2 (16) 6-MP 60 mg/m^2 (28) IT Methotrexate 12.5 mg (3)	**Cycle 1A (21 days):** Dexamethasone 40 mg (8) Cyclophosphamide 300 mg/m^2(6) Doxorubicin 50 mg/m^2 (1) Vincristine 1.4 mg/m^2 (2)[c] IT Methotrexate 12 mg (1) IT Cytarabine 100 mg (1) **Cycle 1B (21 days):** Methylprednisolone 50 mg (6) Methotrexate 1 g/m^2 (1) Cytarabine 3 g/m^2 (4) IT Methotrexate 12 mg (1) IT Cytarabine 100 mg (1)	**Cycle 1 (28 days):** Dexamethasone 40 mg (8) Vincristine 2 mg (4) IT Methotrexate 15 mg (3) IT Cytarabine 40 mg (3) IT Prednisone 40 mg (3)	**Cycle 1 (56 days):** Dexamethasone 40 mg (8) Vincristine 1 mg (4) IT Methotrexate 15 mg (4) IT Cytarabine 40 mg (4) IT Prednisone 40 mg (4)

Consolidation	**HR Block 1 (21 days):** Dexamethasone 20 mg/m^2 (5) Vincristine 1.5 mg/m^2 (2)[c] Methotrexate 5 g/m^2 (1) Cyclophosphamide 200 mg/m^2 (5) Cytarabine 2g/m^2 (2) PEG-asparaginase 2500 IU/m^2 (1) IT Methotrexate[d] **HR Block 2 (21 days):** Dexamethasone 20 mg/m^2 (5) Vincristine 1.5 mg/m^2 (2)[c] Methotrexate 5 g/m^2 (1) Ifosfamide 200 mg/m^2 (5) PEG-asparaginase 2500 IU/m^2 (1) Daunorubicin 30 mg/m^2 (1) IT Methotrexate[d] **HR Block 3 (21 days):** Dexamethasone 20 mg/m^2 (5) Cytarabine 2 g/m^2 (2) Etoposide 100 mg/m^2 (5) PEG-asparaginase 2500 IU/m^2 (1) IT Methotrexate[d] IT Cytarabine[d] IT Hydrocortisone[d] **Reinduction 1 and 2 (63 days):** Dexamethasone 10 mg/m^2 (14) Vincristine 1.5 mg/m^2 (4)[c] Doxorubicin 25 mg/m^2 (4) PEG-asparaginase 2500 IU/m^2 (1) Cyclophosphamide 1 g/m^2 (1) 6-TG 60 mg/m^2 (14) Cytarabine 75 mg/m^2 (8) IT Methotrexate[d]	**Intensification (28 days):** Methotrexate 3 g/m^2 (1) PEG-asparaginase 1000 IU/m^2 (2) **Consolidation 1 (7 days):** Cytarabine 75 mg/m^2 (5) Etoposide 100 mg/m^2 (5) PEG-Asparaginase 1000 IU/m^2 (1) IT Methotrexate 12.5 mg (1) **Consolidation 2 and 4 (7 days):** Cytarabine 75 mg/m^2 (5) Etoposide 100 mg/m^2 (5) IT Methotrexate 12.5 mg (1) **Consolidation 3 (42 days):** Daunorubicin 25 mg/m^2 (4) Vincristine 1.4 mg/m^2 (4)[c] PEG-Asparaginase 1000 IU/m^2 (1) Dexamethasone 10 mg/m^2 (16) IT Methotrexate 12.5 mg (2) Cyclophosphamide 1 g/m^2 (1) Cytarabine 75 mg/m^2 (8) 6-MP 60 mg/m^2 (14)	**Cycle 2A/3A/4A (21 days):** Dexamethasone 40 mg (8) Cyclophosphamide 300 mg/m (6) Doxorubicin 50 mg/m^2 (1) Vincristine 1.4 mg/m^2 (2)[c] IT Methotrexate 12 mg (1) IT Cytarabine 100 mg (1) **Cycle 2B/3B/4B (21 days):** Methylprednisolone 50 mg (6) Methotrexate 1 g/m^2 (1) Cytarabine 3 g/m^2 (4) IT Methotrexate 12 mg (1)[d] IT Cytarabine 100mg (1)[d]	**Cycle 1 (28 days):** Methotrexate 1 g/m^2 (1) Cytarabine 3 g/m^2 (4) IT Methotrexate 15 mg (1) IT Cytarabine 40 mg (1) IT Prednisone 40 mg (1) **Cycle 2 and 3 Pre-HSCT interphase (14 days):** 6-MP 60 mg/m^2 (14) Methotrexate 25 mg/m^2 (2) IT Methotrexate 15 mg (1) IT Cytarabine 40 mg (1) IT Prednisone 40 mg (1)	**Cycle 1/3/5 (28 days):** Methotrexate 1 g/m^2 (1) Asparaginase 10,000 IU/m^2 (1) IT Methotrexate 15 mg (1) IT Cytarabine 40 mg (1) IT Prednisone 40 mg (1) **Cycle 2/4/6 (28 days):** Cytarabine 1 g/m^2 (6)
Maintenance	6-MP 50 mg/m^2 daily Methotrexate 20 mg/m^2 weekly IT Methotrexate[d]	Vincristine 1.4 mg/m^2 [c] every 3 months Prednisolone 60 mg/m^2 for 5 days every 3 months IT Methotrexate 12.5 mg every 3 months 6-MP 75 mg/m^2 Daily Methotrexate 20 mg/m^2 Weekly	Vincristine 1.4 mg/m^2 [c] monthly Prednisolone 60 mg/m^2 for 5 days every month	HSCT	**On alternate month to Dasatinib:** 6-MP 60 mg/m^2 Daily Methotrexate 25 mg/m^2 Weekly **Every 2 months for 3 doses then every 3 months for 4 doses:** Vincristine 1 mg (1) Dexamethasone 40 mg (2)

HSCT: Haematopoietic Stem Cell Transplant; *TKI*: Tyrosine Kinase Inhibitor; *IT*: Intrathecal; *6-MP*: 6-mercaptopurine; *6-TG*: 6-thioguanine; (x) denotes the number of doses of that agent in the cycle

[a]Ponatinib decreased to 15 mg daily once complete molecular remission achieved

[b]Dexamethasone 10 mg/m^2 daily given days 1–14 instead of prednisolone if aged 1–10 years

[c]Vincristine doses capped at a maximum of 2 mg

[d]Intrathecal chemotherapy and cranial irradiation doses and schedule dependant on age and CNS disease risk category

who proceeded to alloHCT in CR1, a conclusion that could not be made in their initial report [53, 105].

The GRAAPH-2005 trial utilised a reduced intensity chemotherapy regimen combined with imatinib followed by either alloHCT if a sibling or 9/10 MUD donor was available or autoHCT for patients without well-matched donors [51]. The 5-year EFS was similar between groups (37.1% vs 45.6%); however, alloHCT was associated with superior relapse-free survival (hazard ratio [HR], 0.69; $P = 0.036$). The group who benefited the most were those with MRD positivity going into transplant. Similar results have been reported in other donor-no donor trials utilising dasatinib, nilotinib, and ponatinib with comparable OS but superior relapse-free survival in the alloHCT group. This highlights the importance of the graft versus leukemia effect in Ph+ ALL [93, 106].

As noted above, the need for universal upfront alloHCT in Ph+ ALL has been challenged with the availability of increasingly potent and effective TKIs. This question is difficult to examine within a randomised controlled study, as donor availability and fitness to proceed to an alloHCT after induction chemo are factors that may confound analyses [107]. The current practice suggests that the benefit of a CR1 alloHCT is greatest in patients who are medically fit and have a well-matched donor. High-risk features, such as persistent MRD or the presence of additional cytogenetic mutations at diagnosis, may also tip the scales in favour of an alloHCT [108, 109]. Historically, outcomes for transplants performed in CR2 compared to CR1 are significantly worse. This may change, given the emergence of effective, less toxic salvage therapies, which may allow for safe transplantation in CR2 for patients who relapse. Blinatumomab, inotuzumab ozogamicin, and CAR-T therapy may change the calculus, and careful sequencing of these either pre- or post-transplant is required. A comprehensive discussion on the specifics of alloHCT is outside the scope of this chapter. In general, myeloablative conditioning is preferred where possible for Ph+ ALL, with total body irradiation and either cyclophosphamide or etoposide being the usual regimens. Reduced intensity conditioning is also a reasonable strategy in patients predicted to have high treatment-related mortality with myeloablative conditioning. However, relapse rates are higher, and early relapse may occur prior to the establishment of a graft versus leukemia effect. Matched sibling or matched unrelated donors are generally preferred where available; however, transplantation using unrelated umbilical cord blood and haploidentical donors has also been shown to be a feasible approach for Ph+ B-ALL [110–112]. AlloHCT using haploidentical donors in particular has shown potential for favourable outcomes [113].

22.3.3.2 Optimisation of Disease Prior to SCT

Relapse post-alloHCT occurs in approximately 25% of Ph+ ALL patients [114] and is more likely to occur in patients with high tumour burden prior to alloHCT, especially those who have not achieved *BCR-ABL1* ≤ 0.1% (MMR) [115]. A number of new therapies, such as blinatumomab, are increasingly being used to induce a deeper MRD response prior to alloHCT, although their long-term benefit is still being elucidated [116–119].

22.3.3.3 Role of TKI Post-SCT?

Because of the significant relapse rates seen in Ph+ ALL following alloHCT, post-transplant TKI therapy has been proposed as a strategy to increase the probability of maintaining long-term remission [114]. While there are minimal high quality clinical trials addressing this question, several retrospective studies have demonstrated a benefit from post-transplant TKI therapy [120–123]. One randomised controlled trial of imatinib maintenance post-alloHCT demonstrated equivalent benefit for both prophylactic and MRD-directed therapy compared to historical data [122]. A recent retrospective study of 171 patients again showed benefit of TKI therapy post-transplant with imatinib being equivalent to newer generation TKIs when used in a prophylactic strategy, but newer agents showing superiority to imatinib when used in an MRD-directed approach [120]. While maintenance TKI therapy post-alloHCT is becoming standard of care for Ph+ ALL, questions remain on practicalities such as the optimal agent, time of initiation of therapy, duration of therapy, and whether a prophylactic or MRD-directed approach is preferable. Tolerance and drug interactions may also pose challenges.

22.4 Therapy for Relapsed/Refractory Disease

22.4.1 Ponatinib

Relapse is a common occurrence in Ph+ ALL and is associated with dismal outcomes [124, 125]. The role of kinase domain mutations in relapse has been described above. The T315I mutation in *BCR-ABL1* is commonly detected in relapsed disease where previous treatment with a TKI has been given. The third-generation TKI, ponatinib, has demonstrated activity in relapsed Ph+ ALL with and without the T315I mutation, with a response rate of 41% of patients in one study. However, the majority of responses were not durable, with progression-free survival at 12 months of only 7% [38].

22.4.2 Asciminib

Asciminib is a TKI currently undergoing clinical trials for CML and Ph+ ALL. This agent binds to the auto-inhibitory myristoyl binding site of BCR-ABL1 away from the ATP-binding pocket where other TKIs bind. The myristoyl N-terminal of ABL1 normally fulfils this function, but is lost with the *BCR-ABL1* fusion, and asciminb restores the auto-inhibitory function of this moiety. The unique shape of the myristoyl binding site makes the action of asciminib highly specific to BCR-ABL1, potentially resulting in less toxicities compared to other TKIs. Furthermore, the different site of action provides high likelihood that this agent will retain activity against many of the common mutations resulting in traditional TKI resistance [126]. A phase 1 trial in heavily pre-treated CML including some patients who had failed ponatinib and/or had the T315I mutation showed promising activity [126]. This has led to investigation of this agent in Ph+ ALL with clinical trials currently in development to examine its use in relapsed and refractory disease.

22.4.3 Blinatumomab

The bispecific T-cell engaging monoclonal antibody blinatumomab has substantially improved outcomes for relapsed/refractory Ph− B-ALL. This drug acts by binding to the CD19 antigen on malignant and non-malignant B-lymphocytes and the CD3+ antigen on T-lymphocytes. This dual binding results in the formation of a link between the malignant B-cell target and the T-cell leading to cytotoxic T-cell-mediated apoptosis of the B-lymphoid cell [127].

In the phase III TOWER study of Ph− ALL, the CR rate was 44% in patients receiving blinatumomab, compared to 25% in patients given "standard-of-care" salvage chemotherapy. Blinatumomab also led to a superior MRD response rate of 33% vs 12%, while transplant rates were similar at 24% for both groups [128]. The efficacy of blinatumomab in R/R Ph+ ALL has since been demonstrated [129]. Despite the promising CR and MRD negativity rate, the response achieved with blinatumomab is generally not durable, with the median OS of 7.7 months in the blinatumomab arm of the TOWER study. As such, blinatumomab should be regarded as a bridge to alloHCT in those who have relapsed/refractory disease.

Blinatumomab is also efficacious in eliminating MRD in patients who have achieved morphological CR after induction chemo, with 78% of patients achieving MRD negative status after 1 cycle of blinatumomab in the BLAST study [130]. Similar observations have been made in Ph+ ALL [129, 131, 132].

Given its efficacy in the relapsed/refractory setting, an upfront chemotherapy-free approach using blinatumomab combined with dasatinib has been advocated. The D-ALBA study using this combination recently reported excellent outcomes, with molecular responses achieved in 68% of patients after two cycles, increasing to 80% after two further cycles of blinatumomab [133]. With a median follow-up of 18 months, the OS and EFS were 95% and 88%, respectively. A subgroup of patients with *IKZF1* alterations plus other genetic abnormalities had inferior responses. While these are very encouraging results, longer term follow-up is awaited. Furthermore, this approach is tempered by a recent study showing that the combination of blinatumomab and TKI in vitro led to inhibition of T-cell leukemic killing through Src inhibition, suggesting that concurrent administration may not necessarily be the optimal approach [134].

22.4.4 Inotuzumab Ozogamicin

Inotuzumab Ozogamicin (IO), an anti-CD22 monoclonal antibody conjugated to the cytotoxic antibiotic calicheamicin, is another prominent therapy used for treating relapsed/refractory B-ALL. As the majority of B-ALL clones express CD22, this agent is active against most Ph+ and Ph− cases. The phase III INO-VATE trial which led to the registration of IO included both patients with Ph+ and Ph− disease [135]. Analysis of patients with relapsed/refractory Ph+ ALL from both a phase 1/2 study and the INO-VATE trial showed significantly higher rates of CR, MRD negativity, progression to alloHCT, and EFS with IO compared to re-induction chemotherapy [136]. Importantly, IO has been associated with increased rates of veno-occlusive disease of the liver (VOD), especially in the context of subsequent alloHCT. This may preclude its wider use in this setting.

As with blinatumomab, there have been attempts to combine IO with TKIs. A recent study of relapsed/refractory Ph+ ALL used bosutinib together with inotuzumab [137] and included patients with kinase domain mutations. Overall response rate was 83% with a subset of patients proceeding to transplant. The combination was well tolerated with rash being the most common grade 3 adverse event and no VOD reported. Negative MRD on flow cytometry was achieved in 67%.

22.5 Future Directions and the Unknown

While a number of significant advances have improved outcomes for patients with Ph+ ALL, many questions remain. As discussed previously, the new generation of studies using chemotherapy-free induction regimens in adults are reporting favourable outcomes, although long-term follow-up is

necessary to gauge the durability of response. The best method to consolidate such a response is also unclear. Although CR1 alloHCT remains the standard of care in many centres, this approach may require re-evaluation if relapse rates continue to fall with incorporation of novel treatments and improved salvage options are developed. Validation of new predictive biomarkers may aid in identifying patients at highest risk of treatment failure, reserving alloHCT for those select few. This may include a more nuanced interpretation of MRD and its kinetics and cytogenetic and molecular abnormalities to better quantify relapse risk [109]. There is already growing data to suggest molecular lesions, such as *PAX5, IKZF1,* and *CDKN2A* alterations, may increase relapse risk. Such knowledge, in turn, may spur discovery of other small molecule inhibitors specific for these genomic lesions.

In patients selected for alloHCT, the optimal donor source and conditioning regimen remains to be determined. With increasing experience in performing haploidentical SCTs, many centres are now reporting excellent outcomes. Similarly, improved effectiveness of post-transplant maintenance may expand the role of reduced intensity conditioning.

The optimal duration of TKI maintenance, either post-alloHCT or as part of a chemotherapy-based regimen, is also unclear. Most contemporary Ph− ALL regimens continue maintenance therapy until at least two years after the initial diagnosis. The COG AALL0031 and AALL0622, Hyper-CVAD, and UKALL14 protocols all include a duration of 2-years of TKI therapy in patients who do not progress to alloHCT; however, common practice is to continue indefinitely [138]. MRD monitoring is likely important in determining when it is safe to cease TKI therapy in patients not undergoing transplant, with prolonged complete molecular remission prior to discontinuation preferable based on a small retrospective study [139]. Continued MRD monitoring post-TKI cessation is still vital with re-institution of TKI therapy and/or consideration of newer agents such as blinatumomab if molecular relapse is identified. Larger prospective studies are needed to better understand this important clinical question.

For future patients with relapsed or refractory disease, a number of new therapies and combinations are on the horizon. In vitro studies combining asciminib with ponatinib in patient-derived xenograft models for ALL show the ability of this combination to overcome compound T315I mutations [140]. Upfront combination therapy consisting of asciminib, dasatinib, and corticosteroids is undergoing assessment, with favourable toxicity on early data [141]. The addition of the BCL-2 inhibitor venetoclax to ponatinib and dexamethasone in patients with Ph+ relapsed or refractory ALL or CML is also currently being investigated (NCT03576547). The safety and efficacy of antibody therapies in the relapsed setting and the enhanced response rates using TKIs frontline have also led to further exploration of novel combinations of TKIs with antibody-based therapy.

Chimeric Antigen Receptor T Cells (CAR-T) have revolutionised the therapy of B-lymphoid malignancies, successfully salvaging multiple relapsed patients who previously had a dismal prognosis. CAR-T cells are genetically engineered human T cells programmed to recognise specific antigens on leukemia cells, inducing a cytotoxic response independent of HLA. CD19-directed CAR-T cells have been approved for use in relapsed/refractory ALL including Ph+ ALL, based on phase II trials showing overall response rates of 81% with CR in 60% of patients [142]. Importantly, MRD negativity by flow cytometry was also achieved in all patients who responded. There are now greater than 100 trials of CAR T-cells in progress, the majority of which are in B-cell malignancies [143]. The combined clinical experience has confirmed high early response rates and highlighted the unique toxicity profile associated with the activation of engineered T cells once target antigens have been encountered. Although the early responses seen with CAR-T cells are impressive, durability of response is still being determined and relapse remains extremely difficult to treat [144]. CD19+ relapses are associated with lack of persistence of CAR-T, while mechanisms of CD19- relapse include altered gene function of CD19 or pre-existing CD19- leukemic cells becoming the dominant clone following selection pressure from CD19-directed therapies [145].

The need for definitive therapy with transplant after CAR-T is debated. AlloHCT has been safely performed post-CAR-T, with reports that attainment of MRD prior may allow attenuation of conditioning to reduce treatment-related mortality. Long term remissions are also seen in the absence of alloHCT, with MRD negativity post-CAR-T and low disease burden at time of infusion felt to be predictive [144, 146].

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Management of Philadelphia Chromosome-Like Acute Lymphoblastic Leukemia (Ph-Like ALL)

23

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Abstract

BCR-ABL1-like or Philadelphia chromosome-like acute lymphoblastic leukemia (Ph-like ALL) ALL is a subset of high-risk (HR) B-ALL associated with high relapse risk and inferior clinical outcomes. Ph-like ALL was first described with a kinase-activated gene expression profile similar to that of Philadelphia chromosome-positive ALL (Ph+ ALL) and frequent *IKZF1* (*Ikaros*) alterations, yet lacking the canonical *BCR-ABL1* oncogene fusion (Mullighan et al., Blood. 2009;360:470–80; Den Boer et al., Lancet Oncol. 2009;10:125–34; Harvey et al., Blood. 2010;116:4874–84). Advances in high-throughput sequencing technologies during the past decade have unraveled the genomic landscape of Ph-like ALL, revealing a diverse array of kinase-activating alterations that may be amenable to molecularly targeted therapies (Tasian et al., Blood. 2017;130:2064–72). Ph-like ALL is now included as a provisional disease entity in the World Health Organization 2016 classification of acute leukemias (Arber et al., Blood. 2016;127:2391–405). Thorough characterization of the epidemiology, clinical portrait, and biology of Ph-like ALL across the age spectrum has subsequently led to current precision medicine trials investigating the therapeutic potential of tyrosine kinase inhibitor-based therapies for children, adolescents, and adults with Ph-like ALL. These efforts have been somewhat challenging to translate given the genomic heterogeneity and diagnostic complexity of Ph-like ALL, and the most optimal treatment paradigm for this high-risk group of patients has yet to be established. This chapter aims to provide a state-of-the-art review of the epidemiology, clinical features, and biology of Ph-like ALL, highlight the challenges in implementing pragmatic and cost-effective diagnostic algorithms in the clinic, and describe the milieu of treatment strategies under active clinical or preclinical investigation that strives to decrease relapse risk and improve long-term survival for patients with this high-risk leukemia subtype.

Keywords

ABL class · Acute lymphoblastic leukemia · Clinical trials · CRLF2 · Genetic testing · Immunotherapy · JAK/STAT · Philadelphia chromosome-like · Precision medicine · Tyrosine kinase inhibitor

23.1 Definition of Ph-Like ALL

Ph-like ALL was originally identified in 2009 via gene expression profiling by two independent groups using different gene classifiers. Researchers in the Children's Oncology Group (COG), St Jude Children's Research Hospital (SJCRH), and University of New Mexico (UNM) via the National Cancer Institute (NCI) TARGET initiative (https://ocg.cancer.gov/programs/target/projects/acute-lymphoblastic-leukemia) utilized Affymetrix gene expression microarray data to identify 257 gene probe sets that defined a distinct gene expression signature of both Ph+ ALL and Ph-like ALL [1], while the Dutch Children's Oncology Group led by den Boer and colleagues at Erasmus Medical Center used hierarchical clustering of 110 probe sets to predict six major pediatric ALL subtypes (*ETV6-RUNX1*, high hyperdiploid, *TCF3-PBX1*, *KMT2A*-rearranged, *BCR-ABL1*, and T-ALL) [2]. Despite sharing only nine common probe sets of seven genes (*CCND2*, *SH3BP5*, *ABL1*, *SOCS2*, *DUSP6*, *LST1,* and *EGFL7*), both gene classifiers identified

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a subset of HR B-ALL patients with poor survival who had frequent deletions of B-cell development and transcription factor genes, such as *IKZF1* [3]. These assays and additional advances in RNA sequencing were further able to define new genetic alterations deregulating tyrosine kinase or cytokine receptor genes in Ph-like ALL, including *CRLF2*, *JAK2*, *ABL1*, and *PDGFRB* [4]. The COG/SJCRH/UNM group subsequently developed the first clinically validated Ph-like ALL screening assay, which measures the expression of 8 or 15 genes in a 384-well low-density array (LDA) microfluidic card to detect the Ph-like ALL signature. This clinical assay is now capable of results return within 24–48 h of sample submission [5]. The LDA card has been used by the COG and other consortia to screen patients with newly diagnosed HR B-ALL for the Ph-like signature and to allocate those with LDA positivity for downstream testing to identify specific Ph-like-associated genetic alterations [6]. It should be emphasized that the most clinically relevant endpoint in patients with Ph-like ALL remains the identification of their specific oncogenic translocations and mutations that activate kinase signaling and may be therapeutically targeted.

23.2 Biology and Genomic Landscape of Ph-Like ALL

In their 2014 landmark paper, Roberts and colleagues described the genomic landscape of Ph-like ALL via a comprehensive genomic analysis of 154 children and young adults with HR B-ALL [7]. The unifying molecular hallmark of Ph-like ALL resides in the heterogeneous spectrum of genetic alterations activating cytokine receptor genes and kinase signaling pathways [7]. These alterations can be subdivided into four distinct genomically defined subsets based upon their underlying kinase-activating lesions: (1) alterations in JAK/STAT pathway genes (predominantly *CRLF2*, *JAK2*, *EPOR, IL7R, and SH2B3*), (2) ABL class alterations (*ABL1*, *ABL2*, *CSF1R*, *LYN, PDGFRA*, and *PDGFRB*), (3) uncommon Ras pathway mutations (*NRAS, KRAS, NF1, PTPN11, and CBL)*, and (4) rare kinase fusions (*NTRK3, PTK2B, and BLNK*) and are described in greater detail below (Table 23.1 and Fig. 23.1) [7, 8]. While *CRLF2* (*cytokine receptor-like factor 2*) rearrangements also occur with lower frequency in children with standard risk (SR) B-ALL [9–11] and in >50% of trisomy 21/Down syndrome (DS)-associated B-ALL [12, 13] (and may or may not have the associated Ph-like gene expression signature), other Ph-like ALL-associated kinase fusions have only extremely rarely been discovered in patients with SR disease.

Deletions of *IKZF1* and other lymphoid transcription factor genes occur commonly in Ph-like ALL, as has been similarly seen in Ph+ ALL [7, 14–17]. *IKZF1* encodes the zinc-finger DNA-binding Ikaros, a transcription factor essential for B-cell lymphoid development. Its alteration results in acquired stem cell-like properties, aberrant bone marrow stromal adhesion, and chemotherapy resistance [18–20]. The most common type of *IKZF1* alteration is intragenic focal deletion of exons 4–7, which results in the dominant-negative Ik6 isoform [20]. In one study, *IKZF1* alterations were detected in 68% of Ph-like ALL compared to 16% of non-Ph-like ALL cases [7]. Inferior clinical outcomes in patients with *IKZF1*-deleted Ph-like ALL have also been reported [7, 21]. Recent studies from European consortia have further described inferior outcomes of patients with the new *IKZF1*^{plus} molecular profile, which is defined by deletion of *IKZF1* co-occurring with one or more deletions in *PAX5, CDKN2A* (heterozygous or homozygous), *CDKN2B* (homozygous only), or the pseudoautosomal region 1 (PAR1) region of the sex chromosomes where *CRLF2* is located and in the absence of *ERG* deletion [22–25]. Stanulla and colleagues reported that the *IKZF1*^{plus} signature conferred the highest hazard ratio for relapse in multivariate analysis and could be incorporated in clinical decision algorithms to refine risk stratification in addition to MRD response [22]. The *IKZF1*^{plus} subgroup in these studies likely contains a high proportion of Ph-like ALL patients, as both populations share adverse clinical and biologic features of higher white blood cell (WBC) at diagnosis, poor prednisone response, positive minimal residual disease (MRD) after induction therapy, and higher frequency of the germline *GATA3* variant rs3824662 reported to predispose to developing Ph-like ALL [22–24, 26].

23.2.1 JAK/STAT Pathway Gene Alterations

Approximately half of the children, adolescents, and adults with Ph-like ALL harbor *CRLF2* rearrangements [9, 27, 28], which leads to *CRLF2* overexpression and increased surface protein expression of the thymic stromal lymphopoietin receptor (TSLPR; encoded by *CRLF2*) detectable by flow cytometry [29]. *CRLF2* alterations occur via two major mechanisms: (1) focal deletion of PAR1 on chromosomes Xp22/Yp11 resulting in the *P2RY8-CRLF2* fusion or (2) translocation to the immunoglobulin heavy chain enhancer region on chromosome 14, resulting in *IGH-CRLF2* rearrangement [12, 30]. Both rearrangements result in overexpression of full-length CRLF2, which heterodimerizes with the IL7R-alpha subunit to form the TSLPR involved in early B-cell development [31–33]. *P2RY8-CRLF2* fusions appear to occur more frequently in younger children and in those with DS-ALL, [13] whereas *IGH-CRLF2* predominates among adolescents and young adults, particularly those of Hispanic/Latino or Native American ancestry [12, 30, 34, 35]. Rarely, activating *CRLF2* F232C point mutations, which typically coexist with *CRLF2* rearrangements, also lead to *CRLF2* deregulation [36]. Half of *CRLF2*-rearranged cases harbor concomitant mutations in *JAK2* or, less commonly,

Table 23.1 Repertoire of Ph-like ALL kinase rearrangements, therapeutic targets, and potential clinical trials

Ph-like genetic subgroups	3′ kinase genes	5′ fusion partner genes	Kinase inhibitors	Clinical trials
JAK/STAT pathway alterations	*CRLF2*	*CSF2RA, IGH, P2RY8*	Ruxolitinib	NCT02420717 (MDACC) NCT02723994 (COG AALL1521) NCT03117751 (SJCRH total XVII) NCT03571321 (University of Chicago)
	JAK2	*ATF7IP, BCR, EBF1, ETV6, GOLGA5, HMBOX1, OFD1, PAX5, PCM1, PPFIBP1, RFX3, SMU1, SNX29, SSBP2, STRN3, TERF2, TPR, USP25, ZBTB46, ZNF274, ZNF340*	Ruxolitinib	
	EPOR	*IGH, IGK, IGL, LAIR1, THADA*	Ruxolitinib	
	TSLP	*IQGAP2*	Ruxolitinib	
	IL2RB	*MYH9*	Ruxolitinib	
ABL class alterations	*ABL1*	*CENPC, ETV6, FOXP1, LSM14A, NUP153, NUP214, RANBP2, RCSD1, SFPQ, SHIP1, SNX1, SNX2, SPTNA1, ZMIZ1*	Dasatinib, imatinib, others	NCT01406756 (COG AALL1131) NCT02143414 (SWOG S1318) NCT02420717 (MDACC) NCT03007147 (COG AALL1631) NCT03117751 (SJCRH total XVII)
	ABL2	*PAG1, RCSD1, ZC3HAV1*	Dasatinib, imatinib	
	CSF1R	*MEF2D, SSBP2, TBL1XR1*	Dasatinib, imatinib	
	PDGFRA	*FIP1L1*	Dasatinib, imatinib	
	PDGFRB	*ATF7IP, EBF1, ETV6, NUMA1, SNX29, SSBP2, TERF2, TNIP1, ZEB2, ZMYND8, ZNF608*	Dasatinib, imatinib	
	LYN	*GATAD2A, NCOR1*	Dasatinib, imatinib	
Other kinases	*NTRK3*	*ETV6*	Entrectinib Larotrectinib	NCT03066661 NCT03834961
	PTK2B	*KDM6A, STAG2, TMEM2*	FAK inhibitors	
	FGFR1	*BCR*	Ponatinib	
	FLT3	*ZMYM2*	FLT3 inhibitors	
	TYK2	*MYB, SMARCA4, ZNF340*	JAK1/3 inhibitor	
	BLNK	*DNTT*		
	CBL	*KANK1*		
	DGKH	*ZFAND3*		

COG: Children's Oncology Group; *SJCRH*: St Jude Children's Research Hospital; *MDACC*: MD Anderson Cancer Center; *SWOG*: Southwestern Oncology Group

JAK1. The most frequently occurring point mutation is R683G in the *JAK2* pseudokinase domain. *JAK1* V658F, which is analogous to the *JAK2* V617F mutation seen in adult myeloproliferative neoplasms, occurs much less frequently in *CRLF2*-rearranged Ph-like ALL [7, 9]. Sequence mutations in *IL7R* and *SH2B3* have also been identified in a small number of *CRLF2*-rearranged cases that lack concomitant JAK mutations [7, 37]. Less commonly (~10% of patients), some children with detected *CRLF2* rearrangements leading to *CRLF2* overexpression (usually *P2RY8-CRLF2* fusions in children with SR B-ALL) do not have the Ph-like expression signature [37]. *CRLF2* overexpression in the absence of specific rearrangement detection has been reported in a small percentage of patients [38].

Kinase fusions involving *JAK2* or *EPOR* rearrangements, each representing approximately 5–10% of Ph-like ALL cases, are also associated with activation of JAK/STAT signaling [7, 9]. Some groups have reported an increased prevalence of *JAK2* fusions among the young adult population compared to children, ~15% vs. 5%, respectively [5, 39]. *JAK2* is a promiscuous 3′ gene in Ph-like ALL with at least 19 different 5′ partner genes reported to date. All *JAK2* fusions are in-frame and disrupt the pseudokinase domain of JAK2, thus relieving from auto-inhibition of the kinase domain and resulting in constitutive activation of JAK/STAT signaling [4, 7, 8]. Several 5′ partner genes in *EPOR* rearrangements have been described, each involving the juxtaposition of the *EPOR* gene to the enhancer region of immunoglobulin heavy (*IGH*) (most common), κ (*IGK*), or λ (*IGL*) loci and leading to deregulated expression of a truncated form of *EPOR* that has been shown to drive Ph-like leukemogenesis and activated JAK/STAT signaling [40]. Insertion and truncation of *EPOR* into the upstream region of *LAIR1* or the *THADA* loci have also been reported in a very small number of patients [40]. As with *JAK2* fusions, the prevalence of *EPOR* rearrangements rises with increasing age with peak prevalence among young adults [7, 40].

Additional mechanisms leading to JAK/STAT pathway activation beyond the aforementioned kinase or cytokine

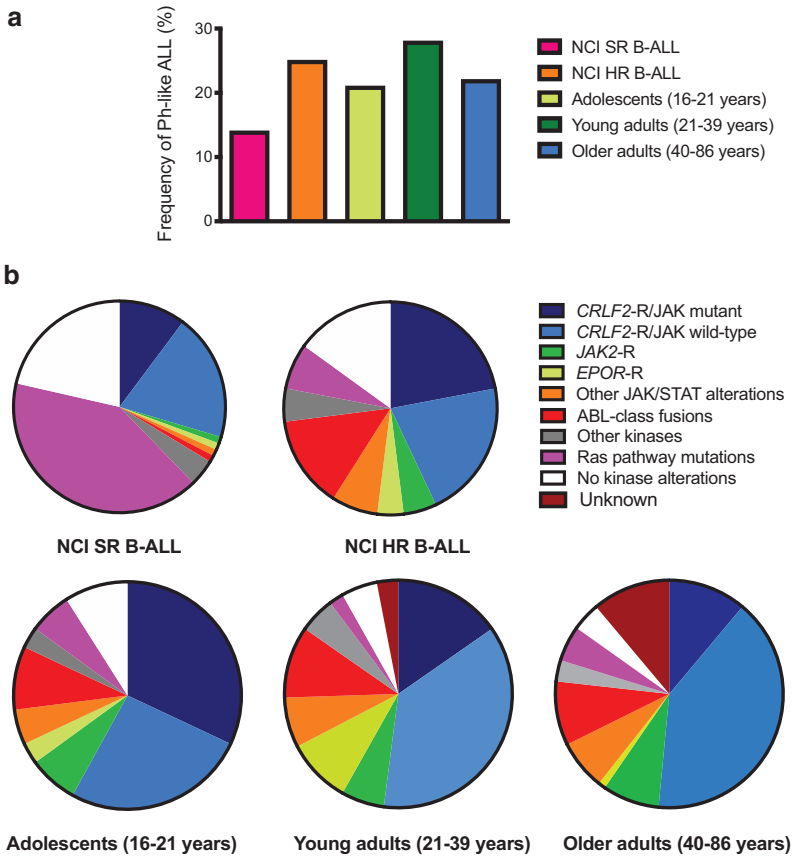

Fig. 23.1 Frequency of Ph-like ALL and genetic subtypes by age group. (**a**) Incidence of Ph-like ALL across the pediatric-to-adult age spectrum. (**b**) Heterogeneity of specific Ph-like ALL genetic alterations across the pediatric-to-adult age spectrum

receptor-activating rearrangements implicate a diverse range of sequence mutations and copy number variations in genes such as *JAK1, JAK3, IL7R, SH2B3, IL2RB*, and *TYK2* [7]. These lesions collectively comprise 14% of children compared to 5% and 7.3% of adolescents and older adults, respectively [4, 7]. Although they lack a kinase-activating rearrangement, these cases often harbor chromosomal rearrangements expressing fusion oncoproteins involving transcription factor genes (*EBF1, PAX5, and ETV6*) and/or epigenetic regulators (*CREBBP, SETD2, and ASXL1*) that merit further study [4].

23.2.2 ABL Class Alterations

The second most clinically relevant subgroup of Ph-like ALL is ABL class alterations, which account for approximately 10% of cases and are mutually exclusive from the *CRLF2* rearrangements and other JAK pathway-activating alterations [7, 27]. Prevalence peaks among children with NCI HR B-ALL at 17% and then decreases to about 10% in young and older adults [7, 27, 37]. ABL class rearrangements involve 3′ *ABL1, ABL2, CSF1R, LYN, PDGFRA*, or *PDGFRB* fusing with multiple 5′ partner genes. Preclinical and clinical studies have shown that ABL class fusion leukemias are sensitive to tyrosine kinase inhibitors, such as imatinib, dasatinib, or ponatinib [7–9, 41].

23.2.3 Ras Pathway Mutations

Approximately 4% of Ph-like ALL patients have activating mutations in Ras pathway genes, including *KRAS, NRAS, NF1, PTPN11*, and *CBL* [7]. Ras pathway mutations are usually subclonal and can occur as the solely detected anomaly or in conjunction with sentinel Ph-like translocations (e.g., *CRLF2*, ABL class, *JAK2*, or *EPOR* fusions) [7, 28]. Ras-activating mutations are also commonly found in hyperdiploid, hypodiploid, *KTM2A*-rearranged, and relapsed ALL and are also often subclonal [42–44]. It is not currently known whether these mutations are pathogenic drivers in childhood ALL.

23.2.4 Rare Kinase Fusions

Other rare fusions involving *NTRK3, BLNK, DGKH, FGFR1, PTK2B, FLT3*, or *TYK2* collectively account for 3% of Ph-like ALL cases [7, 37]. The *ETV6-NTRK3* fusion, which has been reported in other malignancies such as infantile fibrosarcoma and secretory breast carcinoma, can induce an aggressive ALL with in vitro and in vivo sensitivity to TRK inhibitors [45]. Clinical responses to larotrectinib, a pan-TRK inhibitor, in patients with relapsed/refractory B-ALL harboring *ETV6-NTRK3* have been described in single case reports [46, 47]. Functional modeling of other kinase fusions is important to determine their oncogenic role and identify

novel therapeutic targets. As an example, *FGFR1* fusions may be targetable with ponatinib [48] or pazopanib [49].

23.3 Epidemiology and Clinical Picture of Ph-like ALL

The prevalence of Ph-like ALL rises with increasing age and varies by gender, ethnicity, and NCI-defined risk groups. A recent meta-analysis of 15 studies reported that the pooled prevalence of Ph-like ALL across the age spectrum was 15.4% [50]. By age group, the Ph-like subtype comprised 15.6% of B-ALL cases in children aged 1–10 years old, 26.2% in adolescents aged 11–20 years, 25.8% in young adults aged 21–40 years, and 16.9% in adults older than 40 years [7, 27, 28, 50, 51]. Among children and adolescents with B-ALL, Ph-like ALL accounts for 13.6% of NCI SR cases and 22.4% of NCI HR cases [11, 37]. In comparison with Ph+ ALL, Ph-like ALL is three times more common in the pediatric age group [5]. Males are more commonly affected than females across the age spectrum with a male-to-female ratio of 1.5:1 among children and adults [7, 27]. Ph-like ALL also has a predilection for patients of Hispanic/Latino or Native American ethnicity, especially among those with *CRLF2* rearrangements. This phenomenon has been attributed in part to the increased prevalence of germline Ph-like ALL risk variant in *GATA3* (rs3824662) in Hispanic individuals with Native American genetic ancestry [26, 52, 53]. Furthermore, Ph-like ALL patients frequently have adverse clinical features with significantly higher rates of hyperleukocytosis at diagnosis, end-induction MRD positivity, and increased risk of treatment failure and relapse [7, 27, 34, 37, 51, 54]. The Ph-like ALL gene signature may confer an independent adverse risk factor, as shown in some multivariate analyses [27, 51, 55]. Patients harboring *PDGFRB*, *JAK2*, or *EPOR* fusions are notoriously associated with more aggressive disease course and frequent high rates of end-induction MRD or even induction failure [7, 56–59].

The inferior survival of patients with Ph-like versus non-Ph-like ALL patients occurs across the age spectrum, and differential outcomes may exist within the heterogeneous Ph-like ALL subtypes based upon induction chemotherapy response. Children with NCI HR B-ALL and a retrospectively identified Ph-like expression signature treated on the COG AALL0232 phase 3 trial (NCT00075725) had a 5-year event-free survival (EFS) of 63% versus 86% ($P < 0.0001$) of those with non-Ph-like ALL [55]. Importantly, this inferior outcome was detected for patients with Ph-like ALL regardless of the randomized treatment arm assigned, which was in contrast to patients with non–Ph-like ALL with superiority of high-dose methotrexate versus dose-escalating Capizzi-style methotrexate in the first interim maintenance phase [55]. More recent analyses of children with NCI SR Ph-like ALL treated on the COG AALL0331 phase 3 trial (NCT00103285) [60] showed statistically inferior outcomes versus those with SR non-Ph-like ALL with 7-year EFS 82.4% and 90.7% ($P = 0.0022$). However, these patients appear to be salvageable at relapse with no difference in overall survival (OS) (93.2% vs 95.8%, $P = 0.14$) between the two groups [11]. Recent comprehensive analysis of all children with identified *CRLF2*-overexpressing ALL treated on COG SR B-ALL trials AALL0331 and AALL0932 ($n = 77$) or HR B-ALL trials AALL0232 or AALL1131 ($n = 244$) confirmed reasonable clinical outcomes for children with Ph-like SR B-ALL with 5-year EFS 87.2% and OS 94.5%, although EFS was inferior to that of children with non-Ph-like ALL [38]. However, outcomes were quite poor for patients with HR B-ALL with 5-year EFS and OS of 56.3% and 75.4%, respectively. Finally, a recent comprehensive retrospective analysis from the Ponte di Legno group comprised of 14 pediatric oncology cooperative groups studied 122 children with Ph-like ABL class ALL treated with chemotherapy alone (without TKI) and reported high rates of end-induction MRD and poor outcomes despite intensive chemotherapy and often HSCT in first remission. This cohort serves to establish baseline outcomes of children with ABL class fusions in the pre-TKI era with reported 5-year cumulative incidence of relapse, EFS, and OS of 31.0%, 59.1%, and 76.1%, respectively [61].

Data from the CALGB 10403 phase 2 trial (NCT00558519) have shown marked improvement in outcome for adolescents and young adults (AYA) less than 40 years of age when using a similarly intensive pediatric-inspired regimen, although outcomes for Ph-like ALL AYA patients in that trial remained unfavorable with estimated 3-year EFS of 42% compared to 69% ($P = 0.008$) for those with non-Ph-like ALL [62]. Clinical outcomes of patients with Ph-like ALL also worsen with increasing age. Five recent studies focused on defining the incidence and characteristics of Ph-like ALL occurring in young and older adults with B-ALL. Among 49 adults with Ph-like ALL treated at the MD Anderson Cancer Center (MDACC), the 5-year OS was 23% for Ph-like ALL vs 59% ($P = 0.006$) for patients with non-Ph-like ALL [51]. Another study of 194 Ph-like ALL patients from 21 to 86 years old reported 5-year EFS for young adults (21–39 years old), adults (40–59 years), and older adults (60–86 years) of 40.4%, 29.8%, and 18.9%, respectively [27]. Among 88 Ph-negative B-ALL adult patients enrolled on the GIMEMA LAL1913 frontline protocol, 28 (31.8%) patients were identified as Ph-like harboring various kinase fusions. These patients had a significantly lower CR (74.1% vs. 91.5%; $P = 0.044$) and EFS (33.5% vs. 66.2%; $P = 0.005$) compared to non-Ph-like patients despite being treated with a pediatric-inspired and MRD-stratified protocol [63]. Two additional North American and German studies of adult Ph-like ALL cohorts confirmed the poor outcomes of this population [28, 34]. Ph-like ALL patients with concomitant *IKZF1* alterations may have further inferior outcomes compared to those without *IKZF1* alterations, although these analyses have been limited by small patient numbers [7, 21] (Table 23.2).

Table 23.2 Prevalence, clinical features, and treatment outcomes of Ph-like ALL by different study groups

Study group	Study period	Patient population	Prevalence of Ph-like ALL n (%)	Median age at diagnosis (CI)	Median WBC at diagnosis (CI)	Male,%	Ph-like gene signature detection methods	Kinase alteration detection methods	Clinical trials	Treatment outcome	References
Ponte di Legno ALL Working Group	2000–2018	Pediatric B-ALL	ABL class ($n = 122$)	9.7 (mean age)	97.7 (mean WBC)	62	LDA	FISH RT-PCR RNA-seq	Frontline ALL trials without TKI	5-year EFS: 59% 5-year OS: 76.1%	den Boer et al. Lancet Haematol, 2021
UK ALL	2012–2018	Pediatric ALL with slow response to induction ($n = 126$)	ABL class ($n = 21$)	9	35	71	N/A	FISH SNP RNA fusion panel	UKALL2011 Chemotherapy ($n = 8$) Imatinib + chemotherapy ($n = 13$)	4-year EFS: 83.9% (TKI) 4-year OS: 83.9% (TKI) 4-year EFS: 37.5% (no TKI) 4-year OS: 75% (no TKI)	Moorman et al. BJH 2020
GIMEMA	2014–2018	Adult Ph-negative B-ALL ($n = 88$)	$n = 28$, 31,8%	42 (18–65)	3.34 (0.23–347)	68	LDA	FISH MLPA NGS	GIMEMA LAL1913	2-year EFS: 33.5% 2-year OS: 48.5%	Chiaretti et al. Haematologica 2020
MDACC	2010–2012	Adult R/R B-ALL ($n = 53$)	$n = 12$, 22.6%	36 (20–57)	4.2 (1.0–29.3)	67	PAM	WGS RNA-seq	Inotuzumab monotherapy phase 2 trials	1-year EFS: 33% 1-year OS: 33%	Jabbour et al. Blood 2019
AIEOP-BFM	2000–2018	B-ALL ($n = 3854$)	ABL class ($n = 46$)	NA	NA	63	HC	FISH RT-PCR CGH RNA-seq	Chemotherapy ($n = 33$) Chemotherapy + TKI ($n = 13$)	5-year EFS: 62.9% (TKI) 5-year OS: 75.5% (TKI) 5-year EFS: 47.7% (no TKI) 5-year OS: 70.9% (no TKI)	Cario et al. Haematologica 2019
FRALLE CAALL GRAALL EWALL EORTC	NA	Pediatric and adults with B-ALL	ABL class ($n = 24$)	24 (5–72)	30 (4–570)	67	HC	FISH RT-MLPA RQ-PCR BP-PCR RNA-seq	Chemo + TKI ($n = 24$)	3-year EFS: 55% 3-year OS: 77%	Tanasi et al. Blood 2019
CALGB	2007–2012	AYA B-ALL ($n = 131$)	$n = 41$ 31.3%	NA	NA	NA	LDA	LDA for CRLF2 aberration	Pediatric-based chemotherapy Regimen CALGB 10403	3-year EFS: 42% 3-year OS: 63%	Stock et al. Blood 2019

COG	2006–2008	SR B-ALL (n = 1023)	n = 139, 13.6%	NA	15.9 ± 13.7	50	LDA	FISH RT-PCR Sanger sequencing RNA-seq	COG AALL0331 phase 3 for SR B-ALL	7-year EFS: 82% 7-year OS: 93%	Roberts et al. Blood 2018
CALGB ECOG MDACC NILG PMCC SWOG City of Hope	NA	Adult B-ALL (n = 798)	n = 194, 24.3%	40 (21–86)	56.6 (0.2–434)	61	PAM LDA	FISH PCR Sanger sequencing RNA-seq	Multiple trials	5-year EFS: 22.5% 5-year OS: 23.8%	Roberts et al. J Clin Oncol 2017
GMALL	1999–2005	Adult B-ALL (n = 207)	n = 26, 13%	31 (16–59)	NA	74	PAM	FISH Q-PCR Sanger sequencing	Multiple trials	5-year DFS: 19% 5-year OS: 22%	Herold et al. Haematologica 2017
MDACC	2000–2012	Adult B-ALL (n = 148)	n = 49, 33.1%	33.5 (15–71)	17 (1–603)	66	PAM LDA	FISH Targeted NGS	Chemotherapy (hyper-CVAD or augmented BFM)	5-year OS: 23%	Jain et al. Blood 2017
University Pennsylvania University of Michigan	NA	Adult B-ALL (n = 89)	n = 18, 20.2%	43 (19–63)	71.1 ± 31.8 (median: 28.6)	69	LDA	FISH PCR RT-PCR Sanger sequencing Archer FusionPlex	Multiple trials	Median survival: 1.6 years	Tasian et al. Leukemia 2017
JACLS TCCSG CCLSG KYCCSG	NA	Pediatric B-ALL (n = 373)	n = 29, 8%	8.8 (1.9–16)	94.3 (0.6–420)	76	GSEA	Multiplex RT-PCR RNA-seq	Multiple trials	5-year EFS: 48.6% 5-year OS: 73.5%	Imamura et al. Blood Cancer J 2016
HOVON	1993–2009	Adult B-ALL (n = 127)	n = 21, 17%	25 (16–59)	NA	NA	HC	RT-PCR	Multiple trials	3-year EFS: ~25%	Boer et al. Haematologica 2015
COG SJCRH ECOG CALGB MDACC	2000–2007	Children, AYA with B-ALL (n = 1725)	n = 264, 15.3%	NA	106	cHR: 62 A: 73 YA: 82	PAM	FISH RT-PCR WES RNA-seq WGS	Multiple trials	5-year EFS: 58% (cHR), 41% (A), 24% (YA) 5-year OS: 73% (cHR), 66% (ado), 26% (YA)	Roberts et al. NEJM 2014

(continued)

Table 23.2 (continued)

Study group	Study period	Patient population	Prevalence of Ph-like ALL n (%)	Median age at diagnosis (CI)	Median WBC at diagnosis (CI)	Male,%	Ph-like gene signature detection methods	Kinase alteration detection methods	Clinical trials	Treatment outcome	References
SJCRH	2000–2007	Pediatric B-ALL ($n = 344$)	$n = 40$, 11.6%	5.3 (1.3–18.6)	7.1 (1.7–258.3)	68	PAM	FISH RT-PCR Sanger sequencing RNA-seq WGS	MRD-based risk intensification regimen	5-year EFS: 90% 5-year OS: 92.5%	Roberts et al. J Clin Oncol 2014
DCOG COALL	1994–2015	Pediatric B-ALL ($n = 572$)	$n = 94$, 16%	7	44.5	49	HC	FISH CRLF2 mRNA expression by microarray	Multiple trials	5-year CIR: 32%	van der Veer et al. Blood 2013
COG	2002–2011	HR B-ALL ($n = 572$)	$n = 81$, 14%	NA	NA	NA	PAM	Kinome sequencing	COG AALL0232 phase 3 trial for HR B-ALL	5-year EFS: 62.6%	Loh et al. Blood 2013
COG	2000–2003	Pediatric B-ALL ($n = 221$)	$n = 68$, 31%	NA	NA	NA	PAM	SNP PCR	COG P9906	5-year EFS: 26%	Mullighan et al. NEJM 2009
DCOG COALL	1990–2004	Pediatric B-ALL ($n = 154$)	$n = 30$, 19%	NA	NA	NA	HC	RT-PCR	Multiple trials	5-year DFS: 60% (COALL) 5-year DFS: 57% (DCOG)	Den Boer et al. Lancet Oncol 2009

AIEOP-BFM: Associazione Italiana di Ematologia e Oncologia Pediatrica and Berlin-Frankfurt-Münster cooperative groups; *AYA*: adolescents and young adults; *BP-PCR*: breakpoint-specific multiplex polymerase chain reaction; *CAALL*: French Protocol for the Treatment of ALL in Children and Adolescents; *CALGB*: Cancer and Leukemia Group B; *CCLSG*: Children's Cancer and Leukemia Study Group; *CGH*: comparative genomic hybridization; *cHR*: childhood high risk; *CIR*: cumulative incidence of relapse; *COALL*: Childhood Oncology Acute Lymphoblastic Leukemia; *COG*: Children's Oncology Group; *CRLF2*: cytokine receptor-like factor 2; *DCOG*: Dutch Childhood Oncology Group; *DFS*: disease-free survival; *EFS*: event-free survival; *ECOG*: Eastern Cooperative Oncology Group; *EORTC*: European Organisation for Research and Treatment of Cancer; *EWALL*: European Working Group on Adult ALL; *FISH*: fluorescence in situ hybridization; *FRALLE*: French Group for Childhood ALL; *GMALL*: German Multicenter Study Group for Adult ALL; *GRAALL*: French-Swiss-Belgian Group for Research on Adult Acute Lymphoblastic Leukemia; *GSEA*: gene-set enrichment analysis; *HC*: hierarchical clustering; *HOVON*: Hemato-Oncologie voor Volwassenen Nederland; *JACLS*: Japanese Association of Childhood Leukemia Study; *KYCCSG*: Kyushu-Yamaguchi Children's Cancer Study Group; *LDA*: low-density array; *MDACC*: MD Anderson Cancer Center; *MLPA*: multiplex ligation-dependent probe amplification; *MRD*: minimal residual disease; *NILG*: Northern Italy Leukemia Group; *NA*: not available; *OS*: overall survival; *PAM*: prediction analysis of microarrays; PMCC: Princess Margaret Cancer Centre; *RNA-seq*: RNA sequencing; *RQ-PCR*: real-time quantitative polymerase chain reaction; *R/R B-ALL*: relapsed/refractory B-acute lymphoblastic leukemia; *RT-PCR*: reverse transcription polymerase chain reaction; *SJCRH*: St. Jude Children's Research Hospital; *SNP*: single-nucleotide polymorphism; *SR*: standard risk; *SWOG*: Southwestern Oncology Group; *TCCSG*: Tokyo Children's Cancer Study Group; *TKI*: tyrosine kinase inhibitor; *WES*: whole-exome sequencing; *WGS*: whole-genome sequencing

Clinical outcomes among Ph-like ALL patients may also vary according to their underlying kinase-activating alterations, although larger prospective studies are required to confirm these observations. Roberts and colleagues reported that there are significant differences in 5-year EFS among different Ph-like genomic subgroups, with *JAK2/EPOR*-rearranged cases (26.1%) and *CRLF2*-rearranged *JAK2*-mutant cases (38.8%) having dismal prognosis, in contrast to cases harboring other JAK/STAT (68.3%) or Ras pathway mutations (85.7%) having a more favorable outcome ($P < 0.0001$) [7]. The recent Ponte di Legno ALL working group study also highlights the variability in MRD response and clinical outcomes in their cohort of ABL class fusions depending on the specific fusion subtype. Notably, children with *PDGFRB* and *ABL2* fusions appear more likely to have MRD levels greater than 10^{-2} at the end of induction and inferior EFS compared to their counterparts with *ABL1* or the uncommon *CSF1R* rearrangements [61].

23.4 Diagnostic Modalities and Clinical Workflow Algorithms for Ph-Like ALL

The heterogeneous spectrum of kinase-activating alterations, cryptic nature of these genetic aberrations by conventional cytogenetic analysis, and complexity of required testing have rendered Ph-like ALL quite challenging to diagnose via clinical laboratory assays. Several cooperative oncology consortia have adopted different screening strategies largely based on the patient population size to be tested, development of more rapid next-generation sequencing platforms, and availability of therapeutic clinical trials [6].

Clinical diagnosis of Ph-like ALL has involved assessment of the pathognomonic gene expression signature (used by some, but not all, groups) and detection of targetable kinase-activating alterations. Gene expression profiling, the utilized discovery modality in initial European and North American studies [1, 2], is not readily available in the clinic and has now been largely replaced by the TaqMan LDA microfluidic card measuring the expression of 8 or 15-gene panels (*IGJ, SPATS2L, MUC4, CRLF2, CA6, NRXN3, BMPR1B, GPR110, CHN2, SEMA6A, PON2, SLC2A5, S100Z, TP53INP1, and IFITM1*) now used by the COG to determine the Ph-like ALL signature [6, 55, 64]. An integrated score between 0 and 1 is generated from the 8- or 15-gene assay with a score ≥0.5 considered positive for the Ph-like gene signature [37]. Higher LDA score (e.g., >0.7) typically suggests an underlying kinase fusion [6]. This LDA-based approach has provided a rapid and cost-effective screening modality for some groups to identify patients with probable Ph-like ALL who require further detailed genomic characterization to identify targetable kinase-activating alterations and to minimize unnecessary downstream testing for LDA-negative patients. Aside from direct detection of *CRLF2* overexpression and *P2RY8-CRLF2* fusions on the LDA card, this modality is intended only as a screening tool and does not detect other Ph-like kinase-activating lesions. The LDA card is also capable of identifying and "ruling out" patients with Ph+ ALL and *ETV6-RUNX1* ALL who do not require additional Ph-like testing [6].

To enable detection of kinase-activating alterations (the most clinically relevant endpoint in the Ph-like ALL diagnostic work-up), several commercial, research-level, and clinical next-generation sequencing (NGS) platforms have been developed and are replacing prior multiplexed clinical RT-PCR panels [6, 65, 66]. The ArcherDx FusionPlex Heme panel uses anchored multiplex PCR-based enrichment with the ability to detect novel fusions involving 87 genes associated with hematologic malignancies [6]. The FoundationOne Heme panel is a targeted combined DNA and RNA sequencing method capable of fusion and mutation detection in >400 cancer-related genes [67]. Of particular interest, transcriptomic/RNA sequencing (RNA-seq) represents a powerful tool for comprehensive fusion and mutation detection in addition to identifying the Ph-like GEP by hierarchical clustering [6] and is becoming more clinically available. Indeed, RNA-seq is the only single platform capable of fulfilling the two essential aspects of Ph-like ALL's diagnosis, but is currently not considered time- or cost-effective for routine analysis of all patients. Clinical fusion and NGS assays are relatively more cost-effective, but still require a relatively long turnaround time (between 2 and 4 weeks) prior to clinical result reporting.

More rapid testing assays using conventional methods such as fluorescence *in situ* hybridization (FISH) and flow cytometry still retain clinical relevance for the diagnosis of Ph-like ALL. For example, dual color break-apart FISH probes are now commercially available for the canonical Ph-like 3′ kinase genes *ABL1, ABL2, PDGFRB* (will also detect *CSF1R* disruption at 5q32), *CRLF2, JAK2*, and *EPOR* with results typically delivered within 48 h. Since the vast majority of these kinase fusions are exclusively seen with the Ph-like phenotype, an abnormal FISH result might be sufficient to start tyrosine kinase inhibitor (TKI) therapy while waiting for confirmatory molecular testing. The COG has expanded its routine FISH panel to include ABL class FISH probes in its HR B-ALL AALL1732 phase 3 trial (NCT03959085) and its Ph+ ALL AALL1631 phase 3 trial (NCT03007147) in order to facilitate earlier introduction of TKI during induction for patients with identified ABL class alterations. Increased flow cytometric staining of TSLPR also suggests underlying *CRLF2* rearrangement, which is known to occur in over half of Ph-like ALL cases [29]. Flow cytometry availability in most institutions' diagnostic labo-

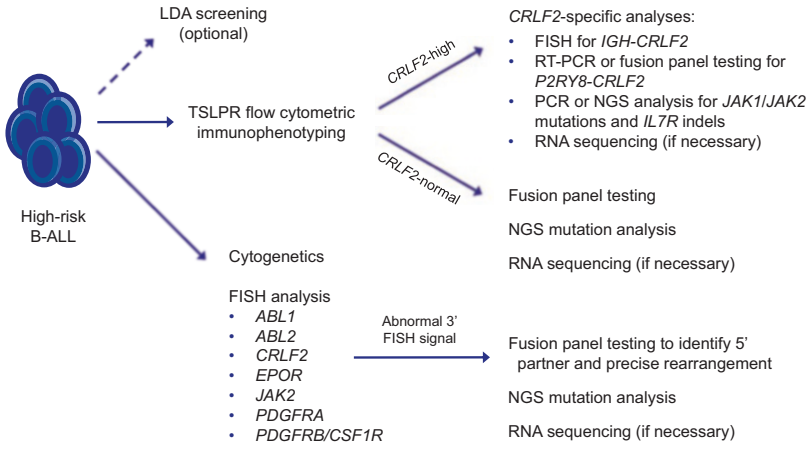

Fig. 23.2 Suggested clinical screening algorithm for Ph-like ALL. *CRLF2*: cytokine receptor-like factor 2; *LDA*: low-density array; *FISH*: fluorescence in situ hybridization; *NGS*: next-generation sequencing; *PCR*: polymerase chain reaction; *RNA*: ribonucleic acid; *RT-PCR*: reverse transcription polymerase chain reaction; *TSLPR*: thymic stromal lymphopoietin receptor. *Adapted from Harvey & Tasian Blood Adv 2020*

ratories and rapid result return within 24 h makes TSLPR immunophenotyping a compelling additional Ph-like screening assay.

In summary, successful identification of patients with Ph-like ALL will most likely benefit from a combined approach of cytogenetic, FISH, and molecular analysis via fusion and NGS testing given the known genetic heterogeneity of this leukemia subtype and ongoing new discovery. Pragmatic clinical screening algorithms will need to be personalized on one's available resources, patient volume, and clinical goals. A suggested diagnostic algorithm for Ph-like ALL is shown in Fig. 23.2 [6].

23.5 Precision Medicine Trials in Ph-Like ALL

23.5.1 Targeted Therapies

The recently characterized genomic landscape of Ph-like ALL has uncovered a myriad of kinase-activating alterations that expand the treatment paradigm of molecularly targeted therapies in ALL and leverage the success story of TKI incorporation for children and adults with Ph+ ALL [15, 68–71]. Despite their heterogeneity, Ph-like ALL-associated genetic alterations commonly converge to activate JAK/STAT, ABL, or Ras signaling pathways. Extensive in vitro and in vivo data have provided compelling evidence to incorporate relevant ABL/PDGFR- or JAK-directed TKIs in combination with chemotherapy to improve these patients' poor outcomes. The efficacy of such precision medicine approaches is currently being prospectively assessed in several clinical trials for children and adults with Ph-like ALL harboring ABL class alterations or JAK/STAT pathway gene lesions.

ABL class alterations phenocopy *BCR-ABL1* and exhibit exquisite sensitivity to imatinib and dasatinib in preclinical models [7, 8, 72]. There is increasing anecdotal evidence demonstrating that the addition of imatinib or dasatinib monotherapy or in combination with chemotherapy can induce remission in patients with relapsed/refractory ALL with ABL class alterations [7, 56–58, 73, 74]. Durable remissions and favorable outcomes have been reported in recent series of Ph-like ABL class patients treated with imatinib or dasatinib in combination with chemotherapy compared to historical controls [75–77]. Based on the increasing clinical experience in Ph-like ALL and the robust demonstration of safety and efficacy in children with Ph+ ALL, the COG AALL1131 phase 3 trial (NCT02883049) was amended in 2016 to add a dedicated treatment arm adding dasatinib to post-induction chemotherapy for Ph-like ALL patients with identified ABL class alterations. AALL1131 was closed to accrual in August 2019, and complete clinical results from this study are not yet available. The international phase 3 EsPhALL2017/COG AALL1631 phase 3 trial (NCT03007147), which is randomizing two different chemotherapy backbones in combination with imatinib for children with Ph+ ALL, will extend eligibility to include patients with Ph-like ABL class fusions in 2021. The St. Jude Children's Research Hospital (SJCRH) Total Therapy XVII ALL (NCT03117751) protocol was activated in early 2017 and incorporates dasatinib beginning in induction therapy for those identified with an ABL class fusion by RNA-seq within 2 weeks of diagnosis [78]. An MDACC phase 2 trial for adults with relapsed/refractory Ph-like ALL and ABL class fusions (NCT02420717) also combined dasatinib with the intensive hyper-CVAD chemotherapy backbone. Results have not yet been reported for these studies.

The largest class of Ph-like kinase-activating alterations constitutes those deregulating JAK/STAT signaling, making

this pathway a major potential therapeutic vulnerability in Ph-like ALL, although JAK inhibitors have been less well studied in patients with ALL to date. Preclinical studies of engineered Ba/F3 cells and patient-derived xenograft (PDX) models harboring a diverse range of JAK/STAT pathway lesions (*CRLF2* rearrangements with or without *JAK* mutations, *JAK2* fusions, *EPOR* fusions, sequence mutations of *IL7R* and/or *SH2B3*) have shown in vitro and in vivo activity to different JAK inhibitors [7, 8, 29, 59, 72, 79–83]. Potent, but sometimes differential, preclinical activity of the JAK1/2 inhibitor ruxolitinib has been reported in Ph-like ALL models with *CRLF2* fusions or *JAK2* fusions, which may be influenced by the level of JAK pathway oncogene addiction or, potentially, by paradoxical JAK2 hyperphosphorylation with prolonged treatment [51, 79, 83]. Subsequent preclinical studies further demonstrated enhanced activity of combinatorial treatment with JAK and PI3K/mTOR inhibitors in Ph-like ALL cell lines and PDX models [80, 81]. Investigating dual pathway inhibition seems quite relevant for Ph-like ALL, as upregulation of alternative signaling pathways is a known mechanism of resistance to single-targeting agents [84–86]. To this end, a recent preclinical study showed that *CRLF2*-rearranged Ph-like ALL cells mediate a complex "BCR-like" signaling characterized by activated SRC family kinase and downstream signaling in the absence of surface μ-heavy chain expression, which may mediate resistance to ruxolitinib monotherapy, but could be overcome by multi-TKI and/or dexamethasone combination [86].

Currently, ruxolitinib is being assessed prospectively in several clinical trials for patients with JAK/STAT pathway-mutant Ph-like ALL. The COG AALL1521 phase 2 trial (NCT02723994) is investigating the efficacy of incorporating ruxolitinib with post-induction chemotherapy for children, adolescents, and young adults with HR Ph-like ALL harboring JAK/STAT pathway lesions [87]. In this study, patients are stratified into four different cohorts based on their underlying Ph-like genetic lesions and by end-induction MRD status to delineate potential differential efficacy for each subset. A soon-to-open phase 1 trial will also assess the safety and tolerability of ruxolitinib in addition to chemotherapy specifically in a Ph-like ALL AYA population ages 18–39 years (NCT03571321). The two previously discussed SJRCH and MDACC trials also have a ruxolitinib treatment arm in combination with chemotherapy for patients with *de novo* or relapsed JAK-mutant Ph-like ALL, respectively.

The enriched prevalence of *IKZF1* deletions in Ph-like ALL opens up another potential therapeutic avenue for this HR patient population, although it is not yet known how these alterations might best be targeted. As above, *IKZF1* alterations are known to mediate aberrant stromal adhesion and therapy resistance in murine models of Ph+ ALL, and it is plausible that such effects could be reversed by retinoic acid compounds or focal adhesion kinase (FAK) inhibitors when combined with TKIs [18, 19].

Targeted inhibitors of apoptotic proteins are promising therapeutic candidates for children and adults with relapsed/refractory acute leukemias and are under early-phase clinical investigation in pediatric trials. Venetoclax, an orally administered selective inhibitor of the anti-apoptotic BCL-2 protein, has shown encouraging efficacy when combined with chemotherapy in 25 children with relapsed/refractory ALL with an overall response rate (ORR) of 56%, including four complete remissions (CRs), four CR with incomplete marrow recovery (CRi), and one CR without platelet recovery (CRp) [88]. The combination of venetoclax with dasatinib or ponatinib appears highly synergistic in Ph+ ALL cell lines and PDX models with demonstrated inhibition of LYN signaling and prevention of downstream MCL-1 upregulation [89]. A recent study demonstrated that dual BCL-2 and MCL-1 inhibition exhibits potent anti-leukemic activity in Ph+ ALL and *CRLF2*-rearranged Ph-like ALL PDX models [90], providing further rationale for potential investigation of BH3-mimetic inhibitors in clinical trials for patients with Ph+ and Ph-like ALL.

23.5.2 Hematopoietic Stem Cell Transplantation

The role of hematopoietic stem cell transplantation (HSCT) in the care of patients with Ph-like ALL remains unclear in the TKI era [91]. Earlier data demonstrated definitive improvement in EFS and OS of children with Ph+ ALL with imatinib or dasatinib in addition to chemotherapy, which also eliminated need for HSCT in most patients [15, 68, 92, 93]. Mirroring this Ph+ ALL experience, it is plausible that TKI addition to chemotherapy could be similarly successful for patients with Ph-like ABL class ALL.

A single-center study recently reported comparable outcomes between children with Ph-like ALL and non-Ph-like ALL (5-year EFS 90.0% vs 88.4%, $P = 0.41$, respectively) using MRD-directed therapy intensification for relevant patients [94]. Consequently, a significant higher proportion of patients with Ph-like ALL underwent HSCT in first remission due to end-induction MRD positivity [94], which is known to occur in two-thirds of children with Ph-like ALL [55]. These results demonstrate the therapeutic efficacy of HSCT in patients with Ph-like ALL and suggest that MRD is also an important outcome predictor in this patient population. Conversely, HSCT in first complete remission did not improve the EFS and OS of children with Ph-like ABL class ALL, and HSCT-related mortality was particularly high (17%) [61]. Another single-center study reported that adult patients with Ph-like ALL fare comparably poorly even

when they achieve post-remission MRD negativity (median OS for MRD+ group 23.0 months vs MRD- group 26.2 months; $P = 0.318$). Decisions for HSCT in first CR for adult patients may be challenged in the current era where pediatric-inspired chemotherapy regimens and access to frontline immunotherapy trials foster high hopes for inducing remission, deepening MRD response, and improving long-term survival [62, 95–98].

23.5.3 Antibody-Based and Cellular Immunotherapy

Major advances in immunotherapy during the past decade have revolutionized the landscape of relapsed leukemia therapy. The CD19xCD3 bispecific T-cell engager antibody blinatumomab, anti-CD22 antibody-drug conjugate inotuzumab ozogamicin, and CD19-redirected chimeric antigen receptor (CAR)-modified T-cell immunotherapy tisagenlecleucel have consecutively received FDA approval for patients with relapsed/refractory B-ALL based on several paradigm-shifting trials [97, 99–101]. Although the above trials did not specifically screen for the Ph-like ALL subtype, it is presumed that a reasonable proportion of these relapsed/refractory, heavily pretreated patients were Ph-like given their known high rates of chemoresistance and relapse. The randomized TOWER phase 3 trial (NCT02013167) showed that treatment with blinatumomab resulted in significantly higher remission rates and longer survival compared to standard chemotherapy among adults with relapsed/refractory Ph-negative B-ALL [97]. Subsequent results from the BLAST phase 2 trial (NCT01207388) demonstrated that the majority (78%) of MRD-positive adult B-ALL patients in first or later CR achieved a complete MRD response following 1 cycle of blinatumomab, which was associated with better outcomes than MRD non-responders [99]. Moreover, single-agent blinatumomab had strong anti-leukemic activity among adults with relapsed Ph+ ALL [102] and was associated with favorable treatment outcomes when compared to an external cohort receiving standard chemotherapy in a propensity score analysis [103]. Favorable safety profiles and anti-leukemic activity with blinatumomab monotherapy were also observed in a heavily pretreated relapsed/refractory pediatric B-ALL cohort [104, 105].

A recent retrospective analysis of 42 adults with R/R B-ALL who had available material for genomic analysis and were treated with blinatumomab monotherapy also showed that Ph-like ALL patients had a high response rate to blinatumomab (16/23; 70%), especially those with Ph-like ALL harboring *CRLF2* rearrangements (12/16; 75%) and non-*CRLF2* rearrangements (4/7; 57%) [106]. Small case series have also reported anecdotal safety and efficacy of combining blinatumomab and dasatinib in small numbers of patients with relapsed/refractory Ph + ALL or Ph-like ABL class ALL [76, 107–109]. The SWOG S1318 phase 2 trial (NCT02143414) is currently comparing the efficacy of blinatumomab with combination chemotherapy versus blinatumomab and dasatinib in older adults with ABL-driven leukemias. The GIMEMA D-ALBA LAL2116 phase 2 trial (NCT02744768) is also currently assessing rates of molecular remission after 2 cycles of blinatumomab and dasatinib consolidation therapy in adult patients with newly diagnosed Ph+ ALL. In an interim analysis with very early follow-up, 60% of study patients achieved a molecular response at the primary endpoint of approximately five months (three months of dasatinib and glucocorticoid chemotherapy followed by two cycles of blinatumomab). Additional improvement to 81% of molecular response after four cycles of blinatumomab was also reported with 1-year disease-free and overall survival of 88% and 95%, respectively, and 24 of the 63 enrolled patients received allogeneic HSCT [110]. Longer-term follow-up is needed to determine if these early favorable outcomes will be sustained. Concerns have been raised with respect to potential antagonism of blinatumomab–dasatinib combination, as dasatinib has been shown to inhibit T-cell function and could potentially abrogate the desired anti-leukemic activity of blinatumomab that requires endogenous T-cell engagement [108, 111, 112]. Correlative functional assays that comprehensively assess potential immunomodulatory effects of dasatinib upon immune cells will provide critical data for future trial design.

In a retrospective analysis of 53 adult patients with relapsed/refractory B-ALL treated with inotuzumab as salvage therapy, 12 patients identified as having the Ph-like subtype had an ORR of 58%, including three with CR (25%) and four with CR (33%) and partial hematologic recovery (CRh) [113]. Five of the seven (71%) Ph-like patients with inotuzumab-induced CR achieved MRD negativity [113]. Inotuzumab at the FDA-approved fractionated adult dosing of 1.8 mg/m^2 per cycle also induced impressive CR response rates among heavily pretreated children with relapsed/refractory B-ALL [114, 115]. In a retrospective study of 51 pediatric R/R B-ALL patients who received inotuzumab via a compassionate use program, the overall CR rate was 67%; three of four Ph-like ALL achieved CR/CRi, one of whom was MRD-negative [115].

Two recent studies showed that CD123 is more commonly expressed in adults with Ph+ ALL [116] and Ph-like ALL [117] compared to Ph-negative B-ALL and that CD123 may represent an additional therapeutic target for these high-risk leukemias. Anti-CD123 targeted immunotherapies (*e.g.*, IMGN-632 and flotetuzumab) have shown promising activity in preclinical models of acute myeloid leukemia (AML) [118–120] and are under current clinical investigation in early-phase trials for patients with relapsed/refractory AML.

Finally, anecdotal reports of CD19 chimeric antigen receptor (CAR) T-cell-induced remission in patients with relapsed/refractory Ph+ and Ph-like ALL have been reported [101]. The current COG AALL1721 phase 2 trial (NCT03876769) assessing the efficacy of tisagenlecleucel in patients with newly diagnosed HR B-ALL with end-consolidation MRD positivity excludes patients treated with kinase inhibitors, however. A planned phase 1 trial based upon promising preclinical data [121] will specifically investigate the clinical safety and preliminary efficacy of TSLPR-redirected CAR T-cell immunotherapy in children, adolescents, and young adults with *CRLF2*-rearranged/over-expressing leukemias, including Ph-like ALL and Down syndrome-associated ALL.

23.6 Conclusions and Future Perspectives

Ph-like ALL is now known to be a relatively prevalent subtype of B-ALL defined by its kinase-activated gene expression signature and associated genetic alterations. Children and adults with Ph-like ALL have historically experienced high relapse rates and inferior clinical outcomes despite best-available conventional chemotherapy. Compelling evidence now exists from an extensive preclinical body of work for incorporation of relevant TKIs in combination with chemotherapy for these high-risk patients, although results from clinical trials testing these strategies are not yet known. Although Ph-like ALL is thrice as common as Ph+ ALL in children and adolescents, its genetic heterogeneity with >70 fusions identified to date [6] represents a limiting factor in designing appropriately statistically powered, randomized controlled trials to assess potential TKI efficacy within the major ABL class and CRLF2/JAK pathway-mutant subsets. As some patients with Ph-like ALL (particularly those with *EBF1-PDGFRB* or *JAK2* fusions) are at high risk of induction chemotherapy failure, future efforts must focus on swift identification of these genetic alterations and TKI addition early in induction therapy. Such strategy has proven successful in children with Ph+ ALL with superior CR rates and MRD negativity with TKI addition mid-induction versus at the beginning of consolidation [15, 68]. Several immunotherapy modalities have now also demonstrated exciting efficacy in children with relapsed/refractory B-ALL. Ongoing and future clinical trials may help to elucidate the potential of such approaches (as monotherapy or combined with TKIs) more specifically in patients with Ph-like ALL.

International collaboration in designing the next generation of Ph-like ALL studies will expedite systematic study of novel treatment strategies that may improve clinical outcomes for this high-risk patient population [122]. Development and implementation of standardized clinical screening strategies among cooperative groups for rapid identification of patients with Ph-like ALL will also be essential for successful and efficient conduction of clinical trials. In parallel, future investigations in Ph-like ALL should also focus on investigating potential mechanisms of TKI resistance, as has been observed in patients with BCR-ABL1-driven chronic myeloid leukemia or Ph+ ALL with emergence of drug-resistant kinase domain mutations after long-term imatinib exposure [123–127]. Similar mutations have also been identified via in vitro saturation mutagenesis screens of Ph-like ALL with *EBF1-PDGFRB* fusions, and resistance mutations likely facilitating clinical relapse in patients with Ph-like ALL have now been reported [128, 129].

In summary, Ph-like ALL illustrates a paradigm of genomic discovery translation into targeted therapeutic approaches and presents an exciting opportunity for new precision medicine opportunities that aim to decrease relapse and improve long-term survival for patients with these high-risk leukemias across the age spectrum.Conflicts of InterestDr. Tasian receives research support from Incyte Corporation for Ph-like ALL studies.

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24 Allogeneic Hematopoietic Stem Cell Transplantation for Acute Lymphoblastic Leukemia

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Abstract

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is currently the standard of care (SOC) for adult acute lymphoblastic leukemia (ALL) patients. In this chapter, we will discuss the indication of allo-HSCT for ALL, donor selection, outcomes of ALL following allo-HSCT, prophylaxis, and prevention of relapse post-transplant, and impact of new immunotherapeutic agents on allo-HSCT.

Keywords

Acute lymphoblastic leukemia · Allogeneic hematopoietic stem cell transplantation

24.1 Introduction

Generally, the treatment of ALL includes induction therapy and post-remission therapy (Chaps. 20–23: Management of ALL). Allogeneic hematopoietic stem cell transplantation (allo-HSCT), combining myeloablative conditioning regimens with beneficial graft-versus-leukemia effect (GVL) mediated by donor T cells, has been the preferred options in the consolidation treatment of acute lymphoblastic leukemia (ALL), especially from human leukocyte antigen (HLA)-matched sibling donors (MSDs) or matched unrelated donors (MUDs), resulting in long-term leukemia-free survival (LFS) 40–80% [1, 2]. However, the shortage of MSDs and limited availability of MUDs (especially non-Caucasians) prevent large populations from benefiting from allo-HSCT [3, 4]. Recently, the rapid development of unmanipulated haploidentical HSCT (HID-HSCT) was confirmed equivalent to MSD or MUD-HSCT [5–9], which open a new era "Every ALL patient has a donor."

Meanwhile, instead of fierce competition with allo-HSCT, new immunotherapeutic agents, such as chimeric antigen receptor (CAR)-T cells and bispecific T-cell-engaging (BiTE) antibody (Chap.25: Immunotherapy for ALL), extended the applications of allo-HSCT in relapsed/refractory (R/R) ALL and enriched the post-transplant strategies for relapse management, which strengthened the critical role of allo-HSCT in the management of ALL.

In the following paragraphs of this chapter, we will focus on the allo-HSCT for ALL and try to address the controversial issues "Allo-HSCT for ALL-who and how?"

24.2 Indication of Allo-HSCT for ALL

The ultimate goal of ALL management is to reduce relapse and non-relapse mortality (NRM) and improve leukemia-free survival (LFS) and, possibly, health-related quality of life (HRQOL) (Chaps. 20–23). Therefore, the transplant decision is weighed against the reduction in risk of relapse and NRM, as well as evaluation of donor availability, depth of remission, comorbidities, and social support ([1]; NCCN guideline: Acute lymphoblastic leukemia. Version 2.2020 [10]; http://www.nccn.org; NCCN guideline: Pediatric acute lymphoblastic leukemia. Version 1.2020 [11]; http://www.nccn.org). According to the definition of the National Comprehensive Cancer Network (NCCN), ALL patients are divided into three subgroups as follows: (1) high-risk ALL (HR) generally refers to older adults (age ≥ 40 years old) or high-risk features, which consist of elevated WBC count (>30 × 10^9/L for B-cell lineage or >100 × 10^9/L for T-cell lineage) or high-risk cytogenetic abnormalities, such as Philadelphia chromosome (Ph) positive, hypodiploidy, t(v;11q23), a complex karyotype (≥5 chromosome abnormalities), or Ph-like type; (2) standard-risk (SR) ALL refers to adolescent and young adults (AYAs, age 15–39) with ALL in the absence of high-risk features; and (3) pediatric patients

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refer to children under age 14 (NCCN guideline: Acute lymphoblastic leukemia. Version 2.2020; http://www.nccn.org; NCCN guideline: Pediatric acute lymphoblastic leukemia. Version 1.2020; http://www.nccn.org). Allo-HSCT is beneficial for patients either in complete remission or refractory/relapsed state.

24.2.1 High-Risk ALL in First Complete Remission

The advantage of allo-HSCT over chemotherapy for ALL was first demonstrated in HR patients. In a randomized trial, allo-HSCT was superior to chemotherapy for patients with high-risk features (such as age ≥35 years and adverse cytogenetics) in terms of 5-year LFS (39% vs. 14%) and overall survival (OS) (44% vs. 20%) [12]. In the pre-tyrosine kinase inhibitor (TKI) era, allo-HSCT was superior to chemotherapy. In MRC UKALLXII/ECOG2993 trial, OS was 44% for MSD-HSCT, 36% for MUD-HSCT, and 19% for chemotherapy [13]. Allo-HSCT remained as standard care for Ph + ALL in the era of TKIs ([14]; NCCN guideline: Acute lymphoblastic leukemia. Version 2.2020; http://www.nccn.org) [15]. Wang J et al. retrospectively reported that for low-risk Ph+ ALL patients (account for 31.6% of all patients studied, defined as WBC <30 × 10^9/L at diagnosis and 3-log reduction of BCR-ABL levels from baseline after two consolidation cycles), there was no significant difference between the allo-HSCT and nontransplant groups for cumulative incidence of relapse (CIR), disease-free survival (DFS), and OS. However, in the intermediate- and high-risk groups (account for 68.4% of all patients), CIR, DFS, and OS rates were significantly better in the transplant arm than in the nontransplant arm, suggesting that allo-HSCT confers significant survival advantages for Ph+ ALL patients compared with TKIs plus chemotherapy [16]. In recent 3–5 years, Ph-like molecular features defined a new high-risk subtype of ALL characterized by kinase-activating alterations, which may account for 20–30% of patients and associated with inferior outcomes compared with non-Ph-like ALL (3–5 year LFS: 22–23% vs. 49–59%) . In addition, achieving minimal (measurable) residual diseases (MRD) negativity does not change inferior long-term outcomes in Ph-like ALL [17–21] (Chap. 23). Therefore, this group of patients might benefit from allo-HSCT while prospective data are needed [22].

24.2.2 Standard-Risk Ph-Negative ALL in First Complete Remission

The advantage of allo-HSCT over chemotherapy for Ph-negative ALL was firstly demonstrated in SR Ph-negative ALL in the UK ALLXII/ECOG E2993 and HOVON trials, which suggested that allo-HSCT with MSD or MUD was beneficial for SR Ph-negative ALL in terms of 5-year OS (62% vs. 52%, $P = 0.02$) and LFS (60% vs. 42%, $P = 0.01$) [23, 24]. Should SR Ph-ALL patients, who have relatively lower CIR and better LFS, pursue haplo-HSCT instead of consolidation chemotherapy in the absence of MSD or MUD? In retrospective study, Yan et al. suggested that haplo-HSCT may be superior to the adult chemotherapy regimen for SR Ph-negative ALL CR1 patients, as indicated by improved 5-year LFS (54% vs. 24%, $P < 0.0001$) [25]. In a multicenter prospective study of young adult Ph-negative ALL patients (aged 18–39 years) without high-risk features in CR1, HID-HSCT resulted in a lower 2-year CIR (12.8% vs. 46.7%, $P = 0.0017$) and better 2-year LFS (80.9% vs. 51.1%, $P = 0.0116$) and 2-year OS (91.2% vs. 75.7%, $P = 0.0408$) than adult chemotherapy (fractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone; hyper-CVAD). HID-HSCT was an independent risk factor related to reduced CIR (HR 0.195, $P = 0.001$), improved LFS (HR 0.297, $P = 0.003$), and OS (HR 0.346, $P = 0.011$) [26]. Therefore, HID-HSCT would be feasible for adult patients with SR Ph-ALL.

More recently, growing evidence suggested that pediatric-inspired regimens might further decrease the CIR of Ph-negative AYA ALL to 12–33%, with encouraging outcomes as 3–5 year LFS 59–73%, OS 60–79%, mainly in the age group of 15–21 years [27–29], which raised the question whether AYA with SR ALL treated with a pediatric-inspired regimen need allo-HSCT in CR1 or not. In a registry-based study, a total of 108 Ph-negative ALL CR1 patients who received a Dana-Farber Consortium pediatric-inspired non-HSCT regimen were matched with age to a cohort of 422 allo-HSCT recipients aged 18–50 years reported to the CIBMTR. The 4-year CIR was comparable between these HSCT and non-HSCT groups (23% vs. 24%; $P = 0.97$), as NRM was higher in the HSCT cohort (37% vs. 6%; $P < 0.0001$), and HSCT was inferior to chemotherapy in terms of LFS (40% vs. 71%； $P < 0.0001$) and OS (45% vs. 73%； $P < 0.0001$) [30]. However, it should be noticed that the NRM in this registry study (37%) was much higher than those in a similar group of patients in other trials (11–16%) [26, 31, 32]. Furthermore, Rytting ME et al. suggested that pediatric-based regimens (ABFM) resulted in similar CIRs compared with those achieved with adult chemotherapy regimen "hyper-CVAD" (37% vs. 38%) [33]. In addition, population-based studies revealed that adult regimens, such as hyper-CVAD, are still used in AYA ALL patients worldwide, even in developed countries such as the USA; only 11% of relatively younger YAs (age 19–29 years) and less than 1% of relatively older YAs (age 30–39 years) with ALL received the pediatric regimens [34, 35]. Therefore, the guidelines tried to recommend the regimens both in adult and pediatric settings (NCCN guideline: Acute lymphoblastic leukemia.

Version 2.2020; http://www.nccn.org; NCCN guideline: Pediatric acute lymphoblastic leukemia. Version 1.2020; http://www.nccn.org). Chinese Society of Hematology also suggests that the decision for allo-HSCT for adolescents who receive a chemotherapy protocol for pediatric patients should be made based on the appropriate guidelines for ALL (age ≤ 14 years) [15]. If a growing number of YA SR ALL patients (age 18–39 years) could receive pediatric-inspired regimens in the near future, it is worthwhile to perform well-designed prospective trials to investigate whether patients could benefit from introduction of allo-HSCT in addition to pediatric-based regimens.

24.2.3 Minimal Residual Diseases for Transplant Decision

In addition to the risk factors at diagnosis, minimal (measurable) residual diseases (MRDs) have emerged as a very powerful and dynamic prognostic tool that has to a large extent replaced traditional risk factors in guiding the decision to transplant [36]. GMALL 06/99 trial identifies a high-risk group of patients with MRD above 10^{-4} detected by quantitative polymerase chain reaction (PCR) in SR Ph-ALL after induction and/or consolidation, which had 3-year relapse rate (RR) of 94% compared with 47% in the remaining patients [37]. Even for the MRD- patients who completed 1 year of chemotherapy, 27% of patients converted to MRD+, and in which, 61% eventually relapsed [38]. These results aroused the question: If we could take MRD status into account for decisions of allo-HSCT? For HR Ph-negative ALL, Dhedin et al. suggested that allo-HSCT was associated with longer LFS in patients with post-induction MRD $\geq 10^{-3}$ but not in good MRD responders [39]. Therefore, the NCCN generally recommends that these patients, especially MRD+ patients and patients with a high risk of relapse, receive allo-HSCT, while the European Cooperative Group for Bone Marrow Transplantation (EBMT) emphasizes the importance of MRD+ status in this decision [1]. Chinese Society of Hematology suggests that adult ALL, no matter MRD status, is recommended to receive allo-HSCT [15].

24.2.4 Pediatric ALL in First Complete Remission

Generally, applications of allo-HSCT of pediatric ALL in CR1 focused on patients with high-risk features (such as positive MRD post-consolidation and unfavorable cytogenetics). Allo-HSCT is generally recommended for pediatric patients with MRD >1% post-induction or MRD >0.1% within 12 weeks after consolidation (NCCN guideline: Pediatric acute lymphoblastic leukemia. Version 1.2020; http://www.nccn.org). In addition, for high-risk T-ALL, Xu et al. reported that children who received transplants during CR1 exhibited a higher LFS (65.7% vs. 26.0%, $P = 0.008$) and lower relapse rate (19.8% vs. 56.7%, $P = 0.014$) compared with those patients who received transplants during non-CR1, indicating pediatric patients with T-ALL in CR1 benefit from HID-HSCT [40]. For pediatric patients with very high-risk Ph-negative B-ALL in CR1, HID-HSCT reduced the CIR (10.9% vs. 46.7%, $P < 0.001$) and improved the LFS rate (81.0% vs. 52.0%, $P = 0.005$) compared to chemotherapy [41]. For pediatric Ph+ ALL patients who received imatinib plus intensive chemotherapy, HSCT still improved the OS and LFS rates and the CIR in the high-risk group [42]. In non-infant children with t(v;11q23)/MLL-rearranged B-ALL, allo-HSCT could also improve LFS (89.5% vs. 52.2%, $P < 0.001$) and reduce CIR (5.3% vs. 74.1%; $P < 0.001$) compared to non-HSCT in CR1 [43].

24.2.5 ALL Beyond CR1

Allo-HSCT is the only potentially curable treatment for R/R ALL. However, the outcomes of allo-HSCT for R/R ALL in active state are suboptimal. The 3- to 5-year OS of allo-HSCT for R/R ALL was only 16–23% [44, 45]. Meanwhile, gaps between CR1 and CR2 were minimal compared to that between CR and R/R state [46, 47]. Therefore, the desired strategy for treating patients with R/R ALL is getting CR2 and then proceeding to allo-HSCT as the definitive curative approach. Chinese Society of Hematology suggests that all patients who exhibit very early or early relapse are candidates for allo-HSCT during CR2. All patients in CR3 are also recommended for allo-HSCT. Moreover, allo-HSCT can be as salvage therapy for refractory or relapsed ALL [15]. New immunotherapeutic agents, such as CAR-T and BiTE antibody, show superiority in re-induction and can be a bridge to allo-HSCT (Chap. 25 Immunotherapy for ALL).

24.3 Donor Selection

24.3.1 Matched Sibling Donors (MSDs) and Unrelated Donors (URDs) in ALL

In the pre-imatinib era, the prognosis of Ph-positive ALL patients who received only chemotherapy was poor. Outcomes with allo-HSCT from either MSDs or URDs appeared similar, and allo-HSCT improved disease control over intensive chemotherapy alone [48]. In the large, international, collaborative MRC UKALL XII/ECOG E2993 trial, both the OS (44% and 36%) and EFS (41% and 36%) for patients with MSDs allo-HSCT and matched URDs allo-HSCT outcomes were significantly improved compared with

those who received only chemotherapy (OS: 19%, EFS: 9%). The incidence of TRM was 27% with MSD allo-HSCT and 39% with matched URD HSCT [13].

The benefit of MSD allo-HSCT in adult patients with standard-risk Ph-negative ALL was also reported by the HOVON cooperative group. The donor arm was associated with a significantly reduced 5-year relapse rate (24% vs. 55%; $P < 0.001$) and a higher 5-year DFS rate (60% vs. 42%; $P = 0.01$) compared with the no-donor arm. In the donor group, the NRM rate at 5 years was 16% and the 5-year OS rate was 69% [23]. In a retrospective analysis of 169 patients who underwent URD HSCT in CR1, the 5-year survival was 39%, which is higher than survival reported in studies of high-risk patients receiving chemotherapy alone, suggesting that URD transplants may be an option for those patients [49].

24.3.2 Haploidentical Donor in ALL

Historically, MSD is superior to alternative donor (MUD, MMUD, and HID) in allo-HSCT treating ALL. However, the shortage of MSD prevents more ALL patients benefit from HID-HSCT. Since NRM has continuously improved with HID-HSCT with either ATG+G-CSF protocol (NRM 13–18%) [50] or PT-CY (NRM 7–22%) [51, 52] compared with early haplo-SCT procedures (30–54%) [53], the advantages of a low CIR and an acceptable NRM resulted in comparable, even better results of HID-HSCT compared with MSD-HSCT.

In the EBMT registry study of HID-HSCT for ALL CR1 with either anti-thymocyte globulin (ATG, 43%) or post-transplant cyclophosphamide (PT-Cy, 57%) as GVHD prophylaxis, the OS, LFS, and GVHD relapse-free survival (GRFS) rates were 52%, 47%, and 40%, respectively, suggesting HID-HSCT as a valid option for high-risk ALL lacking MSD [52]. Later in another registry-based study of HID-HSCT with PT-CY, haplo-HSCT was not associated with worse outcomes compared to MUD 10/10 and MMUD 9/10. For all kinds of ALL in CR1, haplo-SCT and 10/10 matched MUD-SCT resulted in comparable 3-year LFS (49% vs 55%, $P = 0.67$) and OS (54% vs 62%, $P = 0.41$) [7].

For HID-HSCT following ATG + G-CSF protocol, Wang Y et al. compared HID and MSD for HSCT in adults with Ph(−) high-risk ALL in a biological phase III randomized multicenter study. A total of 103 cases received HSCT from HID, and 83 received HSCT from MSD. There were no differences in 3-year DFS (61% vs. 60%, $P = 0.91$) from CR and 3-year OS (68% vs. 64%, $P = 0.56$) from HSCT, TRM (13% vs. 11%, $P = 0.84$), or CIR (18% vs. 24%, $P = 0.30$). Therefore, HID-HSCT is a valid alternative as post-remission treatment for high- and standard-risk adult patients with ALL in CR1 who lack an identical donor [9]. Han LJ et al. retrospectively investigated the outcomes of HID-HSCT in adults with SR Ph-ALL in CR1 and compared these patients to MSD and MUD patients. A total of 127 HID, 144 MSD, and 77 MUD recipients were enrolled in the study. There were no differences in grade III-IV acute graft-versus-host disease (aGVHD), 5-year TRM, CIR, OS, and DFS, or 3-year GRFS [31].

Would MSD always be the first donor choice? Possibly not. In a retrospective study, HID-HSCT was associated with a significantly lower relapse rate than MSD-HSCT (44.8% vs. 19.1%, $P < 0.05$), with no differences in NRM, LFS, or OS between the two groups [51]. For high relapse risk ALL, Chang YJ et al. conducted a phase III biologically randomized trial of ALL, and HID-HSCT reduced the 3-year CIR (23% vs. 47%, $P = 0.006$) and showed better LFS (65% vs. 43%, $P = 0.023$) and OS (68% vs. 46%, $P = 0.039$) compared to MSD-HSCT [50]. Li SQ et al. retrospectively studied Ph+ ALL with a positive pre-transplantation MRD, and HID-HSCT led to a lower 4-year CIR (14.8% vs. 56.4%, $P = 0.021$) and higher 4-year LFS (77.7% vs. 35.9%, $P = 0.036$) compared to MSD-HSCT [53]. These results indicate that HID-HSCT might be superior to MSD-HSCT in patients with high relapse risk ALL. In a multicenter study, 185 acute leukemia patients (≥50 years, 32% ALL) transplanted in the CR1 stage who received HSCT from offspring or MSDs were included to perform 1:1 ratio matched-pair analysis. The lower 3-year (9% vs. 26%; $P = 0.023$) and CIR (6% vs. 17%; $P = 0.066$) resulted in higher OS (85% vs. 58%; $P = 0.003$) and LFS (85% vs. 56%; $P = 0.001$) rates with offspring HSCT than with MSD-HSCT [54]. These data might indicate that a young offspring donor is preferred over an older MSD for patients >50 years old. Taken together, HID-HSCT might exert a stronger GVL effect and result in improved outcomes compared to MSD-HSCT.

The strategies to choose the ideal donor are summarized in Fig. 24.1.

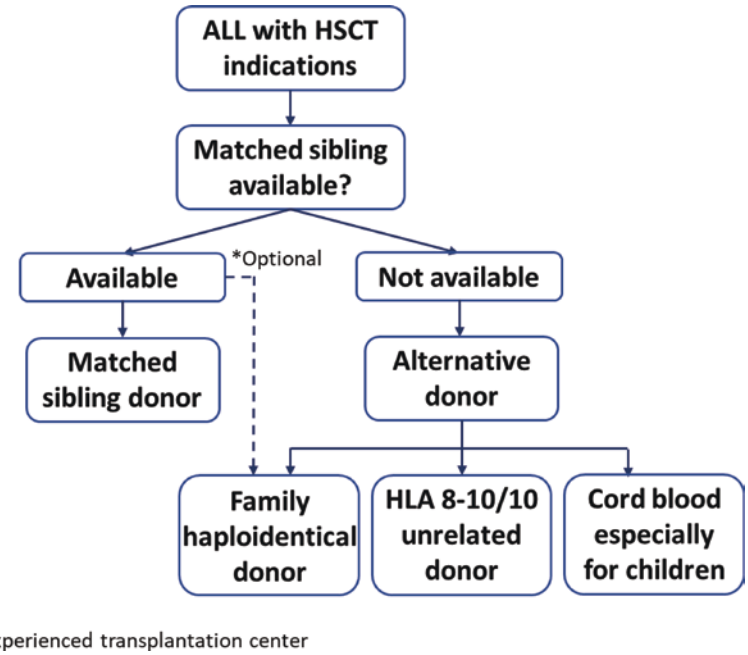

Fig. 24.1 Algorithm for ALL

24.4 Outcomes of ALL Following Allo-HSCT

24.4.1 MRD Before Transplant

Although HID-HSCT is superior to MSD-HSCT in treating ALL with MRD as mentioned above, should positive MRD be eradicated before HID-HSCT? In a cohort including 543 patients with ALL who underwent HID-HSCT, the levels of pre-MRD according to a logarithmic scale were also associated with leukemia relapse, LFS, and OS, except that cases with MRD <0.01% experienced comparable CIR and LFS to those with negative pre-MRD. A risk score for CIR was developed using the variables pre-MRD, disease status, and immunophenotype of ALL. The CIR was 14%, 26%, and 59% for subjects with scores of 0, 1, and 2–3, respectively ($P < 0.001$). Three-year LFS was 75%, 64%, and 42%, respectively ($P < 0.001$). These results indicate that HID-HSCT only overcomes the negative effect of positive pre-HSCT MRD except for low level (FCM < 0.01%) [55]. Therefore, it is better to pursue low-level or negative MRD status even with HID-HSCT. Impact of MRD after CAR-T cells further supported the need for eradicating MRD before bridging to allo-HSCT: Pretransplant MRD recipients had the lowest CIR and best LFS compared to the nontransplant group (17.3% vs. 67.2%; $P < 0.001$) and the pretransplant MRD+ group (17.3% vs. 65.8%; $P = 0.006$) [56].

24.4.2 Conditioning Regimen

Currently, both chemo-based and TBI-based conditioning regimens are frequently used in allo-HSCT. Total body irradiation (TBI) has been considered a standard backbone for myeloablative conditioning in adults with ALL, especially in high-risk patients [1, 57]. However, the use of TBI may be associated with increased risk of late adverse effects, including secondary solid tumors, which should be taken into consideration for AYA patients. Fu et al. compared TBI (770 cGy)/cyclophosphamide (Cy) and busulfan(Bu)/Cy protocol used in the current study in ALL patients following HID-HSCT, and no significant differences were found in the 2-year RR (26.5% vs. 32.3%, $P = 0.742$), 1-year NRM (12.6% vs. 16.2%, $P = 0.862$), 2-year OS (60.2% vs. 57.0%, $P = 0.937$), and 2-year LFS (57.9% vs. 56.6%, $P = 0.845$) [58]. Other studies also suggested that intravenous busulfan in combination with Cy has comparable results to TBI [31, 59, 60]. Thiotepa-based conditioning is also feasible and with LFS 57% and OS 66% [61]. For high-risk ALL in CR1 or beyond CR2, adding etoposide to CY/TBI reduced relapse and improved LFS (HR 0.58–0.76; $P = 0.01$) [62]. In setting of R/R state, donor-derived CAR-T cells are also feasible as part of the conditioning regimens [63–65] (Chap. 25).

GVHD is closely related to the GVL effect post-allo-HSCT. Thought development of acute GVHD (aGVHD) or

chronic GVHD (cGVHD) was associated with lower risk of relapse than no GVHD (HR 0.49–0.69). Increased TRM accompanied by grades III and IV aGVHD (HRs 2.69–3.91) abrogated any protection from relapse might result in an inferior OS. Patients with advanced ALL would benefit only from cGVHD with improved OS (HR 0.69–0.73) [66]. Therefore, the post-HSCT strategies for the balance between GVHD/GVL should be individualized according to the risk of relapse and TRM.

24.4.3 Risk Assessment for Patients Undergoing Allo-HSCT

In a retrospective registry study performed by the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation, a total of 131 ALL patients with detailed information were analyzed aiming to identify risk factors for post-transplant relapse and for OS after relapse. In ALL, risk factors for relapse were disease status different from the CR1 at haplo-HSCT (CR2 vs CR1: HR 2.85, $P = 0.011$; advanced vs CR1: HR 14.28, $P < 0.0001$) and male donor gender (HR 3.64, P = 0.0002) [67]. Peking University Institute of Hematology made a haplo-EBMT risk score with the parameters of the recipient's age, disease stage, interval from diagnosis to transplantation, donor–recipient gender combination, and number of mismatched HLA-A, HLA-B, and HLA-DR loci. The haplo-EBMT risk score was grouped from 0 to 8. The values for OS, LFS, and NRM were worse for an EBMT risk score of 6 (40.0, 40.0, 50.0%) than a score of 1 (83.1, 78.3, 8.4%). Hazard ratios steadily increased for each additional score point, and a higher EBMT risk score was associated with an increased relapse incidence [68]. Chang et al. also explored the combination of EBMT score-modified model and the hematopoietic cell transplantation comorbidity index (HCT-CI) in evaluating risk factors for patients undergoing allo-HSCT. Patients in groups with HCT-CI scores of 0 or 1–2 exhibited similar OS, NRM, and relapse rates, independent of their EBMT score-modified model. In the group in which patients' HCT-CI scores were ≥3, patients with high EBMT score-modified model showed lower OS ($P = 0.003$) and higher NRM ($P = 0.001$) than did patients with low EBMT score. This indicated that risk assessment model can be used to predict outcomes and may improve the stratification of high-risk patients following allo-HSCT.

24.5 Prophylaxis and Prevention of Relapse Post-Transplant

24.5.1 Maintenance with Target Drugs

Prophylaxis with target drugs, such as TKIs, might further improve the outcomes of ALL post-HSCT. In a prospective study of Ph+ ALL, imatinib treatment was scheduled for 3–12 months, until BCR-ABL transcript levels were negative at least for three consecutive tests or complete molecular remission was sustained for at least 3 months. The 5-year probability of LFS was superior in imatinib compared with control (81.5% vs. 33.5%; $P < 0.001$) [69] Maintenance therapy with dasatinib or ponatinib is also feasible post-allo-HSCT [70, 71].

24.5.2 MRD-Guided Pre-Emptive Therapy

Donor lymphocyte infusion (DLI), which induces an immune graft-versus-leukemia (GVL) effect, has been used to treat relapsed leukemia as well as management of MRD+ status after allo-HSCT. Huang's team established a modified DLI protocol [72] that includes the following: (i) the use of G-CSF mobilized peripheral blood stem cell harvests (G-PBSCs) instead of a steady lymphocyte infusion; (ii) the introduction of short-term immune suppressive agents, including cyclosporine A (CSA) or methotrexate (MTX), to further decrease the incidence of GVHD. A study conducted by Wang et al. investigated the effect of the prophylactic use of modified DLI on prevention of relapse following HLA identical transplantation in patients with advanced-stage acute leukemia. Among the patients who received prophylactic DLI, six of 12 patients with ALL survived without relapse. Among the patients who did not receive prophylactic DLI, only one of 25 patients with ALL survived without relapse [73]. In a multicenter, population-based analysis of 932 consecutive patients with advanced-stage acute leukemia after allo-HSCT, Wang et al. reported that the 3-year LFS rates were 56% for patients receiving both prophylactic/preemptive modified DLI and intensified myeloablative conditioning and 30% for those who received neither therapy. Prophylactic/preemptive DLI treatment was linked to significantly higher LFS than non-DLI for ALL patients without increasing the TRM. Prophylactic/preemptive DLI achieved superior outcomes in both MSDs and haplo-HSCT [74]. These studies suggested that the prophylactic/preemptive use of modified DLI can significantly increase survival of patients with advanced-stage, acute leukemia who receive allo- HSCT.

In addition to DLI, interferon-α serves as a powerful tool to prevent relapse post-allo-HSCT. Liu et al. identified the efficacy and safety of preemptive interferon-α (IFN-α) treatment in ALL patients who had MRD after allo-HSCT. The 4-year CIR and NRM after IFN-α treatment were 31.9% and 6.0%. The 4-year probabilities of DFS and OS after treatment were 62.1% and 71.1% [75]. Thus, preemptive IFN-α treatment could protect against relapse and improve long-term survival for ALL patients who had MRD after allo-HSCT.

24.5.3 Therapy Post-Relapse

MRD/GVHD-directed modified DLI is feasible for relapsed acute leukemia relapsing after allo-HSCT. Multiple chemotherapy and DLIs resulted in superior LFS (71% vs. 35%, $P < 0.0001$) and lower CIR (22% vs. 56%, $P < 0.0001$) compared with the control group. In multivariate analyses, no chronic GvHD after therapy (hazard ratio (HR) = 3.56; $P = 0.035$) and a positive MRD test after therapy (HR = 21.04; $P < 0.0001$) were associated with an increased CIR [76]. Sun et al. reported that chemo-DLI resulted in 60.9% of ALL patients with relapse after allo-HSCT achieved CR, which was comparable to that of AML patients [77], indicating that ALL patients who experienced relapse after haplo-HSCT can benefit from chemo-DLI.

24.6 Impact of New Immunotherapeutic Agents on Allo-HSCT

24.6.1 CAR-T May Broaden the Indications of Allo-HSCT in R/R ALL Patients

The emergence of CAR-T cell therapy makes R/R ALL patients more likely to receive allo-HSCT. Because of the high tumor load and malignancy, it is difficult to get CR in the induction phase in R/R ALL patients, and those patients may lose the opportunity to receive allo-HSCT and have a very poor prognosis. New immunotherapeutic agents, such as CAR-T cell therapy and bispecific T-cell-engaging (BiTE) antibodies, are new treatment strategies, which have been proven effective with relatively high CR rate in R/R ALL. Since a series of clinical studies have demonstrated that CAR-T cell therapy shows favorable response rate (ranging from 60% to 90%) in R/R B-ALL [78–81], those patients who achieve CR by CAR-T cell therapy may bridge to allo-HSCT, leading to a considerable outcome. Hu Y et al. reported that a total of 53 r/r B-ALL patients at the first stage received split infusions of anti-CD19 CAR-T cells and 88.7% of the patients achieved CR in first month. In the second stage, 21/47 MRD-CR patients without previous allo-HSCT received consolidative allo-HSCT within three months after CAR-T therapy. Event-free survival (EFS) and relapse-free survival (RFS) were significantly prolonged by allo-HSCT in the subgroups [82]. Pan J et al. also described that a total of 51 patients with B-ALL (42 patients with R/R B-ALL and nine patients with refractory MRD+ B-ALL) were under the treatment of CD19 CAR-T infusion. 90% of R/R patients achieved CR or CRi, and 85% (23/27) of CR/CRi patients bridged to allo-HCT remained in MRD- with a median follow-up time of 206 days, whereas nine of 18 CR/CRi patients without allo-HCT relapsed. In a phase I trial from the Fred Hutchinson Cancer Research Center (FHCRC), 62% of patients with R/R B-ALL who were transplanted while in MRD negativity remained in remission post-CAR-T therapy, compared to only 36% of patients who did not proceed to allo-HSCT [83]. An updated analysis of the trial showed that MRD-negative patients who proceeded to allo-HSCT had superior EFS compared to patients who did not [84]. Therefore, some investigators recommend that allo-HSCT in transplant-naïve R/R B-ALL patients should be considered while in remission after CAR-T cell therapy, which brings more effective therapeutic strategy and may prolong their EFS and RFS. The development of CAR-T may broaden the indication of allo-HSCT in managing R/R ALL, which can benefit these patients who cannot receive allo-HSCT before, so as to achieve long-term survival.

24.6.2 CAR-T in Relapse Post-Allo-HSCT

In the era without new immunotherapy, treatment options are extremely limited for patients with ALL who experience relapse after receiving consolidation with allo-HSCT, the outcomes were sometimes frustrating. Since the extension of new immunotherapy, CAR-T cell therapy can be used as a powerful means of preventing relapse after allo-HSCT. Peking University Institute of Hematology reported 83.3% of MRD-negative CR rate after HSCT donor-derived CAR-T cell infusion in patients with relapsed B-ALL after haplo-HSCT [85]. A study conducted by Huang XJ et al. also confirmed that despite the CR rates (85.7%) of CAR-T being high for relapsed patients after allo-HSCT, cumulative recurrence rate at 18 months was 68.3%, and the OS rate for the CR patients was 30.0% at 18 months, with a median OS of 12.7 months [86]. The study above indicated that for patients who relapsed after allo-HSCT, although a high CR rate was achieved after CAR-T therapy, additional treatment (including a second allo-HSCT) is necessary to further improve long-term efficacy after CAR-T infusion. CAR-T can be also used for patients with MRD positive but no response to DLI post-allo-HSCT. Huang XJ et al. also confirmed that donor-derived CAR-T was effective for patients with MRD but no response to DLI in B-ALL after allo-HSCT with 83.33% of MRD-negative remission. Thus, CAR-T cell therapy can be used as a powerful treatment strategy for relapse after allo-HSCT.

24.6.3 CAR-T Therapy Challenges to Allo-HSCT Indications

The application of new immunotherapy (such as CAR-T) is a challenge for allo-HSCT indications. Allo-HSCT is still the standard care for patients with high-risk ALL in CR1, and CAR-T cell therapy currently cannot replace allo-HSCT as

first-line treatment. However, for standard-risk Ph-negative ALL CR1 patients, the timing for receiving allo-HSCT is controversial, mainly depending on MRD status. In general, MRD positivity at the end of induction predicts high relapse rates and should prompt evaluation for allo-HSCT. With the popularization of CAR-T in clinical application, it is a meaningful question whether MRD + ALL patients need to receive allo-HSCT after CAR-T cell therapy. Meanwhile, whether allo-HSCT could be postponed to CR2 or not after CAR-T therapy for standard-risk Ph-negative ALL with MRD negative in CR1 is also a question that prompts us to discuss. Currently, there are no data available to answer this hypothesis, but it seems that CAR-T cell therapy may be a potential factor that reduces allo-HSCT. In addition, new immunotherapeutic agents could help relapsed patients achieve CR2 post-allo-HSCT, which might be bridged to second transplantation.

24.7 Conclusion and Perspectives

Allo-HSCT plays the key role in curing ALL, and now, it is possible to offer transplant for every patient with HSCT indications, with all available donor sources, including MSD, MUD, and HID. Application of new immunotherapeutic agents such as CAR-T and BiTE antibody strengthened the function of allo-HSCT, which together improve the long-term outcomes of ALL patients.

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Immunotherapy for ALL

25

Wei Sun and Xiao-Jun Huang

Abstract

Acute lymphoblastic leukemia (ALL) is a kind of malignant disease derived from hematologic stem cells. Intensive induction/consolidation chemotherapy followed by allogeneic hematopoietic stem cell transplantation (allo-HSCT) is currently the standard of care (SOC) for adult patients. Recently, several new immunotherapies have shown promising efficacy for relapsed or refractory (r/r) ALL patients in early-phase clinical trials. Based on the outstanding outcomes in the treatment of r/r ALL, immunotherapies are believed to have broad prospects in the next 5 years. In this chapter, we discuss the role of immunotherapy in the clinical biology and treatment of ALL.

Keywords

Acute lymphoblastic leukemia · Allogeneic hematopoietic stem cell transplantation · Immunotherapy · Chimeric antigen receptor T cells · Bispecific T-cell-engaging antibody · Antibody–drug conjugate · Natural killer cells · Donor leukocyte infusion

25.1 Overview

Acute lymphoblastic leukemia (ALL) is a malignant (clonal) disease of the bone marrow in which early lymphoid precursors proliferate and replace the normal hematopoietic cells of the marrow. In recent years, there has been a significant progress in ALL outcomes; some subgroups remain poorly prognosis with early relapse. Faced with these challenges, there is great interest in novel, targeted approaches to therapy. A number of new immunotherapeutic agents have been shown to be very effective in relapsed or refractory (r/r) ALL. Chimeric antigen receptor (CAR) T cells, a form of novel immunotherapy in which adoptive transferring of genetically modified effector T cells, show high response rate in the treatment of r/r ALL. Blinatumomab, a bispecific T-cell-engaging (BiTE) antibody against CD19, and inotuzumab ozogamicin, an anti-CD22 antibody–drug conjugate, have proven to be efficacious based on current clinical trials. In addition, NK cell therapy and CAR-NK cell therapy also show promising outcome in ALL. In this chapter, we discuss the role of immunotherapy in the clinical biology and treatment of ALL.

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25.2 CAR-T Therapy

25.2.1 Overview of CAR-T Cell Therapy

Chimeric antigen receptor (CAR) T cells are T cells that have been genetically engineered to produce an artificial T-cell receptor for use in immunotherapy. CARs typically link an extracellular, antigen recognition molecule comprising antibody domains (a single-chain Fv, scFv, containing the variable domains of the light and heavy chains), a stalk-like region, a transmembrane region, and intracellular signaling domains derived from proximal T-cell signaling machinery [1]. CAR is a core component of CAR-T cells, which enable T cells to recognize tumor antigens in an HLA-independent manner. CAR-T cells can recognize antigens faster and more widely than traditional T-cell surface receptor (TCR) [2] (Fig. 25.1).

In 1989, Gross et al. reported for the first time that chimeric TcR chains composed of immunoglobulin V region and TcR C region could be produced and functionally expressed in T cells. The chimeric receptor provides antibody-like specificity for T cells and is capable of effectively transmitting signals for T cells to activate and perform their effector functions in a non-MHC-restricted manner, which is the prototype of CAR-T cells [3]. Four years later, Eshhar et al. for

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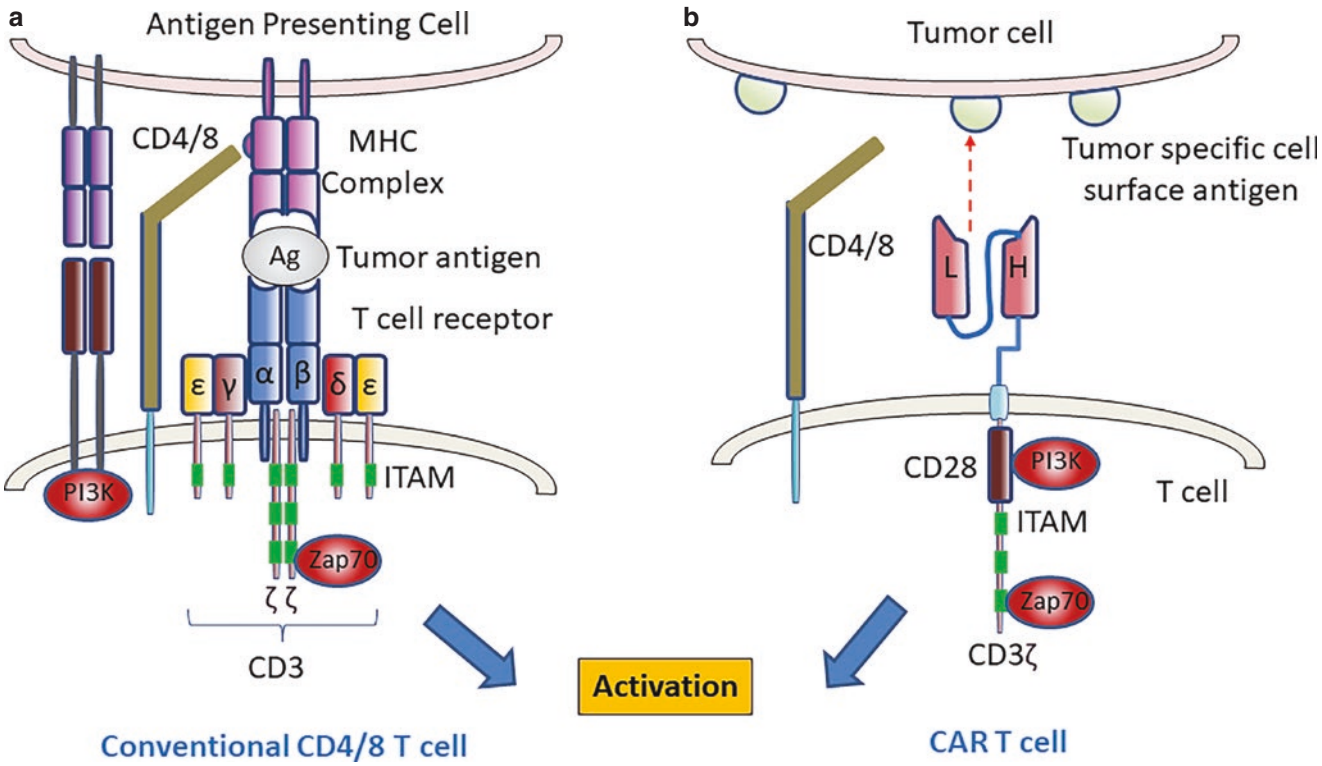

Fig. 25.1 Structure of CARs and T-cell receptors. (**a**) The structure of a T-cell receptor, which consists of heterodimeric and antigen-specific α and β chains that closely associate with the invariant ε, δ, γ, and ζ chains of the CD3 complex. The T-cell receptor binds to the HLA allele that has a bound peptide derived from a tumor antigen on the target cell. (**b**) The structure of a CAR, which includes the single-chain variable fragment (scFv) that binds to tumor antigens, fused to a spacer and transmembrane domain. The intracellular domain contains costimulatory domains, such as CD28 and 4-1BB and the CD3ζ chain, which drive signal activation and amplification of CAR T cells. S–S denotes disulfide bond

the first time combined the single-chain Fv (scFv) of antibody molecules with the constant region domain of TCR to synthesize the first-generation CAR-T cells [4]. These CAR-T cells could trigger IL-2 secretion and mediate the lysis of non-MHC-restricted antigen-specific target cells in response to antigens [5–7]. However, the first generation of CAR-T cells cannot be persistent for a long time, limiting their clinical application [8, 9]. On the basis of the first-generation CAR-T cells, the second-generation CAR-T cells added an ITAM region from the co-stimulatory molecules CD28 or CD137 (4-1BB) in the intracellular segment. The antigen recognition region outside the cell binds to the target antigen, allowing T cells to simultaneously receive antibody stimulation signals and co-stimulation signals [10, 11]. In 2013, the world's first CAR-T cell treatment of adult acute lymphoblastic leukemia (ALL) clinical study was published. The third-generation CAR-T cells simultaneously incorporate two or more co-stimulating domains (usually CD28 and 4-1BB or OX40) into the same CAR [12–14].

From the publication of the world's first clinical study of CAR-T cell therapy for the treatment of adult ALL in 2013 to the FDA approval of Kymriah (tisagenlecleucel) for certain pediatric and young adult patients with a form of progress of CAR-T therapy in 2017, clinical study on CAR-T therapy has sprung up in the United States, China, and other countries in the world in recent years.

25.2.2 Current Status of CAR-T Cell Therapy in ALL

A series of clinical studies have demonstrated that the approved CAR-T cell therapy shows favorable response rate in relapsed or refractory B-ALL. In a single-center phase I–IIa study by Grupp SA et al. in 2014, a total of 30 children and adults received anti-CD19 CAR T-cell therapy tisagenlecleucel, and complete remission (CR) was achieved in 27 patients (90%) [15]. Later in 2018, Grupp SA et al. reported a phase II, single-cohort, 25-center, global study of tisagenlecleucel in pediatric and young adult patients with CD19+ r/r B-cell ALL. The overall remission rate within 3 months was 81% [16]. Studies from other centers have also demonstrated the best rates of CR rate in r/r B-ALL after receiving tisagenlecleucel, ranging from 67% to 93% [15–18]. Fry

et al. reported that patients with acute B lymphocytic leukemia who relapsed after receiving CD19 CAR-T cells were treated with CD22 CAR-T cell therapy. The complete remission rate reached 73%, and the median remission time was 6 months [17]. Hu Y et al. reported that a total of 53 r/r B-ALL patients received split infusions of anti-CD19 CAR-T cells. The overall 1-month remission rate of the 53 patients was 88.7% [19]. Qian C et al. also observed that a total of 10 r/r ALL patients were treated with the second-generation CD19 CAR-T cells. Six patients (60%) achieved complete remission [20]. Thus, CD19 or CD22 CAR-T cell therapy has been proved as a powerful treatment options for r/r B-ALL.

25.2.3 Modification of CAR-T Cell Therapy

Following CAR-T cell therapy, ALL patients have a high risk of relapse. Antigen loss is one of the reasons among tumor escape after CAR-T cell therapy. Multi-specific target CAR-T and humanized CD19 CAR-T may provide strategies for preventing relapse.

One approach to prevent antigen loss following CAR-T cell therapy is to simultaneously target more than one tumor-associated antigen with multi-specific CAR-T cells (bicistronic dual-target T cells, sequential administration of CAR-T cells and cocktail CAR-T cells). For example, one adult patient with r/r B-ALL after HSCT was administered bi-cistronic CAR-T cells targeting both CD19 and CD22. This patient has remained in minimal MRD remission for more than 14 months [21]. In one study, 15 patients with CD19 + CD22+ r/r B-ALL were treated with a cocktail CAR-T cell infusion with CD19 CAR-T cells and CD22 CAR-T cells, and 15/15 (100%) cases achieved CR or CRi, showing technical feasibility, high efficacy, and low toxicities of CD19 and CD22 CAR-T cell cocktail in treating patients with CD19+CD22+ relapsed/refractory B-ALL [22]. Moreover, a group of Chinese researchers also revealed that sequential infusion of anti-CD22 and anti-CD19 CAR-T cell therapy is feasible and safe for r/r B-ALL. A total of 27 patients with r/r B-ALL were enrolled, and 24/27 (88.9%) patients achieved CR or CRi. OS and EFS at 6 months are 79% and 72%. Antigen escape of CD19 and CD22 was not detected in any relapsed patient post-CAR-T cell therapy [23].

One of the possible reasons for CAR-T cell loss is that the scFvs of CD19 CAR in published clinical trials are primarily derived from murine FMC63 or SJ25C1 antibodies, while the immune response induced by murine scFv of the CAR may limit CAR-T cell persistence, thus increasing the risk of leukemia relapse. Xu kailin et al. reported that a number of 18 r/r ALL patients with or without prior murine CD19 CAR-T cell therapy were treated with humanized CD19 CAR-T (hCART19). Among the 14 patients without previous CAR-T cell therapy, 13 (92.9%) achieved CR or CRi on day 30, whereas 1 of the 3 patients who failed a second murine CAR-T cell infusion achieved CR after hCART19s infusion. For CRS, 13 patients developed grade 1–2 CRS, 4 patients developed grade 3–5 CRS, and 1 patient experienced reversible neurotoxicity [24]. This study demonstrated that reducing the immunogenicity of CARs by using humanized scFv may improve the longevity of CAR-T cell persistence and enhance their therapeutic efficacy in patients. They also report using hCART19 to treat two newly diagnosed untreated adults with B-cell ALL. CR was achieved after CAR-T cell infusion. No recurrence was observed with follow-up for 31 and 21 months, respectively [25]. In conclusion, with the approval of CD19 CAR-T cells by FDA in USA, significant therapeutic effects have been shown in CAR-T cell therapy.

25.2.4 Application of Allogeneic CAR-T Cell Therapy

In recent years, more and more researchers focus on allogeneic CAR-T cell therapies, which include HSCT donor-derived CAR-T cells and universal off-the-shelf CAR-T cells. As for HSCT donor-derived CAR-T cells, allo-CAR-T cell therapy can be used as a bridge to HSCT, as conditioning regimen and as a powerful means in preventing relapse after HSCT. Pan J et al. described an approach for "CAR-T cells bridging to HSCT" in r/r B-ALL. A total of 51 patients with B-ALL (42 patients with R/R B-ALL, 9 patients with refractory MRD+ B-ALL) were under the treatment of CD19 CAR-T cell infusion. 90% of R/R patients achieved CR or CRi, and 100% of FCM-MRD+ patients achieved MRD with mild to moderate CRS. 85% (23/27) of CR/CRi patients bridged to allo-HCT remained in MRD with a median follow-up time of 206 days [26]. Therefore, CAR-T cell therapy bridging to HSCT in r/r B-ALL shows high response rate and safety. Similarly, HSCT donor-derived CAR-T cells can be also used as the conditioning regimen under the circumstances of HSCT. A group of researchers reported a case, whom was a 12-year-old girl with CD19 r/r ALL preparing to underwent HSCT. The patient received Flu, Bu, and Cy combined with the same haplo donor-derived CD19-CAR-T cells as the conditioning regimen. The highest level of the allogenic CAR-T cells in blood reached on Days 7, and no blast cells were detected on day +22 after transplantation, suggesting that treatment of r/r ALL with RIC including CD19-CAR-T cells followed by haplo-HSCT was safe and effective [27]. As a powerful means in preventing relapse after HSCT, CAR-T can be used for patients with relapsed B-ALL, for patients with MRD-positive but no response to DLI and for prophylactic infusion in patients with high-risk

B-ALL. For example, Peking University Institute of Hematology reported 83.3% of MRD-negative CR rate after HSCT donor-derived CAR-cell infusion in patients with relapsed B-ALL after haplo-HSCT [28]. Coincidentally, the team also confirmed that donor-derived CAR-T cells was effective for patients with MRD no response to DLI in B-ALL after HSCT with 83.33% of MRD-negative remission, half of the patients currently alive without leukemia. Zhang C, et al. treated two patients of high-risk B-ALL with preventive infusion of donor-derived CD19-CAR-T cells on days 60 and 61 after allo-HSCT. No CRS or GVHD developed, and the CAR-T cells could continually be detected. The patients survived for 1-year and 6-month disease-free, respectively, indicating that prophylactic donor-derived CAR-T cell infusion is effective and safe in high-risk B-ALL after haplo-HSCT [29].

As for universal off-the-shelf CAR-T cells, the most widely studied allogeneic universal adoptive T-cell therapy targeting CD19+ malignancies by scientists is UCART19, which is genetically engineered CD19CAR/RQR8+ TCRαβ-T cells. Anti-CD19 + T-cell activation modules (CD3z + 4-1BB) are designed CAR specific for the tumor, RQR8 (CD20 epitope) is to trigger T-cell destruction by rituximab if required, while TCR knock-out is to prevent GVHD and CD52 knock-out is to confer resistance to alemtuzumab and a longer lymphodepletion. Comparing to autologous CAR-T cells, UCART19 may be more cost-effective and suitable for patients who fail or cannot wait for autologous CAR-T cell manufacturing. In a phase I, open-label, non-comparative study of UCART19 in pediatric patients with r/r B-ALL, preliminary results showed that 6/7 (86%) of the patients achieved CR/CRi on day 28. However, long-term follow-up is not satisfied, with only one patients in molecular remission, while two patients relapsed and died after allo-SCT, one died from post-transplant complications and one relapsed at day 42 but alive, indicating that UCART19 has promising application foreground, but the safety, large-scale feasibility, and efficacy remain to be determined.

25.2.5 Challenges of CAR-T Cell Therapy

It seems that CAR-T cell therapy has a promising therapeutic efficacy and can achieve high response rate in r/r B-ALL. Some issues still need to be addressed when patients are eligible for CAR-T cell therapy. Insufficient lymphocyte apheresis, poor lymphocyte viability, limited expansion ex vivo, and disease progression during the manufacturing process are factors that will prevent patients from receiving CAR-T cell therapy. Furthermore, toxicities such as CRS, on-target off-tumor recognition, and neurotoxicity are inevitable after patients receiving CAR-T cell therapy. Additionally, the most important point is that B-ALL patients have a high risk of disease recurrence following CAR-T cell therapy, with 41% of responders relapsing within 12 months in one study [16]. Park JH, et al. demonstrated that patients with CAR-T infusion have short EFS and OS in a long term, with a median EFS and OS of 6.1 months and 12.9 months, respectively [30]. A study conducted by Huang XJ et al. also confirmed that despite the CR rate is relatively high for relapsed patients after HSCT, cumulative recurrence rate at 18 months was 68.3%, and the OS rate for the CR patients was 30.0% at 18 months, with a median OS of 12.7 months [31]. Therefore, CAR-T cell therapy shows significant short-term effect and favorable response in patients with B-ALL that relapse. However, the long-term outcome is unsatisfied, and other treatments need to be developed for prevention of recurrence. Currently, CAR-T therapy cannot replace HSCT. In conclusion, CAR-T is another platform following chemotherapy and HSCT, which is beneficial to comprehensive therapy.

25.3 Bispecific T-Cell-Engaging (BiTE) Antibody

Bispecific T-cell-engaging (BiTE) antibody is one of the two antitumor therapies which involve T-cell activation in a targeted and MHC-independent way. The other one is adoptive transferring of genetically modified chimeric antigen receptor T cells (CAR-T cells). Theoretically, BiTE and CAR-T cells are infinite in quantity since they could be customized given so many tumor-associated antigens available. BiTE antibody consists of two antigen-binding fragments of the antibodies specific for CD3 and tumor-associated antigen. Upon binding to their respective ligands, tumor cell closely approaches to an simultaneously activated T cells which is triggered by the CD3 binding on the other end of this BiTE [32] (Fig. 25.2). Till recently, this therapy was convinced of its feasibility and efficacy merely in CD19+ B-cell malignance. BiTEs targeting other molecules are being investigated at preclinical stage. As for ALL, there are two BiTE being developed, namely CD20-TDB and blinatumomab (AMG103).

CD20-TDB is a full-length humanized immunoglobulin G1 protein, which targeting CD20 and CD3. In preclinical study of monkey model, a single dose of 1 mg/kg CD20-TDB killed B cells in not only peripheral blood but also lymphoid tissues. Besides, the pharmacokinetic features of this BiTE antibody were comparable to those of conventional monoclonal antibodies [33]. Blinatumomab (AMG103) is a

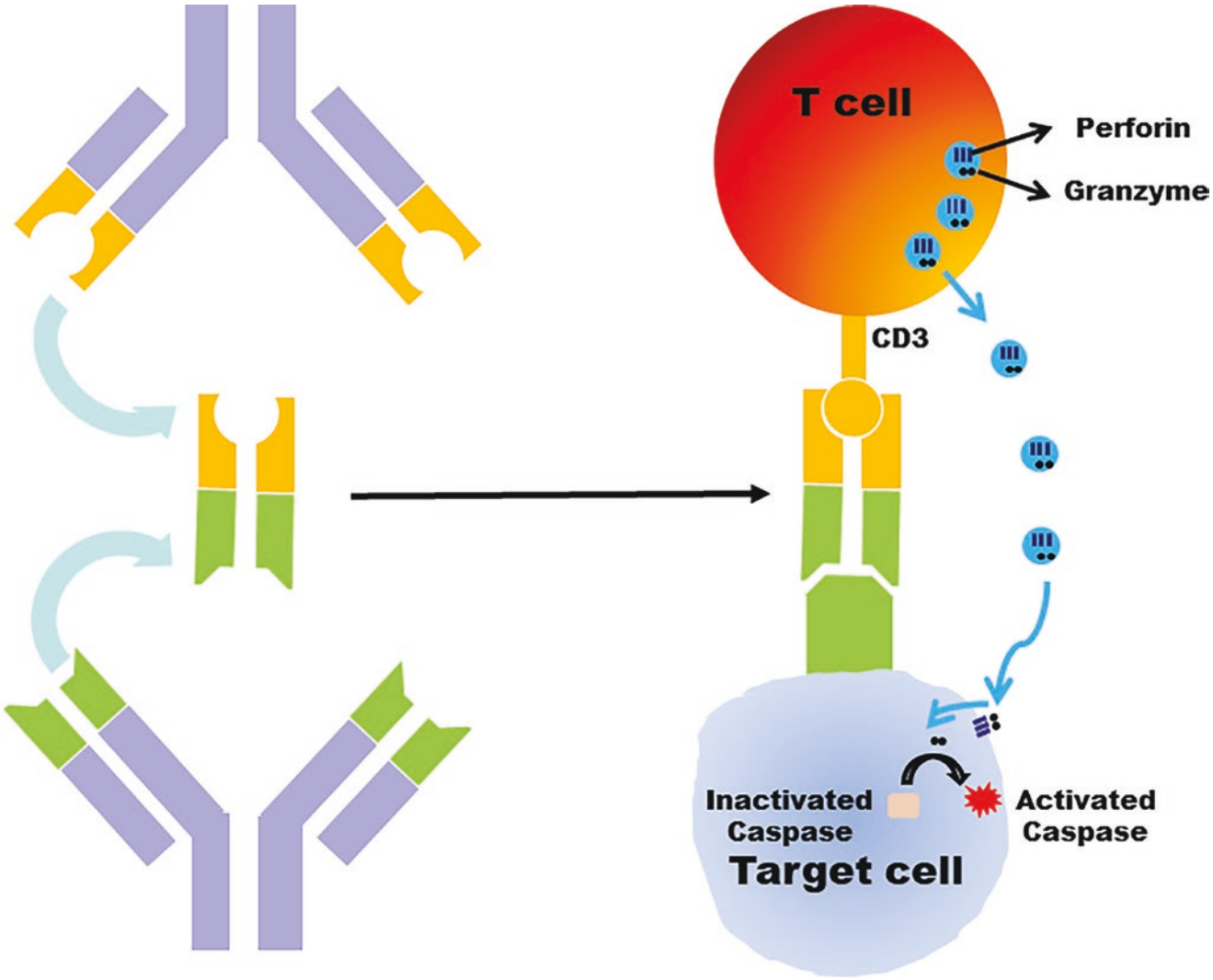

Fig. 25.2 Structure of BiTE antibody. BiTE antibody consists of two antigen-binding fragments of the antibodies specific for CD3 and tumor-associated antigen. Upon binding to their respective ligands, tumor cell closely approaches to an simultaneously activated T cells which are triggered by the CD3 binding on the other end of this BiTE

BiTE targeting on CD19 on B cell and CD3 on T cell. In a phase I clinical trial enrolling MRD-positive B-ALL, blinatumomab exhibited promising response regardless of MRD after chemotherapy [34]. In a following phase II trial enrolling r/r pre-B-ALL patients, blinatumomab improved the efficacy significantly compared to standard therapy, with ORR of 69% and mOS of 9.8 months [35]. In another multicenter phase II trial which contributed to the FDA approval of blinatumomab to treat Ph(−) r/r pre-B-ALL, CR rate was 32%, median remission time was 6.7 months, and 31% of the patients had MRD-negative response, while the toxicity is controllable [36]. The efficacy of blinatumomab in ALL patients has been extensively studied. There are more than 50 trials registered on ClinicalTrial online platform. These include the phase III trial of blinatumomab for the recurrent ALL patients, the phase II trial for recurrent Ph+ ALL, and the first-line treatment for MRD+ ALL. Especially, the phase III clinical trials are listed in Table 25.1.

Table 25.1 Phase III clinical trials of blinatumomab for the recurrent ALL

Clinical trials	Intervention	Patients	Status	N	Phase	Results	Start date	Primary completion date
NCT03476239	Premedication with dexamethasone + blinatumomab	Acute lymphoblastic leukemia	Active, not recruiting	121	III	CR/CRh rate within two treatment cycles: 45.6% (95CI 35.0% to 56.4%) in 90 patients; RFS 12.4 months; MRD response 34.1% (95CI 20.1% to 50.6%) in 41 patients;	2017/10/18	2019/8/21
NCT02013167	Blinatumomab VS standard of care chemotherapy	Relapsed/refractory B-precursor acute lymphoblastic leukemia	Terminated	405	III	OS: 7.7(5.6 to 9.6) VS 4.0 (2.9 to 5.3) months	2014/1/3	2015/12/29
NCT02003222	Blinatumomab+chemotherapy VS chemotherapy	Acute lymphoblastic leukemia|B Acute lymphoblastic leukemia, philadelphia chromosome-negative	Active, not recruiting	488	III	No results available	2013/12/23	2021/6/30
NCT04530565	Steroid+TKI VS steroid+TKI+ chemotherapy VS steroid+TKI + chemotherapy+blinatumomab VS steroid+TKI + chemotherapy +blinatumomab VS steroid+TKI + chemotherapy	B acute lymphoblastic leukemia with t(9;22)(q34.1;q11.2); BCR-ABL1	Not yet recruiting	330	III	No results available	2020/10/14	2028/7/1
NCT02101853	Blinatumomab VS chemotherapy	Recurrent B acute lymphoblastic leukemia	Active, not recruiting	598	III	No results available	2014/12/8	2022/12/31
NCT03914625	Blinatumomab +chemotherapy	B acute lymphoblastic leukemia| B lymphoblastic lymphoma| Down syndrome	Recruiting	6720	III	No results available	2019/6/28	2027/6/30
NCT03643276	Blinatumomab +chemotherapy	Acute lymphoblastic leukemia, pediatric	Recruiting	5000	III	No results available	2018/7/15	2028/7/14
NCT02393859	HC3 chemotherapy VS blinatumomab	Leukemia, acute lymphoblastic	Active, not recruiting	108	III	As of the data cutoff date (July 17, 2019), overall median follow-up time for EFS was 22.4 months	2015/11/10	2019/7/17

25.4 Antibody–Drug Conjugate (ADC)

Antibody–drug conjugate (ADC) is developed under the concept of selective delivery of antitumor agents based on monoclonal antibody (mAb). ADC is composed of three covalently linked components, namely a mAb highly specific to a tumor-associated antigen, a linker that is stable during delivery process while cleavable after being internalized by tumor cell, and a ultratoxic cargo of antitumor effector molecules. These effector molecules could be cytotoxic drugs, immunotoxins [37], radio-pharmaceuticals, and so forth [38] (Fig. 25.3).

The antibody of the ADC likes the guiding system of a missile, precisely aiming to tumor cells. As a consequence, the efficacy of the cytotoxicity is enhanced; on the other hand, the systemic exposure and the resultant toxicity are reduced to a great extent. Straightforward as the concept is, the development of an effective ADC takes extreme efforts. Early ADCs for clinical trials are based on mouse mAbs. Due to immunogenicity, limited potency, and nonspecific targeting, few success was achieved. Thereafter, breakthroughs of all these there aspects were made, and ADCs became real therapeutic weapon. Currently, there are more than 100 ADCs being evaluated in clinical trials around the world [39].

As for ALL, optimal targets for immunotherapy include CD20, CD19, CD52, CD22, and CD10. Currently, CD19- and CD22-based ADC are most studied for ALL [40]. SAR3419 is a humanized ADC targeting CD19. It is composed of a cytotoxic molecule called maytansine. Maytansine could bind to tubulin and prevent microtubule from assembling, thereby inhibiting mitosis [41]. In animal model of CD19 pre-B-ALL xenotransplantation, SAR3419 slows down the disease progression [42]. However, SAR3419 did not exhibit desirable efficacy in clinical trials. In a multicenter, single-arm phase II trial that enrolled r/r ALL patients, only 4 out of 17 patients responded with an ORR of 25.5% and a DOR of merely 1.9 months [43]. Due to relatively low efficacy, this trial was withdrawn prematurely. Another humanized anti-CD19 ADC is named SGN-CD19A. Its cytotoxic agent is monomethyl auristatin F, which also disrupts normal microtubule assembly and leads to G2-M cell cycle arrest. In phase I clinical trial (NCT01786096), SGN-CD19A showed excellent safety to r/r B-ALL and lymphoma

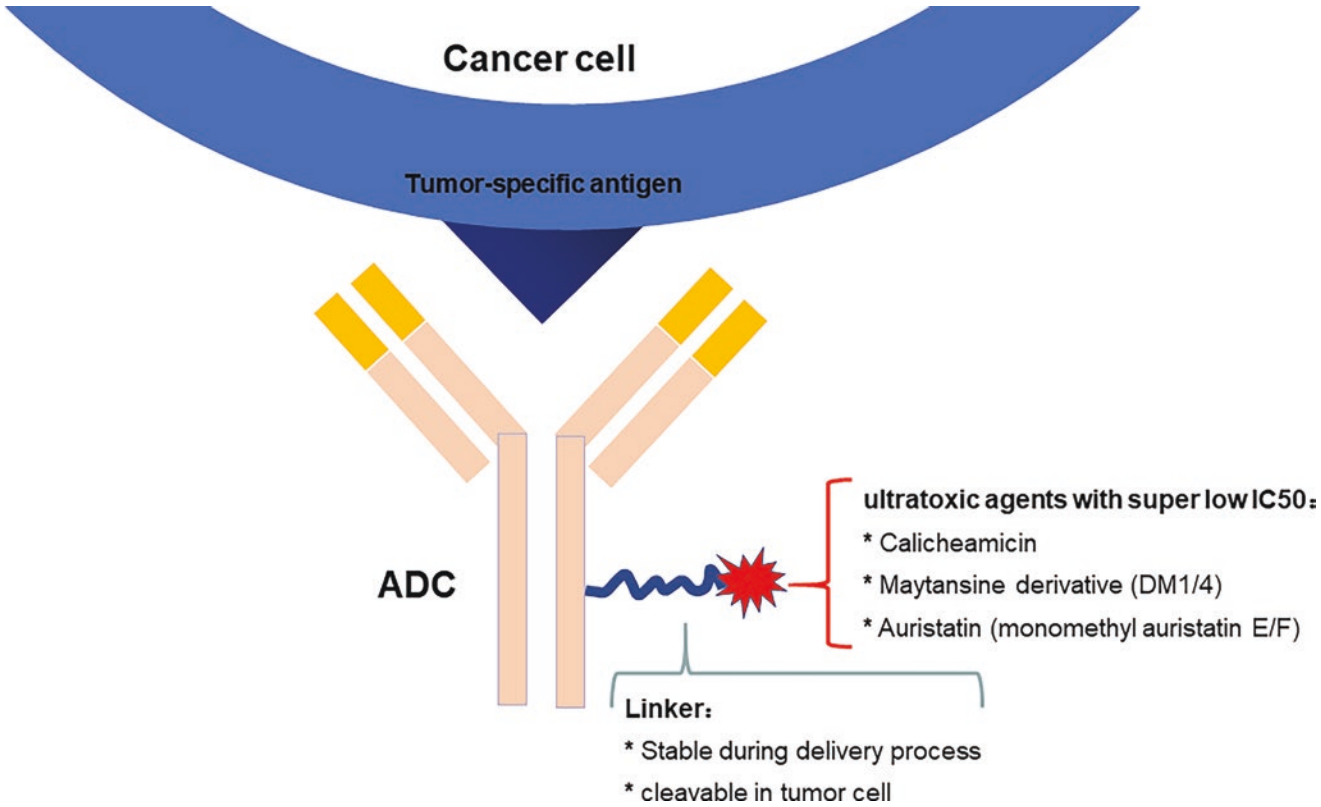

Fig. 25.3 Structure of antibody–drug conjugate (ADC). Antibody–drug conjugate (ADC) is developed under the concept of selective delivery of antitumor agents based on monoclonal antibody (mAb). ADC is composed of three covalently linked components, namely a mAb highly specific to a tumor-associated antigen, a linker that is stable during delivery process while cleavable after being internalized by tumor cell, and a ultratoxic cargo of antitumor effector molecules

patients. The efficacy is satisfactory with ORR of 30% in 33 evaluable patients. A third humanized ADC specific for CD19 is SGN-CD19B. Rather than inhibiting microtubules assembly, the coupling antitumor molecule pyrrolidine diazepine could introduce DNA cross-linkage. In preclinical studies of monkeys, SGN-CD19B exhibits potent efficacy to kill CD20 lymphocytes in both blood and lymphoid tissues. Currently, clinical trials of SGN-CD19B are ongoing attempting to validate its therapeutic values against ALL.

As for CD22, the corresponding ADC is inotuzumab ozogamicin (InO). This humanized ADC is bound to an antitumor agent called calicheamicin. This cytotoxic agent is internalized by tumor cells after the antibody bind to CD22 on cell membrane. Inside the cell, inotuzumab ozogamicin is released to induce double-strand DNA breakage, which causes apoptosis of the cell. In a phase III clinical trial which enrolled 326 r/r ALL patients, the experimental and control group of 1:1 randomization were subjected to InO or standard care of intensive chemotherapy [44]. The complete remission rate of InO and SOC group was 80.7% and 29.4% ($P < 0.001$), respectively. Patients in InO group showed a significant higher MRD-negative rate of 78.4% against 28.1% in the SOC group ($P < 0.001$). Both PFS and OS of InO group were much longer than those of SOC group, with median PFS of 5.0 months, and median OS of 7.7 months comparing to 1.8 months and 6.7 months. Notably, InO significantly enhanced the remission rate of r/r ALL patients, no matter whether the CD22 expression is above or below 90%. Besides, InO showed significant response rate to patients with both low and high tumor burden, unlike blinatumomab, which only responses well to patients with low tumor burden.

25.5 Natural Killer (NK) Cells

Natural killer (NK) cells belong to innate immune system, which exhibit prompting antipathogen or antitumor effect without prior sensitization. Its core functional mechanism is by censoring whether the target cells lost self-ID, namely major histocompatibility (MHC) class I molecules through an membrane-bound inhibitory killer immunoglobulin-like receptor (iKIR). If the target cell does not express self-MHC class I for iKIR to recognize and to produce inhibitory signal, the killing signaling will be unleashed by the activatory receptor on NK cell surface when it binds to the activating ligand to target cells (Fig. 25.4).

Earlier attempts to develop autologous NK cells for therapeutic use have failed due to limited efficacy. Those include in vivo IL-2 stimulation [45] and *ex vivo* stimulation of the NK cells followed by re-infusion of the cells back to the patients [46]. Currently, more research efforts have been

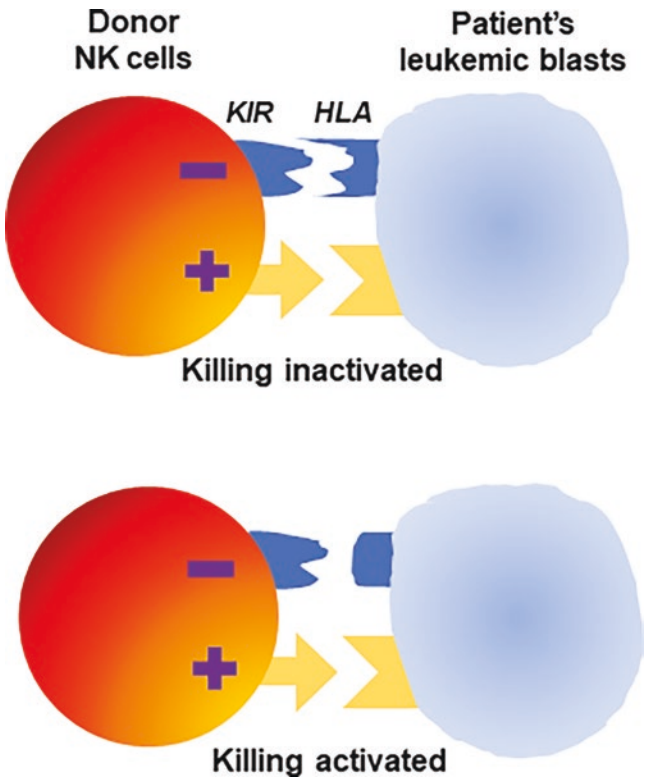

Fig. 25.4 Mechanism of NK cell recognition of target cells. When NK cells recognize self, they are held in check due to an interaction of killer immunoglobulin-like receptors (KIRs) with cognate major histocompatibility complex (MHC) class I molecules. Lack of self-MHC class I molecules on target cells triggers NK cells' cytotoxicity

shift to allogeneic cell therapy, in which NK cells derived from healthy donors were transferred to the patients after being expanded or modified ex vivo. Allogeneic cell therapy is based on the fact that iKIR on the donor NK cells mismatch the KIR ligands (MHC I) on the recipient's leukemic cells. The enhanced efficacy of this KIR-KIRL mismatched NK cells adoptive transfer therapy was firstly confirmed in adult acute myeloid leukemia (AML) [47–51]. For ALL, there are clinical trials being investigated (as the following table shows), but no solid data available yet to support whether the efficacy is improved.

With the remarkable success of CAR-T cells for treating hematological malignancies, there is a rapid growing interest in developing CAR-engineered NK (CAR-NK) cells for hematological malignancy therapy. Similar to CAR-T cells, the goal of CAR-NK cells is to establish a new activation pathway to enhance the antitumor effects of the cells and to improve tumor cell targeting. CAR-NK has the basic framework of CAR-T cells, including an extracellular antigen recognition region, a transmembrane region, and an intracellular signal domain. CD3ζ is a classical intracellular signal segment of the CAR structure [52] and plays an important role in NK cells [53]. CAR-NK generally uses CD3ζ as the first

signal motif (first-generation CAR) and then a costimulatory molecular motif (second-generation CAR), such as CD28 or CD137 (4-1BB) [54], to form an intracellular signal region. Compared to CAR-T cells, CAR-NK cells could offer some significant advantages, including (1) better safety, such as a lack or minimal cytokine release syndrome and neurotoxicity in autologous setting and graft-versus-host disease in allogenic setting, (2) multiple mechanisms for activating cytotoxic activity, and (3) high feasibility for "off-the-shelf" manufacturing.

Currently, clinical-grade NK cells can be manufactured in a large scale from multiple sources including NK92 cell line, PBMCs, UBC, CD34+ hematopoietic progenitor cells (HPCs), and iPSCs. NK-92 cells are an ideal CAR carrier with natural antitumor properties and are easy to cultivate and modify in vitro. NK-92 cells are an ideal CAR carrier with natural antitumor properties and are easy to cultivate and modify in vitro. CD19 CAR-NK-92 approaches are currently under evaluation in CD19+ leukemia (NCT02742727) (NCT02892695). However, compared with CAR-T cells that have been applied in clinical studies, fewer clinical studies have been performed for CAR-NK-92 cells, and little clinical data have been published.

25.6 Donor Leukocyte Infusion (DLI)

Donor leukocyte transfusion (DLI) is designed to induce a graft-versus-leukemia (GVL) effect and is the most vital immunotherapy for relapse after allogeneic hematopoietic stem cell transplantation (HSCT). Evidence shows that DLI could reduce leukemia burden of acute leukemia recurrence after identical sibling donor (ISD) or unrelated donor (URD) HSCT [55, 56]. However, some other studies have reported that DLI alone has a very poor effect in acute leukemia recurrence [57, 58].

Huang's team established a modified DLI protocol [59] that includes the following: (i) the use of G-CSF mobilized peripheral blood stem cell harvests (G-PBSCs) instead of a steady lymphocyte infusion; (ii) the introduction of short-term immune suppressive agents, including cyclosporine A (CSA) or methotrexate (MTX), to further decrease the incidence of GVHD. Impressively, the feasibility and efficacy of the modified DLI were confirmed either for treatment or prevention of relapse after haploidentical HSCT [60–63]. A study conducted by Yan et al. found that modified DLI results in a higher CR rate, 1-year DFS and 1-year OS as compared with chemotherapy alone in patients with AL relapse after allo-HSCT, indicating that modified DLI is more feasible and effective [63]. Pre-emptive DLI based on monitoring of MRD has been explored by researchers to prevent hematological relapse after allo-HSCT. Yan et al. also demonstrated that MRD-directed DLI could significantly decrease the relapse rate without aggravating GVHD [64]. Wang et al. evaluated the efficacy and safety of the prophylactic modified DLI after both HLA-identical and haploidentical transplantation for advanced leukemia, indicating that this approach is effective without any increase in patients with acute GVHD as compared with historical controls [61, 62]. Overall, the available data suggest that modified DLI represents a widely used approach in prophylactic, pre-emptive therapy and therapy for relapse either in HLA-matched HSCT or in haploidentical transplant settings.

25.7 Summary

CAR-T cell immunotherapy holds great promise as a novel cellular immunotherapy against refractory hematologic malignancies. The development of BiTE antibody, ADC, and NK cells, particularly CAR-NK cells, has opened a new pathway to improving the outcome of patients with r/r ALL. Notably, modified DLI offers a new way for prophylaxis and treatment strategies for relapse control in patients with ALL after HSCT. Further investigation of the mechanisms that govern immune escape will provide the rationale to develop powerful immunotherapies for relapse.

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In the Pipeline—Emerging Therapy for ALL

26

Harinder Gill, Cherry Chu, and Yammy Yung

Abstract

Despite medical advances in recent decades with improvement in survival rates (Lenk et al., Cancer Metastasis Rev. 2020;39:173–87; Meyer and Hermiston. Cancer Drug Resist. 2019;2: 313–25), decreased treatment tolerance, persistent minimal residual disease positivity, and subsequent disease recurrence remain issues of concern (Scheffold et al. Venetoclax: targeting BCL2 in hematological cancers. Cham: Springer; 2018. pp. 215–42). Relapses often confer a dismal prognosis with poor outcome. In addition, not all patients possess the capacity to withstand intensive chemotherapy or receive hematopoietic stem cell transplantation (HSCT) due to old age, frail state, or the presence of comorbidities (Sas et al. J Clin Med. 2019;8:1175). Therefore, novel therapies with better safety profile and higher efficacy are of paramount importance in improving relapse rates, disease response, and preventing chemoresistance. We review the novel agents targeting different pathways, receptors, or systems involved in the leukemogenesis of ALL.

Keywords

Acute lymphoblastic leukemia · Targeted therapy · Novel agents · Personalized medicine

26.1 Introduction

Acute lymphoblastic leukemia (ALL) is predominantly a childhood disease [1]. It is the most common malignancy occurring in children and the second most common acute leukemia in adults [2, 3]. B-cell acute lymphoblastic leukemia (B-ALL) accounts for approximately 85% of ALL, whereas T-cell acute lymphoblastic leukemia (T-ALL) constitutes the remaining 15%. Current treatments of ALL mainly comprise cytotoxic chemotherapy including cyclophosphamide, vincristine, doxorubicin, and dexamethasone [4]. Prolonged exposure to chemotherapeutic agents poses threats to the health of patients and is particularly detrimental to the future growth of children. Despite medical advances in recent decades with improvement in survival rates [5, 6], decreased treatment tolerance, persistent minimal residual disease positivity, and subsequent disease recurrence remain issues of concern [7]. Relapses often confer a dismal prognosis with poor outcome. In addition, not all patients possess the capacity to withstand intensive chemotherapy or receive hematopoietic stem cell transplantation (HSCT) due to old age, frail state, or the presence of comorbidities [8]. Therefore, the emergence of novel therapies with lower toxicity, better tolerance, safety profile, and higher efficacy is paramount and necessary in improving relapse rates, disease response, and preventing chemoresistance development in the future.

This following chapter will review the novel agents targeting different pathways, receptors, or systems involved in the leukemogenesis of ALL. Variations in cytogenetic abnormalities in ALL patients may explain the different sensitivities and responses to different novel therapies (Tables 26.1 and 26.2)

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Table 26.1 Different ALL subtypes and their differences in genetic alterations

ALL subtypes	Prognosis	Genetic alteration (s)
B-ALL		
Ph-positive ALL t(9;22)(q34;q11.2)	Poor	BCL-ABL Hyperactivation of JAK/STAT, Akt, RAS pathways CDKN2A/B deletion, IKZF1 deletion and mutations
Ph-like	Poor	ABL rearrangement Hyperactivation of JAK/STAT, PI3K pathways CRLF2 rearrangement CDKN2A/B, IKZF1 deletions
ETV6-RUNX1 t(12;21)(p13;q22)	Good	PAX5 mutation Hyperactivation of JAK/STAT, PI3K pathways
TCF3-PBX1/E2A-PBX1 t(1;19)(q23;p13)	Good	AURKA/B overexpression WNT upregulation
MLL-r ALL t(11q23; variable)	Poor	FLT3 overexpression CDK6 upregulation
Hyperdiploidy (>50)	Good	FLT3, RAS mutations
Hypodiploidy (<45)	Poor	TP53, IKZF, RB1, RAS mutations Hyperactivation of PI3K and RAS pathways
T-ALL		
T-ALL	Poor	PTEN inactivation CDKN2A/B inactivation Overactivation of PI3K, NOTCH1 pathways
ETP-ALL	Poor	FLT3, RAS, DNMT3A mutations

ABL Abelson murine leukemia, *Akt* protein kinase B, *ALL* acute lymphoblastic leukemia, *AURKA/B* aurora kinase A/B, *BCL-ABL* breakpoint cluster region ABL, *CDK6* cyclin-dependent kinase 6, *CDKN2A/B* cyclin-dependent kinase inhibitor 2A/B, *CpG* cytosine-phosphate-guanine, *CRLF2* cytokine receptor-like factor 2, *DNMT3A* DNA methyltransferase 3A, *ETP-ALL* early T-cell precursor ALL, *ETV6-RUNX1* ETS variant transcription factor 6-Runt-related transcription factor 1, *FLT3* FMS-like tyrosine kinase 3, *IKZF1* IKAROS family zinc finger 1, *JAK* Janus kinase, *MLL-r* mixed-lineage leukemia-rearranged, *NOTCH1* neurogenic locus notch homolog protein 1, *PAX5* paired box gene 5, *Ph* Philadelphia chromosome, *PI3K* phosphoinositide 3-kinase, *PTEN* phosphatase and tensin homolog, *RAS* rat sarcoma viral oncogene, *RB1* retinoblastoma 1, *STAT* signal transducer and activator of transcription, *TCF3-PBX1* transcription factor 3-pre-B-cell leukemia transcription factor 1, *TP53* tumor protein p53, *WNT* wingless-related integration site

Table 26.2 Summary of clinical trials of the novel agents in acute lymphoblastic leukemia (ALL)

Drug	Phase	Leukemia	Regimen	Outcome	Adverse events	Trial identifier
PI3Kα/δ inhibitor						
Copanlisib	1 (early)	ALL	Copanlisib	Not yet recruiting	/	NCT04803123
Pan-PI3K inhibitor						
Buparlisib (BKM120)	1	Advanced leukemias	BKM120	Completed • Only one ALL patient was recruited in the study. The cytogenetics of the patient was t(4;11) who underwent progression after treatment	• Neurotoxicity (confusion)	[29] NCT01396499
mTOR inhibitor						
Everolimus (RAD001)	1/2	R/R ALL	Everolimus + hyper-CVAD (cyclophosphamide, vincristine, doxorubicin, dexamethasone)	Completed • 33% achieved CR • 64% response rates in patients treated with everolimus + hyper-CVAD compared with 53% with hyper-CVAD alone • 50% response in T-ALL compared with 39% in B-ALL	• Hyperglycemia • Mucositis • Infections • Transaminitis • Myelosuppression	[37] NCT00968253
	1	Pediatric ALL	Everolimus + re-induction chemotherapy	Completed • 86% of patients achieved CR2 after re-induction • 68% achieved CR2 with low or nondetectable MRD		[38] NCT01523977
	1	T-ALL, T-LBL	Everolimus + chemotherapy (nelarabine, cyclophosphamide, etoposide)	Recruiting		NCT03328104
Rapamycin (sirolimus)	1	R/R ALL	Rapamycin + corticosteroid	Completed (pending results)		NCT00874562
Temsirolimus (CCI-779)	1	Relapsed ALL or nHL	Temsirolimus + dexamethasone, mitoxantrone, vincristine, pegaspargase	Completed • 47% of patients achieved CR or CRi		[39] NCT01403415
	1	R/R childhood ALL, LBL, PTL	Temsirolimus + etoposide, cyclophosphamide	Completed (pending results)		NCT01614197
Sapanisertib	2	B-ALL, Ph-positive B-ALL, Ph-negative B-ALL, R/R ALL, T-ALL	Sapanisertib	Active, not recruiting		NCT02484430
Dual PI3K/mTOR inhibitors						
Dactolisib (BEZ235)	1	ALL, AML, CML-BP	Dactolisib	Completed • Blast reduction in T-ALL and BCP-ALL patients was shown. • Effects shown in small subset of ALL patients • Single-agent effect most prominent in ALL patient		[41] NCT01756118

(continued)

Table 26.2 (continued)

Drug	Phase	Leukemia	Regimen	Outcome	Adverse events	Trial identifier
Tyrosine kinase inhibitors (TKIs)						
Nilotinib (AMN107)	2	Newly diagnosed ALL	Nilotinib + mVPD (vincristine, prednisolone, daunorubicin)	Completed • 92% achieved CHR with median time of 27 days • 53% achieved CMR at the time of CHR	• Jaundice • Serum lipase elevation • Overt pancreatitis	[55] NCT00844298
	1	Pediatric Ph-positive ALL and CML	Nilotinib	Completed • 75% of Ph-positive ALL patients achieved CR • 90.9% of Ph-positive CML patients achieved CHR	• Low-grade fluctuation in hepatic transaminases	NCT01077544
	2	Ph-positive ALL in elderly patients	Nilotinib + chemotherapy	Completed • 97% achieved CHR during induction • 30% achieved CMR after induction • 42% achieved CMR and 29% with undetectable BCR-ABL1 transcript during consolidation		[54] NCT01528085
	N/A	R/R Ph-positive ALL	Nilotinib	Available Access provided by Managed Access Program (MAP)	/	NCT04559555
Asciminib (ABL001)	1	Ph-positive ALL, CML	Asciminib, asciminib + nilotinib/ imatinib/dasatinib	Recruiting	/	NCT02081378
	1	Ph-positive ALL, B-ALL, CML-BP	Asciminib + dasatinib + prednisone	Recruiting	/	NCT03595917
Bosutinib	1/2	CD22-positive, Ph-positive ALL and CML	Bosutinib + inotuzumab ozogamicin	Recruiting	/	NCT02311998
JAK inhibitors						
Ruxolitinib	1/2	TKI-resistant Ph-positive ALL and CML	Ruxolitinib + nilotinib	Completed (pending results)	/	NCT01914484
	2	R/R B-ALL and Ph-like ALL	Ruxolitinib/dasatinib + chemotherapy	Completed (pending results)	/	NCT02420717
	1	Newly diagnosed Ph-positive ALL	Ruxolitinib + dasatinib + dexamethasone	Active, not recruiting	/	NCT02494882
	2	ALL	Ruxolitinib + chemotherapy	Recruiting		[71] NCT02723994
	2/3	ALL, LBL	Ruxolitinib + total therapy XVII	Recruiting	/	NCT03117751
	1	Ph-like ALL, ALL	Ruxolitinib + chemotherapy	Recruiting	/	NCT03571321
	1/2	R/R ETP-ALL	ruxolitinib + LVP (L-asparaginase, vincristine, prednisone)	Not yet recruiting		NCT03613428
g-Secretase inhibitors						
Nirogacestat (PF-03084014)	1	T-ALL, T-LBL	PF-03084014	Completed • 1 out of 3 T-ALL patients achieved CR with full hematologic recovery for 3 months	• Nausea, vomiting	[87] NCT00878189

AL101 (BMS-906024)	1	T-ALL, T-LBL	BMS-906024, BMS-906024 + dexamethasone	Completed • 32% and 37.5% achieved ≥50% and ≥ 98% clearance of bone marrow blasts, respectively • 1 (12.5%) patient achieved CR and 1 (12.5%) achieved PR	• Diarrhea • ALT, AST elevation • Pancytopenia	[88] NCT01363817
Crenigacestat (LY3039478)	1/2	T-ALL, T-LBL	LY3039478 + dexamethasone	Completed • Only 2.8% achieved CR • Overall median EFS of 1.18 months	• GI toxicity (diarrhea, nausea, vomiting) • Hypokalemia	[86] NCT02518113
Anti-NOTCH antibodies						
Brontictuzumab (OMP-52 M51)	1	R/R lymphoid malignancies	OMP-52 M51	Completed	/	NCT01703572
CDK4/6 inhibitors						
Palbociclib (PD-0332991)	1/2	MLL-r ALL, AML	Palbociclib	Recruiting	/	NCT02310243
	1	R/R ALL, AML	Palbociclib, palbociclib + sorafenib/decitabine/ dexamethasone	Recruiting	/	NCT03132454
	1	R/R B-ALL	Palbociclib + dexamethasone	Recruiting	/	NCT03472573
	1	Pediatric R/R ALL, LBL	Palbociclib + chemotherapy	Active, not recruiting	/	NCT03792256
Ribociclib (LEE011)	1	R/R ALL	Ribociclib + everolimus + dexamethasone	Recruiting	/	NCT03740334
SYK inhibitors						
Entospletinib (GS-9973)	1/2	R/R B-ALL	Entospletinib + vincristine + dexamethasone	Terminated due to low response rates	/	NCT02404220
FLT3 inhibitors						
Lestaurtinib (CEP-701)	3	Newly diagnosed ALL	Lestaurtinib + chemotherapy, chemotherapy	Active, not recruiting	/	NCT00557193
Quizartinib (AC220)	1	Pediatric R/R ALL, AML	AC220 + cytarabine, etoposide, methotrexate	Completed • Higher ORR in FLT3-ITD patients than non-ITD patients (43% vs 14%)	• GI toxicity • QTcF prolongation • Hyperbilirubinemia • Hypophosphatemia	[130] NCT01411267
MEK inhibitors						
Selumetinib (MAPK inhibitor)	1/2	Pediatric and adult ALL, R/R ALL	Selumetinib + dexamethasone	Recruiting	/	NCT03705507
Proteasome inhibitors						
Bortezomib	1/2	Pediatric R/R ALL	Bortezomib + chemotherapy	Completed • 64% achieved complete remission with an ORR of 73% but 80% B-precursor patients	• Peripheral neuropathy • Infections • Metabolic derangement	[157] NCT00440726
	3	Newly diagnosed T-ALL, T-LBL	Bortezomib + chemotherapy, chemotherapy	Active, not recruiting	/	NCT02112916
	2	Pediatric ALL	Bortezomib + ALL R3, ALL R3 chemotherapy	Recruiting	/	NCT03590171
Ixazomib (MLN 9708)	1	B-ALL, T-ALL, LBL	Ixazomib + chemotherapy	Completed	/	NCT02228772
	1/2	Pediatric R/R ALL, LBL	Ixazomib + chemotherapy	Recruiting	/	NCT03817320
Carfilzomib	1	Pediatric R/R ALL	Carfilzomib + induction chemotherapy	Recruiting	/	NCT02303821

(continued)

Table 26.2 (continued)

Drug	Phase	Leukemia	Regimen	Outcome	Adverse events	Trial identifier
NAE inhibitors						
Pevonedistat	1	R/R ALL, lymphoblastic nHL	Pevonedistat + VXLD chemotherapy (vincristine, dexamethasone, Peg-asparaginase, daunorubicin)	Recruiting	/	NCT03349281
DOT1L inhibitor						
Pinometostat (EPZ-5676)	1	R/R ALL, AML, MDS, myeloproliferative disorder	EPZ-5676	Completed • 2 patients achieved complete remission • 2 attained clearance of leukemia cutis without BM response • 7 experienced morphologic changes in BM with myeloid differentiation	• Leukocytosis • Cardiac failure • Nausea, constipation • Febrile neutropenia • Hypocalcemia, hypokalemia	[172] NCT01684150
	1	ALL, AML	EPZ-5676	Completed • Only 40% achieved transient reduction in peripheral or BM blasts without objective responses	• Febrile neutropenia • Respiratory failure • ALT elevation	[175] NCT02141828
Hypomethylating agents						
Azacitidine	2	Infant ALL and MLLr-ALL	Azacitidine and combination chemotherapy	Active, not recruiting	/	NCT02828358
Decitabine	1	R/R ALL	Decitabine	Completed	/	NCT00349596
Decitabine	1/2	ALL, precursor B-ALL or T-ALL	Decitabine + vorinostat + chemotherapy	Terminated due to toxicity • 39% achieved CR (CR + CR without count recovery) • 22% had stable disease • 39% experienced treatment-related toxicities	• Infections (invasive fungal infections) • Hypokalemia, hypophosphatemia • Anemia, febrile neutropenia • Cholestasis, steatosis • Hyperbilirubinemia	[181] NCT01483690
HDAC inhibitors						
Panobinostat	2	R/R ALL, AML	Panobinostat	Terminated due to lack of efficacy • No responses observed	• Febrile neutropenia, anemia • Infections • Cardiac disorders • GI events • Elevation of liver transaminases	NCT00723203
Vorinostat	1/2	Infant ALL	Vorinostat + bortezomib + chemotherapy	Recruiting	/	NCT02553460
BRD inhibitors						
Birabresib (OTX015/ MK-8628)	1	ALL, AML, DLBCL, MM	Birabresib	Completed • 2 patients achieved complete remission, while 1 achieved complete remission without platelet recovery	• Diarrhea and nausea • Fatigue • Cutaneous disorders	[196] NCT01713582

BCL-2 inhibitors						
Venetoclax (ABT-199)	1	R/R ALL, LBL	Venetoclax + navitoclax + chemotherapy	Completed • 59.6% and 75% achieved overall complete remission in all and pediatric patients, respectively • 57% and 67% achieved undetectable MRD in all and pediatric patients, respectively	• Febrile neutropenia, thrombocytopenia • Diarrhea, nausea • Hypokalemia • Abdominal pain	[202] NCT03181126
	1	R/R ALL, AML, nHL, neuroblastoma	Venetoclax + chemotherapy	Recruiting	/	[204] NCT03236857
	1	R/R ALL	Venetoclax + chemotherapy	Active, not recruiting	/	NCT03319901
	1/2	R/R T-ALL, B-ALL	Venetoclax + liposomal vincristine	Recruiting	/	NCT03504644
	1/2	R/R Ph-positive ALL or CML	Venetoclax + ponatinib, dexamethasone, rituximab	Recruiting	/	NCT03576547
	1/2	R/R B-ALL and T-ALL	Venetoclax + chemotherapy	Recruiting	/	NCT03808610
MDM2 inhibitors						
Idasanutlin	1/2	ALL, AML, neuroblastoma, solid tumors	Idasanutlin + venetoclax/ chemotherapy	Recruiting	/	NCT04029688
CXCR4 antagonists						
Motixafortide (BL-8040)	2	R/R T-ALL, T-LBL	BL-8040 + nelarabine	Recruiting	/	NCT02763384
Others						
Metformin (activates AMPK)	N/A	R/R Ph-negative BCP-ALL	Metformin during pre-induction with steroids, induction remission, consolidation, and maintenance	Completed • Increased survival in patients treated with metformin (83.3% vs 26.6%)	Tolerable safety profiles	[45] NCT03118128

ALL acute lymphoblastic leukemia, *ALT* alanine aminotransferase, *AML* acute myeloid leukemia, *AMPK* AMP-activated protein kinase, *BCL-2* B-cell lymphoma 2, *BCP-ALL* B-cell precursor ALL, *BCR-ABL* breakpoint cluster region–Abelson murine leukemia, *BM* bone marrow, *BRD* bromodomain, *CDK4/6* cyclin-dependent kinase 4/6, *CHR* complete hematologic remission, *CML* chronic myeloid leukemia, *CML-BP* CML-blast phase, *CMR* complete molecular remission, *CR* complete response/remission, *CRi* CR with incomplete blood count recovery, *CXCR4* C-X-C motif chemokine ligand 4, *DLBCL* diffuse large B-cell lymphoma, *DOT1L* disrupter of telomeric silencing 1-like, *EFS* event-free survival, *ETP-ALL* early T-cell precursor ALL, *FLT3* FMS-like tyrosine kinase 3, *FLT3-ITD* FLT3-internal tandem duplication, *GI* gastrointestinal, *HDAC* histone deacetylase, *JAK* Janus kinase, *LBL* lymphoblastic lymphoma, *MAPK* mitogen-activated protein kinase, *MDM2* mouse double minute 2 protein, *MDS* myelodysplastic syndrome, *MEK* mitogen-activated protein kinase kinase, *MLLr-ALL* mixed-lineage leukemia-rearranged ALL, *MM* multiple myeloma, *MRD* minimal residual disease, *mTOR* mammalian target of rapamycin, *NAE* NEDD8-activating enzyme, *nHL* non-Hodgkin lymphoma, *NOTCH* neurogenic locus notch homolog protein, *ORR* overall response rate, *Ph* Philadelphia chromosome, *PI3K* phosphoinositide 3-kinase, *PR* partial response/remission, *PTL* peripheral T-cell lymphoma, *QTcF* corrected QT interval using Fridericia's formula, *R/R* relapsed or refractory, *SYK* spleen tyrosine kinase, *TKI* tyrosine kinase inhibitor

26.2 Targeting the PI3K/Akt/mTOR Pathway

Hyperactivation of phosphatidylinositol 3-kinase (PI3K) pathway is a common feature in both B-ALL and T-ALL [9, 10]. PI3K is a protein kinase that mediates the downstream signaling molecules such as PDK1 and Akt [11, 12] and activates a series of pro-survival signaling cascades. The phosphorylation of Akt results in the activation of mammalian target of rapamycin (mTOR), which regulates cell survival and migration [10]. Phosphatase and tensin homolog (PTEN) is a tumor suppressor gene that negatively regulates the PI3K/Akt pathway [13]. It is often inactivated in ALL, especially in T-ALL [10, 14, 15]. The inactivation of PTEN results in the constitutive activation of PI3K/Akt/mTOR pathway [16, 17] and subsequently uncontrolled cell proliferation and leukemogenesis (Fig. 26.1).

26.2.1 PI3K Inhibitors

Idelalisib (CAL-101) is a PI3Kδ-(p110δ-) selective inhibitor that effectively reduced p-Akt levels in vitro [18, 19]. It induced cell cycle arrest and caspase-mediated apoptosis in B-cell precursor ALL (BCP-ALL) cell lines [19], with specific sensitivity toward transcription factor 3 (TCF3)-pre-B-cell leukemia transcription factor 1 (PBX1)-positive cells [20]. Pronounced anti-proliferative synergism was demonstrated when it was used in combination with doxorubicin or cytarabine and dexamethasone [19, 21], whereas only modest synergistic activity was seen in combination with vincristine [18]. AS-605240, a PI3Kγ-selective inhibitor, also exhibited synergistic effect with glucocorticoid both in vitro and in vivo in T-ALL cell lines [22]. Copanlisib, a PI3Kα/δ inhibitor, also demonstrated effective anti-proliferative activity in B-ALL cell lines in vitro [23]. It augmented the anti-apoptotic effect when used in combination with cytarabine. This has led to an early phase 1 window trial in relapsed or refractory (R/R) B-ALL (NCT04803123).

Despite the emergence of novel isoform-selective PI3K inhibitors, pan-PI3K inhibitors are associated with higher efficacy and efficiency in treating ALL [24]. ZSTK-474, a pan-PI3K inhibitor, and duvelisib (IPI-145), a δ/γ dual inhibitor, displayed maximal potency in their cytotoxic effects on B-ALL cell lines without significant toxicity to normal B cells. Both agents enhanced the apoptotic events of dexamethasone on B-ALL cells. Other isoform-selective PI3K inhibitors, such as AS-605240 and CAL-101, demonstrated variable degree of, yet inferior

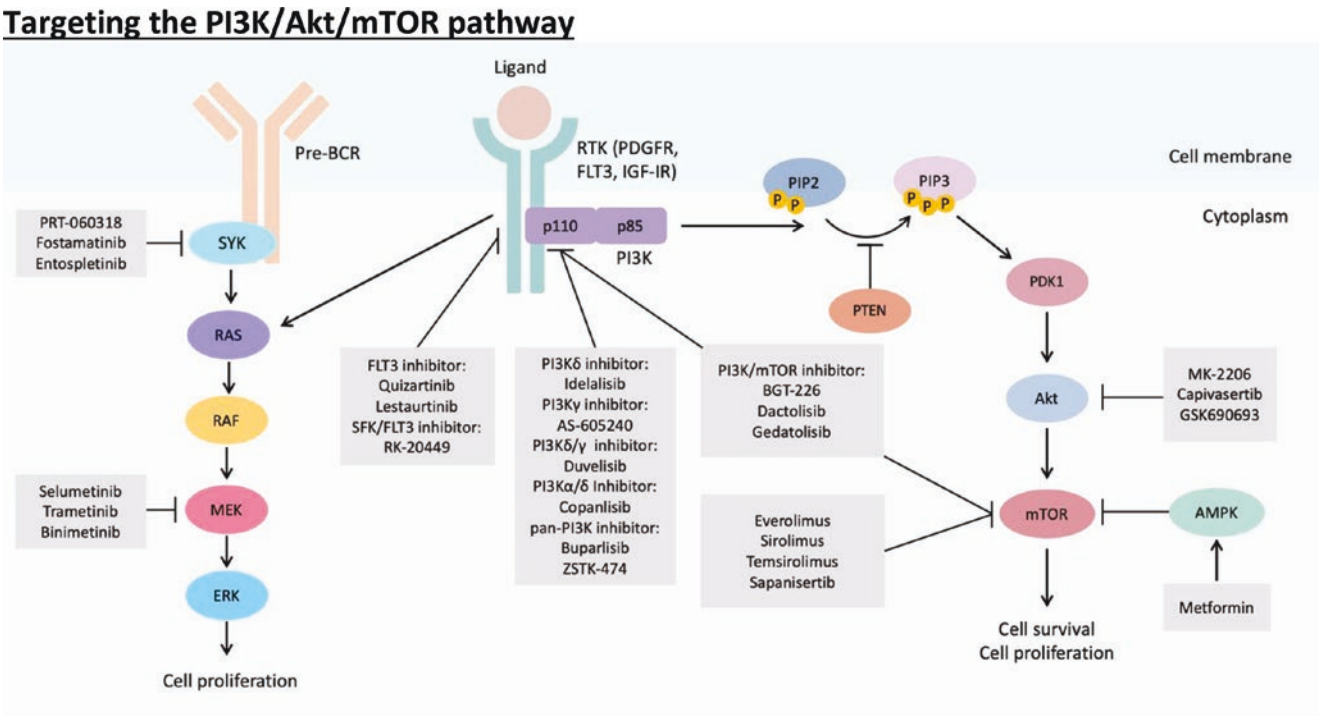

Fig. 26.1 Overview of the novel agents targeting the PI3K/Akt/mTOR, RAS/RAF/MEK/ERK pathways, and the FLT3 receptor. *Akt* protein kinase B, *AMPK* adenosine monophosphate-activated protein kinase, *BCR* B-cell receptor, *ERK* extracellular signal-regulated kinase, *FLT3* FMS-like tyrosine kinase 3, *IGF-IR* insulin-like growth factor 1 receptor, *MEK*: mitogen-activated protein kinase kinase, *mTOR* mammalian target of rapamycin, *P* phosphate, *PDGFR* platelet-derived growth factor receptor, *PDK1* phosphoinositide-dependent kinase 1, *PIP2* phosphatidylinositol 4,5-bisphosphate, *PIP3* phosphatidylinositol-3, 4, 5-triphosphate, *PI3K* phosphoinositide 3-kinase, SDF1, stromal cell-derived factor 1, *PTEN* phosphatase and tensin homolog, *RAS* rat sarcoma viral oncogene homolog, *RAF* rapidly accelerated fibrosarcoma, *RTK* receptor tyrosine kinase, *SYK* spleen tyrosine kinase

potency and sensitivity toward different B-ALL cell lines when compared to ZSTK-474 [25]. ZSTK-474 was also shown to re-sensitize resistant T-ALL cells to nelarabine [26]. Buparlisib (BKM120), a pan-class 1 PI3K inhibitor, downregulated p-Akt, induced apoptosis, and cell cycle arrest in ALL cells in vitro as demonstrated in preclinical studies [27, 28]. It entered a clinical trial in evaluating its safety and tolerability in advanced leukemias (NCT01396499) [29]. Owing to the limited sample size in this trial, the clinical efficacy of buparlisib remains questionable.

Despite limited availability of clinical trials, the promising preclinical results still render PI3K, a potential therapeutic target for future clinical exploration in ALL. Alternatively, PI3K/Akt/mTOR pathway can also be targeted by its downstream signaling molecules such as Akt and mTOR.

26.2.2 Akt Inhibitors

MK-2206, a pan-Akt inhibitor, significantly reduced p-Akt level in both B-ALL and T-ALL cell lines [30, 31]. It led to a remarkable decline in proliferative and metabolic activities regardless of the basal Akt phosphorylation status, suggesting off-target effects of MK-2206 [31]. Capivasertib (AZD5363) and GSK690693, both as pan-Akt inhibitors, exerted cytotoxic effect in B-ALL and T-ALL [32–34].

26.2.3 mTOR Inhibitors

Everolimus, a potent mTOR inhibitor, exhibited compelling preclinical efficacy in vivo [35, 36]. Clinical trials on the addition of everolimus (RAD001) with chemotherapy in R/R ALL demonstrated the feasibility of such combination without significant increase in toxicity (NCT00968253 and NCT01523977) [37, 38]. A phase 1 trial determining its maximum tolerated dose is currently underway (NCT03328104). Rapamycin or sirolimus, another mTOR inhibitor, was also investigated although results are not yet available (NCT00874562). Temsirolimus (CCI-779), the analog of sirolimus, demonstrated excessive toxicity in combination with chemotherapy, which warrants adjustment in drug combination and dosage (NCT01403415 and NCT01614197) [39]. Sapanisertib (TAK-228/MLN-0128), a dual mTORC1/2 inhibitor, is also in progress of evaluating the response of treatment in R/R ALL (NCT02484430).

26.2.4 Dual Inhibitors

BGT-226 and dactolisib (BEZ235), dual PI3K/mTOR inhibitors, demonstrated cytotoxic efficacy both in vitro and in vivo. Although BGT-226 demonstrated greater in vitro cytotoxic effect than everolimus, both agents displayed no superior in vivo effects to everolimus [35]. Further preclinical study on BEZ245 discovered its preferential role in sensitizing T-ALL cells to dexamethasone [40]. A phase 1 clinical trial with BEZ235 in adult patients with R/R acute leukemia has recently been completed (NCT01756118) [41]. It demonstrated clinical efficacy in patients with BCP-ALL and T-ALL despite suboptimal response in most ALL patients. Gedatolisib (PF-05212384/PKI-587), another dual PI3K/mTOR inhibitor, demonstrated a higher potency in comparison with a selective PI3K inhibitor, BYL719, idelalisib, or dual mTORC1/2 inhibitor, vistusertib (AZD2014), in vitro in Philadelphia- (Ph−)-like ALL cells [42]. It also showed synergistic effect with ruxolitinib or dasatinib in vivo with enhanced survival of patient-derived xenograft (PDX) Ph-like ALL models. Both in vitro and in vivo efficacies of PKI-587 were also observed in T-ALL cell lines [43], whereas AZD2014 exhibited greater potency in relapsed ALL cell lines [42].

Surprisingly, metformin, an antidiabetic drug, showed anti-leukemic effect in an open-label, non-randomized clinical trial in patients with Ph-negative BCP-ALL (NCT03118128). It inhibits mTOR via the activation of adenosine monophosphate (AMP)-activated protein kinase (AMPK) [44, 45]. It helped overcome chemoresistance and reduce relapse rates in patients with elevated adenosine triphosphate (ATP)-binding cassette subfamily B member 1 (ABCB1; P-glycoprotein) expressions [45], which is responsible for the efflux of multiple drugs [46].

26.3 Targeting the BCR-ABL1 Fusion

The fusion gene, breakpoint cluster region-abelson murine leukemia 1 (BCR-ABL1) or Ph chromosome at t(9;22)(q34;q11), has a constitutively active tyrosine kinase activity. It is a hallmark in chronic myeloid leukemia (CML) with a breakpoint at p210. The p190 isoform of BCR-ABL1 occurs more frequently in Ph-positive B-ALL [47]. This oncoprotein gives rise to uncontrolled proliferation, impedes cell differentiation, and inhibits apoptosis [48] (Fig. 26.2).

The prevalence of Ph-positive ALL increases with age and is the most frequent subtype encountered in adults [48–50]. The emergence of novel tyrosine kinase inhibitors (TKIs) may help reverse the poor prognostic outcome of BCR-ABL translocation [50]. Imatinib and dasatinib are Food and Drug Administration (FDA)-approved first and second-generation TKIs used in treatment of Ph-positive ALL, respectively. Ponatinib, a third-generation TKI, also received FDA approval for T315-mutated ALL. It targets the T315 mutation in the ATP-binding sites of ABL1 that confers TKI resistance [51, 52]. Despite the success of existing TKIs, the emergence of resistance poses an obstacle to refractory patients. This has led to the development of more novel kinase inhibitors.

Nilotinib (AMN107) is another second-generation TKI. It elicited effective cytotoxicity both in vivo and in vitro in Ph-positive ALL cells [53]. This has led to several clinical trials (NCT01528085 [54] and NCT04559555). Among the completed trials, it demonstrated feasible combination with chemotherapy but no superior efficacy over imatinib or dasatinib in a phase 2 trial (NCT00844298) [55]. In another phase 1 trial with a small sample size, anti-leukemic activity was observed in pediatric R/R ALL treated with nilotinib (NCT01077544) [56].

Asciminib (ABL001), an allosteric BCL-ABL inhibitor, exerts its inhibitory effect via binding to the myristoyl pocket of ABL1 [57]. Several open-label clinical trials investigating the use of asciminib with imatinib/dasatinib/nilotinib and with dasatinib and dexamethasone in R/R Ph-positive ALL or CML are currently underway (NCT02081378 and NCT03595917). Bosutinib, a dual Src/ABL inhibitor, has entered a phase 1/2 trial exploring its combination with inotuzumab ozogamicin (IO) in CD22+ ALL and CML (NCT02311998). Other preclinical validated agents, such as bafetinib and rebastinib, may also provide new insights into future clinical practices in overcoming resistant ALL [58].

26.4 Targeting the JAK/STAT Pathway

Aberration in Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway is often seen in both B-ALL and T-ALL [59]. BCR-ABL1-like or Ph-like ALL, a provisional entity newly characterized by the World Health Organization (WHO), demonstrates similar gene expression profile to that of Ph + ALL apart from the absence of the characteristic BCR-ABL fusion [60–63]. In addition to aberrant ABL rearrangement, it is also associated with overactivation of JAK/STAT, PI3K signaling pathways, cytokine receptor-like factor 2 (CRLF2) rearrangement, and IKAROS family zinc finger 1 (IKZF1) deletions [59, 61, 64]. The aberrations, hence, contribute to uncontrolled proliferation and leukemic survival. Both Ph-positive and Ph-like ALL confer a dismal prognosis with responsiveness toward TKIs [59]. CRLF2, a poor prognostic marker [61, 64], interacts with interleukin-7 receptor (IL7R) to form a heterodimeric receptor for thymic stromal lymphopoietin (TSLP) [65]. Its activation drives downstream signaling, including the JAK/STAT and PI3K pathways [60, 64, 66] (Fig. 26.2).

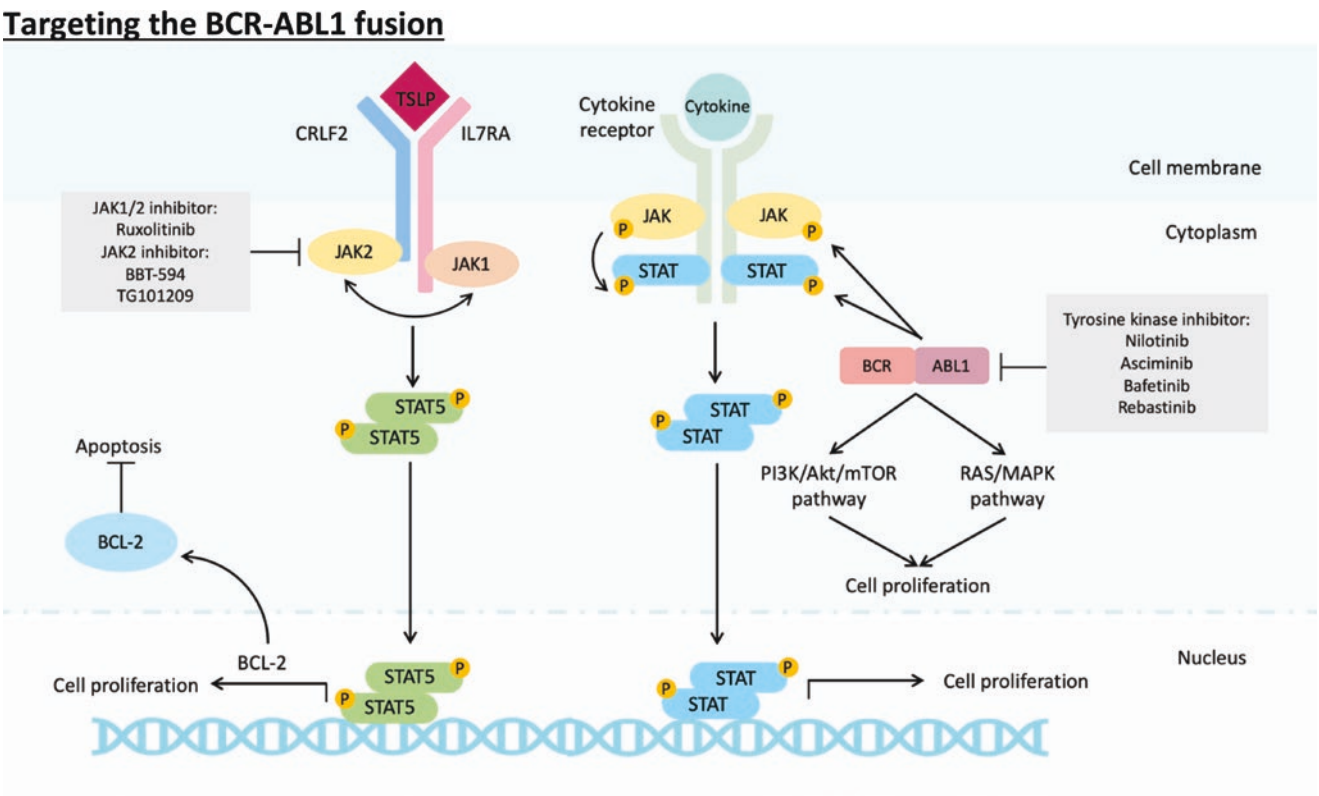

Fig. 26.2 Overview of the novel agents targeting the JAK/STAT pathways and the BCR-ABL1 fusion. *ABL1* Abelson murine leukemia, *Akt* protein kinase B, *BCL-2* B-cell lymphoma 2, *BCR* breakpoint cluster region, *JAK* Janus kinase, *MAPK* mitogen-activated protein kinase, *mTOR* mammalian target of rapamycin, *PI3K* phosphoinositide 3-kinase, *RAS* rat sarcoma viral oncogene

Ruxolitinib (INCB018424), a dual JAK1/JAK2 inhibitor, despite reporting limited effects on T-ALL cell viability as a single agent, it sensitized IL-7-dependent cells to dexamethasone in vitro [67]. Synergistic anti-leukemic effect was shown in vivo with ruxolitinib and venetoclax despite profound gastrointestinal (GI) toxicity [68]. Further exploration and modification of dosing may still render the combination of plausible options in future clinical practices. In addition, synergism was also documented in Ph-like B-ALL cells when the combination of ruxolitinib or BBT-594, a selective JAK2 inhibitor, with AZD2014, a mTOR inhibitor, was adopted [69]. Owing to the limited preclinical efficacy of single-agent ruxolitinib [70], combination therapies with PI3K/mTOR inhibitor, ABL inhibitor, or chemotherapy become the future directions of clinical investigations. This has led to several ongoing clinical trials on various combination therapy with ruxolitinib (NCT02494882, NCT02723994, NCT03117751, and NCT03571321) and two completed trials on TKI and ruxolitinib (NCT01914484 and NCT02420717). Preliminary findings in a phase 2 trial reported ruxolitinib as a safe and tolerable drug when administered concurrently with intensive chemotherapy (NCT02723994 [71]). Another phase 1/2 trial yet to be started will investigate the efficacy of ruxolitinib in combination with LVP (L-asparaginase, vincristine, and prednisone) in treating R/R early T-cell precursor ALL (ETP-ALL) in a clinical trial (NCT03613428).

TG101209, a JAK2 inhibitor, was shown to induce in vitro cell cycle arrest and apoptosis in T-ALL cell lines by the regulation of cell cycle inhibitors and downregulation of BCL-2 anti-apoptotic proteins, respectively [72]. AZD1480, despite limited in vivo and in vitro activity as mono-agent, displayed profound synergistic effect with selumetinib, a MEK inhibitor, in ALL cells harboring JAK mutations [73].

26.5 Targeting the NOTCH Signaling Pathway

Neurogenic locus notch homolog protein 1 (NOTCH1) signaling pathway regulates cellular proliferation and differentiation and is often overactivated in ALL. The NOTCH pathway has a higher prevalence and more well-defined pathogenic role in T-ALL [74–76]. T-ALL is an aggressive disease often associated with high-risk phenotypes such as CNS involvement and leukocytosis [5, 77–79]. NOTCH is a heterodimeric receptor essential for T-cell differentiation and commitment in thymus [76]. Upon activation by its ligands, jagged and delta, it undergoes g-secretase-mediated production of NOTCH intracellular domain (NICD) with subsequent nuclear translocation, resulting in transcriptional activation [80, 81]. About 15% of T-ALL harbors mutation in F-Box and WD repeat domain-containing 7 (FBXW7), a tumor suppressor responsible for the proteasomal degradation of NOTCH1 [77, 78, 82]. Dysregulation in NOTCH1 signaling drives leukemogenesis and may contribute to the development of glucocorticoid resistance [83–85] (Fig. 26.3).

Crenigacestat (LY3039478), nirogacestat (PF-03084014), and AL101 (BMS-906024) are all g-secretase inhibitors (GSIs). All of them have entered clinical trials (NCT02518113 [86], NCT00878189 [87], and NCT01363817 [88]). Results displayed variable degree of anti-leukemic efficacy with GI toxicity as the commonest side effect [86–88], illustrating NOTCH as a promising target in future practices. MRK-560, a selective presenilin-1 (PSEN1) GSI, demonstrated comparable anti-leukemic effect to non-selective GSI and dibenzazepine (DBZ) both in vitro and in vivo without the characteristic detrimental effect on GI tract [80]. DBZ showed synergistic effect in abrogating cell cycle progression with chloroquine (CQ) in T-ALL cells in vitro [89]. Another preclinical study reported chemo-sensitizing ability of GSIs, including GSI IX and GSI-XII on B-ALL cell lines. Despite modest anti-leukemic activity as single agent in vivo, they enhanced the apoptotic activity in B-ALL cells when combined with cytarabine, dexamethasone, and doxorubicin via upregulation of ROS [85].

Brontictuzumab (OMP-52M51), a monoclonal antibody targeting the NOTCH receptor, was identified in a preclinical study to be effective in reducing NOTCH signaling in vivo [90]. It has also been tested in an open-label, phase 1 clinical trial (NCT01703572). However, results are yet to be released.

26.6 Targeting Cell Cycle Regulation

26.6.1 Targeting Cell Cycle Promoters

Cyclin D3 (CCND3) is a downstream signaling molecule of NOTCH1 pathway that is negatively regulated by cyclin-dependent kinase inhibitor 2A (CDKN2A) [91]. It is essential for the activation of cell cycle regulators, CDK4/6, that inactivate retinoblastoma 1 (RB1) via phosphorylation. This results in E2 factor (E2F)-mediated transcriptional activation and subsequent G1-S transition [92, 93]. Deletion of tumor suppressor genes, CDKN2A/B, and hyperactivation of CDK4/6 are common molecular features in ALL [94–97]. CDKN2A/B deletion or hypermethylation accounts for more than 70% of genetic aberrations in T-ALL [98]. On the other hand, the expression of CDK6, a direct target of MLL fusion, is particularly elevated in MLL-r ALL cells owing to its importance in maintaining leukemic survival [99] (Fig. 26.3).

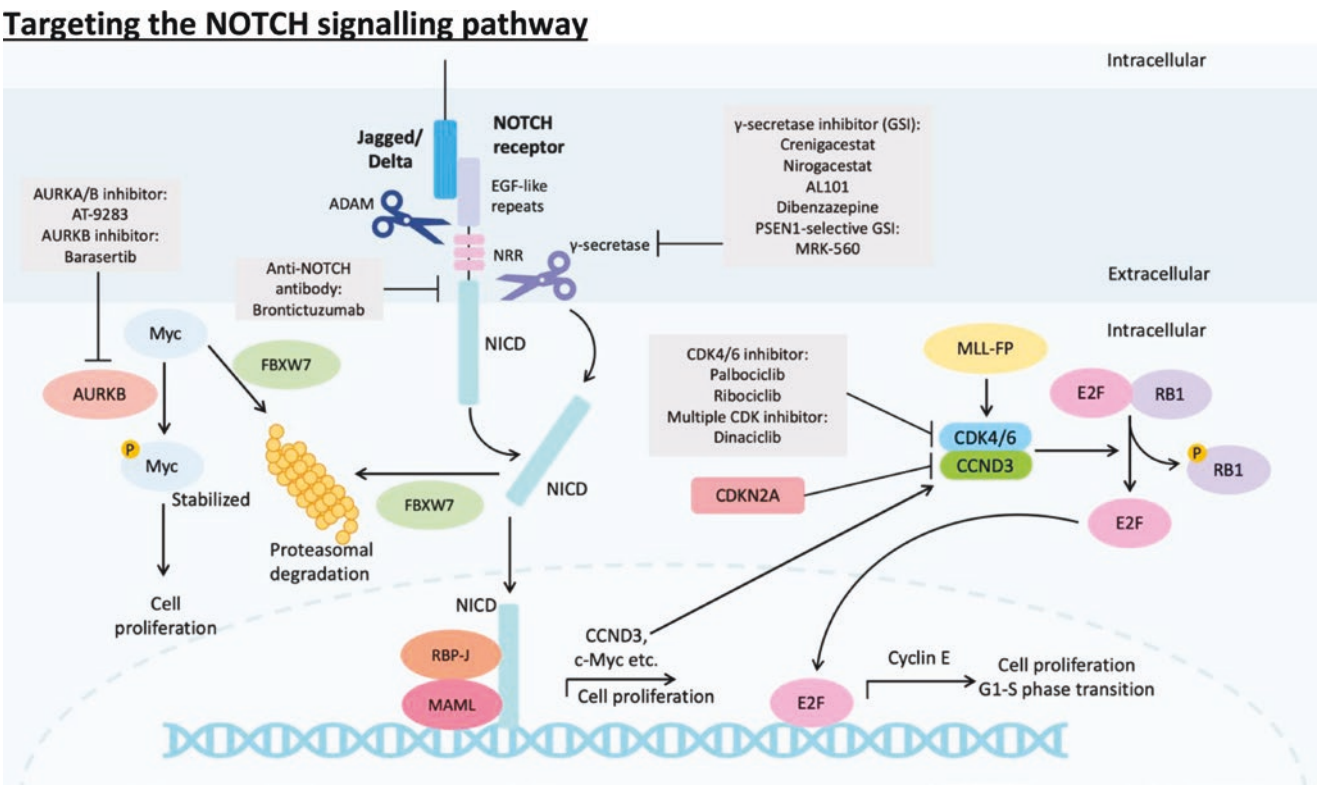

Fig. 26.3 Overview of the novel agents targeting the NOTCH signaling pathway and the cell cycle regulation. *ADAM* disintegrin metalloproteases, *AURKA/B* aurora kinase A/B, *CCND3* cyclin D3, *CDK4/6* cyclin-dependent kinases 4/6, *CDKN2A* cyclin-dependent kinase inhibitor 2A, *Myc* myelocytomatosis oncogene, *E2F* E2 factor, *FBXW7* F-Box and WD repeat domain-containing 7, *RB1* retinoblastoma 1, *MAML* mastermind-like protein, *MLL-FP* mixed-lineage leukemia-fusion protein. *NICD* NOTCH intracellular domain, *NOTCH* neurogenic locus notch homolog protein 1, *NRR* negative regulatory region, *P* phosphate, *RBP-J* recombination signal binding protein-J

Palbociclib (PD0332991), a CDK4/6 inhibitor, was shown to induce G0/G1 cell cycle arrest in ALL cell lines in vitro with pronounced reduction in pRB1 and EZH2 levels in mixed-lineage leukemia-rearranged ALL (MLL-r ALL) cell lines [99]. It also exerted the most potent anti-leukemic effect in T-ALL cells compared to other cell lines as shown in a preclinical study [91]. Several clinical trials experimenting with the single-agent or combination therapy of palbociclib on ALL are undertaking (NCT02310243, NCT03132454, NCT03472573, and NCT03792256). Ribociclib (LEE011), another CDK4/6 inhibitor, exhibited in vitro antagonism with methotrexate (MTX), mercaptopurine, and L-asparaginase in T-ALL cell lines [91]. On the other hand, it synergized with glucocorticoids and everolimus both in vitro and in vivo. This has led to a phase 1 trial evaluating the efficacy of ribociclib with everolimus and dexamethasone in R/R ALL (NCT03740334).

Dinaciclib (SCH727965), a multiple CDK inhibitor, reduced T-ALL cell viability via promoting cell cycle arrest at G2/M phase and inducing apoptosis in vitro. It also demonstrated high efficacy in extending survival in vivo [100]. The favorable preclinical results prompt potential translation of CDK inhibitor into clinical practices.

26.6.2 Targeting the Mitotic Regulators

Aurora kinases A and B (AURKA/B) are mitotic kinases involved in chromosome segregation, spindle formation, and cytokinesis in mitotic cell division, respectively [101]. They are excessively expressed in pediatric ALL, especially in TCF3-PBX1-positive ALL [102–104]. Aberrant increase in AURK expression induces genetic instability, thus promoting leukemogenesis [104]. Myelocytomatosis oncogene (Myc)-driven leukemogenesis is a prominent feature in T-ALL, along with the upregulation of NOTCH signaling pathway. Despite difficulty in direct therapeutic targeting of MYC, its inhibition via AURK inhibition is a promising alternative (Fig. 26.3).

Barasertib (AZD1152), a selective AURKB inhibitor, destabilizes Myc via inhibiting the AURKB-dependent Myc phosphorylation and was shown to reduce Myc levels in T-ALL cells in an in vivo study [105]. It acted synergistically with vincristine in FBXW7-intact T-ALL cells to induce apoptosis and inhibit cell growth [105]. AT-9283, apart from being an AURKA/B inhibitor, also possesses off-target inhibitory effect on FLT-3, JAK2, and ABL. It exerted its growth inhibitory effect on ALL cells in vitro with only lim-

ited toxicity [106]. Synergisms were also observed when it was given together with apicidin, a HDAC inhibitor; 17-AAG, a HSP90 inhibitor; and doxorubicin. They provide rationales for improving drug efficacy as combination therapy in future clinical investigations.

26.7 Targeting the DNA Damage Response (DDR) Pathway

Overexpression of checkpoint kinases 1/2 (Chk1/2) and WEE1 are features in both B-ALL and T-ALL [107, 108]. Chk1 and Chk2 are cell cycle regulators that are activated by ATM and Rad3-related (ATR) and ataxia-telangiectasia-mutated (ATM), respectively. Chk1/ATR and Chk2/ATM mediate the DNA damage response (DDR) by promoting cell cycle arrest upon DNA damage [107, 109, 110]. DDR was found to be protective in cancers and promote tumor progression. It contributes to one of the possible mechanisms of drug resistance development in leukemic cells. The response allows leukemic cells to escape from the cytotoxicity of conventional chemotherapies by repairing the drug-induced DNA damages [110] (Fig. 26.4).

26.7.1 Chk Inhibitor

PF-00477736 (PF) is an ATP-competitive, Chk1-selective inhibitor. It diminished the leukemic cell growth in vivo via induction of replication stress and subsequent apoptosis [108]. The use of PF with bosutinib displayed enhanced apoptotic event in imatinib-resistant ALL cell lines [111].

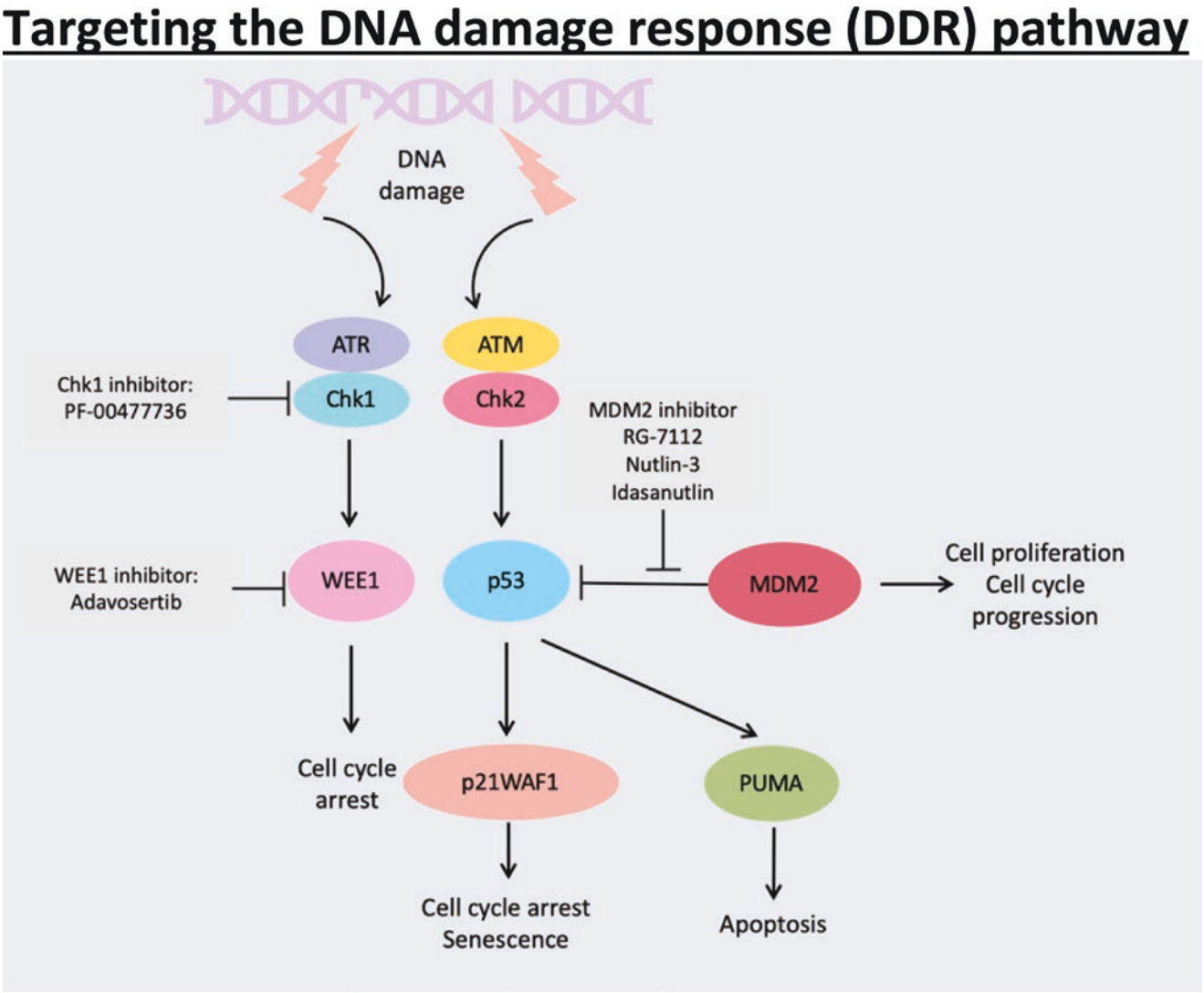

Fig. 26.4 Overview of the novel agents targeting the DNA damage response pathway. *ATM* ataxia-telangiectasia mutated, *ATR* ATM and Rad3-related, *Chk1* checkpoint kinase 1, *Chk2* checkpoint kinase 2, *MDM2* mouse double minute 2 protein, *PUMA* p53 upregulated modulator of apoptosis, *RB1* retinoblastoma, *WEE1* WEE1 kinase

Owing to the absence of significant toxicity and potent preclinical anti-leukemic efficacy in vitro and in vivo, Chk inhibitors provide a strong rationale for future clinical translation.

26.7.2 WEE Inhibitors

Adavosertib (AZD-1775), a WEE1-selective inhibitor, greatly reduced ALL cell viability in vitro [112]. Furthermore, it displayed efficacy in sensitizing ALL cell response to chemotherapy, such as clofarabine, doxorubicin, cytarabine, or vincristine [112, 113]. It also demonstrated ability in overcoming TKI resistance when used along with imatinib, bosutinib, or ponatinib. The successful synergistic combinations of WEE inhibitor and several chemotherapeutic agent pave ways for its use as chemo-sensitizing in future clinical practices.

26.7.3 Combination of Chk Inhibitors and WEE Inhibitors

Simultaneous inhibition of Chk1, Ch2, and WEE by PF and AZD-1775 produced synergistic anti-leukemic effects in ALL cells in vitro, with higher sensitivity in T-ALL cell lines [107, 114]. The combination inhibited the DNA-repairing ability of ALL cells, which was followed by cellular apoptosis. In addition, it was evident in vitro that PF or AZD-1775 or the combination all promoted the cytotoxic activity induced by methotrexate. This prompts future combinatorial use to achieve similar or even superior clinical efficacy with reduced toxicity.

26.8 Targeting the p53-MDM2 Pathway

Murine double minute-2 (MDM2) is an oncogenic protein that negatively regulates the activity of tumor suppressor p53, which mediates cellular apoptosis. It also exhibits cell cycle regulatory effect via interaction with RB and E2F1 [115]. Contrary to most malignancies, wild-type p53 is often found in ALL [116]. Mutation in TP53, a gene-encoding p53 protein, is relatively uncommon. Yet TP53 mutation is frequently mutated in relapsed and low-hypodiploid ALL [75, 117]. Its presence often confers a worse prognosis. The development of novel agents antagonizing the interaction between p53-MDM2 may provide a new strategy in reducing relapses or poor outcomes in future practices (Fig. 26.4).

The following drugs are small-molecule MDM2 inhibitors that constitute the Nutlin family and impede the binding of MDM2 to p53. RG-7112 and nutlin-3 exerted their antiproliferative and pro-apoptotic activities via the activation of p53, subsequently p21WAF1 and PUMA in MLL-r ALL and ETV6-RUNX1-positive cells in vitro, respectively. They also triggered the caspase-dependent apoptosis in vivo [118, 119]. Besides, it acted synergistically with vincristine in vitro, suggesting it is a potential candidate as a combination with chemotherapy in clinical practices. Besides, idasanutlin (RG7388) has demonstrated strong cytotoxic activity against several ALL subtypes including Ph-positive ALL, KMT2A-r ALL, and TCF3-rearranged ALL by stabilizing p53 ex vivo [120]. This has led to a phase 1/2 trial currently underway, evaluating its activity when combined with chemotherapy or venetoclax in R/R solid tumors or acute leukemias including ALL (NCT04029688).

26.9 Targeting the SYK Pathway

Spleen tyrosine kinase (SYK) plays a crucial role in the downstream signaling of pre-B-cell receptor (BCR) [121–123]. It conveys signals to various signaling pathways including PI3K, ERK, and bruton tyrosine kinase (BTK) via phosphorylation of B-cell linker (BLNK) [122]. It is essential for the development of B cells by regulating their proliferation, differentiation, maturation, and survival [124]. Reports showed that the activation of SYK kinase was crucial in the maintenance of growth of various high-risk B-ALL [125]. The use of SYK inhibitor may help reverse the unfavorable prognosis or reduce relapse rate of B-ALL.

PRT-060318 (PRT318), a highly selective SYK inhibitor, demonstrated its superior sensitivity in pre-BCR-positive B-ALL cells over pre-BCR-negative B-ALL cells both in vitro and in vivo [126]. PRT318, fostamatinib (R788), and entospletinib (ENTO)(GS-9973) were all shown to sensitize resistant ETV6-RUNX1 cells to conventional chemotherapy including cytarabine, dexamethasone, and vincristine, with the highest synergism observed in ENTO [127]. Despite preclinical evidence demonstrating the synergism of ENTO with chemotherapy, clinical investigation did not reproduce the results owing to low response rates in R/R B-ALL (NCT02404220) (Fig. 26.1).

26.10 Targeting the FLT3 Signaling Pathway

KMT2A-r ALL is often associated overexpression of FMS-like tyrosine kinase 3 (FLT3) and a poor prognosis [84, 128]. The FLT3 receptor tyrosine kinase (RTK) mediates the differentiation and proliferation of hematopoietic progenitor cells. Upon activation, it regulates various downstream signaling pathways including PI3K and RAS/MAPK pathway [129]. Aberrant expression of FLT3 results in the activation of pro-proliferative cascades and subsequent leukemogenesis (Fig. 26.1).

FLT3 inhibitors have well been investigated in numerous preclinical and clinical studies over a wide range of malignancies. Owing to the upregulation of FLT3 activity in a subset of ALL patients, a few clinical trials have been undertaken to evaluate its efficacy in combination with chemotherapy. Quizartinib (AC220) and lestaurtinib (CEP-701) both showed subclinical response in MLL-r ALL patients (NCT01411267 [130] and NCT00557193 [128]). However, due to the limited number of patient cohort, larger studies are encouraged to assess the efficacy of FLT3 TKI in MLL-r ALL.

On the other hand, RK-20449, a SFK/FLT3 inhibitor, demonstrated the ability to overcome glucocorticoid resistance while exhibiting synergistic cytotoxic effect on MLL-r ALL cells when combined with dexamethasone in vivo [131]. The triple-therapy by the addition of ABT-199, BCL-2 inhibitor to RK-20449, and dexamethasone greatly improved the anti-leukemic effectiveness both in vitro and in vivo.

26.11 Targeting the Wnt/β-Catenin Signaling Pathway

The Wnt/β-catenin pathway is often upregulated in both B-ALL and T-ALL [132]. Upon binding of Wnt to its receptor, frizzled (Fzd), a series of downstream signaling cascades is triggered, resulting in the cytoplasmic accumulation and subsequent nuclear translocation of the transcription factor, β-catenin [133]. This promotes the transcriptional activation of several target genes, including CREB-binding protein (CREBBP/CBP), survivin/BIRC5, c-Myc, and cyclin D1. Precise cellular mechanism contributing to leukemogenesis still remains unclear (Fig. 26.5).

Novel therapies targeting different levels of the Wnt/β--catenin pathway have been developed. By promoting degradation of β-catenin, XAV939 demonstrated modest anti-leukemic effect in ALL cell lines as a single agent and attained synergism when combined with cytarabine both in vitro and in vivo [134]. It is a tankyrase inhibitor and exerts its effect via stabilization of axin that constitutes the β-catenin destruction complex.

The pathway can also be disrupted via inhibiting the interaction between β-catenin and T-cell factor (TCF) that is responsible for transcriptional activation. XX-650-23 is a CBP inhibitor that demonstrated synergy when used in combination with dasatinib in vitro via the inhibition of interaction between CREB and CBP. It also exhibited in vivo efficacy by increasing the disease-free survival in transgenic mouse models [135]. ICG-001, a catenin/CBP inhibitor, disrupted catenin signaling and abrogated proliferation in ALL cells [136]. Despite its lack of cytotoxic effect, it induced differentiation β-catenin/p300 interaction and also sensi-

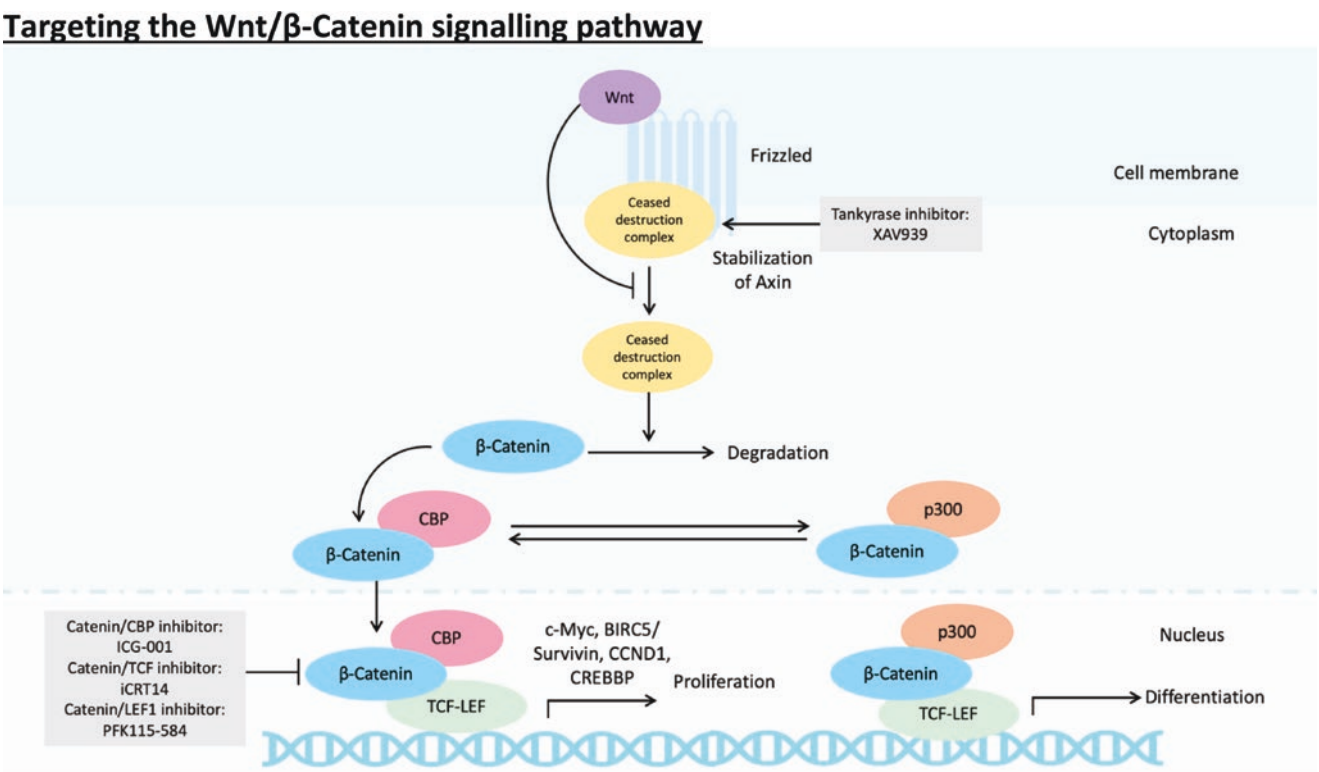

Fig. 26.5 Overview of the novel agents targeting the Wnt/β-Catenin signaling pathway. *BIRC5* baculoviral IAP repeat containing 5, *CBP* cAMP response element-binding protein-binding protein (CREBBP), *CCND1* c-Myc, cellular myelocytomatosis; cyclin D1, *TCF-LEF* T-cell-specific transcription factor/lymphoid enhancer binding factor, *Wnt* wingless-related integration site

tized leukemic cells to chemotherapy. In addition, it also rendered the resistant cell lines susceptible to dasatinib [135]. iCRT14, a β-catenin/TCF inhibitor, caused potent reduction in ALL cell viability both as a single agent or in combination with chemotherapy in vitro. It sensitized the B-ALL cell lines to etoposide, prednisolone, doxorubicin, 6-thioguanine, and cytarabine [137]. PFK115–584, a LEF1/β-catenin inhibitor, exhibited pro-apoptotic and anti-proliferative effect on T-ALL cell lines with downregulation of Myc protein in vitro and significantly reduced leukemic burden in vivo [138]. The promising synergistic preclinical data prompts future use of these drugs in preventing the incidence of R/R ALL.

26.12 Targeting the RAS/RAF/MEK/ERK (MAPK) Pathway

The mitogen-activated protein kinase (MAPK) pathway is critical in regulating numerous cellular functions including cell division, proliferation, survival, differentiation, apoptosis, migration, and metabolism [139, 140]. It mediates a series of downstream cascades involving RAS, RAF, MEK, and ERK proteins [141]. Dysregulation of this pathway can drive leukemogenesis, with the highest frequency occurring in high hyperdiploid ALL. Ras mutation often confers a poor prognosis and often contributes to chemoresistance [142, 143] (Fig. 26.1).

Selumetinib (AZD6244), a MEK inhibitor, demonstrated synergism with dexamethasone in ALL with RAS mutation in a preclinical study [144]. The combination resulted in significant downregulation of anti-apoptotic MCL, phosphorylation of ERK, and upregulation of pro-apoptotic BIM. In MLL-r ALL cells, concomitant administration of MEK inhibitor, selumetinib, trametinib (GSK212), or binimetinib (MEK162) with prednisolone displayed pronounced synergistic effect in vitro [145, 146]. This has led to the SeluDex trial (NCT03705507), which is currently in progress. Moreover, it has been discovered that the addition of ATR inhibitor, AZ-20, produced synergistic in vivo pro-apoptotic effect in MLL-r ALL cell lines with RAS mutation [147]. The discovery of combination between MEK inhibitor and glucocorticoids provide new insights into future clinical practices to overcome resistant disease.

26.13 Targeting the Autophagy Pathway

ETV6-RUNX1 (formerly known as TEL/AML1) gene fusion results from the chromosomal translocation of t(12;21)(p13;q22) and is often associated with good prognosis [148, 149]. It is the most frequent form of genetic abnormalities in childhood BCP-ALL [150–153]. The presence of fusion proteins is essential for the survival of ETV6-RUNX1-positive cells, with elevation of Vps34 (autophagy regulator) activity [153].

Hydroxychloroquine (HCQ), an autophagy inhibitor, significantly reduced the cell viability in ETV6-RUNX1-positive BCP-ALL cells in vitro compared to the modest effect in ETV6-RUNX1-negative BCP-ALL cells [153]. It was also demonstrated to sensitize ETV6-RUNX1-positive cells to L-asparaginase. This paves the way for future clinical investigation in targeting the autophagy pathway in ALL.

26.14 Targeting the Ubiquitin–Proteasome System

The ubiquitin–proteasome system (UPS) regulates the homeostasis of cellular proteins [154]. It mediates the clearance of misfolded or damaged proteins via polyubiquitination and subsequent proteasomal-mediated degradation. Alteration in UPS induces endoplasmic reticulum (ER) stress and activates unfolded protein response (UPR). Phosphorylation of eukaryotic initiation factor 2α (eIF2α) ensues, followed by termination of translation and increase in pro-apoptotic protein and C/EBP homologous protein (CHOP) expression [3, 155] (Fig. 26.6).

Bortezomib (BTZ), a selective 26S proteasome inhibitor (PI), when combined with valproic acid (VPA), a potent histone deacetylase (HDAC) inhibitor, exhibited synergistic anti-leukemic effect in BCP-ALL cells in vivo [156]. Past clinical trial demonstrated the effective combination of BTZ with chemotherapy in R/R BCP-ALL patients (NCT00440726 [157]). Several clinical trials assessing the safety and tolerability of proteasome inhibitors in R/R ALL are currently underway (NCT02112916, NCT02303821, and NCT03590171). Besides, carfilzomib (CFZ), a second-generation irreversible PI, reported synergism in pro-apoptotic activities with vorinostat in T-ALL cell lines in vitro [158]. Currently, a phase 1 trial is undertaking studying the use of CFZ with chemotherapy in R/R ALL (NCT02303821). Another phase 1 evaluating the use of ixazomib (MLN9708) with chemotherapy in treating ALL has just completed (NCT02228772) [159]. This has led to the opening of a phase 1/2 trial on ixazomib (NCT03817320). VLX1570, a deubiquitinase (DUB) inhibitor, was shown to induce ER stress in ALL cells in vitro. It phosphorylated eIF2α and subsequently inhibited transcriptional initiation. It also demonstrated anti-proliferative effect in ALL cells in vivo, which prompts further studies for its future use as therapeutic agents.

26.15 Targeting the NEDD8 Conjugation Pathway

Neural precursor cell expressed developmentally downregulated protein 8 (NEDD8), activated by NEDD8-activating enzyme (NAE), is an essential component involved in the ubiquitin-mediated proteasomal degradation [160]. It mediates the neddylation of the cullin proteins, which are crucial for the proteolysis of proteins involved in the regulation of a range of cellular processes including cell growth, survival, autophagy, and apoptosis [161]. Dysregulated protein turnover results in apoptosis of ALL cells with induction of ER stress via upregulation of mTOR and UPR/eIF2α pathway [162] (Fig. 26.6).

Pevonedistat (MLN4924) is a first-in-class inhibitor of NAE. It demonstrated limited efficacy as a single agent, although exhibited anti-leukemic potential in eliciting ER stress both in vivo and in vitro [160]. Owing to the paradoxical and compensatory activation of MEK/ERK pathway in response to the cytotoxicity of pevonedistat, it is often associated with recurrence and unfavorable outcomes [162]. Nevertheless, the limitation can be overcome by the co-administration with a MEK inhibitor, selumetinib. TAS4464, another NAE inhibitor, exhibited higher potency and durability in inhibiting neddylation [161]. A clinical trial is currently underway, investigating the efficacy of pevonedistat with chemotherapy in R/R ALL or lymphoblastic NHL in adolescents and young adults (NCT03349281).

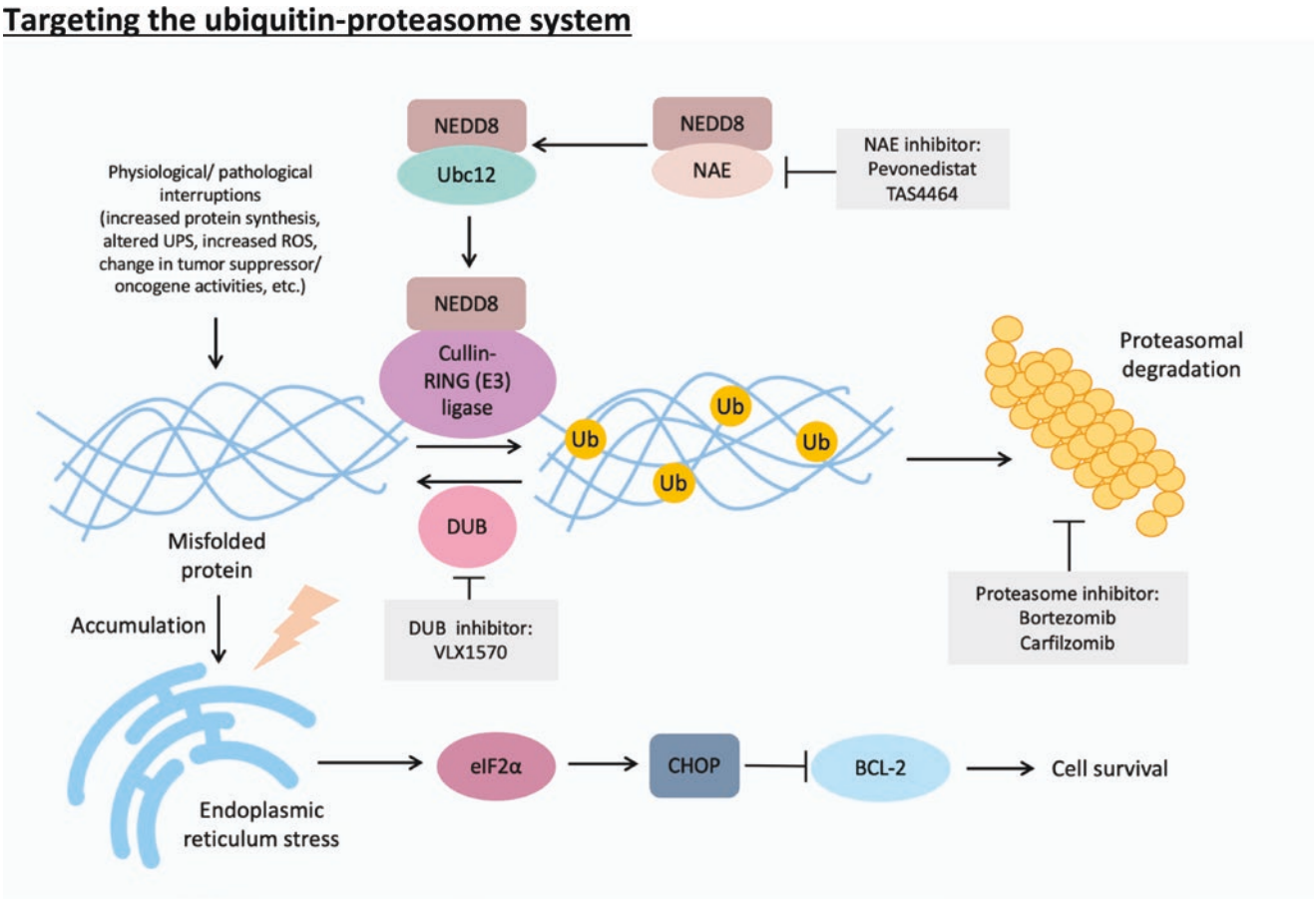

Fig. 26.6 Overview of the novel agents targeting the ubiquitin–proteasome system and the NEDD8 conjugation pathway. *BCL-2* B-cell lymphoma 2, *CHOP* CCAAT/enhancer binding protein (C/EBP) homologous protein, *DUB* deubiquitinating enzyme, *ER* endoplasmic reticulum, *eIF2α* phosphorylation of eukaryotic initiation factor 2α, *NAE* NEDD8-activating enzyme, *NEDD8* neural precursor cell expressed developmentally downregulated protein 8, *Ubc12* ubiquitin–conjugating enzyme 12, *UPS* ubiquitin–proteasome system

26.16 Targeting the Epigenetic Regulation

Epigenetic modification in DNA or histone via methylation and acetylation is an essential oncogenic event in the development of ALL [163]. It can either result in transcriptional activation or repression depending on the genes involved. Aberrant gene expression eventually leads to leukemogenesis.

26.16.1 Histone Methylation

Histone-lysine N-methyltransferase 2A (KMT2A, formerly known as mixed-lineage leukemia, MLL) is a H3K4 methyltransferase responsible for epigenetic regulation of gene transcription [164, 165]. Chromosomal translocations harboring KMT2A gene at 11q23.3 can be found in both acute myeloid leukemia (AML) and ALL [166]. It is the most common genetic subtype in infant ALL [77, 167], with KMT2A-AFF11 at t(4;11)(q21;23) accounting for approximately 60% of the KMT2A rearrangement [165]. Apart from AFF11 (AF4), partner fusion genes with KMT2A in ALL also include MLLT1 (ENL), MLLT3 (AF9), and ELL. [166]. Fusion with KMT2A results in the loss of SET domain and subsequently its catalytic function [164]. The fusion partner genes can often interact with disrupter of telomeric silencing 1-like (DOT1L), a H3K79 methyltransferase, via both direct and indirect pathways [168]. The resultant transcriptional activation of DOT1L promotes the expression of KMT2A target genes that favor leukemic transformation [169]. In general, KMT2A-rearranged ALL (KMT2Ar-ALL; MLLr-ALL) confers a poor prognosis with high-risk clinical features and a long-term survival rate of less than 60% [165, 169, 170], and they are particularly amenable to epigenetic regulators [171] (Fig. 26.7).

Pinometostat (EPZ-5676) is a first-in-class small-molecule DOT1L inhibitor [172]. It demonstrated effective in vitro anti-leukemic activity in most cell lines with MLL-r cell lines compared to non-MLL-r cell lines [173]. It also exhibited in vivo superior potency and selectivity over another DOT1L inhibitor, EPZ004777. Besides, the stability of anti-apoptotic gene BCL-2 is maintained by KMT2A-AFF1 via methylation. Co-administration of BCL-2-selective inhibitor, ABT-199, and DOT1L inhibitor, SGC0946, reported synergistic anti-proliferative activity in MLL-r cell lines in vitro [174]. The safety and efficacy of EPZ-5676 in R/R MLL-r leukemia have been evaluated in several clinical trials (NCT01684150 and NCT02141828). Only modest and transient anti-leukemic activities were observed; hence, pinometostat is not a particularly useful monotherapy agent despite tolerable safety profile. However, further clinical studies are required to validate its efficacy [172, 175].

BIX-01294 is a G9a-selective inhibitor antagonizing the activity of H3K9 methyltransferase. It diminished proliferation in T-ALL cell lines. It abrogated cell cycle progression and induced apoptosis via upregulation of CDK inhibitors, p15 and p21, and downregulation of pro-survival signal, BCL-2 [176]. The promising preclinical result opens a new path for future clinical development of histone methylation inhibitors (Fig. 26.7).

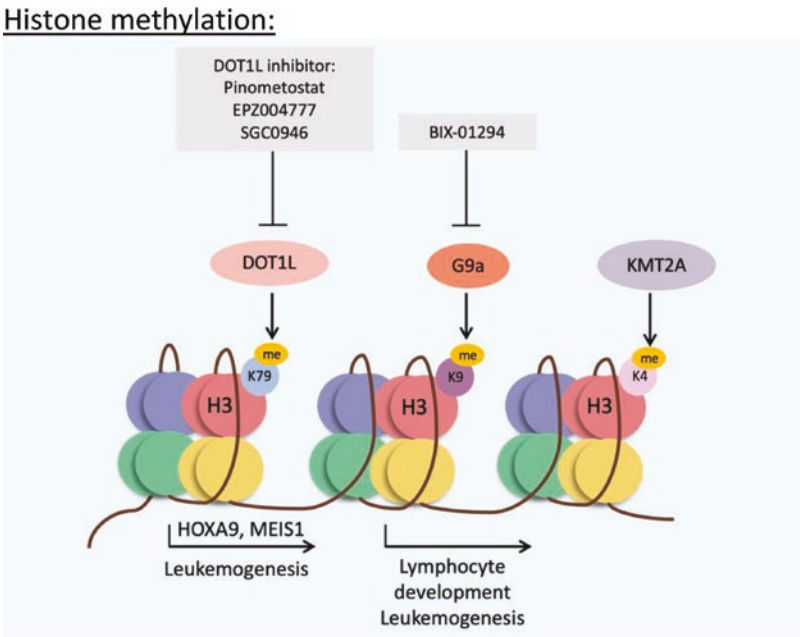

Fig. 26.7 Overview of the novel agents targeting histone methylation. *DOT1L* disrupter of telomeric silencing 1-like, G9a, also known as EHMT2, euchromatic histone-lysine N-methyltransferase 2, *H3* histone H3, *HOXA9* homeobox A9, *KMT2A* histone-lysine N-methyltransferase 2A, *K4* lysine K4, *K9* lysine K9, *K79* lysine 79, *me* methyl group, *MEIS1* myeloid ecotropic viral insertion site 1

26.16.2 DNA Methylation: Hypomethylating Agents (HMAs)

DNA methylation is generally associated with transcriptional inhibition via covalent addition of methyl group to the cytosine-phosphate-guanine (CpG) islands of the gene promoter region by DNA methyltransferases (DNMTs) [163]. Significant hypermethylation of promoter CpG islands (CGIs) is often noted in ETV6/RUNX1 hyperdiploid B-ALL, which contributes to its potential susceptibility to HMAs [177]. Besides, the major genes involved in the leukemogenesis of ALL include CDKN2B, IKZF1, TP73, SALL4, DLX, and DLC [98, 178]. Silencing of these genes via DNA hypermethylation leads to dysregulated cell differentiation, proliferation, repair, and survival (Fig. 26.8).

Azacitidine (Aza) and decitabine (Dec) are both cytosine nucleoside analogs targeting against DNMTs. Remarkable anti-proliferative and anti-metabolic activities were observed in ALL cell lines treated with either drug in vitro, with higher potency in Dec [179]. Combination of HMA with cytarabine or doxorubicin results in augmented anti-leukemic effect in vitro in MLL-positive BCP-ALL cell lines. This led to a phase 2 clinical trial in progress, investigating the combination of Aza with chemotherapy in pediatric ALL and MLL-r ALL (NCT02828358). Interestingly, the use of Dec with foretinib, RTK inhibitor, also exhibited synergism in ALL cell lines in vitro [180]. The use of Dec has been evaluated in R/R ALL (NCT00349596), but results are yet to be published. Combination of Dec with HDAC inhibitor, vorinostat, is also investigated. Yet, its use is restricted due to pronounced infectious toxicities despite potent clinical efficacy (NCT01483690 [181]). Future development of clinical strategy in minimizing toxicity and optimizing disease response is required.

26.16.3 Histone Acetylation

Histone deacetylase (HDAC) mediates the deacetylation of histone and non-histone proteins. It is involved in transcrip-

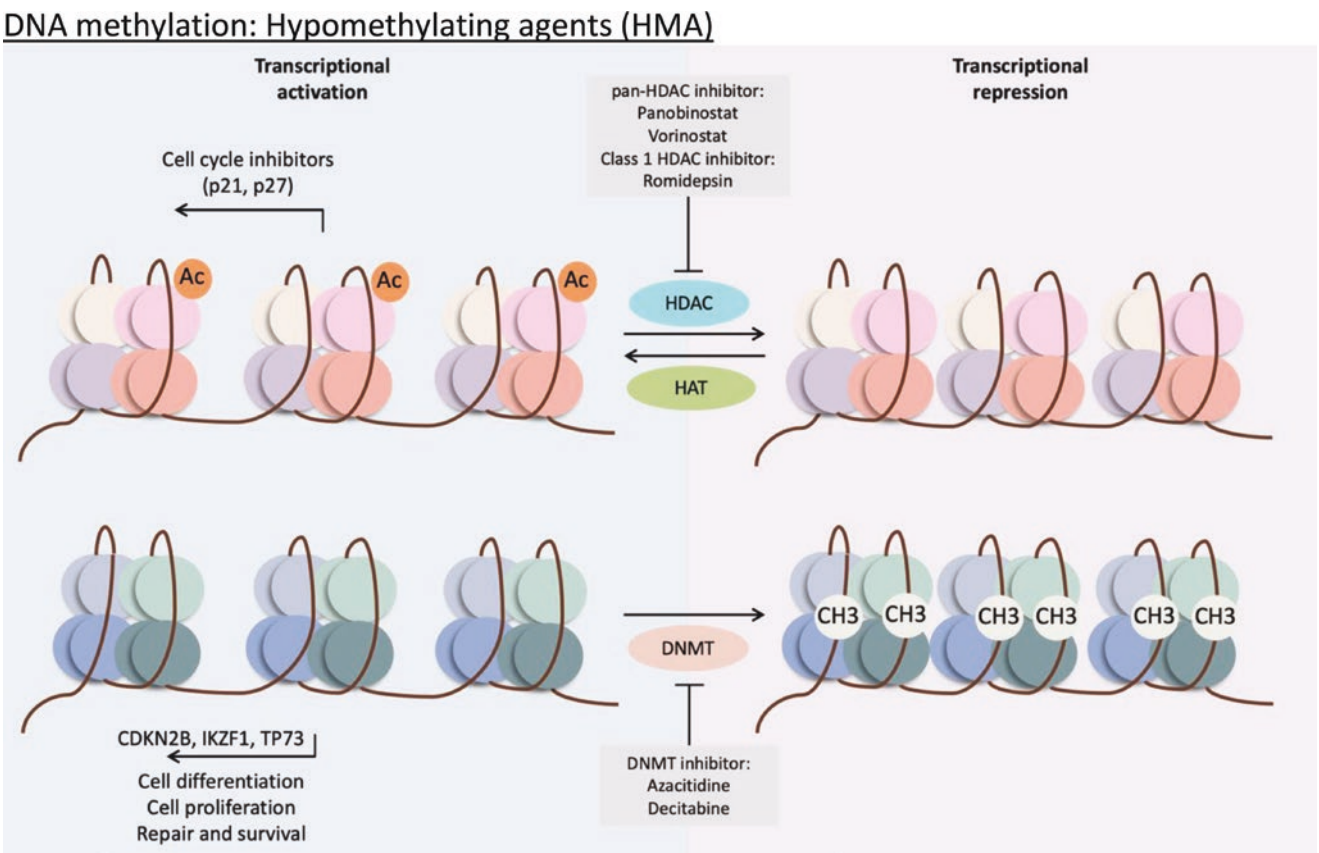

Fig. 26.8 Overview of the novel agents targeting histone acetylation and DNA methylation. *Ac* acetylation, *CDKN2B* cyclin-dependent kinase inhibitor 2B, *CH3* methyl group, *DNMT* DNA methyltransferase, *HAT* histone acetyltransferase, *HDAC* histone deacetylase, *IKZF1* IKAROS family zinc finger 1

tional repression of various genes including tumor suppressor genes and subsequent initiation of leukemogenesis [182–184]. Aberration of DNA deacetylation is commonly noted in ALL with elevated expression of HDACs, significant association with T-ALL and initial leukocytosis at presentation, and poorer response to glucocorticoids [6, 184] (Fig. 26.8).

Panobinostat (LBH589) is a pan-HDAC inhibitor that robustly downregulated BRE1 complex (RNF20, RNF40, and WAC), an essential component in the maintenance of MLL-r ALL cell lines, both in vitro and in vivo [171]. The leukemic cell viability is compromised as a result of the loss of the E3 ligase activity of BRE1. In treated BCP-ALL cell models, reduction in cell viability was also observed with upregulation of cell cycle inhibitors (p21 and p27) and downregulation of cell cycle promoters (c-Myc and CDK4) [182]. It was shown to shrink leukemic burden in B-ALL PDX models in vivo [185]. Preclinical data showed promising results, yet clinical trial showed otherwise, which it is terminated due to lack of efficacy (NCT00723203). Vorinostat, another pan-HDAC inhibitor, when used cooperatively with a plant derivative quinacrine, induced cytotoxicity in T-ALL cells both in vitro and in vivo [183]. In vitro study showed potential reversal of steroid resistance with vorinostat via re-expression of BIM protein, a target substrate of glucocorticoid (GC), selectively in cells with BIM CpG methylation [186, 187]. The promising results of preclinical studies deem HDACi a strong candidate for future therapeutic use in ALL. Currently, several clinical trials are underway investigating the application of vorinostat with bortezomib and chemotherapy (NCT02553460).

Romidepsin (FK288), a selective class 1 HDAC inhibitor, when used in combination of low-dose cytarabine, significantly increased the survival and reduces leukemic burden in MLL-r xenografted mice [188]. Despite the ineffectiveness when using as monotherapy, romidepsin significantly augmented the effect of low-dose cytarabine and reduced myelosuppression. The combination also resulted in profound improvement in survival of xenografts. In vivo study demonstrated selective HDAC inhibition in inhibiting growth in B-ALL cell lines with HDAC1/2-specific inhibitors [185]. This paves the way for future drug development with increasing specificity.

26.17 Targeting the BET Protein

The bromodomain (BRD), extra-terminal (BET) protein family, recognizes the acetylated lysine residues present on the surface of histones and non-histone proteins [189]. It manipulates the expression of oncogenes via binding. Bromodomain 4 (BRD4) constitutes the BET family protein and is a key component in the recruitment of positive transcription elongation factor complex (P-TEFb) to the acetylated chromatin for transcriptional activation [190]. Activation of target genes such as c-Myc and BCL-2 results in leukemogenesis [191]. BRD4 is often overexpressed in T-ALL [192].

JQ-1, a BET inhibitor, exhibited potent anti-proliferative effect in vitro in B-ALL cell lines, especially those with CRLF2 rearrangement [191, 193]. It increased anti-apoptotic activities by downregulating Bcl-2 and reducing CDK2 and CDK4 expression [191]. It was also demonstrated to interfere with the glycolytic pathway of leukemic cells via inhibition of c-Myc expression and its targeted genes (PKM2 and LDHA). Other in vivo and in vitro studies also elucidated the downregulation of c-Myc by JQ-1 and showed synergistic effect between JQ-1 and dexamethasone [194]. Particular sensitivity was shown in cell lines with CRLF2 rearrangement in an in vitro study. Besides, birabresib (OTX015/MK-8628), a BRD2/3/4 inhibitor, also elucidated its anti-leukemic effects via downregulation of c-Myc expression in ALL cell lines in vitro [195]. This has led to a dose-finding trial on the use of birabresib in patients with hematologic malignancies including ALL (NCT01713582) [196].

I-BET151, a BRD2/4 inhibitor, demonstrated growth inhibitory effect in vitro in MLL-AF4-positive ALL cells with cell cycle arrest at G0/G1 phase [197]. It demonstrated ability to overcome GC resistance in MLL-r ALL cell lines. In addition, synergistic activity was exhibited when combined with pan-HDAC inhibitor, givinostat (ITF2357) or panobinostat (LBH589). ARV-825, a BRD4 degrader, demonstrates anti-proliferative effect via CRBN-mediated proteasomal degradation (Fig. 26.9). Pronounced cytotoxic effects were seen with the use of ARV-825, which was more superior to that of JQ-1, dBET1 in T-ALL cell lines in vitro [192].

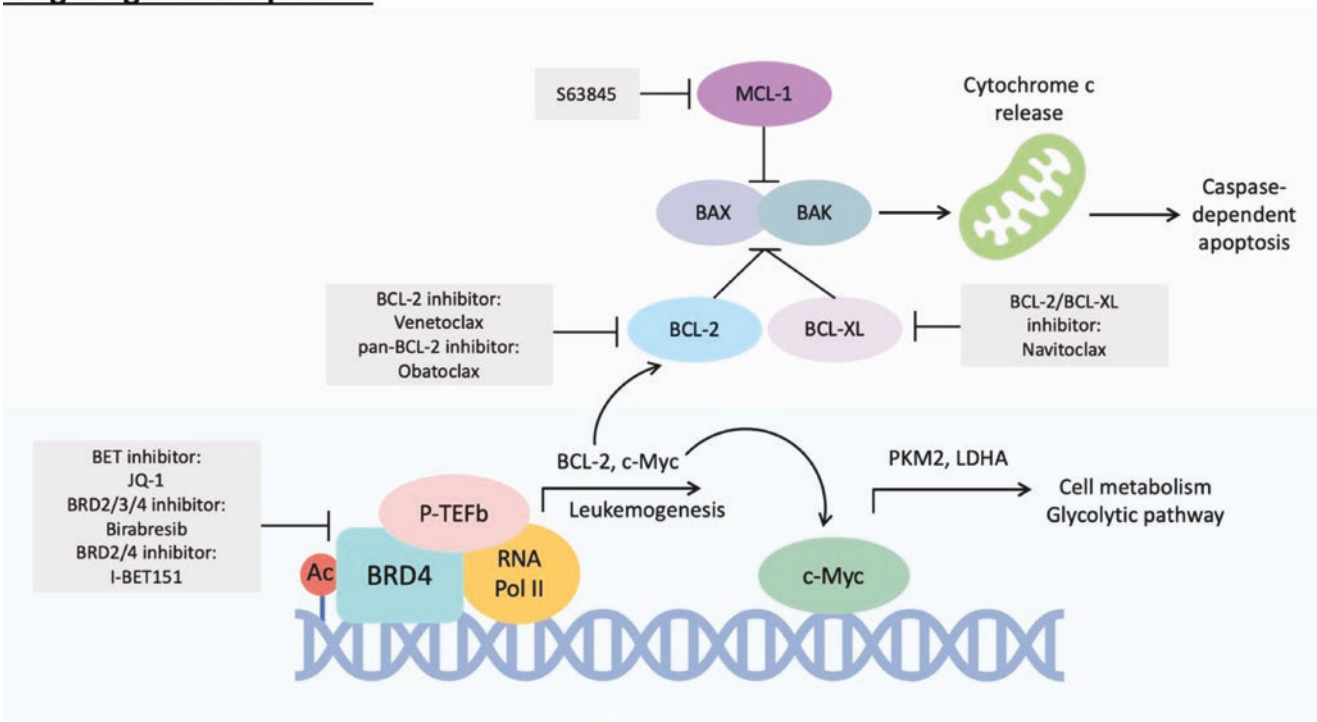

Fig. 26.9 Overview of the novel agents targeting the BET proteins and BCL-2 family proteins. *Ac* acetyl group, *BAK* Bcl-2 antagonist/killer, *BAX* Bcl-2-associated X, *BCL-2* B-cell lymphoma 2, *BCL-XL* B-cell lymphoma-extra-large, *BET* the bromodomain, *c-Myc* cellular myelocytomatosis oncogene, extra-terminal, *BRD* bromodomain, *LDHA* lactate dehydrogenase A, *MCL-1* induced myeloid leukemia cell differentiation protein MCL-1; *PKM2* tumor M2-pyruvate kinase, *P-TEFb* positive transcription elongation factor b, *RNA Pol II*: RNA polymerase II

26.18 Targeting the Mitochondrial Pathway of Apoptosis

26.18.1 Targeting BCL-2 Family Proteins

BCL-2 family protein consists of a group of pro-apoptotic and pro-survival proteins responsible for the regulation of intrinsic apoptosis. It is often overexpressed in a wide range of malignancies, including ALL. However, its dependence showed heterogeneity across different ALL subtypes [198]. The BCL-2 family can be subdivided into two groups: pro-apoptotic (BAX, BAK, BH3-only subfamily, etc.) and pro-survival (BCL-2, BCL-XL, MCL-1, etc.). Activation of BAX/BAK is a crucial step in inducing apoptosis via mitochondrial regulation and caspase activation [199]. BCR-ABL1 fusion alters the balance of BCL-2 family proteins, with a shift toward anti-apoptotic events with upregulation of MCL-1 and reduction in BH3-only proteins [7, 199] (Fig. 26.9).

Venetoclax (ABT-199), a BCL-2-selective inhibitor, exhibited strong single-agent effect in Ph+ B-ALL, MLL-r ALL, and TCF3-HLF-positive ALL subtypes [198, 200]. Synergistic result was obtained when it was combined with nilotinib, but not asciminib [200]. It also demonstrated superior in vivo activity when used in conjunction with a dual BCL-2/BCL-XL inhibitor, navitoclax (ABT-263), than monotherapy in most ALL subtypes including T-ALL and ETP-ALL [198]. In Ph-like ALL, its combination with dasatinib or ruxolitinib provided strong preclinical evidence of synergism [201]. Amplified cytotoxic effect was achieved with the co-administration of venetoclax or ABT-737 with chemotherapy, with most potent response observed with L-asparaginase [174].

A phase 1 trial on the combination therapy with venetoclax, navitoclax, and chemotherapy in R/R ALL has just been completed (NCT03181126) [202]. The study illustrated the shift in BCL-2/BCL-XL dependence as possible resistance mechanism to single BCL-2/BCL-XL inhibitors in ALL patients. Therefore, the concomitant inhibition of BCL-2 and BCL-XL was speculated to overcome such resistance and was associated with pronounced response rates and clinical tolerability [202]. In addition, venetoclax also exhibited synergism with S63845, a MCL-1 inhibitor, in enhancing TKIs and conventional chemotherapy [203].

Currently, several clinical trials are in progress exploring the combination of venetoclax with liposomal vincristine (NCT03504644), or chemotherapy (NCT03808610, NCT03236857 [204], and NCT03319901) in R/R ALL. Another phase 1/2 trial is also underway in investigating its combination with ponatinib and dexamethasone in Ph-positive R/R ALL or CML (NCT03576547).

Obatoclax (GX15-070), a pan-BCL-2 inhibitor, induced cell death in in vitro ALL cells via downregulation of MCL-1, with induction of both apoptosis and autophagy. It can overcome resistance in ALL cells with high level of MCL-1 expression, which cannot be reproduced in ABT-737 or ABT-263 [205]. It also demonstrated GC-sensitizing ability as most GC-resistant cells have high level of MCL-1 expression. This prompts future clinical investigations into BCL-2 inhibitor with broader coverage with higher efficacy. Cooperative results could be obtained when GX15–070 combined with dactolisib [206].

26.19 Targeting Bone Marrow Microenvironment (BMM)

Bone marrow (BM) niche plays a vital role in sustaining the development of ALL [207]. In ALL, there is aberrant proliferation of lymphoid precursor cells in the bone marrow of patients. The crosstalk between leukemic cells and bone marrow stromal cells (BMSCs) is essential for the optimal survival and proliferation of leukemic cells. BMM also provides sites for escape and protection that subsequently contribute to the emergence of drug resistance and relapse in ALL. Disruption of the interaction in BMM may help restore sensitivity and reverse the dismal outcomes in ALL patients.

26.19.1 Targeting the CXCL12/CXCR4 Axis

C-X-C chemokine receptor type 4 (CXCR4) is a receptor for its ligand, CXCL12 (also known as stromal cell-derived factor 1 (SDF-1)). Elevated expression of CXCR4 can be observed in both B-ALL and T-ALL [208–210]. The CXCL12 secreted by BMSCs creates a chemotactic attraction for CXCR4-bearing leukemic cells and promotes their homing to BMM. Its expression largely correlates with the risk of relapse with increased expression in R/R disease [210, 211] (Fig. 26.10).

Plerixafor (AMD3100) and AMD3465 are both CXCR4 antagonists, interfering with the communication between resistant ALL cells and BMM. They help promote the mobilization of leukemic cells into peripheral circulation and increase their exposure to chemotherapy. They exhibited potent single-agent anti-leukemic activities in B-ALL and

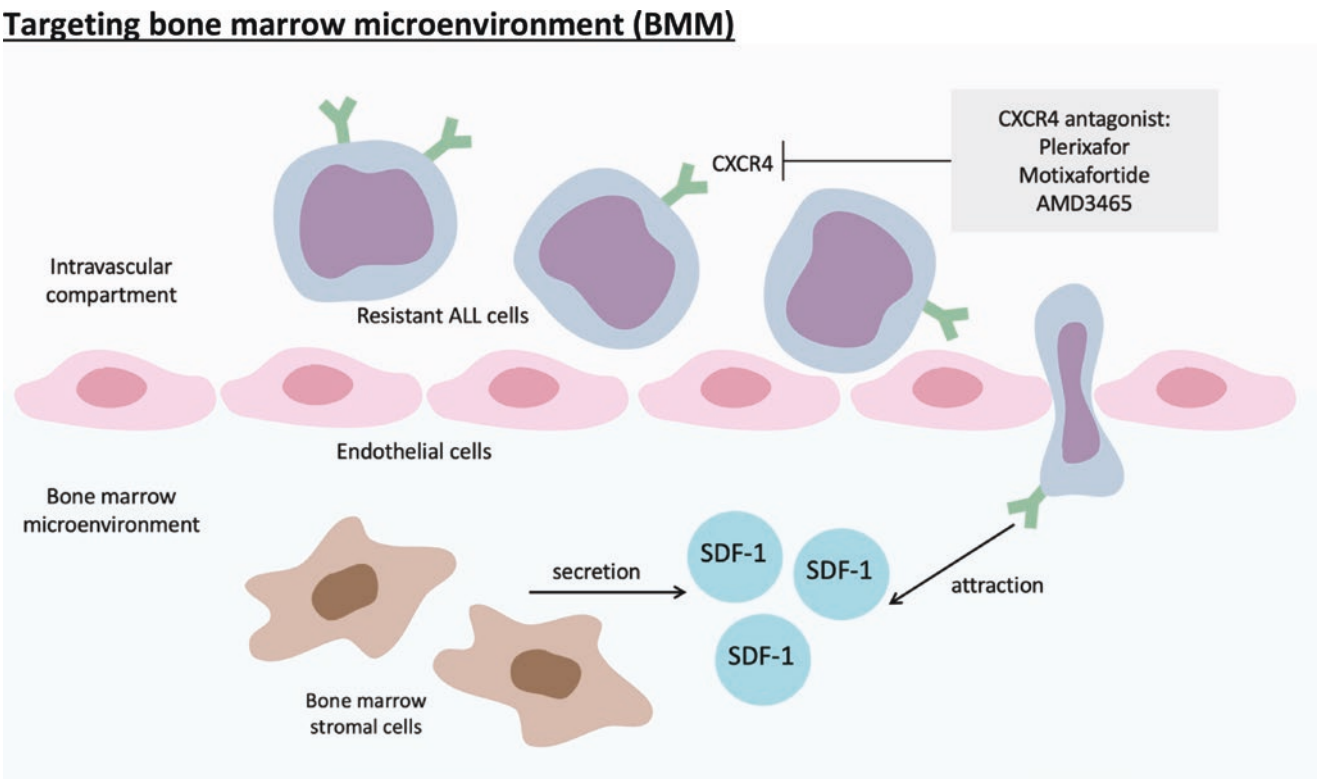

Fig. 26.10 Overview of the novel agents targeting the CXCL12/CXCR4 axis in the bone marrow microenvironment. *ALL* acute lymphoblastic leukemia; *CXCR4* C-X-C chemokine receptor type 4; *SDF-1* stromal cell-derived factor 1, also known as CXCL12

T-ALL cells in vivo, respectively [210, 212]. In an in vitro preclinical study, AMD3100 sensitized ALL cells to the cytotoxic activity of vincristine via upregulation of pro-apoptotic protein, BAX [210]. Apart from chemosensitization to vincristine, B-ALL cells treated with plerixafor or motixafortide (BL-8040/BKT140), a peptide CXCR4 inhibitor, also demonstrated increased susceptibility to dexamethasone and cyclophosphamide [209]. The use of BL-8040 and nelarabine in R/R T-ALL/LBL is currently underway (NCT02763384). POL5551, a novel CXCR4 antagonist, is a protein epitope mimetic. In an in vitro study, it markedly reduces SDF-1-mediated chemotaxis and undermines the ERK signaling cascades via inhibition of SDF-1-induced phosphorylation [213]. It profoundly sensitized ALL cells including high-risk subtypes, MLL-r ALL, to cytarabine and rendered them vulnerable to chemotherapy-induced apoptosis. These positive preclinical results provide new directions for future clinical investigation in using CXCR4 antagonist to overcome chemoresistance.

26.20 Immunotherapy

The treatment paradigm in ALL has been refined and optimized by the incorporation of novel immunotherapeutic options [214–216]. It can be classified into five categories according to their mechanism of actions. These include naked monoclonal antibodies, antibody–drug conjugates (ADCs), bispecific T-cell engagers (BiTEs), chimeric antigen receptors (CARs), and immune checkpoint inhibitors.

26.21 Naked Monoclonal Antibodies

Naked monoclonal antibodies recognize and bind to specific cell surface antigens on leukemic blasts to activate cell death by antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and direct apoptosis [214–217]. To date, naked monoclonal antibodies have become more significant in targeting B lymphocyte cell surface receptors, while their efficacy in T-ALL remains a field to be explored [216]. Common targeted B-cell surface markers include CD19, CD20, and CD22 [215–218], while antigens that could be directed in T-ALL include CD38 and CD52 (Fig. 26.11).

26.21.1 Anti-CD20 Monoclonal Antibodies

CD20 is a B-cell-specific non-glycosylated transmembrane phosphoprotein present on both normal and neoplastic B lymphocytes with the exception of HSCs, pro-B cells, and terminally differentiated plasma cells [215, 217–220]. CD20 is expressed on 30–50% of precursor B-ALL [219, 221–224]

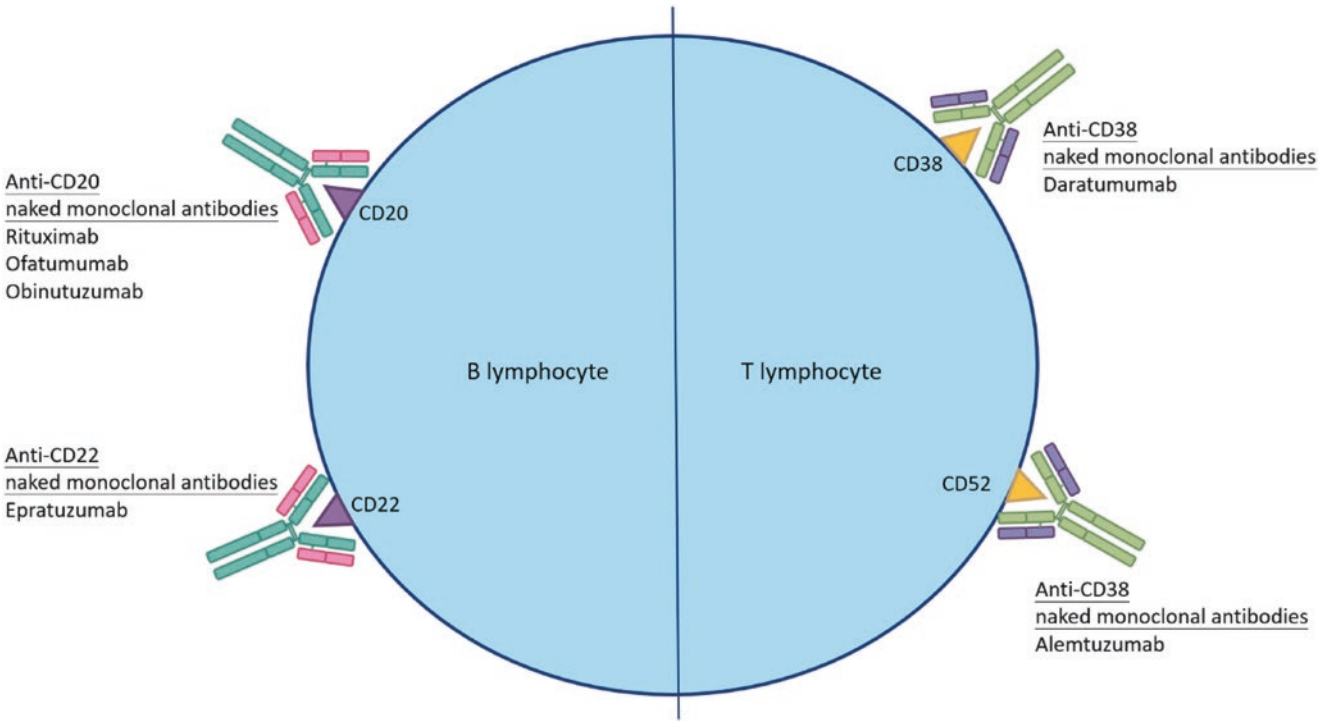

Fig. 26.11 Naked monoclonal antibodies therapy in ALL

and on almost all mature B-ALL leukemic blasts [214]. Clinically, its expression is generally associated with worse prognostic outcome in B-ALL [1, 222, 223]. It is also stably expressed as neither would it be internalized or released upon binding of antibodies, nor would it circulate in plasma freely [218, 224]. More importantly, CD20 lost on HSCs and plasma cells allows conservation of stem cell pool and significant reduction of off-target adverse effects [224]. All these make anti-CD20 antibody an attractive and plausible immunotherapeutic option to target B-cell malignancies via interrupting cellular differentiation and progression and regulating apoptosis [1, 217]. Hence, the use of anti-CD20 naked monoclonal antibodies has been explored in B-ALL.

Rituximab is a first-generation chimeric monoclonal anti-CD20 antibody that has been extensively investigated and incorporated as an adjunct with chemotherapy in the treatment of mature B-cell non-Hodgkin lymphomas to improve survival and prognosis [220, 225]. In view of the promising results, the efficacy of rituximab is explored in B-ALL. In the phase 3 Group for Research on Adult Acute Lymphoblastic Leukemia 2005 (GRAALL-2005) clinical trial, addition of rituximab to conventional chemotherapy offered longer event-free survival (EFS) by diminishing relapse risks despite no significant differences in early clearance of blasts [225]. Hence, this enhances the appeal of rituximab to be included in frontline chemotherapy for B-ALL patients, especially those who are below 60 years old [216, 218] (Table 26.3).

Ofatumumab is a second-generation naked monoclonal antibody against CD20 [1, 226, 227]. Different from rituximab, it anchors to the small extracellular epitope of CD20, which enhances ADCC and CDC by augmenting complement 1q binding to ofatumumab-opsonized B lymphocytes [1, 223, 226, 228]. The unique binding is also tighter, resulting in a longer half-life and a slower dissociation rate than that of rituximab [1, 224]. Ofatumumab has been evaluated in several phase 2 clinical trials in combination with hyper-CVAD (hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone) [226–228] (Table 26.3). Durable complete remission (CR) and OS were demonstrated [226–228]. The exciting results were not only limited to adults, but also manifested in 18 to 39-year-old young patients. Ofatumumab–hyper-CVAD was shown to have comparable outcomes in terms of CR and OS when compared to the pediatric-specific protocol in the Cancer and Leukemia Group B (CALGB) 10,403 prospective clinical trial, signifying a possible alternative for younger population [228, 229] (Table 26.3). Hence, ofatumumab serves as a significant novel alternative to rituximab in frontline B-ALL therapy.

Obinutuzumab (GA101) is a glycoengineered anti-CD20 antibody, which possesses stronger binding affinity to immune cells for ADCC [230]. To date, the effect of obinutuzumab has only been appraised in preclinical trials [231]. Increased ADCC and cell death were displayed in rituximab-resistant B-ALL murine models, encouraging future clinical studies on obinutuzumab use in B-ALL [216, 231].

26.21.2 Anti-CD22 Monoclonal Antibodies

CD22 is a common marker found on >90% B lymphoblasts in B-ALL and is internalized rapidly upon binding of antibodies [214, 215, 230, 232]. Epratuzumab, a naked anti-CD22 monoclonal antibody, has been investigated in relapsed CD22-positive B-ALL in the pediatric population [233]. In the phase 2 Children's Oncology Group (COG) Study ADVL04P2, it was demonstrated that epratuzumab was well-tolerated with modest MRD response, yet, it failed to yield significant therapeutic effects to induce second CR in relapsed B-ALL when comparing to conventional chemotherapy regimens [233] (Table 26.3). Similar results were displayed in another phase 2 study, which adopted the hyper-CVAD backbone in R/R B-ALL in young adults [234] (Table 26.3). A phase 3 international study for children experiencing relapsed B-ALL was underway (NCT01802814). As a short-lived minimal residual response (MRD) was noticed in both clinical trials, it might suggest the inclusion of epratuzumab in frontline settings to bridge patients to HSCT [230, 234]. Furthermore, in view of the rapid internalization property of CD22, a possible future direction is to strengthen the effect of epratuzumab by cytotoxic drug conjugation [230].

26.21.3 Anti-CD38 Monoclonal Antibodies

CD38 is preferentially expressed on activated T cells, thymocytes, and terminally differentiated B cells with minimal expression on normal myeloid and lymphoid cells [216], hence making it a possible therapeutic target in T-ALL. Although a high level of CD38 expression is not associated with survival benefits, it is associated with worse prednisolone response and increased MRD levels [235], further enhancing the appeal of anti-CD38 monoclonal antibodies.

Daratumumab is an anti-CD38 monoclonal antibody that induces ADCC, CDC, and antibody-dependent cellular phagocytosis (ADCP) [236, 237]. In preclinical PDX models, daratumumab was revealed to be efficacious in pediatric T-ALL, including ETP-ALL, a T-ALL subtype that confers a more dismal prognosis [238]. Its efficacy was further validated in significant MRD eradication in patients with CD19-/CD22-negative T-ALL [239]. This provides the groundwork for daratumumab use in relapsed or MRD-positive T-ALL. This paved the way to a phase 2 clinical study in R/R

Table 26.3 Clinical trials results of novel monoclonal antibodies in B- and T-ALL

Cell surface marker	Novel agent	Clinical status of ALL	Age group	Outcome and observations	Adverse effects	Clinical trial phase	References
Naked monoclonal antibodies							
CD20	Rituximab	Ph-negative CD20-positive precursor B-ALL	Adults	1. Longer EFS contributed by decreased relapse risks 2. No significant difference in OS 3. Higher complete remission rate	• Slightly increased risks of infections	GRAALL-2005, phase 3	[225]
	Ofatumumab	CD20-positive B-ALL	Adults	1. Longer CRD and OS 2. Attainment of MRD negativity after induction 3. Similar toxicity profile when compared to hyper-CVAD ± rituximab1.1.	• NS	Phase 2	[226]
		Ph-negative CD20-positive B-ALL	Adults		• Infusion reaction • Infections during induction and consolidation • Hypokalemia • Hyperglycemia		[227]
			Adults, young adults				[228]
CD22	Epratuzumab	CD22-positive relapsed B-ALL	Pediatrics to young adults (2–30 years old)	1. No significance in increasing CR2 rates compared to conventional chemotherapy 2. Non-significant MRD responses	• Infections • Neutropenic fever • Hyperglycemia • Elevated ALT levels	Phase 1/2	[233]
		CD22-positive R/R B-ALL	Young adults	1. Higher CR/CRp but disease improvement was short-lived 2. Improved MRD responses	• Pancytopenia • Well-tolerated, no severe toxicity related to epratuzumab	Phase 2	[234]
CD52	Alemtuzumab	R/R ALL	Adults	1. Some achievement of CR 2. Transient response with frequent disease progression	• Opportunistic infections (pulmonary aspergillosis, CMV reactivation) • Fever and chills • Skin rash • Bronchospasm	Phase 2	[242]

Table 26.3 (continued)

Cell surface marker	Novel agent	Clinical status of ALL	Age group	Outcome and observations	Adverse effects	Clinical trial phase	References
Antibody–drug conjugates							
CD22	Inotuzumab ozogamicin	R/R BCP-ALL	Adults	1. Significant lengthened PFS and OS compared to standard intensive chemotherapy 2. Vital role in bridging patients to HSCT Safety and tolerability in high disease burden patients 3. Improved CR and CRi rates regardless of leukemic blasts in BM and disease burden 4. Achievement of sustained MRD negativity 5. Higher CR and HSCT rates compared to blinatumomab 6. Similar OS with blinatumomab	• Myelosuppression (thrombocytopenia, anemia, neutropenia) • Hepatotoxicity (VOD/SOS, hyperbilirubinemia) • Infections and infestations	INO-VATE, phase 3	[252] [253] [256]
		Newly diagnosed Ph-negative ALL	Elderly (≥60 years old)	1. Improved PFS and OS in inotuzumab ozogamicin-mini-HCVD regimen 2. Decreased death rates and improved tolerability compared to hyper-CVAD regimen	• Hepatotoxicity (VOD/SOS, hyperbilirubinemia) • Infections during the course of induction and consolidation • Hyperglycemia • Hypokalemia	Phase 2	[257]
		R/R Ph-negative ALL	Adults	1. Improved PFS and OS in inotuzumab ozogamicin-mini-HCVD regimen 2. Increased CR and MRD negativity	• Myelosuppression (thrombocytopenia) • Hepatotoxicity (VOD/SOS, hyperbilirubinemia)	Phase 2	[258]
		R/R BCP-ALL	Pediatrics (1 to <18 years old)	1. Sustained CR rate and high MRD negativity 2. Increased EFS and OS 3. Similar efficacy in high-risk CG patients	• Hepatic events (raised ALT level, no SOS) • Myelosuppression (thrombocytopenia, anemia, neutropenia) • Pulmonary infections	ITCC-059, phase 1/2	[260]
	Moxetumomab pasudotox	Chemo-refractory ALL	Children (6 months to 25 years old)	1. Improved CR and MRD response to bridge patients to HSCT 2. Transient response and disease progression in KMT2A-rearranged infant ALL patients due to decreased CD22 expression	• Elevated ALT, AST levels • CLS (edema, hypoalbuminemia, weight gain) • Myelosuppression (thrombocytopenia, anemia, leukopenia) • HUS/TMA	Phase 1	[261]
		R/R B-ALL	Pediatrics (6 months to <18 years old)	1. Modest CR compared to phase 1 study 2. Termination of trial due to inadequate response	• CLS (weight gain) • Ocular dysfunction and visual blurring most likely related to CLS • HUS	Phase 2	[263]
		R/R B-ALL	Adults	1. Modest clinical activity in terms of CR, EFS, OS	• Transaminitis • CLS • HUS	Phase 1	[262]

CD19	Coltuximab ravtansine	R/R B-ALL	Adults	1. Transient (<2 months) response and lack significant therapeutic efficacy 2. Early discontinuation of clinical trial	• GI events (nausea, diarrhea) • Myelosuppression (thrombocytopenia, neutropenia, leukopenia) • Low-grade ocular toxicity • Peripheral motor neuropathy	Phase 2	[265]
	Denintuzumab mafadotin	R/R B-ALL	Adults	1. Sustained MRD negativity 2. Achievement of CR, especially in Ph-positive patients.	• Fever, nausea, fatigue, headache • Ocular events (visual blurring, superficial microcystic keratopathy)	Phase 1	[268]
	Loncastuximab tesirine	R/R B-ALL	Adults	1. Minimal achievement in CR 2. Early discontinuation of clinical trial	• Nausea • Neutropenic fever • Reversible elevated liver enzymes (AST, ALT, total bilirubin, GGT)	Phase 1	[269]
CD25	Camidanlumab tesirine	R/R ALL	Adults	1. Early discontinuation of clinical trial 2. The specific adverse effects (polyradiculopathy/ Guillain–Barré syndrome) of camidanlumab tesirine in R/R cHL were not seen	• Transaminitis • Neutropenic fever • Nausea, fatigue	Phase 1	[273]

(continued)

Table 26.3 (continued)

Cell surface marker	Novel agent	Clinical status of ALL	Age group	Outcome and observations	Adverse effects	Clinical trial phase	References
Bispecific T-cell engagers (BiTEs)							
CD3/ CD19	Blinatumomab	Ph-negative BCP-ALL	Adults	1. Significant improved OS, EFS, and CR compared to conventional chemotherapy	• CRS • Neurologic events	TOWER, phase 3	[277]
		R/R Ph-positive BCP-ALL	Adults	1. Significant CR and MRD response compared to conventional chemotherapy 2. Equally significant hematological and molecular response in BCR-ABL1 with or without T315I mutation 3. Similar RR and RFS in across age groups	• Fever (neutropenic and non-neutropenic) • Headache	ALCANTARA, phase 2	[278]
		MRD-positive BCP-ALL	Adults	1. Significant MRD response 2. Better MRD response in CR1 patients than that of CR2/3 patients due to presence of fewer aggressive subclones 3. Longer RFS in both HSCT candidates and patients who did not undergo HSCT	• Fever, headache • Myelosuppression (neutropenia, leukopenia, thrombocytopenia, anemia) • Neurologic events (tremor, aphasia, dizziness)	BLAST, phase 2	[279]
		CD19-positive R/R BCP-ALL	Pediatrics (>28 days to <18 years old)	1. Significant CR and MRD response, especially in patients with lower blast burden 2. Equally significant RR regardless of prior HSCT and relapses provided that leukemic blasts are CD19-positive 3. Efficacious in patients with trisomy 21, implying the effectiveness to patients who are at high risk of toxicity in intensive chemotherapy	• Neurologic events (headache, reduced level of consciousness, seizure) • CRS	Expanded access protocol	[282]
		R/R CD19-positive ALL	Adults	1. Significant MRD-negative CR rate with blinatumomab combined with immune checkpoint inhibitors (PD-1 inhibitor nivolumab, CTLA-4 inhibitor ipilimumab)	• Infusion reactions • Elevated AST, ALT levels	Phase 1	[283]

Ph-negative Philadelphia chromosome negative, *EFS* event-free survival, *OS* overall survival, *GRAALL-2005* Group for Research on Adult Acute Lymphoblastic Leukemia 2005, *CRD* complete remission duration, *MRD* minimal residual disease, *NS* not stated, *CR* complete remission, *R/R* relapsed or refractory, *CRp* partial complete remission, *INO-VATE* INotuzumab Ozogamicin trial to inVestigAte Tolerability and Efficacy, *BCP* B-cell precursor, *PFS* progression-free survival, *HSCT* hematopoietic stem cell transplantation, *VOD/SOS* veno-occlusive disease/sinusoidal obstructive syndrome, BM bone marrow, *CRi* complete remission with incomplete hematological recovery, *mini-HCVD* low-intensity regimen modified from hyper-CVAD, *ITCC-059* Innovative Therapies for Children With Cancer in Europe-059, *EFS* event-free survival, *CG* cytogenetics, *CLS* capillary leak syndrome, *HUS/TMA* hemolytic uremia syndrome/thrombotic microangiopathy, GI gastrointestinal, AST aspartate aminotransferase, ALT alanine transferase, *GGT* gamma-glutamyl transferase, *cHL* classical Hodgkin lymphoma, *CRS* cytokine release syndrome, *RR* response rate, *RFS* relapse-free survival

B-ALL and T-ALL patients (NCT03384654). Interestingly, all-trans retinoic acid (ATRA), the standard agent used in acute promyelocytic leukemia, could potentiate daratumumab via the upregulation of CD38 on tumor cells, providing another possible therapeutic strategy [240, 241].

26.21.4 Anti-CD52 Monoclonal Antibodies

CD52, a glycoprotein connected to cell membrane via a phosphatidylinositol glycan linkage, is expressed on numerous cell types including mature B and T lymphocytes and is involved in T-cell activation [217, 218, 230, 236]. Alemtuzumab, a recombinant humanized monoclonal antibody against CD52, induces CDC, ADCC, and apoptosis upon CD52 binding [236]. Alemtuzumab has been explored to target CD52 on T lymphocytes for the treatment of T-ALL. However, its efficacy was only transient and was coupled with multiple adverse effects in phase 2 clinical trial, greatly impairing the clinical significance of alemtuzumab in T-ALL therapy [219, 230, 242] (Table 26.3).

26.22 Antibody–Drug Conjugates (ADCs)

ADC is a developing chemoimmunotherapeutic strategy with a higher potency than monoclonal antibodies [243, 244]. It comprises three major units: a monoclonal antibody, a cytotoxic payload, and a linker to connect the above two molecules together [244] (Fig. 26.12a). The monoclonal antibody first binds to specific cell receptors on leukemic cells. Then, the complex would be internalized into the cell where the cytotoxic payload is cleaved or degraded to induce leukemic cell death [244] (Fig. 26.12b). This mechanism not only minimizes off-target effects by inducing selective cytotoxicity to leukemic cells, but also prevents toxicities from free-drug administration [243, 245].

26.22.1 Anti-CD22 ADCs

Inotuzumab ozogamicin targets CD22, a B-lineage cell surface marker that is ubiquitously expressed on B lymphoblasts, and is one of the most promising ADCs to date [214,

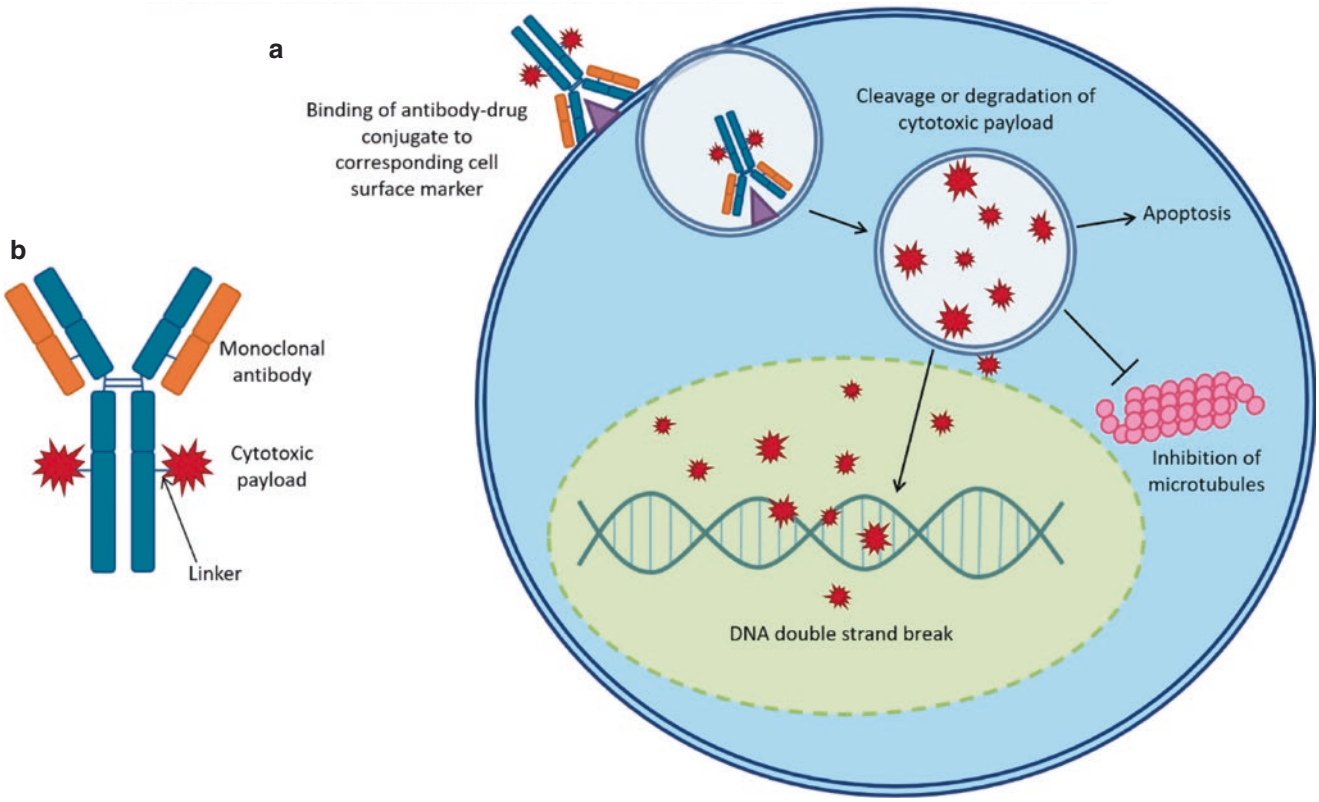

Fig. 26.12 Structure and mechanism of actions of antibody–drug conjugates in ALL. (**a**) shows the structure of antibody–drug conjugate. It comprises mainly three components: monoclonal antibody, cytotoxic payload, and the linker that links the above two together. (**b**) shows the mechanism of actions of antibody–drug conjugate. Upon binding of antibody–drug conjugate to the specific cell surface marker, the cytotoxic payload would be either cleaved or degraded depending on the nature of the linker. Then, the cytotoxic payload would be released to exhibit either one of the following actions: inhibit microtubules, DNA double-strand breaks, or direct cellular apoptosis

215, 243, 246, 247]. It is recently approved by the FDA in 2017 for R/R B-ALL and has an off-label compassionate use in the pediatric population [246, 248–250]. Inotuzumab ozogamicin is an immunoconjugate that consists of CD22-directed IgG4 monoclonal antibody attached to the potent cytotoxic payload calicheamicin [214–218, 230, 232, 243, 245–247, 249, 251]. As previously mentioned, CD22 is internalized rapidly upon antibody binding, which allows the delivery of calicheamicin to the intracellular compartment, causing DNA double-strand breaks and cell death by binding to the minor DNA groove [215–218, 230, 247]. It has been evaluated extensively in the phase 3 INotuzumab Ozogamicin trial to inVestigAte Tolerability and Efficacy (INO-VATE) trial as salvage therapy for R/R CD22-positive BCP-ALL in adults [252–256] (Table 26.3). Promising long-term outcomes including profound lengthening of progression-free survival (PFS) and OS were elucidated [252] irrespective of disease burden [253] and race [255]. Inotuzumab ozogamicin serves as an imperative stepping stone to HSCT to improve the dismal survival outcomes of R/R B-ALL patients [252–256]. Hepatotoxicity is a specific adverse effect of inotuzumab ozogamicin, and it should be monitored closely [251, 254]. HSCT conditioning regimens comprising more than two alkylating agents are associated with increased hepatic events and hence should be avoided if inotuzumab ozogamicin is administered [254].

In light of the favorable results, the use of inotuzumab ozogamicin was explored in the elderly and pediatric populations [257–259] (Table 26.3). Compatible with the INO-VATE trial, improved OS and PFS were exemplified in the elderly population using inotuzumab ozogamicin combined with mini-HCVD (a low-intensity regimen protocol modified from hyper-CVAD) in frontline settings [257, 259]. Similar superior outcomes were also established in the R/R ALL adult population with the same therapeutic combination [258]. In the pediatric population, sustained OS and event-free survival (EFS) were evidenced in the phase 1/2 Innovative Therapies for Children with Cancer in Europe-059 (ITCC-059) study [260]. The durable promising outcomes have made inotuzumab ozogamicin a bona fide in B-ALL therapy. A phase 3 clinical study in high-risk B-ALL was underway (NCT03959085).

Moxetumomab pasudotox (CAT-8015, HA22) is a recombinant immunotoxin consisting of an anti-CD22 monoclonal antibody fused to the *Pseudomonas aeruginosa* exotoxin A [232, 261–263]. Clinical significance was demonstrated in both pediatric and adult population. Modest clinical activity was seen, suggesting the potential of moxetumomab pasudotox in overcoming chemotherapy resistance in a phase 1 clinical trial in pediatrics and adults [261, 262]. However, the efficacy was modest and limited by capillary leak syndrome and hemolytic uremia syndrome, which were constantly observed adverse effects of moxetumomab pasudotox in early clinical trials [261–263].

26.22.2 Anti-CD19 ADCs

CD19 is a signature of pre-B and mature ALL that positively regulates and enhances the activation of B lymphocytes by decreasing the signaling threshold [230, 264]. As anti-CD19 is rapidly internalized upon antibody binding, it is a more ideal agent in ADCs instead of naked monoclonal antibodies [230]. Several anti-CD19 ADCs were developed, but further researches are required to optimize and increase their applicability.

Coltuximab ravtansine (SAR3419), an anti-CD19 antibody conjugating to cytotoxic molecule maytansinoid [264], has been evaluated in a phase 2 clinical trial [265]. Despite coltuximab ravtansine being generally well-tolerated, the trial is terminated due to modest outcomes [265] (Table 26.3). Although the limited efficacy might be contributed by the study design with R/R patients as participants, the outcomes observed were still inferior to other ADCs such as inotuzumab–ozogamicin [265]. Recently, huB4-DGN462 is developed. It exploits the same anti-CD19 antibody in coltuximab ravtansine with conjugation to an ultra-potent DNA-alkylating payload. Encouraging anti-leukemic activity was displayed in preclinical studies [266]. Further clinical evaluations are envisaged.

Denintuzumab mafodotin (SGN-CD19A) is another anti-CD19 ADC comprised an anti-CD19 antibody connected to an anti-mitotic payload, resulting in cellular arrest at G2-M phase and apoptosis [218, 230, 267]. Preclinical trials exhibited the activity of denintuzumab mafodotin in pediatric ALL xenografts regardless of the ALL subtypes such as the presence of Philadelphia chromosomes and precursor B-ALL [267]. A phase 1 study for R/R B-ALL was carried out [268]. Similar to coltuximab ravtansine, denintuzumab mafodotin has a generally safe profile and some encouraging outcomes were displayed, which warrant more in-depth studies [268] (Table 26.3).

Loncastuximab tesirine (ADCT-402) is a recently developed ADC composed of SG3199, a pyrrolobenzodiazepine (PBD) dimer-containing toxin, incorporated into the anti-CD19 antibody [215, 269, 270]. Upon antibody binding, the highly potent PBD toxin was cleaved and cross-linked with DNA to induce apoptosis [270, 271]. Excellent preclinical dose-dependent response was observed in terms of cellular arrest and disease regression [270]. Yet, the significant efficacy was not consistent with the phase 1 clinical trial in R/R B-ALL [269] (Table 26.3). The overall clinical activity of loncastuximab tesirine was less significant in B-ALL than that of other B-cell malignancies such as diffuse large B-cell lymphoma (DLBCL), and the phase 1 study was discontin-

ued [269]. Yet, the data were still preliminary and were only limited to heavily pre-treated B-ALL patients. The efficacy might be possibly enhanced via combination with different treatments [269].

26.22.3 Anti-CD25 ADC

CD25, the α-subunit of interleukin-2 receptor (IL-2R), is highly expressed on activated T and B lymphocytes and regulatory T cells [215, 244, 272]. As CD25 expression is associated with adverse prognosis such as induction failure and increased relapse risks [215, 272, 273], CD25-targeting ADC is a feasible rationale to improve OS. Camidanlumab tesirine (ADCT-301) has a similar mechanism as loncastuximab tesirine, which both of them are conjugated to a PBD-dimer payload [273, 274]. Potent cytotoxic effects were depicted in preclinical murine xenograft models [274]. Yet, the phase 1 clinical trial in R/R CD25-positive ALL was discontinued due to limited efficacy and slow accrual [273] (Table 26.3). Although monotherapy camidanlumab tesirine failed to yield meaningful results, further clinical evaluations could be performed in combining it with other regimens [273].

26.23 Bispecific T-Cell Engager (BiTE)

Being the first-in-class and only FDA-approved BiTE [275], blinatumomab (AMG103), a CD3/CD19 BiTE, is a remarkable breakthrough in ALL treatment. It is a fusion protein formed by the coalescence of CD19, a widely expressed B-cell marker on leukemic blasts, with T-cell co-receptor CD3 by a glycine–serine linker [215, 232, 276]. Upon simultaneous binding of CD19 and CD3, leukemic B lymphocytes will be brought in close proximity to cytotoxic T lymphocytes, resulting in cellular apoptosis via the release of cytotoxic perforins and granzymes by T cells [276] (Fig. 26.13).

Blinatumomab has been appraised comprehensively in a myriad of clinical trials [277–283] (Table 26.3). In the phase 3 TOWER trial, durable improvements in OS, EFS, and CR were revealed in comparison with conventional chemotherapy in Ph-negative BCP-ALL [277]. Its efficacy was not limited to Ph-negative subtypes. In the phase 2 ALCANTARA study, blinatumomab successfully yielded higher CR rate in R/R Ph-positive BCP-ALL patients irrespective of T315I mutation [278], offering a plausible strategy to overcome TKI resistance in Ph-positive B-ALL. Moreover, the response rates and relapse-free survival (RFS) were consistent across age groups, which help bring survival benefits to non-

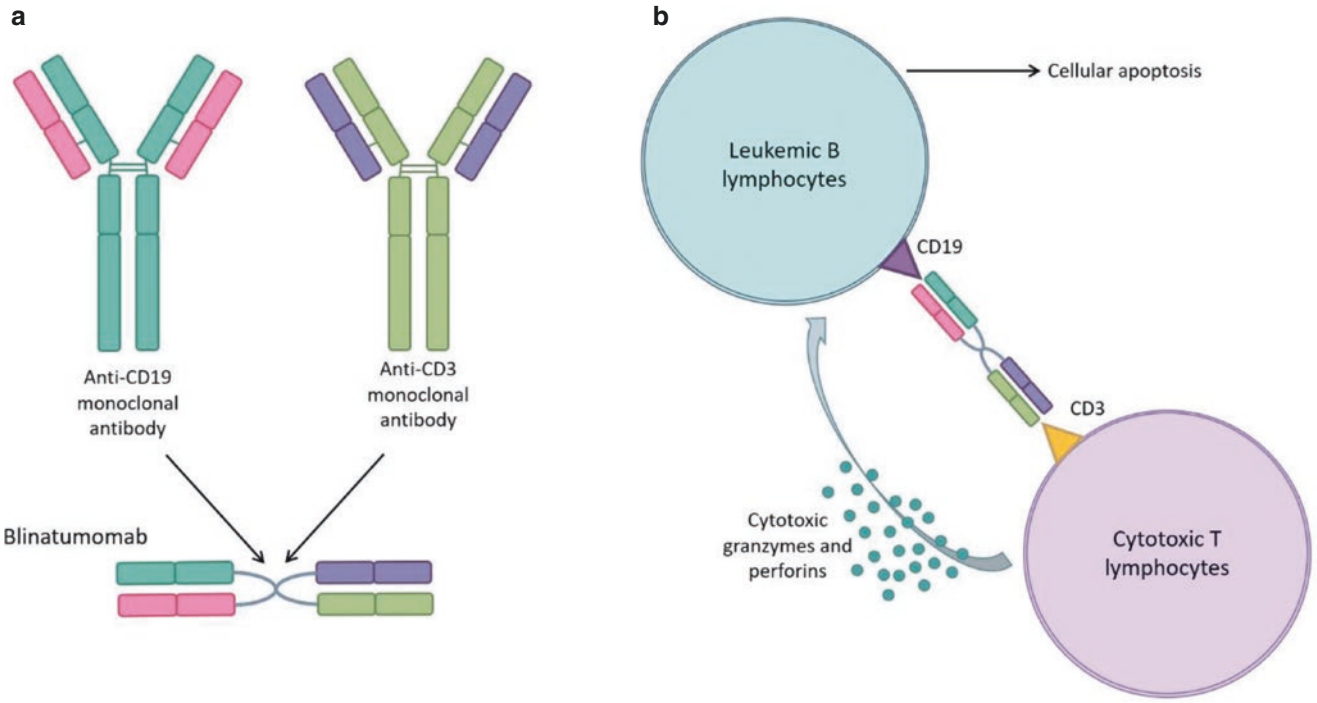

Fig. 26.13 Structure and mechanism of actions of blinatumomab. (**a**) shows the structure of blinatumomab, which allows specific binding to both CD19 and CD3. (**b**) shows the mechanism of actions of blinatumomab. Upon binding to leukemic B cells and cytotoxic T lymphocytes by blinatumomab, cytotoxic granzymes, and perforins are released by T cells to induce cellular apoptosis of malignant B cells

transplant-eligible older patients [278]. Co-administration of TKIs and blinatumomab investigations is underway (NCT03263572 and NCT03147612). In light of persistent MRD positivity in ALL patients, the phase 2 BLAST clinical study was commenced [279]. Meaningful MRD responses were yielded, especially in patients in first CR, suggesting the effectiveness of blinatumomab in overcoming MRD positivity in chemotherapy-resistant patients. Early administration of blinatumomab was also proposed to induce early MRD response [279, 281]. Furthermore, the application of blinatumomab was explored in the pediatric population in the expanded access protocol RIALTO trial [282] (Table 26.3). Sustainable CR and MRD responses were reinforced and exemplified in the pediatric populations. Due to the promising results, blinatumomab was actively evaluated in multiple trials using different therapy combination in different populations to maximize efficacy. Combination therapy was not limited to blinatumomab with chemotherapy or TKIs, but also extended to co-administration of blinatumomab and immune checkpoint inhibitors [283] (Table 26.3).

26.24 Chimeric Antigen Receptors (CARs)

CAR-T cell therapy is an emerging novel modality in the treatment of ALL via the production of genetically engineered antigen receptors on cytotoxic T lymphocytes directed against leukemic blasts [217, 219, 230, 247, 249, 284, 285]. It comprised an extracellular single-chain variable fragment (scFv) domain for antigen binding, a hinge domain, a transmembrane domain, and a CD3ζ intracellular signaling domain for release of cytotoxic granules to induce apoptosis [230, 285] (Fig. 26.14). To boost the potency and proliferation of CAR-T cells, a co-stimulatory domain is tailored to optimize CAR-T activity. CD28 and CD137 (4-1BB) are commonly selected co-stimulatory molecules to augment immunogenicity and enhanced persistence of CAR-T cells, respectively [230]. There is growing interests and applications of CAR-T therapy in B-ALL, while its application in T-ALL remains exploratory [236]. The limited use in T-ALL can be contributed by the lack of efficacy as CAR-T cells recognize "self" as "tumor" and induce fratricide, as well as T-cell aplasia and immunodeficiency caused by cytotoxic effects if CAR-T [236].

26.24.1 CD19 CAR-T Cells

CD19 is a widely expressed cell surface marker on B lymphocytes, making it an attractive target for CAR-T therapy in B-ALL [286]. Tisagenlecleucel is recently FDA-approved for refractory or second or later relapse BCP-ALL patients below 25 years old [286]. As mentioned above, co-stimulatory molecules are added to enhance efficacy of CAR-T therapy, and this has been demonstrated in clinical trials (Table 26.4). A CAR-T therapy, which made use of CD28/CD3ζ signaling

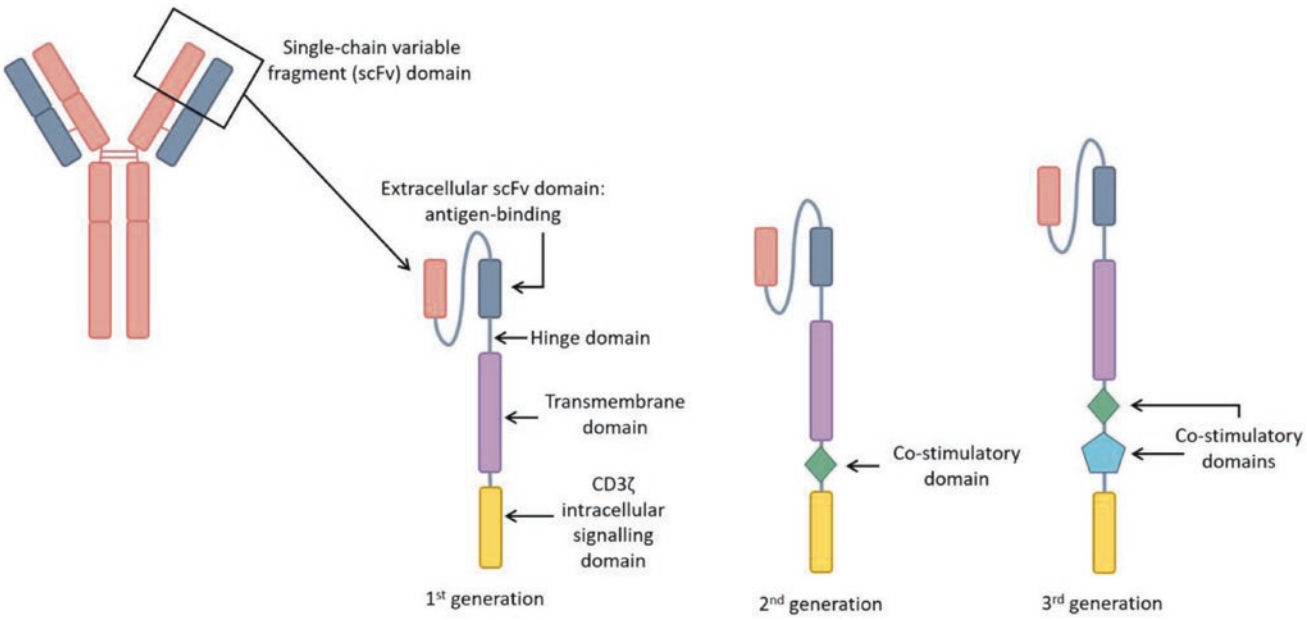

Fig. 26.14 Structure and different generations of CAR-T cells. The CAR-T cell consists of an extracellular antigen binding, a hinge domain, a transmembrane domain, and an intracellular signaling domain. In second- and third-generation CAR-T cells, addition of co-stimulatory molecules helps to increase efficacy of CAR-T therapy

Table 26.4 Clinical trials of CD19 chimeric antigen receptors T cells

Co-stimulatory molecule/signaling domain	Subtype of ALL	Age groups	Observations and outcomes	Adverse effects	Clinical trial phase	References
CD28/CD3ζ	R/R B-ALL	Adults	1. Significantly longer EFS and OS in patients with smaller disease burden 2. No association was observed between CAR-T cell level and OS, reflecting persistence of CAR-T cells is not required for sustained remission duration 3. Relapse was not always prevented, especially in the presence of high disease burden	• CRS (fever, tachycardia, hypotension, respiratory distress, hypoxemia) • Neurotoxic events (confusion, disorientation, aphasia, encephalopathy, seizure)	MSKCC, phase 1	[287]
		Children and young adults (≥12 months to <27 years old)	1. Achievement of 90% of MRD-negative CR 2. Positive correlation between CD19 antigen load with persistence of CAR-T cells and B-cell aplasia 3. Successful engraftment and MRD negativity with the use of lymphodepleting agents (fludarabine and cyclophosphamide) 4. Relapse in around half of the patients mostly due to emergence of CD19-negative blasts		Phase 1/2	[288]
CD137 (4-1BB)/CD3ζ	R/R B-ALL	Children and young adults (≥3 to <21 years old)	1. Durable >80% ORR with 80% 6-month RFS 2. Persistence of CAR-T cells due to the use of CD137 co-stimulatory domain 3. Majority of toxic events occurred in the first eight weeks upon CAR-T infusion	• CRS (fever, tachycardia, hypotension, respiratory distress, hypoxemia) • Neurotoxic events (confusion, disorientation, aphasia, encephalopathy, seizure)	ELIANA, phase 2	[289]

R/R relapsed/refractory, *B-ALL* B-cell acute lymphoblastic leukemia, *CRS* cytokine release syndrome, *EFS* event-free survival, *OS* overall survival, *MSKCC* Memorial Sloan Kettering Cancer Centre, *MRD* minimal residual disease, *CR* complete remission, *ORR* overall response rate, *RFS* relapse-free survival

pathway, was evaluated in a phase 1 clinical trial in adult R/R B-ALL [287]. As CD28 co-stimulatory domain was used, the potency of CAR-T cells was enhanced, which allowed rapid apoptosis of leukemic blasts. This was evidenced in the study on the independent relationship between remission duration and the level of CAR-T cells. However, relapse might not be effectively prevented in high disease burden situation as leukemic cells were not completely eliminated within a short period of time [287]. In another phase 1/2 clinical trial in R/R B-ALL children, positive results were also shown [288]. Relapse due to emergence of CD19-negative blasts was a common observation shared by both studies, implying that although CAR-T therapy was effective in eradicating CD19-positive leukemic cells, this provided room for proliferation and expansion of CD19-negative cells [287, 288]. ELIANA is a phase 2 trial that paved CAR-T therapy's way to FDA approval [289]. Durable remission was displayed, yet dire consequences and admission to intensive care units could also be resulted if high-grade cytokine release syndrome or neurotoxic events occur [289]. In the analysis of quality of life (QOL) of CAR-T patients in ELIANA trial, despite the seemingly sustained improvements in QOL in some patients, it should be noted that the situations of critically ill patients could not be assessed [290]. Hence, CAR-T therapy should be considered case by case to maximize clinical benefits and minimize risks.

26.24.2 CAR-T Therapy in T-ALL

The development of CAR-T therapy in T-ALL remains preliminary due to fratricide of CAR-T cells and immunodeficiency due to T-cell deficiency [236, 247]. Several T-cell markers were being evaluated to identify a suitable target. Pan-T cell markers such as CD5 and CD7 might lead to inadvertent fratricide, and the CRIPR-Cas9 technology was applied to edit and modify T cells to prevent this off-target effect [236]. A phase 1 clinical trial on CD7-CRISPR-Cas9-modified CAR-T cells was proposed (NCT03690011). As for T-cell receptor β constant 1/2 (TRCB1/2), their uses were more relevant to mature T cells as only certain subtypes of T-ALL express TCR [291]. CD1a is a recently identified pos-

sible target for CAR-T due to its selective expression on cortical T-ALL while sparing HSCs [291, 292]. However, as CD1a is also expressed on Langerhans cells, CD1a-targeting might result in localized immunodeficiency and autoimmunity [291]. Therefore, CAR-T development in T-ALL is still an arena to be explored and investigated.

26.25 Immune Checkpoint Inhibitors

Immune checkpoint inhibitors are rational yet ineffective immunotherapeutic strategies in the treatment of ALL. The programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) axis was exploited in ameliorating MRD in adult ALL. Pembrolizumab, an anti-PD-1 inhibitor, was evaluated in a phase 2 clinical trial, and clinical significance was barely seen [293]. Although PD-1/PD-L1 inhibitor monotherapy was found ineffective, it might be used as an adjunct with blinatumomab [283, 294]. T-cell anergy and blinatumomab resistance could be relieved by antagonizing the overexpressed PD-L1 on lymphoblasts [294]. The efficacy of combined treatment could be further confirmed upon completion of larger sample size clinical trials.

26.26 Conclusion

There is a gradual shift of treatment paradigm in ALL over the past few decades due to the emergence of targeted therapies and immunotherapies. The incorporations of the novel therapeutic agents into conventional treatment have exhibited promising outcomes. Owing to the genetic heterogeneities B-ALL, its investigations are more established than that of T-ALL by targeting specific genetic alterations. Despite the numerous preclinical studies with compelling results, some novel agents still remain experimental in clinical trials with suboptimal efficacy and unacceptable toxicities. To fill the treatment void, optimization of treatment dosage and combination is required. More mature novel agents with minimized toxicity, improved efficacy, and the capacity to reverse chemoresistance are anticipated.

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Inherited/Genetic Predisposition to MDS and AML

27

Lucy A. Godley

Abstract

Recognition of the importance of germline predisposition to hematopoietic malignancies, especially to the myeloid malignancies, is increasing with dramatic expansion in the number of involved genes. Diagnosis of germline predisposition has important implications for the clinical management of patients, choice of allogeneic stem cell donor, and surveillance strategies for patients and their relatives who share the deleterious variant. We now understand that there is extensive overlap in genes that confer risk for myeloid malignancies with those that confer risk for lymphoid malignancies, immunodeficiencies, and solid tumors, with more intersections likely to be uncovered in the future. Germline genetic testing should be considered standard of care now for anyone aged 40 or younger diagnosed with myelodysplastic syndrome given the high likelihood of identifying a deleterious variant. The increasing use of molecular profiling of malignant cells provides an opportunity to identify individuals without striking personal and/or family histories of cancer given how often predisposition genes are mutated somatically in these diseases and are therefore included on gene panels. The future will likely bring the identification of more risk genes, recognition of factors that promote leukemia development and development of diagnostic and surveillance guidelines for germline mutation carriers.

Keywords

Germline DNA · Germline predisposition · Myeloid malignancy · Inherited cancer · Deleterious variant

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27.1 Introduction

Inherited predisposition to cancer has been appreciated for many years, but has focused historically more on susceptibility to solid tumors. Now, there is increasing awareness of the heritability of the hematopoietic malignancies, with most known about inherited risk for myeloid malignancies [1–4]. As deleterious variants are identified in genes that confer risk for hematopoietic malignancies, we recognize extensive overlap in risk for solid tumors (Fig. 27.1). In this chapter, I will review key features of germline susceptibility genes for myeloid malignancies, highlighting unique aspects of each syndrome. I will also discuss the complexity of clinical testing and how it is possible to discern individuals likely to have germline predisposition alleles from molecular analysis of malignant cells.

Several terms will be used repeatedly throughout this chapter, which are important to define at the outset. The terms "inherited" and "germline" have a subtle distinction in meaning: "Inherited" means that an allele has been passed from a parent to child. However, "germline" means that the allele is present in the germ cells of an individual and is not necessarily inherited from the parents. "De novo" DNA variants arise spontaneously within germ cells and are not inherited. Importantly in the context of this chapter, de novo variants in *SAMD9*, *SAMD9L*, and *GATA2* arise fairly commonly in young children and confer risk to myeloid malignancies. Within the field of human genetics, there is a preference for the term "variant" as opposed to "mutation," since variations in DNA sequence are not necessarily deleterious. DNA variants are assigned clinical significance using a five-tier system standardized by the American College of Medical Genetics and the Association of Molecular Pathology that includes pathogenic (P), likely pathogenic (LP), variant of uncertain significance (VUS), likely benign, or benign categories [5, 6]. It is important to remember that germline variants can be deleterious [P or LP], benign, or of uncertain significance, so the germline nature of a variant

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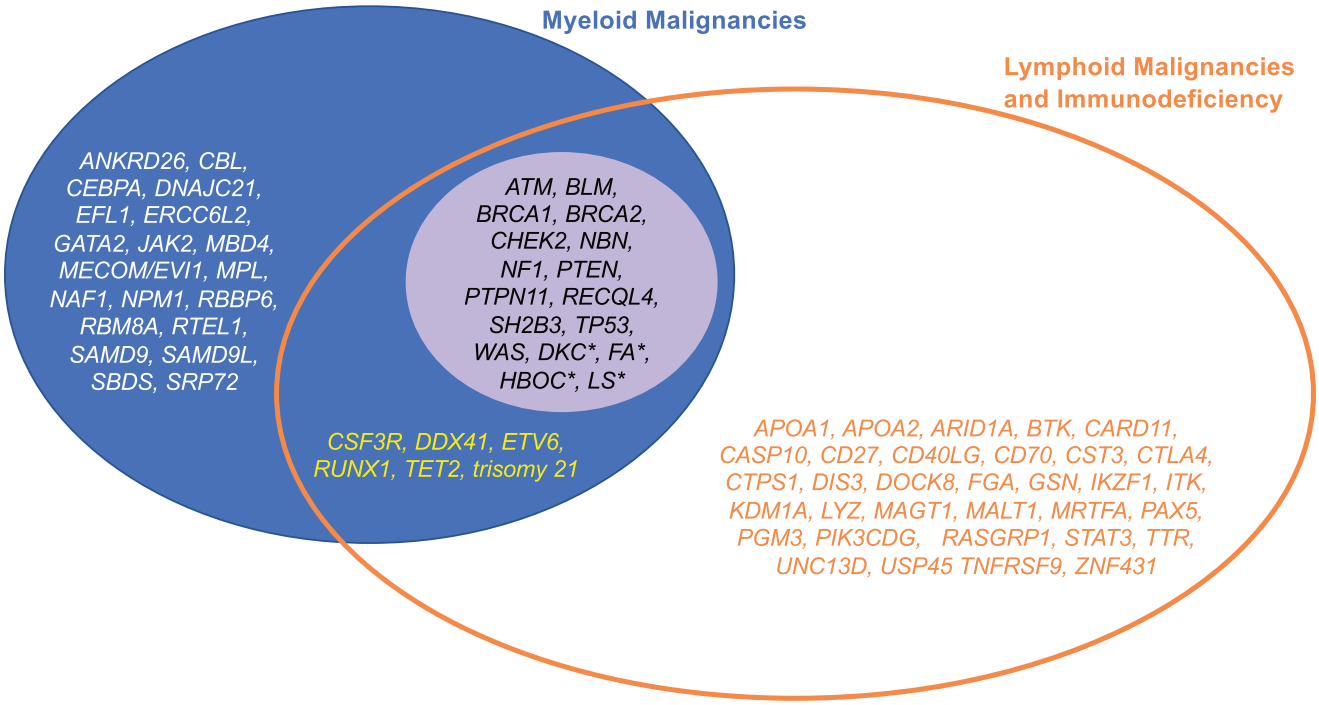

Fig. 27.1 Predisposition genes that confer risk for hematopoietic malignancies show a great degree of overlap with those that increase the risk for the development of solid tumors. Genes that confer risk for myeloid malignancies are shown in the solid blue circle: white, those genes that confer risk only to myeloid malignancies; yellow, those genes that confer risk for hematopoietic malignancies, both myeloid and lymphoid; black within purple circle, those genes that confer risk for hematopoietic and solid tumors; orange, those genes that confer risk for lymphoid malignancies or immunodeficiency

should not be equated with being P/LP. The word "mutation" typically refers to P or LP variants.

27.2 Germline Predisposition to Myelodysplastic Syndrome (MDS)

MDS is a clonal bone marrow malignancy characterized by ineffective hematopoiesis, bone marrow dysplasia, peripheral blood cytopenias, and an increased risk of transformation to acute myeloid leukemia (AML). MDS is generally considered to be a disease of the elderly, since disease incidence rates rise significantly with age: in those <40 years old (yo), 0.14 per 100,000; 40–49 yo, 0.62 per 100,000; 50–59 yo, 1.95 per 100,000; 60–69 yo, 7.14 per 100,000; 70–79 yo, 20.05 per 100,000; and in those >81 yo, 35.49 per 100,000 [7–9]. To date, the strongest associations between germline predisposition mutations and development of hematopoietic malignancies are seen for MDS. Collectively, studies show that there is a tight correlation between the age of MDS diagnosis and likelihood of finding a deleterious germline variant in a particular gene, with germline *SAMD9/SAMD9L* mutations found in the youngest individuals, germline *GATA2* mutations in children, adolescents and young adults, mutations in DNA repair and telomere biology genes in adults diagnosed at 40 yo or younger, and germline *DDX41* mutations seen in elderly individuals (Fig. 27.2) [10–15].

27.2.1 Deleterious Germline *SAMD9* Variants (OMIM 610456, 617053, and 619041) [12, 14, 16, 17]

Defined clinically, MIRAGE syndrome is characterized by adrenal hypoplasia and insufficiency, growth restriction, intellectual impairment, genital phenotypes [including microphallus, cryptorchidism, and hypospadias], enteropathy, infections, and risk for MDS [12]. Invasive infections are often the cause of death by 10 yo [12]. These individuals have been identified with germline heterozygous *sterile alpha motif domain-containing protein 9* (*SAMD9*) mutations. Most germline *SAMD9* mutations are gain-of-function missense that is prone to somatic reversion, but some are loss-of-function frameshift or nonsense alleles that do not undergo somatic reversion [16]. Importantly, many of these germline alleles are de novo within the family [12, 14]. *SAMD9* and its paralogue *SAMD9-like* (*SAMD9L*) are located next to one another on chromosome 7q21. *SAMD9*

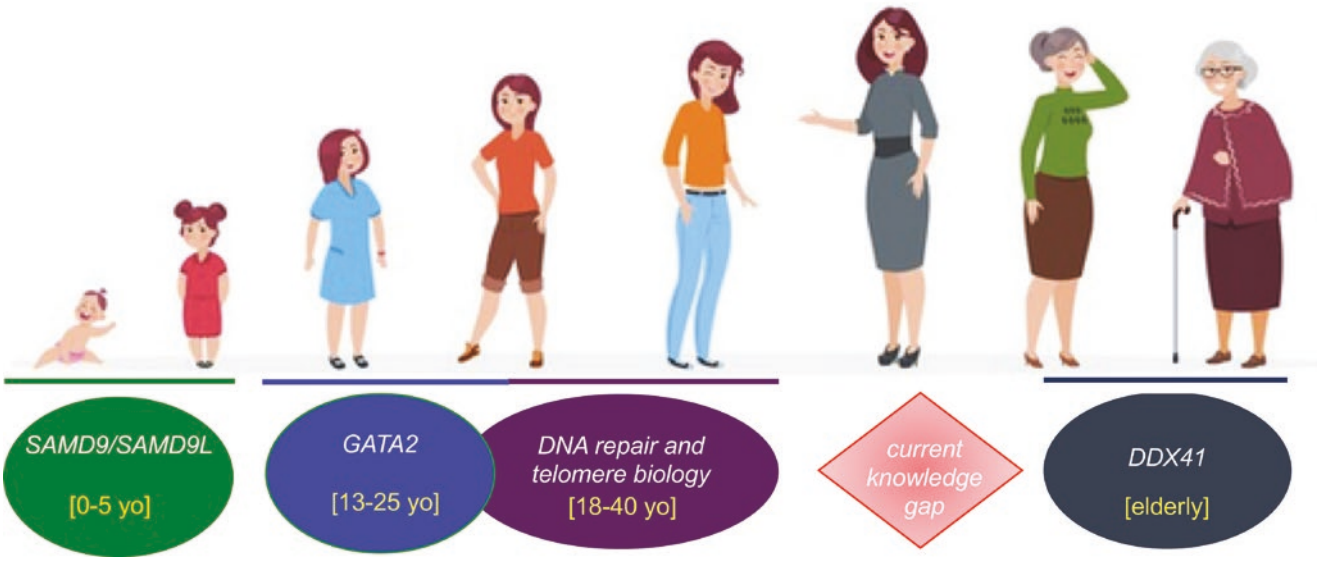

Fig. 27.2 Genes commonly implicated in germline predisposition to myeloid malignancies change with age of MDS presentation. In the youngest age range of less than 5 years old (yo), deleterious germline variants in *SAMD9* or *SAMD9L* predominate, followed by those in *GATA2* in children, adolescents, and young adults, with those in DNA repair and telomere biology genes in young adults, and in *DDX41* in the elderly. Currently, we lack data on which germline predisposition alleles confer risk in those diagnosed with MDS older than 40

encodes an IFN and TNF-α responsive protein that mildly restricts growth and is involved in endosome fusion, development, and growth factor signal transduction [14]. The MDS that develops in germline mutation carriers often shows −7, with loss of the chromosome containing the germline gain-of-function allele, which has the tendency to resolve spontaneously (see Sect. 27.2.3 below) [12, 14]. Because young children are usually diagnosed when they are diagnosed with MDS, and due to the tendency for the malignant cells to lose the gain-of-function mutation-carrying chromosome 7, recognition of these mutations is much more likely if true germline DNA is used for testing. Making this diagnosis via molecular testing of affected hematopoietic tissue is highly unlikely for this reason.

27.2.2 Deleterious Germline *SAMD9L* Variants (OMIM 611170, 159550, and 252270) [11, 14, 16–18]

Ataxia-pancytopenia syndrome is an autosomal dominant disorder featuring cerebellar ataxia, cytopenias, and predisposition to bone marrow failure and myeloid malignancies, with *SAMD9L* identified as the causative gene [11]. Like germline mutations in *SAMD9*, germline *SAMD9L* mutations are usually gain of function, and the MDS that arises is often characterized by monosomy 7 [11, 14, 16–18].

27.2.3 "Adaptation by Aneuploidy" Seen in Germline *SAMD9/SAMD9L* Mutation Carriers (OMIM 619041) [12, 14]

A unique feature of the MDS that most often arises in germline *SAMD9/SAMD9L* mutation carriers is that the loss of the mutation-carrying chromosome 7 occurs as an adaptation to the heightened growth-restricting properties of the germline gain-of-function variants, termed "adaptation by aneuploidy" [12]. As noted above, the loss of the mutant allele in hematopoietic tissues, occasionally to levels lower than 5%, renders the diagnosis more difficult and essentially requires true germline DNA for diagnosis [12, 14]. Complete disappearance of the monosomy 7 clones is documented and called "transient monosomy 7 syndrome" [12, 14]. The presence of a monosomy 7 clone is dangerous though due to its propensity for progression to malignancy through acquisition of somatic driver mutations [12, 14]. Somatic revertant mosaicism can also be seen in these syndromes, with expansion of corrected clones that arise either through: (i) acquired truncating *SAMD9/9L* mutations that occur on the germline mutant allele to neutralize it or (ii) via uniparental disomy of 7q that occurs through duplication of the wild-type allele and non-homologous recombination [12, 14]. These somatic revertants can result in long-term remission and normal hematopoiesis [12, 14].

27.2.4 Deleterious Germline *GATA2* Variants (OMIM 137295, 601626, 614286, 614038, and 614172) [13, 14, 19–21]

Pediatric groups have screened MDS patients younger than 18 yo based solely on age at diagnosis and found that 7% have deleterious variants in *GATA2* and in 37% of children with MDS and monosomy 7 [13, 14]. Germline *GATA2* mutations can occur de novo or can be inherited and are heterozygous loss-of-function variants that often affect the DNA-binding function of the C-terminal zinc finger [14]. Other types of *GATA2* mutations include intronic variants affecting the +9.5 kb enhancer element [22–24]; synonymous variants that introduce a new splice donor causing nonsense-mediated decay and selective loss of the mutated *GATA2* mRNA [25–27]; or those that result in disrupted spacing between the first and second zinc finger [28]. The MDS that develops can be hypocellular with peripheral blood cytopenias or even hypercellular if advanced and can feature several cytogenetic abnormalities: monosomy 7, der(1;7), or trisomy 8 [14]. More details about the phenotypic presentations of individuals with germline *GATA2* deficiency are given in Sect. 27.3.3 below.

27.2.5 MDS in Young Adults

The frequency of germline predisposition in adults diagnosed with MDS at 40 yo or younger is estimated conservatively at 19% and 15% for those in the same age range diagnosed with aplastic anemia (AA) [15]. Most of the affected genes involve DNA repair and telomere biology pathways [15]. Importantly, variants affecting non-protein encoding DNA regions comprise 20% of the variants, including *TERC*, the RNA component of telomerase, and the promoter of *ANKRD26* [15], emphasizing the importance of using comprehensive approaches and not relying on panels designed for prognostication. Moreover, copy number variants account for 10% of causative variants [15], further emphasizing the importance of using a testing platform capable of detecting these types of DNA rearrangements.

In a similar study of MDS patients diagnosed at 45 yo or younger and AA patients diagnosed at 40 yo or younger who underwent allogeneic stem cell transplant, panel-based testing identified a causative germline genetic variant in about 14% of MDS patients and 5% of those with AA [29]. Similarly, physical stigmata of known germline syndromes were present in only about a quarter of adults, and only about half of germline mutation carriers had a positive family history to suggest an underlying inherited cancer risk syndrome [29].

Taken together, these data indicate that comprehensive germline genetic testing should be offered to anyone diagnosed with MDS at 40 yo or younger merely based on their age of disease diagnosis, regardless of physical examination findings or family history.

27.3 Germline Predisposition to AML

Systematic examination of germline predisposition alleles in patients presenting with acute myeloid leukemia (AML) has not been performed yet, as has been done for those with MDS. Despite this current knowledge gap, there are some important features of particular germline susceptibility syndromes that should be noted and are given in separate sections below.

27.3.1 Deleterious Germline *DDX41* Variants (OMIM 608170) [1, 2, 4, 30, 31]

A basic tenet of germline predisposition is that diagnosis at an age much earlier than the average signals a likelihood of having a causative germline susceptibility allele, as described in the previous section. However, many have assumed that this also means that there is little contribution of germline alleles to the development of disease at an age expected from population incidence studies. This latter assumption is incorrect, as deleterious germline *DDX41* variants demonstrate. The average age of diagnosis of a myeloid malignancy in those with a germline *DDX41* mutation is 65–70 yo, which is the same as the median age of MDS diagnosis in the general population [31, 32]. Interestingly, men with germline *DDX41* mutations progress to malignancy more often than women [33, 34], although the molecular basis for this observation is not yet delineated.

27.3.2 Deleterious Germline *RUNX1* Mutations/Familial Platelet Disorder (FPD) (OMIM 151385 and 601399) [35–37]

Germline predisposition to myeloid malignancies specifically was first identified for those with FPD, which found to have deleterious germline variants in *RUNX1* [38]. These individuals experience life-long platelet dysfunction, both in function and number, as well as risk for myeloid malignancies most frequently, followed by T-cell acute lymphoblastic leukemia (ALL), and occasionally B-cell lymphomas [1, 2, 35, 39]. *RUNX1* variant curation rules for germline alleles are now being used throughout the world [36, 37], which should improve consistency in how variants are classified.

Because the etiology of FPD was discovered first, we have the longest duration of prospective monitoring for these

at-risk individuals, which has facilitated serial collection of peripheral blood samples over time to measure the frequency of acquisition of clonal mutations in hematopoietic stem and progenitor cells (HSPCs) in the process now referred to as clonal hematopoiesis (CH). Such studies have shown that about 30% of germline *RUNX1*-mutated patients have CH prior to leukemia development, where *BCOR* mutations predominate [40, 41]. Additional somatic mutations in the leukemias involve several genes recurrently, including what was the wild-type *RUNX1* allele, *ASXL1*, *FLT3*, *GATA2*, *PHF6*, *SRSF2*, and *WT1* [40–44]. The hope is that if a common molecular progression can be defined, at-risk individuals could be followed prospectively with molecular profiling and given treatment, possibly including pre-emptive allogeneic stem cell transplantation [45], in advance of overt malignancy. However, more natural history studies will likely need to be published before such a strategy will be adopted throughout the world.

27.3.3 Deleterious Germline *GATA2* Variants (OMIM 137295, 601626, 614286, 614038, and 614172) [19–21, 24, 46, 47]

As noted in Sect. 27.2.4, deleterious *GATA2* variants are commonly seen in children and young adults diagnosed with MDS, especially in the context of del [7] [13, 19, 20]. The phenotype of people with germline *GATA2* mutations is quite pleomorphic, which challenges diagnosis. Common presentations derive from immunodeficiency, with recurrent infections by fungal, viral, and bacterial species, especially non-tuberculous *Mycobacteria* [48, 49]. Additional phenotypes include lymphedema, panniculitis/vasculitis, deafness/tinnitus, thrombosis/embolism, urogenital abnormalities, and pulmonary alveolar proteinosis[48, 49]. These individuals also have an elevated risk for bone marrow failure and/or myeloid malignancies [19, 20, 49], with acquisition of *ASXL1*, *EZH2*, and *N/KRAS* mutations [50]. Protocols at the National Institutes of Health are pioneering the use of allogeneic stem cell transplantations for germline *GATA2* mutation carriers based on a variety of presenting conditions, not restricting the therapy to those who have developed malignancy [51].

27.3.4 Deleterious Germline *CEBPA* Variants (OMIM 116897) [52]

Molecular assessment of *CEBPA* occurs commonly in AMLs, since the identification of bi-allelic *CEBPA* mutations confers a relatively good prognosis and therefore has prognostic value [52]. The two alleles show a distinctive pattern of mutation in AMLs: Typically there is one mutation at the 5′ end of the gene and another at the 3′ end of the other allele. Importantly, approximately 10% of people with bi-allelic *CEBPA*-mutant AMLs inherit one of those alleles as a germline allele, typically the one at the 5′-end [52], although rare 3′-end germline mutations exist [53]. For this reason, germline genetic testing is prudent for all individuals diagnosed with AMLs which have bi-allelic *CEBPA* mutations. To date, only myeloid malignancies and typically AMLs have been diagnosed in those with germline *CEBPA* mutations. Interestingly, among patients with bi-allelic *CEBPA*-mutant AMLs, survival appears to be the longest for those with a germline mutation [54]. This observation suggests that the presence of the germline mutation in the supporting bone marrow mesenchymal stromal cells (MSCs) confers chemosensitivity, a hypothesis worthy of further study.

The penetrance of germline 5′-end *CEBPA* mutations is virtually 100% [52], but appears to be lower for those with a germline 3′-end mutation [53]. Given the very high penetrance of developing AML with a 5′-end germline mutation, pre-emptive allogeneic stem cell transplantation using a wild-type donor is worthy of consideration [45]. However, others argue that because bi-allelic *CEBPA*-mutant AMLs are so chemosensitive, and since first remissions can last years, allogeneic stem cell transplantation should be used only if AML re-emerges. Importantly, the term "relapse" should be used carefully, since the acquired *CEBPA* mutation from the initial AML serves as a molecular marker, and these acquired mutations are distinct in AMLs that re-emerge in germline *CEBPA*-mutant carriers, identifying them as independent primary AMLs [54].

27.4 Key Aspects of Germline Testing

27.4.1 Who Should Be Tested?

Criteria that outline which patients should undergo germline testing for predisposition to hematopoietic malignancies are beginning to be described for patients with particular diagnoses [1, 2, 4, 49, 55–58]. Consideration of a germline susceptibility syndrome should be given when (i) an individual is diagnosed with a malignancy at a unusually early age compared to the general population, such as anyone diagnosed with MDS at 40 yo or younger [14, 15]; (ii) an individual who has a history of multiple malignancies; (iii) the physical examination of a patient reveals features consistent with a germline predisposition syndrome [59]; (iv) there is a notable family history of bleeding, cytopenias, and/or the presence of a hematopoietic or young-onset (e.g., <50 yo) solid tumor within two generations of the proband; or (v) a P or LP variant in a known germline predisposition gene is identified on tumor-based molecular testing [60]. It is important to rec-

ognize that the diagnosis of a hematopoietic malignancy at an advanced age does not exclude the potential for a germline predisposition syndrome, since the average age of diagnosis of a myeloid malignancy in those with a deleterious germline *DDX41* variant is the same as the median age of diagnosis in the general population, about 65 yo [31].

27.4.2 When Will Be Testing All Patients with a Myeloid Malignancy and Their Allogeneic Stem Cell Donors?

As recognition of hematopoietic malignancy predisposition syndromes increases and germline testing becomes more widespread [55, 57, 58], universal germline testing for patients with particular diagnoses may become the standard of care at diagnosis and other key points in treatment [61]. Already, the decision to use a related hematopoietic stem cell donor for allogeneic transplantation is a critical time when the identification of a familial cancer predisposition syndrome becomes extremely important [62]. However, it is important to remember that all individuals have germline genetic variants, and all hematopoietic stem cell donors have the potential of introducing a deleterious germline variant. Germline predisposition testing as standard practice at diagnosis of a myeloid or other hematopoietic malignancy would facilitate evaluation of potential related hematopoietic stem cell donors, since the presence of a germline predisposition syndrome within a particular family would already be known when donors were being considered.

27.4.3 Use of True Germline DNA

27.4.3.1 Why Is It Critical to Use True Germline DNA?

It is critical to use true germline DNA when performing clinical testing, because the use of DNA obtained from hematopoietic tissues can easily yield false negative results due to somatic reversion, with the erroneous conclusion that the individual/family does not have a deleterious germline variant. Many studies have shown that somatic reversion events occur in many gene contexts, particularly in bone marrow failure and DNA repair deficiency syndromes: *ADA* [63, 64], *BLM* [65], *BRCA1/2* [66–68], *CARD11* [69], Diamond-Blackfan anemia [70–72], *DOCK8* [73], Dyskeratosis congenita [74, 75], Fanconi anemia [76–79], *IL2RG* [80], *SAMD9* [81, 82], *SAMD9L* [18], and *WAS* [83–85]. Therefore, given the numerous genes in which somatic reversion has been documented, the use of true germline DNA for clinical testing for germline predisposition to hematopoietic malignancies is essential.

27.4.3.2 How Do You Obtain True Germline DNA?

There are two ways to determine that a variant is germline: (i) identify the variant at a high variant allele frequency (VAF) in true germline DNA, and cutoff used by many clinical laboratories is a VAF >30% in a DNA source considered to be germline; (ii) identify the variant in at least two related individuals at a high VAF (again, generally >30%). Germline tissue is not easy to obtain in quantities that allow comprehensive testing, but can be obtained from cultured skin fibroblasts. Cultured skin fibroblasts provide several advantages as follows: (i) They can be collected easily at the time of a bone marrow biopsy or from a skin punch biopsy; (ii) they grow easily in culture or from frozen viable aliquots, so that regenerating more sample from a particular individual rarely requires re-biopsy; and (iii) they serve as an excellent source of material for research. For clinicians who do not have direct access to clinical laboratories that grow skin fibroblasts, several commercial laboratories provide this service. Additional sources of germline DNA include hair bulbs and bone marrow-derived mesenchymal stromal cells, which are non-hematopoietic [86]. However, the amount of DNA obtained from hair bulbs is limited, which can complicate downstream testing. Some clinical laboratories may not accept MSCs as germline material.

27.4.4 Use Testing That Is Comprehensive

Comprehensive testing for germline predisposition includes assessment of single nucleotide and copy number variants in all of the genes that can confer risk for hematopoietic malignancies [87]. Unfortunately, few academic or commercial laboratories provide comprehensive testing [87], making it challenging for busy clinicians to order appropriate testing. Many of the assays offered by reputable laboratories are incapable of detecting all of the mutation types that confer germline susceptibility and/or are missing key genes [87]. To complicate matters, many of these laboratories accept hematopoietic tissues for germline testing without strong and obvious explanations that this testing is inherently flawed, as explained above. Therefore, clinicians may be completely unaware that the testing they are ordering is not actually comprehensive and will give patients and families the impression that they have had adequate testing.

27.4.5 Carefully Interpret Molecular Profiling Data from Leukemia Cells in Patients Without Significant Personal/Family Histories

As outlined above, the presence of a strong personal and/or family history of cancer signals the need for germline predisposition testing in certain individuals. However, many patients present with a hematopoietic malignancy without fulfilling criteria for clinical germline predisposition testing. As part of their clinical assessment for their disease, molecular profiling of tumor cells may be performed to make diagnostic, treatment, and prognostic decisions. Because many of the genes that predispose to inherited hematopoietic malignancies are also mutated somatically in these tumors, many molecular profiling platforms contain at least some of the genes that confer inherited cancer risk. Although care providers are not advised to rely on these tests exclusively for germline genetic testing for the reasons outlined above, when molecular profiling is performed on tumor cells, consideration should be given regarding the potential germline nature of the identified alleles [60]. Variants found from molecular profiling of tumor cells can represent: somatic changes that are unique to the tumor; changes reflective of clonal hematopoiesis (CH) and are derived from the clonal expansion of hematopoietic stem or progenitor cells that have acquired somatic mutations over time; or germline variants that are present in all of the non-germ cells in the person's body. The VAF associated with each variant cannot be used to determine the somatic versus germline nature of an allele. Although ideally a germline variant has a VAF ~0.5 if heterozygous and ~ 1.0 if homozygous, the VAF of a somatic mutation can also be found within this range, depending on the amount of tumor present, the genomic changes present within the tumor cells, including loss of heterozygosity (LOH), copy number variations (CNVs) like insertions or deletions, and sequencing artifacts especially with low sequencing depths. For these reasons, when a deleterious variant is found in a gene known to confer germline risk for cancer at a VAF >30%, patients should be cautioned about the possibility of the variant as a germline allele and offered appropriate testing. Importantly, certain gene alleles have only been seen to date as germline alleles, and these should warrant particularly close attention: truncating *DDX41* variants [31, 33, 34, 88] and CHEK2 I200T and del1100C. For other alleles, such as *RUNX1* or *TP53* variants, variant etiology is difficult to surmise, since the same allele can be somatic or germline.

Sequential molecular profiling over time may yield more information, especially when disease status changes [60]. Following the VAFs of potentially germline alleles across multiple testing is an efficient way to identify potential germline variants using data already collected for diagnostic and prognostic purposes. Although conditions such as persistent disease, CH, CNVs, and LOH may yield a stable VAF ~50%, persistence of a variant in sequential testing can suggest its germline nature, especially after a patient has entered a clinical remission.

27.5 Conclusions

With increased recognition of the importance of germline predisposition to myeloid and other hematopoietic malignancies, physicians, genetic counselors, and other healthcare providers will be challenged to diagnose and manage affected patients and their relatives. Currently, we lack sufficient knowledge about the natural history of many of these predisposition syndromes, the factors that drive development of overt malignancy, clinical guidelines and management recommendations, and optimal surveillance strategies. Based on the high likelihood of identifying a germline predisposition syndrome in MDS patients diagnosed at age 40 or younger, germline genetic testing in these individuals should now be considered standard of care. Widespread germline testing that is comprehensive and easily accessible to at-risk individuals is critical, and testing of potential germline mutation carriers based on deleterious variants seen in malignant cells is likely to become more commonplace in the near future. Ultimately, the design and implementation of strategies that delay or prevent development of malignancies in at-risk individuals based on a molecular understanding of disease pathogenesis will lead hopefully to an improved quality of life for members of these families.

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Clonal Hematopoiesis and Its Functional Implications in MDS/AML

28

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Abstract

Clonal hematopoiesis (CH) is now a specifically recognized as a precursor myeloid neoplasm and comprised clonal hematopoiesis of indeterminate potential (CHIP) and clonal cytopenia of undetermined significance (CCUS). Various cohort studies have confirmed the association between CH and the subsequent development of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). In addition, there is recent evidence to show that CH is associated with highlighted inflammatory responses and may influence non-hematological disease states in the general population and in patients with hematological malignancies.

Keywords

Clonal hematopoiesis · Clonal hematopoiesis of indeterminate potential · Clonal cytopenia of undetermined significance

28.1 Introduction

Clonal hematopoiesis (CH) is now specifically recognized as a precursor myeloid neoplasm and comprised clonal hematopoiesis of indeterminate potential (CHIP) and clonal cytopenia of undetermined significance (CCUS) [1, 2]. CHIP is defined as the presence of somatic mutations associated with myeloid neoplasms with a variant allele frequency (VAF) of ≥2% (≥4% for X-linked gene mutations in men) without a preexisting hematological disorder or cytopenia [1]. CCUS is defined as the presence of somatic mutations associated with myeloid neoplasms with a variant allele frequency (VAF) of ≥2% (≥4% for X-linked gene mutations in men) with unexplained cytopenia [1]. Significant dysplasia is typically absent, and the diagnostic criteria for any defined myeloid neoplasm are not met. Cytopenia is defined as follows: hemoglobin <13 g/dL for men/<12 g/dL for women and/or absolute neutrophil count (ANC) $<1.8 \times 10^9$/L and/or platelet count $<150 \times 10^9$/L [1]. CHIP is associated with increased risk of hematological neoplasms, cardiovascular events [3], and adverse survival outcome. It is postulated that CHIP derives from $Lin^-CD34^+CD38^-$ hematopoietic stem cell (HSC) compartment and precedes hematological neoplasms, notably myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) [4–7]. Individuals with CHIP have expanded clonal population of cells carrying somatic mutations, some of which may be putative oncogenic drivers. Individuals with CHIP have an absolute risk of 0.5–1% per year of developing hematological neoplasms [8]. This is 4 to 15 times the risk in healthy individuals without CHIP [8]. In addition to the malignant potential, CHIP clones are associated with chronic inflammation and end-organ damage via the effect of engrafted pro-inflammatory monocytes with altered epigenetic control [9–11].

28.2 Clonal Hematopoiesis and Hematological Malignancies

CHIP is an age-related phenomenon, and large cohort studies have confirmed the association between CH and the development of hematolological malignancies [12–15]. The presence of CH is postulated to be a biomarker of stressed or genomically unstable hematopoietic system that is prone to malignant transformation. There is considerable evidence to show the role of "hematopoietic stressors" such as genotoxic agents in the development of CH and the subsequent progression to hematological malignancies. There is strong preclinical and clinical evidence that cytotoxic chemotherapy selects hematopoietic stem progenitors cells (HSPCs) carry-

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ing mutations in the DNA damage-response genes *TP53* and *PPM1D* [12]. The implicated cytotoxic agents comprise platinum group of chemotherapy and etoposide. Inflammatory cytokines may also contribute as a stressor by promoting expansion of hematopoietic stem and progenitor clones carrying mutations in epigenetic modifiers, for example, *TET2*. Ribosomal biogenesis stress is another factor strongly implicated in the progression of CH to MDS or AML [12]. This was exemplified in patients with Shwachman–Diamond syndrome (SDS), where HSPC clones carrying *TP53* mutations are selective expanded.

In general, lymphoid malignancies are more common than myeloid malignancies despite the presence of gene mutations more commonly associated with myeloid malignancies. In a large cohort study by Jaiswal et al. involving 3341 patients, approximately 4% of individuals with CH due to somatic mutations develop a hematological malignancy after a median follow-up of 8 years [16]. The approximate risk of developing a hematological malignancy in individuals with CHIP is 0.5% per year. Following the exposure to cytotoxic therapy, CH is associated with an increased risk of developing a therapy-related myeloid neoplasm (t-MN). Retrospective case–control studies have demonstrated a ten-fold increase in the risk of t-MN in patients with CH at the time of treatment of the primacy malignancy [17, 18]. In patients undergoing autologous hematopoietic stem cell transplantation (HSCT), the cumulative incidence of t-MN was 14.1% in patients with CH versus 4.3% in patient without CH at the time of autologous HSCT [19]. In another large cohort of cancer patients undergoing chemotherapy, radiotherapy, or immunotherapy, 75 patients (0.8%) developed t-MN [20]. The presence of CH was significantly associated with an increased risk of t-MN with a hazards ratio of 6.9 and median time to development of t-MN of 26 months. Risk factors for the development of hematologic malignancies in individuals include the specific genes mutated, the clone size of the mutated gene, and the presence of multiple gene mutations [12]. The specific genes associated with a higher risk of developing myeloid malignancies (mostly MDS or AML) include *TP53*, the spliceosome genes (*U2AF1*, *SF3B1,* or *SRSF2*), *IDH1/2*, *RUNX1,* and *PHF6*. Specific variants associated with higher risk of myeloid malignancies include the *DNMT3A* variants R882C/H, R729W, R326C, R320, R736H/C Y735C, W860R, R771*, R598*, and P904L; the *SRSF2* P95R/H/L variants; and the *SF3B1* K700E and K666N variants, *JAK2*V617F, *IDH2* R140Q, and *GNB1* K57E. A variant allele frequency (VAF) of >10% is generally associated with an increased risk of hematologic malignancies or t-MN in patients with CH. In CCUS, i.e., patients with CH and cytopenia without morphologic dysplasia, the 5-year probability of developing a myeloid malignancy was 82% compared to 9% of patients with idiopathic cytopenia without CH (also known as idiopathic cytopenia of undetermined significance). In individual with CH involving specific genes such as *SF3B1* or involving multiple genes, the 5-year probability of developing a myeloid is in excess of 90%. Specific red cell morphologic features such as increased red cell distribution width (RDW) or increased mean corpuscular volume (MCV) are associated with an increased risk of developing myeloid malignancies in patients with CH.

28.3 Clonal Hematopoiesis and Non-hematological Diseases

In CHIP, somatic mutations translate into mutated immune effector cells such as natural killer cells, monocytes, and granulocytes. Mutations may occasionally be seen in B lymphocytes and rarely T lymphocytes. In non-hematological diseases where chronic inflammation is involved, these mutated immune effector cells may influence the disease states. Loss-of-function mutations in the DNA methylation genes *DNMT3A* and *TET2* are the most common genetic aberrations seen in CHIP. An increasing role of *DNMT3A* and *TET2* mutations is being reported. Tet2-deficient murine macrophages were more likely to express chemokine inflammatory cytokines than wild-type murine macrophages [21]. In addition, murine mast cells that lack *Dnmt3a* exhibit heightened allergic responses in conjunction with higher levels of interleukin (IL)-6, tumor necrosis factor (TNF)-α, IL-13, and immunoglobulin (Ig)-E. Similar to *Tet2*-deficient mice, the expression of Cxcl1, Cxl2, and IL-6 is also increased in mice with mutated Dnmt3a [21]. Patients carrying somatic *DNMT3A* mutations generally have increased levels of IL-6 in circulation. There is paucity of data on the impact of CHIP with *SF3B1*, *SRSF2,* and *ASXL1* mutations on immune effector cells.

CHIP harboring somatic *DNMT3A*, *TET2,* and *ASXL1* mutations is associated with increased risk of incident coronary artery disease (CAD) and ischemic stroke, which accounted for up to 40% of the mortality in CHIP due to non-hematological diseases. CHIP mutations with a VAF of more than 10% are more likely to develop CAD. The impact of *DNMT3A*, *TET2,* and ASXL1 mutations on the development of CAD is similar. The impact of mutations in spliceosome genes (*SF3B1*, *SRSF2,* and *U2AF1*) and DNA-damage response genes is less well studied.

Epidemiological studies have consistently shown an association between MDS and autoimmune phenomena that could be associated with the presence of somatic mutation seen in CHIP. Other diseases with putative inflammatory etiology include type 2 diabetes mellitus, chronic obstructive airway disease, and age-related neurodegenerative diseases such as Alzheimer's disease and Parkinson disease [21].

28.4 Impact of Clonal Hematopoiesis on Cellular Therapy

In recipients allografted with donor CHIP, a twofold increase in the risk of chronic graft-*versus*-host disease (GVHD) was observed [22]. Despite the wide belief that older donors are more likely to harbor CHIP, a recent pilot study has demonstrated that pathogenic mutations are far more prevalent in younger adult donors than previously appreciated [23]. Donor-derived malignancies are well reported in allogeneic hematopoietic stem cell transplantation (allo-HSCT) recipients. This is enhanced by the proliferative stress required to re-establish hematopoiesis in the recipient during engraftment. In addition, post-HSCT immunosuppression may negatively affect immunological surveillance and promote oncogenic transformation. Small case series have indicated that CHIP may lead to the development of donor cell leukemia, which is a rare complication affecting 0.1% of allo-HSCT recipients [24–29]. In addition, case series have indicated that cytopenia occurred in recipients of allograft harboring *DNMT3A* mutations [29]. In autologous HSCT, CHIP may result in poor stem cell mobilization and increase the risk of subsequent therapy-related MDS or AML. In chimeric antigen receptor T-cell (CAR-T) therapy, the presence of CHIP may alter its effectiveness. CHIP is especially prevalent in patients with lymphomas receiving CAR-T therapy. Possible effects include impact on CAR T-cell expansion, persistence, and function, the occurrence of cytokine release syndrome, and cytopenia [30].

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Therapy-Related MDS/AML and the Role of Environmental Factors

29

Maria Teresa Voso and Giulia Falconi

Abstract

Therapy-related myeloid neoplasms (t-MNs) include acute myeloid leukemia (AML), myelodysplastic syndromes (MDS), and myelodysplastic/myeloproliferative neoplasms (MDS/MPN), onsetting in patients treated with cytotoxic therapy (chemotherapy and/or radiation therapy) for a primary cancer or an autoimmune disorder.

t-MN accounts for approximately 10–20% of newly diagnosed cases of AML or MDS and can occur at any age. The risk of developing a t-MN is determined by complex interactions between the nature and dose of the chemotherapy agents and radiation intensity. Inherited risk factors and environmental exposures may then contribute to the accumulation of somatic mutations in hematopoietic stem cells and t-MN onset. Recent advances in deep sequencing techniques have shed light on the pathogenesis of t-MN, identifying clonal hematopoiesis of indeterminate potential (CHIP) as a frequent first step in the multi-hit model of t-MN. CHIP is often detectable at the time of the primary cancer diagnosis prior to any cytotoxic treatment, probably setting the fertile genomic premalignant state for secondary leukemogenesis. The pathogenesis of t-MN is then a multifactoral process, where the type of cancer therapy, the aging process, and the individual exposures may favor additional hit development, such as the acquisition of TP53 mutations and unfavorable karyotype abnormalities.

Patients with t-MN generally have poor prognosis (5-year overall survival <10%) and are often refractory to standard treatment strategies, with the exceptions of t-AML with recurrent translocations, including t-APL (acute promyelocytic leukemia) and core-binding factor t-AML, who should receive conventional treatment according to age and performance status. Other t-MN patients should be considered candidates for HSCT, if eligible, since this is the only potentially curative treatment. However, not all patients may benefit from transplantation, such as patients with TP53 mutations, that account for about 30–40% of all t-MN cases. The unfavorable prognosis of t-MN indicates the need for new pharmacological approaches, such as CPX-351, or venetoclax in combination with hypomethylating agents, monoclonal antibodies as magrolimab, or targeted drugs against pathogenic mutations.

Keywords

Predisposition · Exposition · Microenvironment · Acquired lesions

Abbreviations

AML	Acute myeloid leukemia
ASXL1	Additional sex combs like 1
BM	Bone marrow
BMM	Bone marrow microenvironment
CHIP	Clonal hematopoiesis of indeterminate potential
CNA	Copy number alterations
cnLOH	Copy neutral loss of heterozygosity
CR	Complete response
DAMP	Damage-associated molecular pattern
DE	Distally exposed
DNMT3A	DNA methyltransferase 3A
HSC	Hematopoietic stem cells
HSCT	Hematopoietic stem cell transplantation
HSPC	Hematopoietic stem/progenitor cells
KMT2A	Histone-lysine N-methyltransferase 2A
MDS	Myelodysplastic syndromes
MDS/MPN	Myelodysplastic/myeloproliferative neoplasms

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MSC	Mesenchymal stem cells
OS	Overall survival
PE	Proximally exposed
RUNX1	Runt-related transcription factor 1
SF3B1	Splicing factor 3B subunit 1
SIR	Standardized incidence ratio
SNP	Single nucleotide polymorphisms
SNV	Single nucleotide variants
TET2	Tet methylcytosine dioxygenase 2
TLR4	Toll-like receptor 4
t-MN	Therapy-related myeloid neoplasms
TP53	Tumor protein p53
VAF	Variant allele frequency

29.1 Introduction

Therapy-related myeloid neoplasms (t-MN) include according to the 2008 WHO classification [1] and its 2016 revision [2] therapy-related acute myeloid leukemia (AML), myelodysplastic syndromes (MDS), and myelodysplastic/myeloproliferative neoplasms (MDS/MPN). They are a late complication of cytotoxic therapy (chemotherapy and/or radiation therapy) used in the treatment of both malignant (solid or hematological) and non-malignant (mostly autoimmune) diseases.

t-MN is an emerging problem of our aging societies, where the newer therapeutic drugs and the ameliorated cancer management protocols are improving the life expectancy of cancer patients [3]. This means that an increasing number of patients treated with chemotherapy and/or radiotherapy are at risk of developing late treatment-related complications, such as cardiac and pulmonary disease, and therapy-related myeloid neoplasms. t-MNs are generally characterized by poor prognosis (5-year overall survival <10%) [4] and refractoriness to standard treatment strategies [5].

The damage induced to DNA by cytotoxic therapy is a direct and often desired consequence of the action mechanism of many anticancer drugs. In some cases, however, this damage can have "off-target" manifestations and determine the development of permanent alterations in the bone marrow (BM) stem cells. Furthermore, individual susceptibility and exposure to additional toxic events, such as environmental pollution and/or unhealthy behavioral habits, may increase the damage to these cells. When the DNA damage exceeds the thresholds tolerable by the mechanisms of repair and cells escape apoptosis, clonal alterations in the hematopoietic stem cells (HSCs) and/or in the bone marrow microenvironment (BMM) can develop and confer a replicative advantage responsible for the development of a hematological neoplasm, in this case related to the therapy [6].

29.2 Epidemiology

A t-MN should be suspected whenever a patient with a history of chemotherapy, radiotherapy, or immunosuppressive therapy presents with clinical or morphological features of MDS or AML. t-MN accounts for approximately 10–20% of newly diagnosed cases of AML or MDS, with a current incidence of 0.13 per 100,000 men and women in the United States [3, 7]. The median age of t-MN patients at the time of diagnosis is 65 years, but the disease can occur at any age [3]. Chemotherapy treatment increases the incidence of t-MN by 4.7-fold, and a younger age at the time of exposure further increases this risk [7].

t-MN is a rare condition; less than 1–2% of patients exposed to cytotoxic drugs and radiotherapy develop a t-MN [7–9]. t-MN can occur following cytotoxic therapy for nearly all tumor types consistent with expanded use of cytotoxic agents in the twenty-first century [10]. Currently, the most common primary malignancies with a t-MN-associated risk are breast cancer and, among hematological malignancies, non-Hodgkin lymphoma [3, 7]. Patients with a prior bone or soft-tissue sarcoma are also at particularly high risk for t-MN, up to 11% of patients treated with high-intensity chemotherapy develop t-MN [11]. Moreover, another growing at-risk population is solid organ transplant recipients, who have a standardized incidence ratio (SIR) of 4.6 for MDS and 2.7 for AML [12].

Regarding the latency period between exposition to anticancer drugs and development of t-MN, it may vary from some months up to 10 years, depending on the age at the time of primary malignancy diagnosis, the kind of cytotoxic treatment used, the cumulative dose, and dose intensity [9, 13]. Patients diagnosed with a solid tumor have an increased risk of t-MN within 9 to 12 months after treatment. This risk peaks at 2 years and returns to a population baseline risk in 10–15 years [14]. On the contrary, the development of a t-MN after a primary hematological malignancy follows a broader time course that peaks at 5 years and does not return to baseline even after 15 years [6].

29.3 Pathogenesis

The risk of developing a t-MN is determined by a complex interaction between the nature and dosage of the chemotherapeutic agent, the radiation intensity, inherited risk factors, environmental exposures, the accumulation of somatic mutations in the HSC, comorbidities and the age of the patient, and finally stochastic events [6] (Fig. 29.1).

The contribution of the main drivers of t-MN development is described in the following sections.

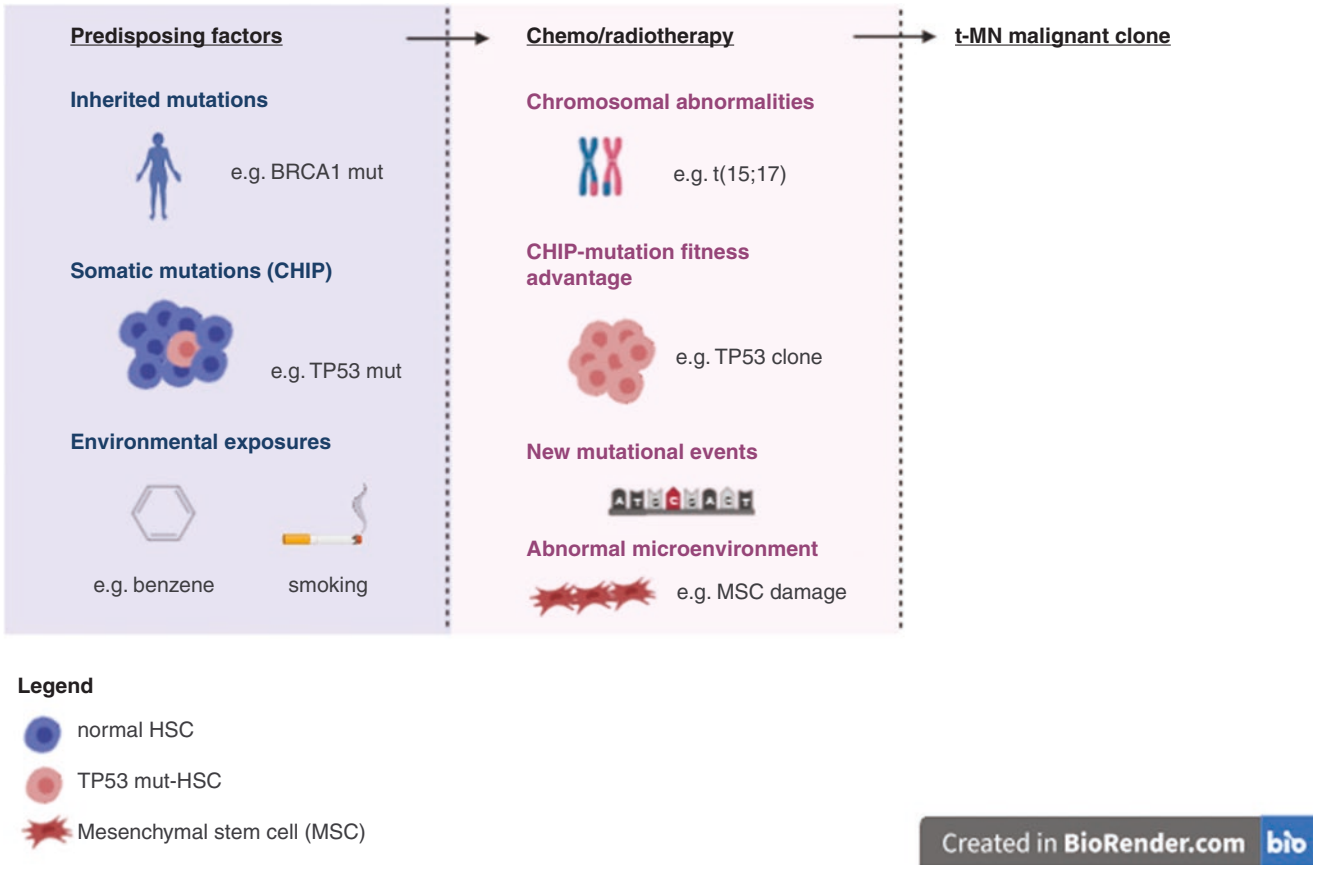

Fig. 29.1 Schematic representation of t-MN pathogenesis

29.3.1 Cytotoxic Therapy

By definition, t-MNs have a strong extrinsic driver, which is previous chemotherapy and/or radiotherapy, which makes them a formidable model of in vivo leukemogenesis.

The chemotherapeutic agents used to treat primary cancers, most frequently associated with t-MN development are alkylating agents and topoisomerase II inhibitors. The former, including nitrogen mustards, nitrosoureas, and alkyl sulfonate, are compounds that add an alkyl group to the guanine base of the DNA molecule. They induce a direct DNA damage through the formation of intra- or inter-strand cross-linking, the induction of abnormal base pairing, and double-strand breaks in DNA. Topoisomerase II inhibitors, such as etoposide, doxorubicin, idarubicin, and mitoxantrone, are chemical compounds blocking the action of topoisomerases II, preventing the repair of single- and double-stranded DNA breaks, thus impairing apoptosis and cell death.

Historically, t-MN was been categorized into two clinical subsets according to the nature of the cytotoxic therapy used for the treatment of primary disease (alkylating agents and/or radiation therapy *vs* topoisomerase II inhibitors). Table 29.1 shows the characteristic of these main 2 t-MN subtypes.

The first subtype, typical of patients who received alkylating agents and/or radiation therapy, is the most common, accounting for approximately 70% of t-MN patients, and characterized by:

- The loss of part of chromosome 5 (del(5q)) and/or either part or all of chromosome 7 (del(7q) or –7)
- A long latency period (on average 5 years)
- The onset as MDS, which often progresses rapidly to AML with multilineage dysplasia
- A poor prognosis (median survival of 8 months)

The second subset of t-MN, typical of patients who received topoisomerase II inhibitors, has instead characterized by:

- Translocations involving histone-lysine N-methyltransferase 2A (*KMT2A*) at 11q23.3 or runt-related transcription factor 1 (*RUNX1*) at 21q22.1
- A short latency period (in the range of 1–2 years)
- The onset as overt leukemia without an antecedent MDS phase
- A favorable response to intensive induction therapy

Table 29.1 Classification of t-MN according to the nature of the cytotoxic therapy used for the treatment of primary disease

	Alkylating agents	Topoisomerase II inhibitors
Cytogenetics	del(5q), −7/del(7q)	t(11q23.3), t(21q22.1), t(15;17)
Frequency	About 70% of t-MN patients	About 30% of t-MN patients
Latency	5–7 years	1–2 years
Presentation	MDS	AML

Currently, this classification is less actual since the treatment of primary tumors has moved from monotherapy to combination therapy regimes. However, many of the listed aspects are still recognizable frequent in t-MN.

Damage to the hematopoietic progenitor DNA, linked to cytotoxic therapy, plays an important role for t-MN development, as demonstrated by the global sequencing studies performed in patients with *NPM1*-mutated AML who become *NPM1* wild type at relapse [15]. In these cases, the process of secondary leukemogenesis is supported by an increase in transitions and transversions at the time of hematological recurrence and the appearance of new karyotypic aberrations and somatic mutations. Moreover, recent evidence showed that mutations are selected differentially based on exposures, with radiation, platinum, and topoisomerase II inhibitors preferentially selecting for mutations in DNA damage response genes (*TP53, PPM1D, and CHEK2*) [16].

However, in addition to the type of treatment, also the number of therapy cycles and the overall exposure dose are risk factors to t-MN development, as evidenced by higher risk of developing a t-MN in patients who require multiple lines of treatment, than in patients who maintain remission after first-line treatment [17, 18].

29.3.2 Inherited Risk Factors

For many years, t-MN has been considered only a consequence of DNA damage induced by cytotoxic therapy in normal hematopoietic stem or progenitor cells. Nonetheless, over the years, increasing scientific evidence has shown that exposure to cytotoxic agents as the sole cause of t-MN is unsatisfactory for the following reasons:

- Up to 10% of patients have more than one cancer before t-MN diagnosis.
- <1–2% of patients exposed to cytotoxic drugs and radiotherapy develop a t-MN [7–9].
- In some patients, second cancers have a latency of 10 years or more.
- t-MN are inherently chemoresistant.
- HSC with acquired somatic mutations may exist years before treatment of the primary disease. Chemotherapy and/or radiotherapy may then promote clonal selection of these pre-existing mutant HSCs and favor t-MN development [19].

Analysis of the frequency of polymorphic variants in the past and of germline variants more recently is one of the main area of interest in t-MN pathogenesis. Genetic factors known to predispose to the development of t-MN are discussed in the next sections.

29.3.2.1 Single-Nucleotide Polymorphisms (SNP)

Analysis of the frequency of polymorphic variants in genes implicated in the pathways of cell detoxification, DNA repair, nucleotide synthesis, and apoptosis has been one of the main areas of study in this field.

Enzymes involved in phase I cell detoxification (cytochrome P 450, NAD(P)H quinone oxidoreductase, etc.) and phase II (mainly glutathione S transferase) play a key role in modifying and/or degrading some of the drugs commonly used in cancer therapy. Polymorphic variants in these enzymes can modulate the intensity and effectiveness of drugs and may play a role in predisposing the patient to the development of t-MN. In particular, polymorphisms in cell detoxification enzymes, such as those of the cytochrome P450 (*CYP3A4* and *CYP3A5*), *NQO1*, *GSTT1*, *GSTM1*, *GSTA1,* and *GSTP1* have been described as risk modulators in the development of t-MN [20–27], though their role has not been confirmed yet.

Another class of genes studied in the field of individual susceptibility for t-MNs is the genes involved in DNA repair, and in particular *XRCC1*, *XRCC3*, *XPD,* and *RAD51*, which participate in the nucleotide excision repair system and the homologous repair of double-stranded DNA breaks or apoptosis-related genes as BCL2L10. Again, results were variable in different reports [21–23, 28].

So far, higher frequency of SNP in detoxification and DNA-repair enzymes, alone or in association, have been reported in t-MN, but none has been validated as significant risk factor in large patient groups. Moreover, the complexity of the interactions between the different metabolic pathways, the heterogeneity of anticancer treatments, and the lack of adequate control cohorts represent the main barrier to define the real contribution of these SNP to the development of t-MN.

29.3.2.2 Germline Single-Nucleotide Variants (SNV)

About 16–21% of cancer survivors who developed t-MN have a germline mutation associated with inherited cancer

susceptibility genes. Germline mutations in several breast and ovarian cancer susceptibility genes such as *BRCA*, tumor protein p53 (*TP53)*, partner and localizer of BRCA2 (*PALB2*), and checkpoint kinase 2 (*CHEK2*) were been identified in 47 breast cancer survivors who developed a t-MN [29]. Other studies have corroborated the role of germline mutations in *BRCA1*, *BRCA2*, and *TP53* and Fanconi anemia genes in patients with t-MN [19, 30, 31].

Fanconi anemia (FA) is a childhood syndrome characterized by chromosomal instability, developmental abnormalities, aplastic anemia, and predisposition to cancer, particularly gynecological, head and neck, and gastrointestinal. FA is associated with the occurrence of biallelic loss-of-function mutations in the family of *FANC* genes, comprising 16 DNA repair genes. Carriers of germline homozygous mutations of at least five out of the sixteen FA genes, including *FANCD1/BRCA2, FANCJ/BRIP1, FANCN/PALB2, FANCO/RAD51C,* and *FANCQ/ERCC4*, are at a higher risk to develop cancer. In t-MN setting, our group showed that germline FA gene variants were frequent in t-MN patients, with six out of 37 patients (16%) carriers of at least one genomic variant [31].

Many of the known t-MN predisposition genes encode components of the DNA damage response, which suggests a model whereby individuals with these germline mutations are particularly susceptible to cytotoxic chemotherapy and/or radiation due to deficiencies in DNA repair, genomic instability, and/or insufficient cell cycle arrest and apoptosis. Supporting this hypothesis, *BRCA1*-deficient mice exhibit cytopenias, genomic instability and bone marrow failure, and a subset of these mice develop a myeloid neoplasm [32].

An alternative but not mutually exclusive model is that genetically susceptible patients are at risk for the independent development of a second malignancy, as evidenced by the extended latency period to t-MN development in patients with *BRCA1* or *BRCA2* mutations of 133 months [29]. In addition, among patients with germline mutations in any cancer susceptibility gene, about half had either normal karyotype or balanced recurring translocations [33], which are cytogenetic findings more typical of de novo AML.

Familial susceptibility in t-MN is a fascinating subject of study not yet fully explored. Although most cases of MDS or AML are sporadic, it is becoming clear that a subgroup of cases is associated with germline mutations and is familial [34]. The presence of germline variants or predisposition syndromes should be considered as part of the diagnosis and management of t-MN. This concept has been reiterated by the addition of a section on myeloid neoplasms with germline predisposition in the 2016 revision of the WHO classification [2].

Finally, a family history of solid or hematological malignancies should be taken into consideration when selecting the stem cell donor for allogeneic transplantation. The stem cells of a donor "carrier" of a germline mutation, placed in a context of "immunosuppression," may give rise to secondary leukemias after allogeneic transplantation [35].

29.3.2.3 Clonal Hematopoiesis of Indeterminate Potential (CHIP)

Clonal hematopoiesis of indeterminate potential (CHIP) refers to the presence of clonal mutations in the peripheral blood at a variant allele frequency (VAF) of at least 2%, in the absence of any known cytopenias or hematological disorders [36, 37]. CHIP is relatively common in healthy populations, and its incidence increases with age (up to 30% of subjects over 80 years) and is associated with higher odds of cardiovascular mortality and of developing hematological malignancies with a risk of progression of about 0.5–1% per year vs. <0.1% in non-CHIP carriers [38, 39].

The genes mutated in CHIP, such as DNA methyltransferase 3A (*DNMT3A*), additional sex combs like 1 (*ASXL1*), Tet methylcytosine dioxygenase 2 (*TET2*), splicing factor 3B subunit 1 (*SF3B1*), and *TP53*, are also recurrently mutated in myeloid malignancies. Mutations affecting these genes were found almost 10 years before the diagnosis of AML, with a progressive increase in the VAF of mutations observed in serial samples prior to AML diagnosis, but not in age-matched controls with detectable mutations [5].

In the context of t-MN, CHIP has been shown to be frequent at the time of the primary cancer diagnosis, representing a pre-malignant state that can be triggered by the exposure to DNA damage due to cytotoxic agents. Clonal hematopoiesis as a risk factor for t-MN has been reported in 30–70% of patients developing a t-MN after a lymphoproliferative disorder or other hematologic malignancies [40–42]. The positive predictive value of CHIP for the development of t-MN was 27–35%, and the negative predictive value was 89–98%, thereby providing the first potential biomarker for t-MN. Mutations in RUNX1, TP53, SRSF2, and TET2 genes were been more commonly observed in patients who developed a t-MN compared to disease-matched controls [42].

In the last years, the results of these studies paved the way for an alternative t-MN pathogenesis. Indeed, cancer treatment may favor and select pre-existing CHIP lesion instead of directly being responsible for their development, as we thought until last decade.

29.3.3 Bone Marrow Niche: Focus on the Mesenchymal Stem Cells

Recent studies have highlighted the role of a complex bidirectional crosstalk between HSCs and the BMM in normal hematopoiesis, as well as in the pathogenesis of myeloid diseases [43]. Emerging data suggest that alterations of mesenchymal stem cells (MSCs), important components of the

BMM [44], may play a role in the pathogenesis of myeloid neoplasms both *de novo* and therapy-related [45–50], although the mechanisms are not yet fully understood.

One of the major evidence supporting the crucial role of BM-MSCs in the initiation and progression of myeloid malignancies derives from in vivo models, where DICER1 deletion in the stromal compartment leads to the development of MDS and then overt leukemia in mice [51].

Bone marrow MSC isolated from patients with *de novo* MDS/AML exhibits decreased proliferative and clonogenic capacity, altered morphology, increased senescence, impaired immunoregulatory properties, reduced osteogenic differentiation, abnormal expression of surface adhesion molecules, altered chemokine/cytokine production, and reduced ability to support HSC growth and differentiation [45–50]. This last functional defect may account in part for the poor results of HSC transplantation in patients with t-MN and high-risk MDS and AML. MSCs isolated from MDS patients display an impaired differentiation program and are essential for the propagation of MDS HSC (Lin-, CD34+, and CD38-) in orthotopic xenografts [49]. Similarly, healthy MSCs adopt MDS MSC-like molecular features when exposed to hematopoietic MDS cells [49].

These data show that bone marrow stromal changes and the formation of a malignant niche are not merely a consequence of the malignant process but contribute directly to the pathogenesis of the disease.

In this context, the role of cytotoxic therapy is complex and not yet clear. Cytotoxic therapy exerts several effects on the BMM, including a pro-inflammatory response with the consequent release of inflammatory cytokines and the release of reactive oxygen species by MSCs with resultant genotoxic damage to HSCs [52]. The haploinsufficiency of two del(5q) genes (EGR1 and APC), together with *TP53* knockdown, in a mouse model, produces a high frequency of myeloid diseases following concurrent treatment of both hematopoietic cells and the BM stroma with an alkylating agent, but not after treatment of either alone [52].

Moreover, cytotoxic therapy creates an environment that selects for pre-existing mutant clones at the expense of normal HSCs. In this context, DNA damage-induced competition led to a selective clonal advantage of HSCs and hematopoietic progenitor cells with reduced p53 function in mouse bone marrow chimaeras—reminiscent of the CHIP phenotype—via growth arrest and senescence-related gene expression in cells with higher p53 activity [53].

29.4 Genetic and Cytogenetic Profile of t-MN

Molecular alterations typical of t-MN have not been identified. The spectrum of genomic abnormalities in t-MN parallels that of *de novo* myeloid neoplasms, with the key distinction that t-MN, is markedly skewed toward high-risk abnormalities (Fig. 29.2). Deletions of chromosomes 5 (del(5q)), monosomy or deletions of chromosomes 7 (−7/del(7q)), and complex karyotype and *TP53* mutations, carrying an adverse prognosis, are all profoundly over-represented in t-MN compared with *de novo* counterparts. In contrast, favorable-risk abnormalities such as the t(8;21) or intermediate-risk normal karyotypes are considerably underrepresented in t-MN. However, t-MN and *de novo* AML with the same karyotype abnormality are indistinguishable at the genetic level.

The data of the "Italian Network of Secondary Leukemia" on 212 t-MN patients reported karyotype alterations in 66% of patients: 11% was classified as a favorable risk karyotype, 50% intermediate, and 39% unfavorable. Chromosomes 5 and 7 alterations and complex karyotypes were the most frequent aberrations identified in t-MN patients [4].

Despite previous exposition to chemotherapy and/or radiation therapy for primary diseases, t-MN presents the lowest mutational burdens across human cancers and is characterized by a lower number of SNV than other tumors. Moreover, unlike *de novo* AML and MDS, a mutational profile charac-

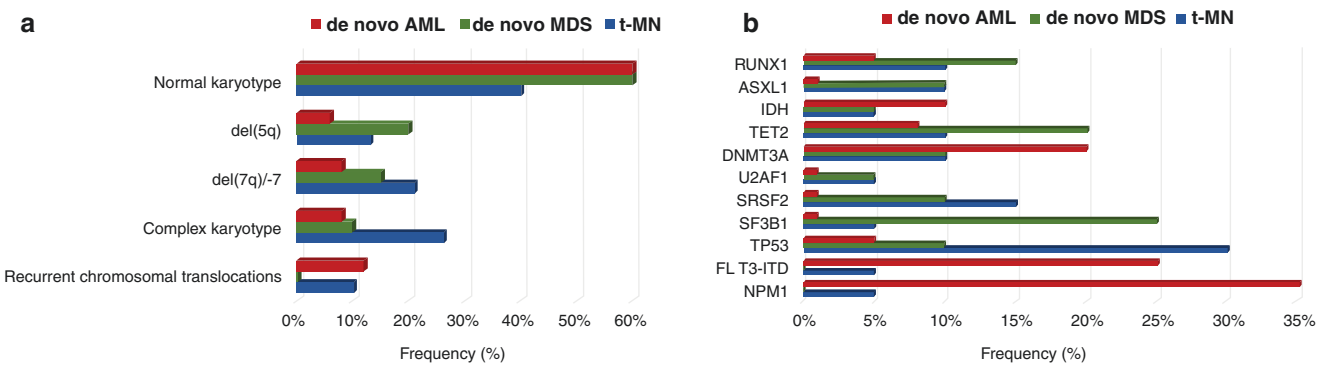

Fig. 29.2 (**a**) Comparison of cytogenetic profiles in myeloid neoplasms, *de novo* and therapy-related. (**b**) Comparison of mutational profiles in myeloid neoplasms, *de novo* and therapy-related (data from [4, 19, 22, 23, 54, 55])

terizing t-MN has not been identified, except for the high frequency of mutations in the *TP53* gene. *TP53,* located on chromosome 17p13, encodes the p53 tumor suppressor protein, an integral transcription factor able to respond to DNA damage by activating transcriptional programs for DNA repair and activating apoptosis when necessary [56]. *TP53* mutations are observed in more than 50% of solid tumors [57] but occur in only ~5–10% of *de novo* MDS/AML (Cancer Genome Atlas Research [54]). In comparison, *TP53* is the most commonly mutated gene in t-MN, occurring in approximately 30% of cases [19, 58]. Historically, it was thought that this selective enrichment was addicted to the direct mutagenic effects of cytotoxic therapy on hematopoietic stem/progenitor cell (HSPC) DNA. However, more recently, increasing evidence suggests that *TP53* mutations are detectable with a very low VAF in 60–70% of t-MN patients at the time of the primary malignancy before any exposure to therapy, and after treatment, these chemotherapy-resistant pre-leukemic clones preferentially expand [19, 40]. Moreover, *TP53* clones precede the development of cytogenetic abnormalities in t-MN as demonstrated by the presence of sub-clonal chromosome 5 and 7 copy number alterations (CNAs) in *TP53*-mutated patients many years before the diagnosis of t-MN [59]. Chemotherapy and/or radiation creates an environment that selects for pre-existing mutant clones at the expense of normal, healthy HSCs.

There are at least two mechanisms by which *TP53* mutations occur in t-MN: 1) *TP53* mutant HSCs or the chemotherapy itself may directly induce DNA damage and leukemogenic *TP53* lesions and 2) *TP53* clones are present in patients before the onset of chemotherapy as CHIP and chemotherapy promotes clonal selection of pre-existing clones [19, 40, 41].

No mutational hotspots have been identified in *TP53*, and mutations are spanned throughout the entire gene. Among *TP53*-mutated patients, 72.5% had a single *TP53* mutation, 26.5% had two, and 1% had three genomic hits affecting TP53 [59]. Approximately half of the patients with one *TP53* mutation had loss of the wild-type allele by deletion or copy neutral loss of heterozygosity (cnLOH). In contrast, only 13% of patients with more than one *TP53* mutation had a concomitant allelic imbalance at the *TP53* locus [59]. *TP53* mutations are associated with complex and monosomal karyotype, few co-occurring mutations, high-risk presentation, and poor prognosis [60–64].

Other mutations found at high frequency in t-MN are those of RNA splicing genes (*SRSF2, SF3B1, U2AF1,* and *ZRSR2*), with an overall frequency of 25.6%, and are very frequently mutually exclusive [65]. Mutations in *FLT3* and *NPM1* genes are less frequent in t-MN than in *de novo* forms, while those in *SETBP1* were found in 6.2% of patients, associated with −7/del(7q) in 75% of cases [22, 23, 55].

29.5 Environmental Factors

Myeloid neoplasm due to environmental exposure is traditionally classified as t-MN.

The more traditional definition of environmental factors includes exposures to poisons, for professional or personal reasons (increased risk of t-MN for people living in industrial vs rural areas). One known leukemogenic agent is benzene, a solvent used in the rubber industry, chemical plants, shoe manufacturing, oil refineries, and gasoline-related industries, and some cleaning products, detergents, and paints. Long-lasting exposure to benzene has been shown to significantly increase the risk of t-MN [66], although this risk has been decreasing due to better and safer working conditions. Smoking also increases the risk of MN, with a specific pathogenetic effect also related to the presence of ASXL1 mutations [16].

Exposure to large amounts of high-energy radiation significantly increases the risk of leukemia. Exposure to atomic bomb explosions is a paradigm for this. The "advantage" in these cases is that the amount and type of exposure are measurable, and a cause-effect relationship may be better studied.

In t-MN diagnosed in atomic bomb survivors, genetic aberration profiles differ from those observed in t-MN. In particular, in MDS in proximally exposed (PE) cases (<1·5 km from the hypocenter), chromosomal translocations and inversions were more frequent than in MDS in "distally exposed patients" (DE), with increased frequency of structural alterations in chromosomes 3, 8, and 11. A significantly higher frequency of copy number loss for 11q was observed in the PE vs DE group, associated with the presence of biallelic alterations of ATM. The mutational profile was characterized by a mutation burden similar to other MDS, but significantly fewer mutations in genes of the DNA methylation pathway were observed in PE patients [67, 68]. TP53 mutations have similar frequencies in PE patients and t-MN. These data show that ATM alterations and the related DNA damage–repair defect may play a major role in MDS onsetting following atomic bomb exposure.

29.6 Clinical Characteristics and Treatment

29.6.1 Prognosis

The outcome of t-MN patients is poor, with a 5-year survival of 10% [4]. In the largest study carried out by a single institution, which recruited 303 t-MN patients between 1972 and 2001, the median survival was 8.0 months [33]. Two decades later, a modest improvement in survival (14.6 months) was reported in the Italian multicenter registry including 277

patients recruited between 1999 and 2013 [4]. t-MN patients are more frequently characterized by high-risk karyotypes, comorbidities, and poor performance status than *de novo* AML patients. Nevertheless, t-MN and *de novo* AML are biologically similar in older patients. Patients over 60 years, with a high-risk karyotype and treated with intensive therapy, had a similar outcome, when adjusted for these confounding factors [69]. On the contrary, for younger patients and those with favorable-risk karyotypes, t-MN is an independent adverse prognostic factor [69–71].

Unfavorable prognostic factors are patient-related factors, as older age at the time of t-MN diagnosis, comorbidities, and status of the previous malignancy. Unfavorable disease-related factors include poor and very-poor risk karyotypes, and *TP53* mutations, which are present in about 30% of patients with t-MN [4, 59, 60, 62, 72, 73]. Patients with *TP53* mutations had median overall survival (OS) of 4.6 months compared to 35.6 months in non-mutated cases. In a multivariate analysis adjusting for various factors, including de novo/secondary AML and cytogenetic risk group, *TP53* mutations were independently associated with reduced OS [60]. Even in the complex karyotype cohort, patients with *TP53* mutations had an inferior outcome, with 3-year OS 0% *versus* 27.9%. [62]. Moreover, *TP53* mutations were associated with significant reduction in complete response (CR) rates, and ~70% out of the few patients that achieve CR and underwent a hematopoietic stem cell transplantation (HSCT), relapsed within 6 months and have a 3-year OS of 15% [74, 75]. Recent evidence shows that the number of *TP53* mutations also influences the prognosis. The presence of more than one *TP53* mutation is an unfavorable prognostic factor, whereas outcome of patients with only one *TP53* mutation did not differ from *TP53* wild-type patients [59].

29.6.2 Treatment

As for *de novo* myeloid neoplasms, therapeutic options available for the treatment of t-MN include supportive care, hypomethylating agents, and conventional chemotherapy. However, t-MN patients are frequently frail due to the therapies received for the primary disease. Various factors related to the previous therapy can complicate the management of patients with t-MN: chronic immunosuppression in individuals undergoing solid organ transplantation, depletion of the hematopoietic reservoir due to chemotherapy, which translates into more severe and prolonged cytopenias, use of G-CSF support, and variable organ dysfunctions.

To date, all eligible t-MN patients should be considered candidates for HSCT, that it is the only potentially curative treatment able to significantly improve patient survival [4]. Despite this, less than 20% of t-MN patients undergo HSCT, due to poor performance status and advanced age. For those patients who receive a transplant, outcome is inferior to in *de novo* forms, with a 38% overall survival at 5 years and 24% at 10 years [76]. These data have been confirmed by a recent study including 178 s-/t-AML patients [77]. A risk stratification model by Litzow and colleagues, performed on 868 patients with t-MDS and t-AML undergoing HSCT, showed that only patients with a maximum of two risk factors (age over 35, unfavorable cytogenetics, active disease at the time of transplantation, and type of donor) had a significant benefit from transplantation [78].

Moreover, emerging molecular data indicate that not all patients may benefit from transplantation. MDS patients with *TP53* mutations are the emblematic example, with a median overall survival of just 4.6 months after HSCT [79]. This is a big issue, considering that up to 40% of t-MNs are *TP53*-mutated [80]. The addition of decitabine to transplant regimens may benefit patients with *TP53*-mutant AML and MDS [81].

The unfavorable prognosis of t-MN, also in the context of HSCT, indicates the need for new pharmacological approaches. One of the most promising new drugs, approved by the US Food and Drug Administration for the treatment of newly diagnosed sAML in 2017, is CPX-351, a liposomal combination of daunorubicin and cytarabine, at the ratio of 5:1 [82], whose liposomal formulation allows internalization within leukemic cells more efficiently than the combination of conventional daunorubicin and Ara-C, and a prolonged activity. This drug is included among standard-intensity chemotherapy drugs. The phase III study demonstrated the greater efficacy of CPX-351 compared to conventional "3 + 7" therapy in sAML patients in the age group between 65 and 70 years, particularly in therapy-related forms [82, 83]. CPX-351 significantly improved median OS versus 7 + 3 regimen (9.56 vs 5.95 months) and overall remission rate (47.7% vs 33.3%) [83].

In the context of non-intensive care, the hypomethylating agents (HMAs) azacitidine and decitabine have been used in t-MN with results similar to *de novo* diseases [84, 85]. Efficacy of HMA significantly increases when combined with hypomethylating agents, and venetoclax, a potent oral inhibitor of the BCL2 protein, has been recently approved in elderly patients with AML [86]. The combination of azacitidine and venetoclax significantly improved overall survival compared to the combination of azacitidine and placebo (14 months vs 9.6 months) and the incidence of complete remission (36.7% vs 17.9%) [87].

A promising drug thus far is an inhibitor of the mutated *TP53* protein, APR-246, a prodrug that is converted to methylene quinuclidinone and binds covalently to the mutant p53 core domain, restoring the upregulation of apoptotic transcriptional programs. In a phase II study, APR-246 was com-

bined with azacitidine to treat patients with *TP53* mutant MDS and AML, *de novo* and therapy-related. The combination of APR-246 with azacitidine had an overall response rate of 100%, with 82% of *TP53*-mutated patients achieving CR. Responses were accompanied by deep molecular remissions with a median VAF of 0.3% in NGS-negative patients [88, 89]. These data will have to be validated by the ongoing phase III study.

A newer, promising drug is the antibody magrolimab (Hu5F9-G4), which blocks CD47, a macrophage immune checkpoint and "don't eat me" signal on cancer cells. Magrolimab induces tumor phagocytosis and eliminates leukemia stem cells (LSCs). Azacitidine (AZA) synergizes with magrolimab by inducing "eat me" signals on leukemic blasts, thereby enhancing phagocytosis. Magrolimab + AZA has been shown to be clinically effective in AML and MDS, including TP53-mutant AML (Sallman et al., ASH [90]).

All these approaches show significant advantage, in particular when used as a bridge to HSCT, where remission status at the time of transplant is a significant prognostic factor for patients' outcome.

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Prognostic Indicators in MDS and CMML

30

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Abstract

Various prognostic scoring systems have been developed over the last 2 decades aiming to better risk stratify myelodysplastic syndrome (MDS) and chronic myelomonocytic leukemia (CMML). Molecular alterations are increasingly important in the current era of personalized medicine. In this chapter, we review the evolution of prognostic models in MDS and CMML and the development of personalized prognostic assessment in these disorders.

Keywords

Myelodysplastic syndrome · Chronic myelomonocytic leukemia · Prognosis · Prognostic models

30.1 Myelodysplastic Syndrome

30.1.1 Introduction

Myelodysplastic syndrome (MDS) is a clonal hematopoietic disorder characterized by ineffective hematopoiesis with dysplasia and cytopenia in at least one lineage [1, 2]. The disease per se is associated with an indefinite risk of progression to secondary acute myeloid leukemia (sAML) in approximately 20–30% of the patients [3]. The disease is characterized by clinical heterogeneity and constitutes one of the most common hematologic malignancies [4]. It displays a male preponderance with a ratio of 1.26:1 and has a median age of diagnosis at 70 years [5–7]. The incidence of MDS each year is about 4.5 per 100,000 individuals [2]. There is currently no curative measure for MDS patients except for allogeneic hematopoietic stem cell transplantation (allo-HSCT). Cytogenetic abnormalities may be observed in up to 50% of the MDS patients with 5q deletion as the most prevalent aberration identified [8, 9]. Monosomy 7 acts as the second most common abnormality.

The diagnosis of MDS is made based on the morphologic features in peripheral blood [10], bone marrow (BM) aspiration, and trephine biopsy. MDS patients are also classified under the World Health Organization (WHO) classification with a total of seven subtypes (Table 30.1). Initial risk stratification of patients at diagnosis is essential in guiding future

Table 30.1 Classification of myelodysplastic syndrome (MDS)

The FAB classification (1982)	The WHO classification (2016)
RA	MDS-SLD
RARS	MDS-MLD
RAEB	MDS-RS • MDS-RS-SLD • MDS-RS-MLD
RAEB-T	MDS with isolated del(5q)
CMML	MDS-EB • MDS-EB-1 • MDS-EB-2
	MDS-U • With 1% peripheral blood blasts • With SLD and pancytopenia • Based on defining cytogenetic abnormality
	RCC (provisional)

CMML: chronic myelomonocytic leukemia; *del(5q)*: 5q deletion; *FAB*: French American British classification; *MDS*: myelodysplastic syndrome; *MDS-EB*: MDS with excess blasts; *MDS-MLD*: MDS-multilineage dysplasia; *MDS-RS*: MDS-ring sideroblasts; *MDS-SLD*: MDS-single lineage dysplasia; *MDS-U*: MDS, unclassifiable; *RA*: refractory anemia; *RAEB*: refractory anemia with excess blasts; *RAEB-T*: RAEB in transformation; *RARS*: RA with ring sideroblasts; *RCC*: refractory cytopenia of childhood

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treatment decisions for patients [11]. The International Prognostic Scoring System (IPSS-R) serves as the standard prognostic model in MDS. Despite the availability of multiple therapeutic options, complete eradication of the disease is difficult. Rather, hampering the progression to sAML and improving the overall survival of patients become the primary goals in the treatment of MDS. Besides, anemia is the major manifestation in MDS patients. Risk of infection or hemorrhagic event may also be present, impeding the normal functioning of patients. Thus, treatment also aims to improve the quality of life of patients with alleviation of symptom burden.

30.1.2 Classification and Prognostication of MDS (Table 30.1)

The French American British (FAB) classification was introduced in 1982. It divided MDS into five subtypes based on the presence and number of blasts, ring sideroblasts, monocytes, and dysplasia [12]. In 1999, a new classification model has been formulated by WHO with a total of seven subgroups. Subsequently, revisions were made in 2001, 2008, and 2016 [1, 13] with additional consideration of the presence of cytopenia and cytogenetic abnormalities [13].

30.1.3 Prognostic Scoring Systems in MDS

The development of prognostic scoring system (PSS) helps in predicting the overall survival (OS) and risk of leukemic transformation in MDS patients with varying risk groups. This assists in treatment decision in clinical practice (Fig. 30.1). The FAB or WHO classification models can also provide a certain degree of prognostic significance in MDS patients. Nevertheless, it does not suffice as a well-established prognostic model. This has led to the development of several PSSs. Continuous effort and investigations were given to improve the discriminatory power of existing system so as to better differentiate the patients based on a number of clinical, pathologic, and molecular parameters in the hope to predict the outcome and estimate the prognosis of MDS patients (Tables 30.2 and 30.3).

Numerous PSSs have been proposed over the past decades, namely the International PSS (IPSS), Revised IPSS (IPSS-R), WHO classification-based PSS (WPSS), MDA General Risk Model (MDAS), MDA Low-Risk PSS (MDA LR-PSS), and molecular IPSS-R (MIPSS-R) [14] (Tables 30.2 and 30.3). The several prognostic models share common parameters including cytopenia, marrow blast percentage, and cytogenetic evaluation. Owing to the heterogeneity of the disease itself and the clinical course of MDS patients, the management plan is usually personalized to individual patients according to their risk groups.

30.1.4 International Prognostic Scoring System (IPSS)

The International Prognostic Scoring System (IPSS) is first introduced by Greenberg et al. in 1997 and is extensively recognized for the prognostication in de novo MDS or primary MDS patients [15, 16]. The scoring system is constitutive of marrow blast percentage, number of cytopenia, and the cytogenetic subtype [15]. These prognostic markers were determined based on investigation results with selection of parameters that exhibited the most significant impact on AML progression and survival of patients. However, it neglected the severity of cytopenia, the presence of adverse karyotypic abnormalities,

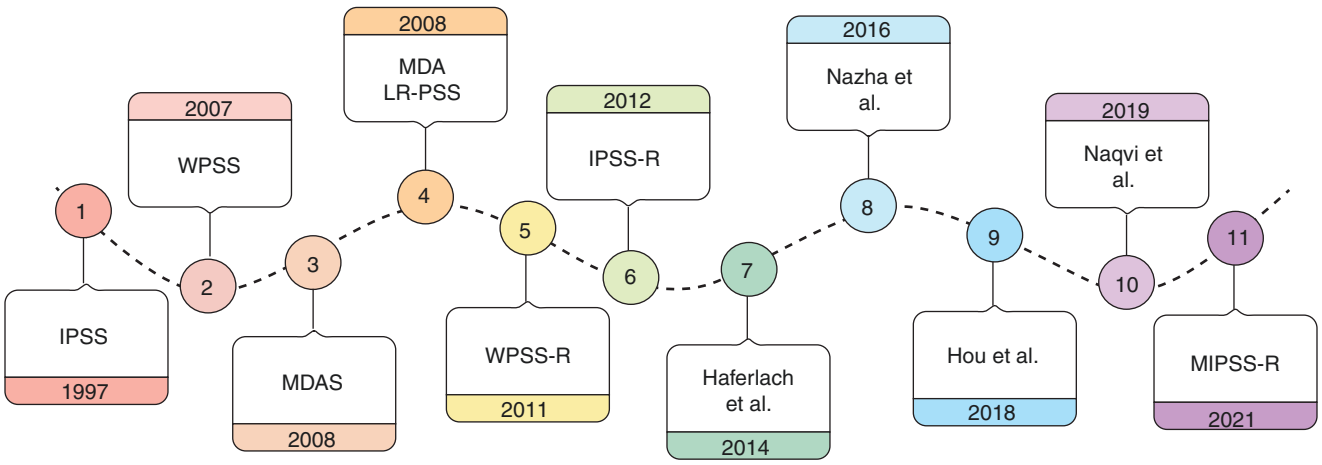

Fig. 30.1 Timeline of the development of different prognostic models in MDS. *IPSS*: International Prognostic Scoring System; *IPSS-R*: Revised IPSS; *MDA LR-PSS*: MD Anderson Low-Risk Prognostic Scoring System; *MDAS*: MD Anderson General Risk Model; *MIPSS-R*: molecular IPSS-R; *WPSS*: WHO Classification-Based Prognostic Scoring System; *WPSS-R*: Revised WPSS

Table 30.2 Prognostic indicators used in different prognostic scoring systems of MDS [29]

Criteria	IPSS (1997)	WPSS (2007, 2011)	MDAS (2008) [25]	MDA LR-PSS (2008) [26]	IPSS-R (2012) [28]	MIPSS-R [30]
Bone marrow blasts						IPSS-R criteria
Blasts	<5%, 5–10%, 11–20%, 21–30%	WHO category (RA/ RARS/5q-), RCMD/ RCMD-RS, RAEB-1, RAEB-2)	5–10%, 11–29%	≥4%	≤2%, >2–<5%, 5–10%, >10–30%	
Cytopenia						
Neutropenia	Number of cytopenia	/	Leukocytosis (>20 × 10^9/L)	/	ANC <0.8 × 10^9/L	
Anemia		Severe anemia (Hb <9 g/dL in men; Hb <8 g/dL in women)	Hb <12 g/dL	Hb <10 g/dL	Hb <8 g/dL	
Thrombocytopenia		/	Plt <30 × 10^9/L, 30–49 × 10^9/L, 50–199 × 10^9/L	Plt <50 × 10^9/L	Plt <50 × 10^9/L	
Cytogenetics						
Cytogenetics	Cytogenetics (three categories, six abnormalities)	Cytogenetics (three categories)	Cytogenetics (four categories)	Unfavorable cytogenetics (non-diploid or non-del(5q))	Cytogenetics (five categories, 16 abnormalities)	
Gene mutation	/	/	/	/	/	Gene mutation (number of gene mutations, presence of SF3B1 mutation)
Other prognostic markers						
Age	/	/	Y (60–64, ≥65)	Age (≥60)	/	/
Performance status	/	/	Y (≥2)	/	/	/
Prior transfusion	/	/	Y	/	/	/
Biochemical parameters	/	/	/	/	Serum ferritin, lactate dehydrogenase, and β2-microglobulin	/
Risk categories	4; low, intermediate—1, intermediate—2, high	5; very low, low, intermediate, high, very high	4; low, intermediate—1, intermediate—2, high	3; category 1, category 2, category 3	5; very low, low, intermediate, high, very high	5; very low, low, intermediate, high, very high

Note: Y, yes

ANC: absolute neutrophil count; *Hb*: hemoglobin; *IPSS*: International Prognostic Scoring System; *IPSS-R*: Revised IPSS; *MDA LR-PSS*: MD Anderson Low-Risk Prognostic Scoring System; *MDAS*: MD Anderson General Risk Model; *MIPSS-R*: molecular IPSS-R; *Plt*: platelet; *RA*: refractory anemia; *RARS*: refractory anemia with ringed sideroblasts; *5q–*: myelodysplastic syndrome with isolated del(5q) and marrow blasts less than 5%; *RCMD*: refractory cytopenia with multilineage dysplasia; *RCMD-RS*: refractory cytopenia with multilineage dysplasia and ringed sideroblasts; *RAEB-1*: refractory anemia with excess of blasts 1; *RAEB-2*: refractory anemia with excess of blasts 2; *SF3B1*: splicing factor 3b subunit 1; *WPSS*: WHO Classification-Based Prognostic Scoring System; *WPSS-R*: Revised WPSS

and excess blasts, which may result in the underestimation of the disease risk and prognosis [17]. It also failed to include patients with therapy-related MDS (t-MDS) or secondary MDS and myeloproliferative variant of chronic myelomonocytic leukemia (CMML-MP) (Tables 30.2 and 30.3).

30.1.5 WHO Classification-Based Prognostic Scoring System (WPSS)

The WHO classification-Based Prognostic Scoring System (WPSS) evolved as a prognostic tool in 2007 for de novo MDS [18]. It included the 2001 WHO classification of

Table 30.3 Application of different prognostic scoring systems [18, 25, 31]

Criteria	IPSS	IPSS-R	WPSS	MDAS [25]	MDA LR-PSS [26]	MIPSS-R
De novo MDS	Y	Y	Y	Y	Similar to that of IPSS	Similar to that of IPSS-R
t-MDS	N	Y	N	Y		
CMML-MD	Y	Y	Y	Y		
CMML-MP	N	N	N	Y		
Time point	Untreated	N	Dynamic (untreated and treated)	Dynamic (untreated and treated)		

NOTE: Y, yes; N, no

CMML: chronic myelomonocytic leukemia; *CMML-MD*: CMML-myelodysplastic variant; *CMML-MP*: CMML-myeloproliferative variant; *IPSS*: International Prognostic Scoring System; *IPSS-R*: Revised IPSS; *MDA LR-PSS*: MD Anderson Low-Risk Prognostic Scoring System; *MDAS*: MD Anderson General Risk Model; *MDS*: myelodysplastic syndrome; *MIPSS-R*: molecular IPSS-R; *t-MDS*: therapy-related MDS; *WPSS*: WHO Classification-Based Prognostic Scoring System; *WPSS-R*: Revised WPSS

MDS as one of the criteria of prognostication. Studies have discovered additional prognostic marker such as transfusion requirement for building better prognostic system. Transfusion dependency is defined by the requirement of ≥1 red blood cell (RBC) transfusion every 8 weeks over the course of 4 months. The WHO criteria and transfusion dependency exhibited high hazard ratio (HR) of 2.1 and 1.8, respectively, and rendered the aforementioned parameters good prognostic indicators for prediction of adverse outcome in patient survival [18, 19]. The result is concordant with another study conducted by Germing, U. et al. in which the median survival for transfusion-independent and transfusion-dependent patients was 97 and 44 months, respectively [20]. This may be attributable to the potential secondary iron overload caused by recurrent red cell transfusion. The toxicity of free iron is detrimental to cells and may ultimately lead to heart failure, liver damage, or even multiple organ failure [21]. In addition, Malcovati, L. et al. also demonstrated transfusion dependency as a negative prognostic indicator in OS and leukemia-free survival (LFS) with a HR of 2.16 and 2.02, respectively [19]. However, the need of RBC transfusion in patients may be dependent on the clinician's subjective judgment. The validity of such prognostic criteria is being questioned [22]. This has led to the amelioration of the model in 2011 to revised WPSS (WPSS-R). It introduced the prognostic parameter "severity of anemia" in place of "transfusion dependency." The definition of severe anemia is determined by the study performed by Malcovati L. et al. with HR of 5.56 in men with hemoglobin <9 g/dL and HR of 5.35 in women with Hb <8 g/dL [23, 24]. Moreover, WPSS also demonstrated superiority over IPSS with its dynamic property in assessing patient prognosis at different time points of the disease (Tables 30.2 and 30.3).

30.1.6 MD Anderson General Risk Model (MDAS)

In 2008, the MD Anderson Cancer Centre (MDACC) proposed a new risk model, MDA General Risk Model (MDAS), with the inclusion of the IPSS system, performance status, age, degree of abnormal cell count in all three cell lineages, marrow blast percentage, karyotypic abnormalities, and presence of prior transfusion as prognostic indicators [25]. Komrokji, R.S. et al. validated the complementarity of MDAS to IPSS with successful recognition of IPSS "low-risk" patients as MDAS "high risk" with poorer outcome. It also exhibited wider coverage of target patients suitable for model with inclusion of the myeloproliferative variant of CMML (CMML-MP) (Tables 30.2 and 30.3).

30.1.7 MD Anderson Low-Risk Prognostic Scoring System (MDA LR-PSS)

Despite the development of various models, existing models still preclude the discrimination of low-risk MDS patients with poor prognosis where early therapeutic interference may potentially be beneficial [26]. Garcia-Manero et al. introduced a Low-Risk Prognostic Scoring System (LR-PSS) refined from IPSS in 2008. This enabled the further differentiation of low-risk MDS into three categories [26, 27]. Arnan Sangerman Montserrat et al. validated its prognostic value in IPSS-R very low/low/intermediate risk MDS [27]. LR-PSS took patient's age into consideration, along with hemoglobin, platelet count, marrow blast percentage, and the presence of unfavorable cytogenetics [26]. Pharmacologic intervention for low-risk MDS (LR-MDS) patients are not of routine practice unless when disease pro-

gresses. Better risk stratification helps minimize unnecessary mortality from low-risk group with adverse prognostic factors (Table 30.3).

30.1.8 Revised International Prognostic Scoring System (IPSS-R)

With the collaborative effort from researches and studies, Greenberg et al. made refinements to the initial IPSS and formulated a revised model, IPSS-R, in 2012 [28]. The IPSS-R encompasses the addition of more thorough discrimination of the categories of marrow blast percentage, karyotypic abnormalities, and depth of cytopenias (Table 30.4). This was accompanied by the amalgamation of several prognostic variables including age, performance status, serum ferritin, lactate dehydrogenase (LDH), and β2-microglobulin prediction of survival [28].

The revised model results in recategorization with upshifting of patients in IPSS lower-risk groups to IPSS-R higher-risk groups. The redistribution demonstrated more accurate differentiation of the IPSS-R model. This greatly minimizes the risk of mis-stratification, which may falsely refrain the patients from receiving prompt therapeutic interventions.

Table 30.4 Revised International Prognostic Scoring System (IPSS) assessment criteria [33]

Criteria	Score
Hemoglobin (g/dL)	
≥10	0
8–<10	+1
<8	+1.5
Absolute neutrophil count (×10^9/L)	
≥0.8	0
<0.8	+0.5
Platelets (×10^9/L)	
≥100	0
50–<100	+0.5
<50	+1
Bone marrow blasts (%)	
≤2	0
>2–<5	+1
5–10	+2
>10	+3
Cytogenetic risk group	
−Y or del(11q)	0 (very good)
Normal karyotype, del(5q), del(12p), del(20q), or double including del(5q)	+1 (good)
+8, +19, del(7q), i(17q), or other single/double independent clone	+2 (intermediate)
−7, inv(3)/t(3q)/del(3q), double including −7/del(7q), or complex (three abnormalities)	+3 (poor)
Complex >3 abnormalities	+4 (very poor)

30.1.9 Prognostic Values of Different Prognostic Scoring System

Despite the emergence of several prognostic models, IPSS-R remains the standard of prognostication in MDS [32]. To analyze the predictive value and discriminatory power of different PSSs, several prognostic indices have been established to determine the accuracy and performance of the prediction model in assessing the overall survival and leukemic transformation of patients. The prognoses of MDS patients with risk stratification as demonstrated by different PSSs are as follows (Tables 30.5, 30.6, 30.7, 30.8, and 30.9).

Several predictive indices used in evaluating the prognostic power of different PSSs include Harrell's c-index (concordance index, CI), Somer's D value, the Dxy value, and the Akaike information criteria (AIC). The CI value varies between 0.5 and 1. The closer it is to 1, the better the discriminatory power of the model. The higher Somers' D and Dxy value or the lower the AIC value, the better the goodness of fit of the prognostic model.

Treatment modalities vary between low-risk MDS (LR-MDS) [36] and HR-MDS patients. A more conservative approach is adopted in the former risk group by observation and symptomatic control, while a more intensive intervention is used in the latter risk group with the use of hypomethylation agents (HMAs) and allo-HSCT. There is no clear dichotomic boundary for the definition between LR- and HR-MDS. According to the OS and leukemic transformation, it is proposed that LR-MDS should include IPSS-R very low/low/intermediate risk categories, while HR-MDS comprises high/very high-risk groups [31]. On that account, it is crucial to accurately stratify patients into their respective risk groups to offer the best appropriate management.

In LR-MDS, Zeidan, A.M. et al. showed a superior discriminatory ability of LR-PSS in risk stratification of low-risk MDS with higher CI and lower AIC values than IPSS-R and IPSS (CI: 0.74 vs 0.64, 0.64; AIC: 8110 vs 8147, 8150, respectively). They still fail to recognize a significant subgroup of patients with poor OS [37], albeit the better predictive ability of IPSS-R than IPSS, MDAS, WPSS, and revised WPSS [33].

Table 30.5 IPSS risk category and prognosis [15]

Risk category	IPSS score	Median OS (year)	AML/25% (year)
Low	0	5.7	9.4
Intermediate 1	0.5–1	3.5	3.3
Intermediate 2	1.5–2	1.2	1.1
High	≥2.5	0.4	0.2

AML: acute myeloid leukemia; *AML/25%*: time for 25% of patients to undergo leukemic transformation; *IPSS*: International Prognostic Scoring System; *OS*: overall survival

Table 30.6 WPSS risk category and prognosis [18]

Risk category	WPSS Score	Median OS (months)	AML progression at 2 years (%)	AML progression at 5 years (%)
Very low	0	141	3	3
Low	1	66	6	14
Intermediate	2	48	21	33
High	3–4	26	38	54
Very high	5–6	9	80	84

AML: acute myeloid leukemia; *OS*: overall survival; *WPSS*: WHO Classification-Based Prognostic Scoring System

Table 30.7 MDA LR-PSS risk category and prognosis

Risk category	MDA LR-PSS score	Median OS [26, 34]	Median OS (year) [27]	4-year survival (%) [26]	Rate of AML progression (%) [27]
Category 1	0–2	80.3	7.1	65	10
Category 2	3–4	26.6	5.7	33	15
Category 3	5–7	14.2	2.8	7	27

AML: acute myeloid leukemia; *MDA LR-PSS*: MD Anderson Low-Risk Prognostic Scoring System; *OS*: overall survival

Table 30.8 MDAS risk category and prognosis

Risk category	MDA Risk Model score	Median survival (months) [25]	Median survival (months) [35]	3-year survival (%) [25]	Rate of AML progression (%) [35]
Low	0–4	54	61	63	5.6
Intermediate 1	5–6	25	28	34	15.4
Intermediate 2	7–8	14	15	16	38
High	≥9	6	8	4	49.6

AML: acute myeloid leukemia; *MDAS*: MDA Risk Model

Table 30.9 IPSS-R risk category and prognosis [28]

Risk category	IPSS-R Score	Median (year)	AML/25% (year)	Mortality (%)
Very low	≤1.5	8.8	NR	27
Low	>1.5–3	5.3	10.8	40
Intermediate	>3–4.5	3.0	3.2	55
High	>4.5–6	1.6	1.4	71
Very High	>6	0.8	0.73	86

AML/25%: time for 25% of patients to undergo leukemic transformation; *IPSS-R*: Revised International Prognostic Scoring System; *NR*: not reached

In general, IPSS-R stands out as the best discriminatory model for prognostication with more effective differentiation of risk groups. Lorand-Metze I et al. showed a higher Somer's D value in IPSS-R than IPSS in OS and AML transformation (0.41 vs 0.39 and 0.55 vs 0.53, respectively) [33]. Greenberg et al. have shown a higher Dxy value in IPSS-R than IPSS (OS: 0.43 vs 0.37; leukemic progression: 0.52 vs 0.48) [28]. De Swart L et al. demonstrated higher CI values in IPSS-R than IPSS in OS and disease progression (OS: 0.632 vs 0.583; disease progression: 0.724 vs 0.636) [38]. In addition, D. Moreno Berggren et al. also concluded with similar findings with higher CI in OS (0.74 vs 0.71). Besides, the age-adjusted version of IPSS-R (IPSS-RA) displayed superior predictive power in OS than IPSS-R but showed otherwise in disease progression (OS: CI of 0.655 vs 0.632; disease progression: CI of 0.689 vs 0.724, respectively) [38]. Hence, age adjustment will only be applicable in prediction of survival. On the other hand, IPSS-R only showed slight advantage over WPSS in OS prediction (CI: 0.74 vs 0.73) [39]. The AIC value also displayed superiority of IPSS-R (2343) over other prognostic models (WPSS: 2361, IPSS: 2364) [40]. Neukirchen J et al. demonstrated higher Dxy value in IPSS-R (OS: 0.391 in IPSS-R vs 0.335 and 0.320 in WPSS and IPSS, respectively; AML progression: 0.435 in IPSS-R vs 0.410 and 0.343 in IPSS and WPSS, respectively), while Pfeilstöcker, M. et al. also reproduced the same results with Dxy values in OS (0.43 in IPSS-R vs 0.41 and 0.37 in WPSS-R and IPSS, respectively) [36, 41].

30.1.10 Limitations of Current Prognostic Scoring System

Despite the extensive research in refining existing PSSs and determining the discriminatory power of PSSs, time-dependent attenuations of predictive potential and hazards of prognostic systems are exemplified by the study conducted by Pfeilstöcker M. et al. It is essential to establish systems with better stability. It is observed that age adjustment of the PSS enhanced the prognostic power and stability in survival prediction [36]. Prognosis of MDS patients also exhibits variance in populations with differing races and ethnicities [42]. Nonetheless, their impacts on MDS are still poorly defined, which require more evidence and investigations.

Despite the presence of well-established risk stratification model, the clinical courses of MDS patients of the same risk category still exhibit a variable degree of diversity. Karyotypic abnormalities alone may not be adequate in view of the presence of a normal cytogenetic profile in about half of the MDS patients [2, 43]. The prognosis of these patients may not be appropriately assessed in existing models. Thus, additional genetic profile of the patients should also be obtained to better delineate patient's risk and prognosis and address the limitations of existing systems. This has led to the formulation of various proposals with incorporation of mutational genes.

30.1.11 The Molecular Genetics of MDS

The advent of high-throughput sequencing, also known as the next-generation sequencing [13] has led to more in-depth molecular understanding on disease pathogenesis and impact [13]. Somatic mutation is a common feature in MDS. Recent studies and investigations allow detection of genetic aberrations in approximately 90% of MDS patients with the presence of at least one mutation [30, 44–46]. Patients with MDS carry a median of nine somatic mutations (with driver and non-driver mutations included) [47]. The number of mutation varies with the subtypes of MDS and also increases with disease progression [46–48]. More than 30 mutations have been discerned in the pathogenesis of MDS. The genomic instability and heterogeneity contribute to the complexity of the disease [49].

Mutations occur in genes encoding for splicing factors (e.g., SF3B1, SRSF2 U2AF1, and ZRSR2), epigenetic regulators (e.g., TET2, ASXL1, DNMT3A, EZH2, and IDH1/2), cohesion complex (e.g., STAG2), transcription factors (e.g., GATA2 and RUNX1), and genes involved in signal transduction (e.g., TP53 and RAS), etc. [50–52]. Genetic aberrations in splicing factors account for the most frequently encountered mutations in MDS [51]. Different mutations have discrete prognostic impacts on the survival and risk of leukemic transformation in MDS patients [53]. A great number of studies have been done to evaluate the hazard ratios (HRs) of the commonly mutated genes in MDS (Table 30.10). Some act as independent prognostic gene, and some exhibit combinatorial prognostic impact in patients. SF3B1 is considered a favorable prognostic marker [10, 54], whereas IDH1/2 and DNMT3A are negative prognostic genes that confer dismal prognoses [55]. PRPF8 mutation, although uncommon, is associated with a more aggressive clinical course of disease in MDS [56]. In addition, SRSF2 and U2AF1 are also markers of poor prognosis. The former is observed in patients with pronounced cytopenia, dysplasia, and excess blasts and

Table 30.10 Hazard ratios of different prognostic genes in predicting overall survival (OS) and leukemia-free survival (LFS) in MDS [45, 53]

Function/pathway	Genes	OS (HR)	LFS (HR)	Reference(s)
Signal transduction	RAS	2.76	7.04	[61]
Cohesin complex	STAG2	2.45	/	[34]
DNA methylation	TET2	1.13	/	[62]
	DNMT3A	1.654	4.624	[55]
Histone/chromatin modification	IDH1/2	1.62	2.21	[63]
	IDH1	2.21	2.65	
	ASXL1	1.45	2.20	[64]
	EZH2	2.47	/	[65]
	SETBP1	1.808	/	[66]
RNA splicing	SRSF2	1.78	1.89	[67]
	ZRSR2	/	1.48	[68]
	SF3B1	0.58	0.63	[68]
	U2AF1	1.60	No difference	[69]
Transcription factor	TP53	3.12/2.67	/	[34, 58]
	RUNX1	1.43	1.88	[70]

ASXL1: additional sex combs like 1; *DNMT3A*: DNA methyltransferase 3A; *EZH2*: enhancer Of zeste 2; *HR*: hazard ratio; *IDH*: isocitrate dehydrogenase; *MDS*: myelodysplastic syndrome; *RAS*: rat sarcoma; *RUNX1*: Runt-related transcription factor 1; *SETBP1*: SET binding protein 1; *SF3B1*: splicing factor 3b subunit 1; *SRSF2*: serine and arginine-rich splicing factor 2; *STAG2*: stromal antigen 2; *TET2*: Tet methylcytosine dioxygenase 2; *TP53*: tumor protein 53; *U2AF1*: U2 small nuclear RNA auxiliary factor 1; *ZRSR2*: zinc finger (CCCH-type), RNA binding, motif and serine/arginine-rich 2

associated with high risk of leukemic transformation [46]. On the other hand, the latter exhibited high RUNX1 co-mutation risk and subsequent risk of AML evolution [57]. Besides, the presence of specific mutations may also affect the morphologic presentation and treatment response. For instance, TP53, RUNX1, and NRAS mutation are associated with higher marrow blast percentage, whereas the presence of TET2 mutation predicts potential response to DNA methyltransferase (DNMT) inhibitors [51, 58]. SF3B1 on the other hand may also predict response to luspatercept, a recently Food and Drug Administration (FDA)-approved agent for treating LR-MDS with transfusion-dependent anemia [59, 60]. Furthermore, Montalban-Bravo G et al. also demonstrated the association of the number of mutations with response to therapy. The presence of specific mutation may contribute to variations in response duration [34].

30.1.12 New Scoring System with Molecular Integration

In 2021, S. Gu et al. constructed a novel prognostic system with integration of molecular profile named molecular IPSS-R (S. Gu et al. MIPSS-R) (Table 30.11). A higher area under curve (AUC) of the receiver operating characteristic curve is observed in MIPSS-R (0.790) than IPSS-R (0.731) [30].

In 2018, Montalban-Bravo et al. have also established similar prognostic model with addition of molecular basis to IPSS-R (Montalban-Bravo et al. MIPSS-R) (Table 30.12).

Table 30.11 Gu et al. MIPSS-R [30]

Risk category	S. Gu et al. MIPSS-R score	Median OS (months) quintile	Median OS (months) Validation cohort
Very low	1.28–2.24	>60	75.1
Low	2.33–3.93	>60	34.5
Intermediate	4.02–4.34	44.41	24.2
High	4.57–5.30	11.68	24.2
Very high	5.62–8.59	5.92	16.3
MIPSS-R score = mutation score × 1.047 + IPSS-R × 0.64			

IPSS-R: Revised International Prognostic Scoring System; *MIPSS-R*: Molecular IPSS-R; *OS*: overall survival

Table 30.12 Montalban-Bravo et al. MIPSS-R [34]

Risk category	Montalban-Bravo et al. MIPSS-R score	Median OS (months)	Median OS (months) Validation cohort
Low	0–0.5	NR	NR
Intermediate	1–2	29	54
High	2.5–3.5	12	13
Score: IPSS-R intermediate risk +0.5, high/very high risk +1.5, TP53 mutation +1, ≥3 mutations +1			

IPSS-R: Revised International Prognostic Scoring System; *MIPSS-R*: Molecular IPSS-R; *NR*: not reached; *OS*: overall survival; *TP53*: tumor protein 53

The prognostic ability of the Montalban-Bravo et al. MIPSS-R is validated and demonstrated a higher discriminatory potential than IPSS-R with a higher Dxy of 0.46 (vs 0.43) [34].

Apart from the aforementioned new models, there are a number of studies conducted over the past few years in the hope to optimize existing prognostic models with integration of molecular markers [30, 71]. Nazha et al. utilized only three mutations (EZH2, SF3B1, and TP53) in generating their model [72]. Hou et al. developed a novel prognostic risk model which incorporated five mutation genes namely ASXL1, CBL, DNMT3A, IDH2, and TP53 [71]. On the other hand, Haferlach et al. incorporated 14 prognostic genes along with age, gender, and IPSS-R in producing their prognostic tools [45]. Naqvi et al. have also created a novel prognostic system with C-index of 0.822 with the incorporation of TP53 mutation status, IPSS-R, and Adult Comorbidity Evaluation-27 (ACE-27) [44].

The advancement of molecular techniques provides genetic insights into the development of novel prognostic systems in recent years. Nonetheless, continuous inputs from studies and investigations are required for more detailed exploration of existing and additional prognostic markers to formulate a comprehensive PSS for more accurate risk stratification of patients. This enables better treatment planning without delay and ultimately improves the survival of patients.

30.2 Chronic Myelomonocytic Leukemia (CMML)

30.2.1 Introduction

Chronic myelomonocytic leukemia (CMML) is a rare, clonal hematopoietic stem cell disorder characterized by clinical and genetic heterogeneity with features of both MDS and myeloproliferative neoplasm (MPN) [73]. It was originally classified under MDS by the FAB classification in 1982 (Table 30.1). In 1994, CMML was differentiated under FAB classification into two subtypes, namely the dysplastic type (CMML-MD, $<13 \times 10^9$/L) and the proliferative type (CMML-MP, $\geq 13 \times 10^9$/L) [74, 75]. Until 2001, the WHO re-classified it under a new entity of MDS/MPN overlap syndromes [76]. The new entity encompasses CMML, atypical chronic myeloid leukemia (CML), juvenile myelomonocytic leukemia (JMML), MDS/MPN with ring sideroblasts, and thrombocytosis (MDS/MPN-RS-T) and MDS/MPN unclassifiable (MDS/MPN-U). CMML accounts for the most common subtype [60]. Evidence provided by E. Schuler et al. in 2014 allowed more precise prognostic differentiation of CMML in survival [77]. Thereafter, in the revised 2016 WHO classification, CMML has been further categorized

into three subtypes based on the amount of blasts in peripheral blood (PB) and bone marrow (BM) (Table 30.13).

The diagnosis of CMML is made by the presence of persistent absolute monocytosis (≥1 × 10^9/L), relative monocytosis with ≥10% of white blood cells (WBCs) in PB and dysplastic features in BM. Blasts in PB and BM should not exceed 20%. In case of eosinophilia, presence of PDGFRA/B, or PCM1-JAK2 and FGFR1 rearrangement should be excluded. When all other causes of monocytosis are excluded, without meeting the WHO criteria for Philadelphia chromosome-positive CML, polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF), CMML can then be confirmed [79]. Patients with CMML have a male predominance (2.7:1) similar to that of MDS with an incidence of 0.35 in 100,000 individuals [78, 80, 81]. It remains the most prevalent entity among MDS/MPNs [82]. The median age of diagnosis is around 71–73 [83]. It exhibits clinical diversity with inherent propensity of leukemic progression of 15% over the course of 3–5 years [83].

Table 30.13 2016 WHO Classification of CMML [78]

Subgroup of CMML	Criteria	Median survival (months) [77]
CMML-0	Blasts in PB: <2% and Blasts in BM: <5%	31
CMML-1	Blasts in PB: 2–4% and/or Blasts in BM: 5–9%	19
CMML-2	Blasts in PB: 5–19%, Blasts in BM: 10–19% and/or presence of Auer rods	13

BM: bone marrow; *CMML*: chronic myelomonocytic leukemia; *PB*: peripheral blood

30.2.2 Prognostic Scoring Systems in CMML

Prognostic assessment is a major challenge in CMML [84]. The prognostic models used for CMML in early days were equivalent to those used for prognostication in MDS (IPSS, IPSS-R, and MDAS) (Fig. 30.2). However, the introduction of IPSS in 1997 was primarily built on a population of MDS patients with inclusion of the dysplastic variant of CMML (CMML-MD) only [85]. It does not embody the capacity to produce a well-fined risk stratification in CMML patients. In addition, the lack of exploitation of the criteria renders the model unsuited for prognostication in CMML [84]. Together with the WHO re-classification of CMML as a novel entity in 2001, several CMML-specific prognostic models have been designed to enhance prognostic prediction in survival of CMML patients. In 2002, Onida et al. have introduced the MD Anderson Prognostic Scoring System (MDAPS) model for CMML. The model was later revised by Beran M. et al. to MDAPS-M1 in 2007 [86, 87] with substitution of marrow blasts with LDH level as independent prognostic factor owing to the higher-risk ratio (RR) of LDH (1.64 vs 1.46) [86]. Despite the modification, there was almost no variation in survival exhibited between the two models (Table 30.14). In 2008, Kantarjian et al. proposed the MDAS model for MDS with inclusion of the myeloproliferative variant of CMML (CMML-MP) [25]. In 2012, IPSS was revised to

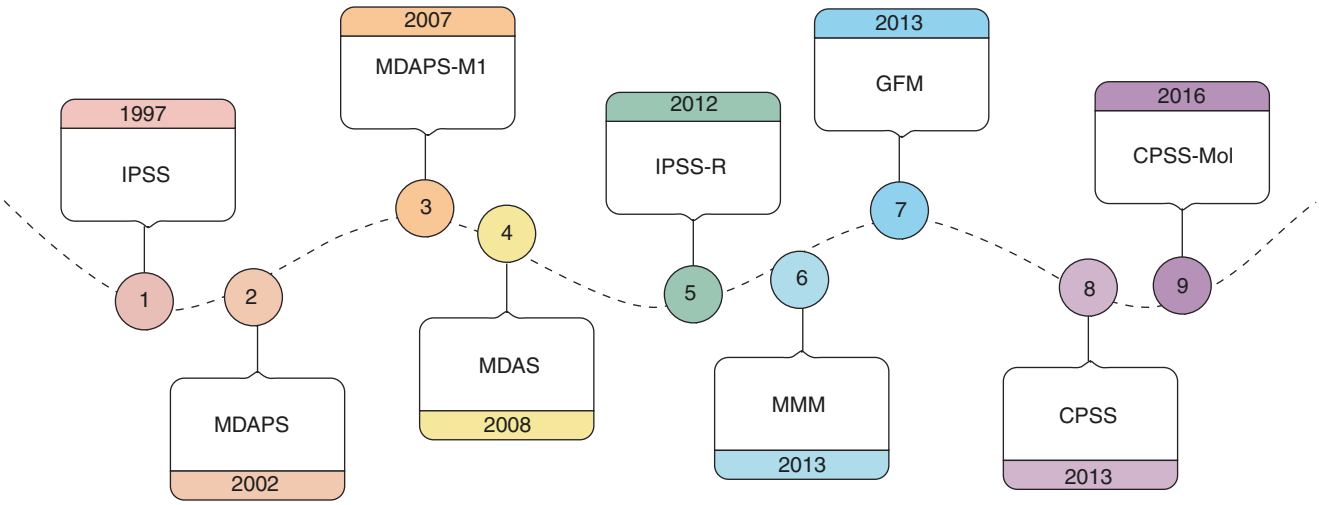

Fig. 30.2 Timeline of the development of different prognostic models in CMML. *CMML*: chronic myelomonocytic leukemia; *CPSS, CPSS*: CMML-Specific Prognostic Scoring System; *CPSS-Mol*: Molecular CPSS; *GFM*: GroupeFrançais des Myélodysplasies; *IPSS*: International Prognostic Scoring System; *IPSS-R*: Revised IPSS; *MDAPS*: MD Anderson Prognostic Scoring System; *MDAS*: MDA General Risk Model; *MMM*: Mayo Molecular Model

Table 30.14 Risk category and prognosis of MDAPS and MDAPS-M1 in CMML (2002, 2007)

MDAPS risk category	Score	Median survival (MDAPS) (months)	Median survival (MDAPS M1) (months)
Low	1	26.3	26.3
Intermediate 1	2	15.7	15.7
Intermediate 2	3	9.8	9.8
High	4	5.9	4.9

CMML: chronic myelomonocytic leukemia; *MDAPS*: MD Anderson Prognostic Scoring System

Table 30.15 Risk category and prognosis of MDAS in MDS and CMML (2008) [25]

MDAS risk category	Score	25% survival (months)	Median survival in CMML-MP [25]
Low	0–4	51	33
Intermediate 1	5–6	31	19
Intermediate 2	7–8	18	12
High	≥9	8	8

CMML: chronic myelomonocytic leukemia; *CMML-MP*: myeloproliferative variant of CMML; *MDAS*: MDA General Risk Model; *MDS*; myelodysplastic syndrome

Table 30.16 Risk category and prognosis of CPSS in CMML (2013) [75]

Risk category	Score	Median (months)	AML/25%[a] (months)	Mortality[a] (%)
Low	0	72	95	45
Intermediate 1	1	31	40	75
Intermediate 2	2–3	13	11	90
High	4–5	5	4	100

AML: acute myeloid leukemia; *AML/25%*: time for 25% of patients to undergo leukemic transformation; *CMML*: chronic myelomonocytic leukemia; *CPSS*: CMML-Specific Prognostic Scoring System
[a]At 5 years

IPSS-R. Nonetheless, its prognostic power was not improved with inferior predictive ability compared to the novel model, CMML-specific prognostic scoring system (CPSS), developed by E. Such et al. in 2013 [75]. Despite the development of various prognostic models specific to CMML (MDAPS and CPSS), no consensus has yet been reached for a universally recognized risk stratification for prognostication of CMML (Tables 30.14, 30.15, and 30.16). Table 30.14 summarizes the prognostic criteria for several CMML prognostic models.

30.2.3 Incorporation of Gene Mutations into Prognostic Models in CMML

Cytogenetic aberrations are detected in over 30% of CMML patients [88], whereas gene mutation is observed in 90% of the patients [89]. Alteration in epigenetic regulators accounts for the most common type of genetic mutations in more than 60% of CMML patients [24]. The genes most frequently mutated are TET2 followed by ASXL1, DNMT3A, and EZH2 [12, 24]. Contrary to the genetic profile in MDS, splicing factor genes including U2AF1 and SF3B1 are less common [79]. The identification of ASXL1, RUNX1, NRAS, and SETBP1 as independent prognostic markers formed the basis of the development of a new model, molecular CPSS (CPSS-Mol), from the existing CPSS by Elena et al. in 2016 [90] (Tables 30.17, 30.18, 30.19, and 30.20).

Table 30.17 Risk category and prognosis of CPSS-Mol [83]

CPSS-Mol risk category	Risk factor(s)	Median OS (months)	Rate of AML progression (%)
Low	0	NR	0
Intermediate 1	1	64	3
Intermediate 2	2–3	37	21
High	≥4	18	48

AML: acute myeloid leukemia; *CMML*: chronic myelomonocytic leukemia; *CPSS*: CMML-Specific Prognostic Scoring System; *CPSS-Mol*: molecular CPSS; *NR*: not reached; *OS*: overall survival

Various prognostic tools have been developed over the past years before the emergence of CPSS-Mol. In 2013, Patnaik et al. proposed the Mayo molecular model (MMM), while Itzykson et al. introduced the GroupeFrançais des Myélodysplasies (GFM). Both included the assessment of ASXL1 mutational status (Tables 30.21, 30.22 and 30.23).

Table 30.18 Prognostic value of gene mutations in CMML [91]

Gene	OS (HR)
RUNX1	2.32
NRAS	2.19
SETBP1	2.00
ASXL1	1.77
CPSS cytogenetic risk groups	1.54

ASXL1: additional sex combs like 1; *CMML*: chronic myelomonocytic leukemia; *CPSS*: CMML-specific prognostic scoring system; *HR*: hazard ratio; *NRAS*: neuroblastoma RAS viral oncogene homolog; *OS*: overall survival**;** *RUNX1*: Runt-related transcription factor 1; *SETBP1*: SET binding protein 1

Table 30.19 CPSS-Mol genetic risk score

Score	CPSS genetic risk	ASXL1	NRAS	RUNX1	SETBP1
0	Low Normal, isolated—Y	Not mutated	Not mutated	Not mutated	Not mutated
1	Intermediate Other abnormalities	Mutated	Mutated	/	Mutated
2	High Trisomy 8 (+8), complex karyotype (≥3), Abnormalities of chromosome 7	/	/	Mutated	/

ASXL1: additional sex combs like 1; *CMML*: chronic myelomonocytic leukemia; *CPSS*: CMML-specific prognostic scoring system; *CPSS-Mol*: molecular CPSS; *NRAS*: neuroblastoma RAS viral oncogene homolog; *RUNX1*: Runt-related transcription factor 1; *SETBP1*: SET binding protein 1

Table 30.20 CPSS-Mol genetic risk group [91]

Genetic risk group	Score
Low	0
Intermediate 1	1
Intermediate 2	2
High	≥3

CMML: chronic myelomonocytic leukemia; *CPSS*: CMML-specific prognostic scoring system; *CPSS-Mol*: molecular CPSS

Table 30.21 Risk category and prognosis of MMM [83, 92]

MMM risk category	Risk factor(s) (Table 30.14)	Hazard ratio (HR)	Median survival (months)
Low	0	/	97
Intermediate 1	1	1.9	59
Intermediate-2	2	3.4	31
High	≥3	6.2	16

MMM: Mayo molecular model

Table 30.22 Risk category and prognosis of GFM [93]

GFM risk category	Score	Median OS (months)	Median LFS (months)
Low	0–4	NR	56.0
Intermediate	5–7	38.5	27.4
High	8–12	14.4	9.2

GFM: GroupeFrançais des Myélodysplasies; *LFS*: leukemia-free survival; *OS*: overall survival

Table 30.23 Criteria in each prognostic model of CMML

CPSS	MDAPS (2002) [87]	MDAPS-M1	MMM (2013)	GFM (2013) [93]	CPSS-Mol
WHO subtypes CMML-1 vs CMML-2	ALC >2.5 × 10^9/L	ALC >2.5 × 10^9/L	AMC >10 × 10^9/L	WBC >15 × 10^9/L (+3)	WBC ≥13 × 10^9/L (+1)
FAB subtypes CMML-MD vs CMML-MP	Hb <12 g/dL	Hb <12 g/dL	Hb <10 g/dL	Hb <10 g/dL in women (+2) Hb <11 g/dL in men (+2)	Transfusion dependency (+1)
CMML cytogenetic risk group	BM blasts ≥10%	LDH >700 U/L	Plt <100 × 10^9/L	Plt <100 × 10^9/L (+2)	BM blasts ≥5% (+1)
RBC transfusion dependency	Circulating IMC >0%	Circulating IMC >0%	Circulating IMC	Age > 65 (+2)	Intermediate 1 genetic score (+1)
			ASXL1 mutation	ASXL1 mutational status (+2)	Intermediate 2 genetic score (+2)
					High-risk genetic score (+3)

AMC: absolute monocyte count; *ALC*: absolute lymphocyte count; *ASXL1*: additional sex combs like 1; *BM*: bone marrow; *CMML*: chronic monomyelocytic leukemia; *CMML-MD*: CMML-dysplastic variant; *CMML-MP*: CMML-proliferative variant; *CPSS, CPSS*: CMML-specific prognostic scoring system; *CPSS-Mol*: molecular CPSS; *FAB*: French American British; *GFM*: GroupeFrançais des Myélodysplasies; *Hb*: hemoglobin; *IMC*: immature myeloid cells; *LDH*: lactate dehydrogenase; *MDAPS*: MD Anderson Prognostic Scoring System; *MDAS*: MDA General Risk Model; *MMM*: Mayo molecular model; *Plt*: platelet; *RBC*: red blood cell; *WBC*: white blood cell; *WHO*: World Health Organization

30.2.4 Predictive Ability of Different Prognostic Models in CMML

To discriminate between the prognostic values of different risk stratification models, several investigations have been undertaken. In a study, E. Such et al. demonstrated a higher CI value of CPSS than IPSS-R (0.69 vs 0.60 in OS; 0.68 vs 0.64 in risk of AML evolution) [94]. Conversely, Padron et al. showed otherwise with marginally higher CI in predicting OS and LFS in IPSS-R in comparison with that of CPSS. (OS: 0.640 vs 0.639; LFS: 0.647 vs 0.640) [95]. It was also shown that CPSS in general has a higher predictive potential than most proposed models including MDAS, MMM, IPSS, and MDAPS (CI in OS: 0.639 vs 0.636, 0.618, 0.611, and 0.597, respectively) in the prediction of OS. It displayed better prognostic ability than IPSS, MMM, and MDAPS (CI in leukemia-free survival (LFS): 0.640 vs 0.620, 0.615, and 0.604, respectively), while exhibiting slight inferiority in LFS prediction to MDAS (CI in LFS: 0.640 vs 0.641). Besides, the discriminatory powers were also differentiated among models with molecular integration. CPSS-Mol outperformed other prognostic models including MMM and GFM with AIC values of 630, 832, and 880, respectively, and CI values of 0.73, 0.66, and 0.62, with MMM being superior to GFM [91].

30.2.5 Impact of Risk Stratification on Treatment Decision

Risk stratification of patients provides guidance to treatment and clinical trials. Treatment decision is developed according to patient fitness, the predominant symptoms present, either as cytopenic or proliferative, and the risk categories identified. Therefore, the management of CMML is often tailor-made to address patients' needs individually [96].

In general, the mainstay of management in lower-risk CMML patients is supportive care and observation, whereas higher-risk patients are potential candidates for the use of HMAs with azacitidine, decitabine, or HSCT [97]. Supportive treatment includes control of symptom burden with correction of anemia with erythropoiesis-stimulating agents (ESAs), blood transfusion, iron chelation, or cytoreduction with hydroxyurea (HU) in CMML-MP. Allo-HSCT serves as the only curative treatment in CMML. Yet, not all high-risk candidates are considered fit for transplantation [60]. The only FDA-approved drug in the management of CMML is HMAs. However, the current pharmaceutical agents have minimal disease-modifying effects [79], posing a major treatment challenge. This prompts further exploration on the pathogenesis of the disease for recognition of potential novel therapeutic targets.

Apart from providing basis for treatment planning, cytogenetic and molecular genetic profiles may assist the prediction of treatment response. For instance, Duchmann et al. have shown that ASXL1 mutation is associated with lower overall response rates in patients treated with HMAs [98]. It was also demonstrated that TET2 mutation in the absence of ASXL1 mutation is a positive prognostic marker for higher rate of complete remission [98, 99]. The presence of t(5;12) demonstrated by Germing et al. may indicate treatment response to imatinib, a tyrosine kinase inhibitor [84] in CMML.

30.3 Conclusion

All in all, the collective efforts of many studies and investigations on the molecular landscape of disease contribute to the huge progress in the development of prognostic systems in both MDS and CMML. However, the existing prognostic systems still lack comprehensive coverage of the prognostic factors involved in disease pathogenesis. Molecular translation into clinical practices is yet to be fully accomplished. Continuous investigations are essential to improve the models and better differentiate patients into different risk groups for treatment guidance in the future.

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Treatment Algorithm of Myelodysplastic Syndromes

31

Anne Sophie Kubasch and Uwe Platzbecker

Abstract

Myelodysplastic syndromes (MDSs) are a diverse group of myeloid neoplasms that result in ineffective hematopoiesis, various degrees of bone marrow dysplasia, peripheral cytopenias, and an increased risk of progressing to acute myeloid leukemia (AML). MDS is driven by structural chromosomal changes and somatic mutations in neoplastic myeloid cells, which are supported by an inflammatory bone marrow microenvironment.

Higher-risk MDS (HR-MDS) patients are given more invasive treatments to alter the course of the disease and prevent disease progression, while supportive care such as regular red blood cell transfusions or erythropoiesis-stimulating agent (ESA) is the main strategy to enhance the quality of life and anemia symptoms for lower-risk MDS (LR-MDS) patients. However, existing MDS treatments are not curative, and many patients experience relapse or resistance to first-line treatment. Apart from participating in a clinical trial, there are typically no additional treatment options available. Therefore, there is an unmet need for new, more effective, and tolerable MDS management strategies.

Keywords

Treatment · MDS · ESA · Luspatercept · HMA

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31.1 Introduction

Anemia and anemia-related symptoms are prevalent among patients with MDS, and they can have a detrimental impact on health-related quality of life (QoL) [1–3]. Am observational study of patients with lower-risk MDS identified that hemoglobin levels were the most crucial independent predictor of health-related QoL, and they were associated with fatigue levels [3].

The treatment goals for patients with MDS are divided into two main directions: altering the natural course of the disease to HR-MDS or AML and improving peripheral blood values (such as increasing hemoglobin levels and decreasing bleeding and infection rates) [3, 4]. The treatment decision for newly diagnosed and relapsed/refractory MDS patients depends on several factors, including risk classification, comorbidities, individual treatment goals and preferences, social support, and eligibility for hematopoietic stem cell transplantation (according to HCT-CI) [5]. IPSS low or INT-1 risk patients are classified as lower-risk MDS patients, as well as those with IPSS-R very low, low, or intermediate classification up to 3.5 points [5, 6]. IPSS INT-2 or high- and IPSS-R intermediate (>3.5 points), high, or very high-risk patients are classified as higher-risk MDS patients [5, 6].

Intermediate-risk IPSS-R patients represent a group with widely variable disease courses and highly divergent clinical outcomes [6, 7]. Factors associated with adverse survival like age over 66 years, peripheral blood blasts over 2%, and a history of RBC transfusion are additional stratification factors that enable the classification of MDS IPSS-R intermediate-risk patients into two prognostic subgroups (int-favorable vs int-adverse) with significantly different outcomes [5–7].

The primary treatment goals for LR-MDS patients are to improve hematologic function to prevent complications (such as bleeding and severe infections), reduce the burden of transfusion, and enhance QoL [4, 5]. In contrast for patients with HR-MDS, the main treatment priorities are delaying disease progression, improving overall survival,

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and considering HCT as a potential cure of MDS if the patient is eligible [5, 6]. Regardless of individual risk, all patients should undergo regular clinical monitoring including supportive care, psychosocial assistance, and close QoL monitoring [5, 6, 8]. Supportive care comprises administering RBC transfusions to alleviate symptomatic anemia, iron chelation to manage iron overload, antibiotics to treat bacterial infections, and platelet transfusions to address bleeding events [4, 5].

31.2 Summary of Current Available Treatment Options

31.2.1 Treatment Options for Patients with Lower-Risk MDS

31.2.1.1 Erythropoiesis-Stimulating and Maturing Agents (ESAs and EMAs)

Currently, erythropoiesis-stimulating agents (ESAs) such as recombinant erythropoietin (EPO) and glycosylated forms such as darbepoetin are generally considered the primary treatment for anemia in LR-MDS [9–11]. Within 3 months of initiating therapy, approximately 30–60% of patients experience an erythroid response, which is defined as a 1.5 g/dL increase in hemoglobin levels in transfusion-independent patients or a significant reduction or elimination of transfusion requirement in transfusion-dependent patients [9, 10, 12]. The EPOANE trial, which resulted in the licensing of EPO alfa (in the EU) for the treatment of lower-risk MDS with anemia, demonstrated that erythroid hematologic improvement (HI-E, IWG 2006 [13]) was achieved in 32% of patients treated with EPO compared to 4% of patients who received a placebo [11].

Between week 16 and week 24, the percentage of patients who were dependent on transfusions decreased from 54% to 25% [9–11]. However, primary resistance to erythropoiesis-stimulating agents (ESAs) is common, and even among responders, relapse occurs in 70% of cases, likely due to the loss of sensitivity of erythroid progenitors to ESA. Previous studies have shown that the median response duration to ESA treatment is 18 to 24 months, and while response was associated with improved survival, it did not have an impact on the progression of acute myeloid leukemia (AML) [10, 14]. The wide variation in clinical response rates and duration can be attributed to several biological and clinical factors that allow for the selection of patients with the highest likelihood of successful treatment. Patients with low baseline endogenous EPO levels (≤200 U/L), low (≤2 RBC units per month) or no need for RBC transfusions, normal cytogenetics, marrow blasts <5%, and only a few (≤2) somatic mutations tend to have a better response to ESAs [14, 15].

The "Nordic Score" [14], a predictive tool for response to erythropoiesis-stimulating agent (ESA) therapy, indicates that patients with low pre-treatment endogenous EPO levels (<500 IU/L) and a transfusion burden of less than four units within an 8-week period have a 74% probability of responding to treatment [9]. In addition to low endogenous EPO levels and a low transfusion burden, a low International Prognostic Scoring System-Revised (IPSS-R) score has also been identified as a predictive factor for response to ESAs. According to a previous study that examined the effect of IPSS-R on response rates, the erythroid response rates for the very low, low, intermediate, and high-risk groups were 85%, 68%, 48%, and 31%, respectively [16]. Additionally, the addition of granulocyte-colony stimulating factor (G-CSF) may increase response rates in 20–30% of cases for patients who did not respond or lost response to single-agent ESA treatment [4].

Luspatercept (ACE-536), an erythroid maturation agent (EMA), is a promising new treatment for patients with lower-risk MDS and RS/SF3B1^{mut} who require RBC transfusions but are refractory, intolerant, or unlikely to respond to ESAs [17, 18]. Luspatercept is composed of specific activin receptor fusion proteins that contain the extracellular domain of activin receptor IIA (ActRIIA) linked to the human immunoglobulin G1 (IgG1) Fc domain [17, 19, 20]. The activin receptor ligands, which are members of the TGF-β superfamily, negatively regulate erythropoiesis by inducing apoptosis and cell-cycle arrest in erythroblasts, leading to the inhibition of erythroid differentiation [19]. Luspatercept inhibits the TGF-β pathway by binding to select TGF-β superfamily ligands to reduce aberrant Smad2/3 signaling. This inhibition promotes late-stage erythropoiesis, such as differentiation of erythroblasts into RBCs [17, 19, 21, 22].

In the phase II (PACE-MDS) study, luspatercept demonstrated promising results in increasing hemoglobin levels in lower-risk MDS patients with transfusion-dependent anemia [20]. Patients were administered subcutaneous injections of luspatercept at a dose of 1–1.75 mg/kg every 3 weeks for up to 5 cycles. Out of 49 patients, 30 (61%) showed a response in terms of hemoglobin improvement (HI-E) and 16 out of 29 patients (55%) achieved RBC transfusion independence (RBC-TI) [20]. The PACE study demonstrated that patients with a lower transfusion burden, RS presence, *SF3B1* mutation, and lower serum EPO levels exhibited a better response to luspatercept [20]. In 2022, long-term (up to 5 years) efficacy and safety results of the phase II PACE-MDS study were published [23]. Comparing RS and non-RS patients, RBC transfusion independency (RBC-TI) for 8 weeks or longer was observed in 52% (22 out of 42), and 35% of patients, respectively [23]. Additionally, patients with a low transfusion (LTB) dependency reached an RBC-TI for 8 weeks or longer in 72% of cases compared to 27% in

patients with high transfusion burden (HTB) [23]. Of particular importance are the high response rates (HI-E) in patients without transfusion requirements (NTB), which were achieved in 71% of cases, underscoring the future importance of luspatercept also in this subgroup of MDS patients [23]. The results of the phase III COMMANDS study (NCT03682536), evaluating the efficacy and safety of luspatercept versus ESA in ESA naive, LR-MDS patients with RBC transfusion dependency, are eagerly awaited.

The encouraging results from the PACE study led to the initiation of a phase III MEDALIST study, which was a placebo-controlled randomized trial of luspatercept in 229 transfusion-dependent lower-risk MDS patients who were refractory or not eligible for ESA and had RS or *SF3B1* mutation [17]. Out of the 153 patients who received luspatercept, 58 (37.9%) achieved the primary endpoint of RBC transfusion independence (RBC-TI) for a minimum of 8 weeks compared to only 10 of the 76 patients (13.2%) receiving placebo ($P < 0.0001$). Additionally, 43 of the 153 (28.1%) patients receiving luspatercept achieved the key secondary endpoint of RBC-TI for at least 12 weeks during weeks 1–24 compared to 6 of the 76 (7.9%) patients receiving placebo ($P = 0.0002$) [17]. During the first 24 weeks, 81 of the 153 (52.9%) patients receiving luspatercept achieved hemoglobin improvement (HI-E) compared to only 9 of the 76 (11.8%) patients receiving placebo. Over weeks 1–48, 90 patients (59%) in the luspatercept group achieved HI-E compared to only 13 (17%) in the placebo group. The median duration of the longest single continuous period of response to luspatercept was 30.6 weeks [17].

Importantly, the percentage of patients who responded to luspatercept treatment was not affected by the *SF3B1* allelic burden or the number of somatic mutations present at baseline [17]. Moreover, luspatercept was generally associated with low-grade toxicity, with the most common treatment-related adverse events (AEs) being fatigue, diarrhea, asthenia, nausea, and dizziness. These AEs were mostly of grade 1 or 2 intensity [17].

Based on the promising results from the phase III MEDALIST study, the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) recently approved luspatercept for the treatment of adult patients with IPSS-R very low-, low-, and intermediate-risk MDS with RS or *SF3B1* mutation or with myelodysplastic/myeloproliferative neoplasm with RS and thrombocytosis (MDS/MPN-RS-T) who have anemia failing an ESA and require at least two RBC units over 8 weeks [17]. The starting dose for luspatercept is 1 mg/kg administered subcutaneously once every 3 weeks. If there is no noticeable reduction in RBC transfusions after two doses (6 weeks), the dosage should be increased to 1.33 mg/kg and, if needed, further increased to a maximum of 1.75 mg/kg after two additional consecutive doses [17, 19].

31.2.1.2 Lenalidomide

At first diagnosis, approximately 50% of patients with de novo MDS present with cytogenetic abnormalities, where del(5q) occurs in approximately 5–10% of patients. ESAs have also been the primary therapeutic option for patients with del(5q) who have symptomatic anemia and low RBC transfusion burden. However, many of these patients initially present with excessive EPO levels, which predict only a short or lack of response to ESAs. Thus, the preferred treatment for these del(5q) patients currently remains the immune modulatory drug (ImiD) lenalidomide, which has demonstrated clinical activity in 60–75% of lower-risk MDS patients with del(5q) and a median response duration of 2 years. Lenalidomide can not only reduce transfusion requirements but also reverse cytogenetic abnormalities in around 45% of patients with the 5q31 deletion [24, 25]. The German LEMON5 study revealed that patients with del5q and a TP53 mutation had lower response rates (RBC-TI: 50% vs. 75%) and lower survival rates with lenalidomide treatment compared to those without a TP53 mutation [16]. These patients were also found to have a higher risk of leukemic progression. In lower-risk MDS patients without del(5q), response rates to lenalidomide were only around 25% [26]. Although not yet approved in non-del(5q) MDS patients, lenalidomide has also demonstrated clinical activity to induce long-lasting RBC-TI in 20–30% of this subgroup of patients [27, 28]. A phase III study was conducted to evaluate the efficacy of lenalidomide in non-del(5q) MDS patients with anemia who were either ineligible for or refractory to ESAs. The study found that 27% of lenalidomide-treated patients achieved RBC-TI for a minimum of 8 weeks, while only 2.5% of placebo-treated patients achieved the same outcome ($P < 0.001$) [28].

31.2.2 Treatment Options for Patients with Higher-Risk MDS

31.2.2.1 Hypomethylating Agents (HMAs)

For patients with HR-MDS, there are low-intensity therapies available such as hypomethylating agents (HMAs) [29]. Subcutaneous azacitidine (AZA), intravenous decitabine, and oral cedazuridine/decitabine are examples of HMAs that are commonly used as a first-line treatment option for HR-MDS(4,5)patients who are not suitable for AML-like chemotherapy and/or allogeneic hematopoietic stem cell transplantation (HCT), as well as for fit with HR-MDS patients who do not have a donor [30, 31]. Another common approach to "bridge" HR-MDS patients from the time of diagnosis to allogeneic HSCT is a treatment with HMAs such as AZA due to its anticipated moderate toxicity profile. The results of the VIDAZALLO trial indicate that treating patients with AZA induction followed by allogeneic HSCT

compared to continuous AZA without transplant resulted in enhanced survival with HSCT (3-year OS 49% vs 22%) [32].

Both AZA and decitabine (DAC) are cytosine analogs that were developed in the 1960s with different modes of action [33]. While DAC can be integrated into DNA strands, AZA integrates itself into both DNA and RNA chains. In 1993, a phase II trial was conducted using 75 mg/m2 of AZA for 7 days every 28 days, over six cycles, involving 43 patients with HR-MDS [34]. The trial yielded a 49% response rate, and subsequent phase II and III trials have closely replicated the results. In 2009, a phase III, multicenter open-label trial named AZA-MDS-001 led to the approval of AZA usage in patients with up to 30% bone marrow (BM) blasts [29]. The 358 randomly assigned patients with HR-MDS received, based on the investigator's choice, either AZA or conventional care regimens (CCR) such as intensive chemotherapy (ICT), low-dose cytarabine (LDAC), or best supportive care (BSC). The responses included a 49% hematologic improvement (HI) rate among AZA-treated patients and 29% of patients showing either complete remission (CR) or partial remission (PR) [29]. The AZA-treated patients had a median overall survival of 24.5 months, while the conventional care group had a median overall survival of 15.0 months demonstrating that AZA significantly extended the survival of patients with HR-MDS compared to conventional care. The AZA-MDS-001 study led to FDA and EMA approval of AZA for MDS patients who are not eligible for allogeneic HCT [29].

A randomized phase III trial conducted in 2011 included 233 HR-MDS patients over 60 years, not eligible for ICT and compared low-dose DAC 15 mg/m^2 IV for 3 days, in 6-week cycles, to best supportive care (BSC) only. The trial showed that there was no significant difference in median overall survival (OS) between DAC and BSC (10.1 vs. 8.5 months, respectively) [35]. However, progression-free survival (PFS) was significantly longer in patients treated with DAC compared to BSC (median PFS 6.6 vs. 3.0 months, respectively) [35]. The overall response rate (ORR) in the DAC arm was 34% (including HI), with 13% achieving complete response (CR), 6% partial response (PR), and 15% hematological improvement (HI) [35]. The trial confirmed the activity of DAC in HR-MDS, and the absence of a clear survival benefit prevented EMA approval, but the FDA approved DAC in 2010 [36]. Independent poor prognosis factors for OS, PFS, and progression into AML were found to be IPSS high risk, poor cytogenetics, ECOG 1 or 2 and less than 3 months of MDS duration [35]. Administered continuously until disease progression, HMAs have a median response duration of approximately 1 year. Nevertheless, a small group of patients experienced prolonged responses, in some rare cases lasting for over 3–4 years [35, 36].

Gene mutations, such as TET2 in the absence of ASXL1, have been identified as possible predictors of response to HMA treatment [37]. Nevertheless, until today response to HMAs cannot reliably be predicted and seems to occur independently of clinical variables. A large French study of 282 patients with HR-MDS or low blast AML (≤30% marrow blasts) found that no previous treatment with LDAC, blast count <15%, and normal karyotype independently predicted better response to AZA [38]. In contrast, shorter HMA response duration and reduced OS were independently predicted in the presence of complex karyotypes, poor ECOG performance status, the appearance of circulating blasts in peripheral blood, and red blood cell (RBC) transfusion dependence [38]. A basic predictive score was formulated by merging these variables, which categorized patients into three distinct groups with markedly dissimilar survival rates (i.e., low, intermediate, high). Furthermore, the investigation revealed that attaining HI (especially HI-E) in individuals who did not achieve complete or partial remission following AZA therapy was linked to better overall survival [38].

Bores et al. developed the European ALMA score (E-ALMA) which divides patients into three risk groups based on their ECOG score, white blood cell count (WBC) before treatment initiation, and cytogenetic abnormalities (normal or abnormal) [39]. The risk groups (favorable, intermediate, unfavorable) have varying survival rates and response rates to AZA [39].

In 2020, FDA approved the oral combination of decitabine and cedazuridine for patients with HR-MDS. The new agent was investigated in HR-MDS/CMML patients within two open-label, randomized, crossover trials (ASTX727-01-B and ASTX727-02 [40, 41]). The results showed an CR rate of 18–21% and a median duration of CR between 7.5 and 8.7 months with a toxicity- and safety profile like other HMAs [40, 41]. Although AZA or DAC is only in the US approved for treating LR-MDS, it is also frequently used in this therapeutic setting worldwide.

Various clinical trials are currently exploring the use of a combination of venetoclax, which inhibits BCL-2, with HMAs in patients with first-line and/or refractory diseases following HMA failure [42, 43]. The FDA has designated the combination of venetoclax and AZA as a breakthrough therapy for patients with untreated HR-MDS. The preliminary findings from a phase Ib study, which examined the combination of venetoclax (administered for 14 days) and AZA (administered for 28 days) in untreated higher-risk MDS patients, indicated an ORR of 77%, a median PFS of 17.5 months, and a median response duration of 14.8 months [44]. The results of the phase III combinational study are eagerly awaited.

31.2.3 Treatment Options for Patients with Hypoplastic MDS

The decreased marrow cellularity is a distinguishing feature of hypocellular myelodysplastic syndrome (hMDS), which

can make it challenging to differentiate from aplastic anemia (AA) using typical morphological criteria [45]. The term "hypocellularity" describes in patients younger than 70 years less than 30% and in patients older than 70 years less than 20% cellularity in the bone marrow [45]. Profound immune dysregulation is an increasingly recognized feature of patients with hypoplastic MDS, contributing to ineffective hematopoiesis and driving disease progression. Immunosuppressive therapy with antithymocyte globulin (ATG, either horse or rabbit), with or without addition of cyclosporine (CSA), has been investigated in several clinical trials, showing trilineage response rates ranging from 14% to 70% [46]. Patients identified with a higher likelihood of responding to immunosuppressive therapeutic approaches are those with hypoplastic marrow, short duration of transfusion dependence, MDS with single-lineage dysplasia with no RS, trisomy 8, HLA type DR15, age younger than 60 years, and females [4, 47].

The NCCN gives clear instructions that patients who have hypocellular marrow, STAT-3 mutated cytotoxic T-cell clones, or paroxysmal nocturnal hemoglobinuria clone positivity and are younger than 60 years old with less than or equal to 5% bone blasts should receive immunosuppressive therapy with ATG with or without cyclosporine A [31]. According to the ELN, individuals who are below the age of 60, have less than 5% marrow blasts, normal cytogenetics, and transfusion dependence and are ineligible for hematopoietic growth factors, should be evaluated for ATG along with 6 months of oral cyclosporine if they have a hypoplastic bone marrow [48].

31.3 Summary

In conclusion, because patients with MDS continue to have very few approved therapy options, there is still a high clinical need for new targeted therapeutic approaches. Enrolling patients in clinical trials provides not only access to new therapies, but also potential for improved outcomes and quality of life, even in the absence of other approved treatment options. As our understanding of the molecular mechanisms of MDS continues to expand, there is higher opportunity for the development of personalized, targeted therapies to improve outcomes for our patients.

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Treatment Algorithm of CMML and Other Adult MDS/MPN Subtypes

32

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Abstract

Myelodysplastic syndrome/myeloproliferative neoplasm (MDS/MPN) overlap syndromes are now recognized as distinct entities in recent World Health Organization classifications. Aside from juvenile myelomonocytic leukemia, MDS/MPNs including chronic myelomonocytic leukemias and rare subtypes such as atypical chronic myeloid leukemia or MDS/MPN with ring sideroblasts and thrombocytosis occur in older adults. Each entity harbors a distinct clinical presentation and molecular profile, but their prognosis remains overall poor. Different risk-scoring systems have been established which are yet to be integrated with therapeutic algorithms. The only curative therapy remains allogeneic hematopoietic stem cell transplantation (HSCT), but few patients are eligible due to their age and comorbidities. Because of their low incidence, few clinical trials have been conducted in MDS/MPNs, and aside from azacitidine in a subset of CMMLs, no drug is labeled for these entities. Therapeutic decisions in MDS/MPNs thus often rely on small retrospective series or case reports and aim to alleviate symptoms, with limited hope to alter the disease's natural history. Thus, MDS/MPNs remain an unmet medical need. In this chapter, we review the epidemiology, diagnostic, and prognostic criteria of each MDS/MPN entity and propose therapeutic algorithms to guide the management of these rare but high-risk patients.

Keywords

MDS · MPN · JMML · aCML · MDS/MPN-RS-T · MDS/MPN-U · HSCT · HMAs

32.1 Introduction

Myelodysplastic syndrome/myeloproliferative neoplasm (MDS/MPN) overlap syndromes have been individualized since the 2001 World Health Organization (WHO) classification as a distinct group of myeloid neoplasms. Each MDS/MPN entity is defined by the association of myelodysplastic and myeloproliferative features. According to the 2016 WHO classification, MDS/MPNs include chronic myelomonocytic leukemias (CMMLs), by far the most frequent entity, atypical chronic myeloid leukemia (aCML), MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T), MDS/MPN unclassified (MDS/MPNu), and juvenile myelomonocytic leukemias (JMML) [1]. Leaving aside JMML which has a clearly distinct epidemiology and pathogenesis, whether adult MDS/MPN entities represent a continuum in the clinical and molecular spectrum of clonal myeloid disorders, or a heterogeneous collection of unrelated malignancies remains disputed, although recent reports suggest the former [2].

First restricted to cytomorphology, diagnostic criteria of MDS/MPN entities have evolved to integrate cytogenetics and genetics (Table 32.1). Recurrent cytogenetic alterations and gene mutations also play an increasing role to assess disease risk and to guide therapy.

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Table 32.1 MDS/MPN entities according to WHO 2016 classification

	CMML			aCML	MDS/MPN-RS-T	MDS/MPN-U
Criterion	CMMML-0	CMML-1	CMML-2			
Monocytes ($\times 10^9$/L)	>1 (>10%)			<10%		
Neutrophils ($\times 10^9$/L)						
IMC		<10%		>10%		
WBC ($\times 10^9$/L)				>13		>13
Platelets ($\times 10^9$/L)					>450	>450
Basophils				<2%		
Blasts (PB)	<2%	3–5%	5–19%	<20%	<1%	
Blasts (BM)	<5%	5–10%	10–19%	<20%	<5%	<20%
RS >15%	No			No	Yes	No
Dyplasia	≥ 1 lineage			Dysgranulopoiesis		≥ 1 lineage
t(9;22)/*BCR-ABL1*	No			No	No	No
PDGFRA/B rearrangement	No			No	No	No

IMC: Immature myeloid cells; *WBC:* White blood cells, *PB:* Peripheral blood; *BM:* Bone marrow; *RS:* ring sideroblasts

32.2 Chronic Myelomonocytic Leukemias

32.2.1 Epidemiology

Chronic myelomonocytic leukemia (CMML) is the most frequent myelodysplastic/myeloproliferative neoplasm defined by the 2016 WHO classification [1]. Previously grouped with myelodysplastic syndromes (MDS), CMMLs accounted for up to 20% of MDS cohorts. CMML incidence ranges between 3.5 and 4.1/100,000 inhabitants per year in the United States (US) and Europe [3, 4]. These figures may be an underestimation because lower-risk CMMLs are often revealed by monocytosis only, whose discovery is often incidental.

As in MDS, CMML is mostly diagnosed in older adults, with a median age at diagnosis of 72 years. CMML is rare before the age of 50 years. CMML patients younger than 65 years still have poor outcome, with a 5-year survival of 45% [5]. CMML has a marked male predominance (M/F ratio 2.3) which currently remains mostly unexplained [4].

The etiology of CMML remains unknown in most cases and is thought to result from aging of the hematopoietic system [6]. In some series, up to 10% of CMML cases occurred in patients with antecedent exposure to chemotherapy or radiations compatible with the empirical definition of therapy-related CMML [7]. A statistical association between CMML and prior history of infections or autoimmune/auto-inflammatory disorders has been reported [8]. Finally, molecular data suggest that CMML can arise from a clonal hematopoiesis of indeterminate significance (CHIP) or from a bona fide MDS [9, 10]. Collectively, these data suggest that CMML results from myeloid clonal expansion favored by "inflammaging." CMML monocytes may in turn accelerate this process, forming a vicious circle [11, 12].

In large CMML series, median overall survival (OS) varies between 2 and 3 years, with a cumulative risk of transformation into acute myeloid leukemia (AML) of 25–30%. However, these figures hide a broad spectrum of clinical presentation and disease history [4].

32.2.2 Presentation and Diagnosis

32.2.2.1 Clinical Presentation

CMML diagnosis is often incidental with the discovery of monocytosis on a systematic complete blood count (CBC) in an asymptomatic patient. Clinical presentation is very heterogeneous. Some patients can also be referred for the investigation of constitutional symptoms (fatigue, diffuse bone pain, and night sweats), splenomegaly (30% of cases), inflammatory or auto immune manifestations, the discovery of extramedullary myelomonocytic infiltrates (skin lymph nodes, gingiva, kidneys, pericardium, or other sites) [13–15], or cytopenias.

Rare but potentially severe complications include lysozyme nephropathy [16], CNS involvement [17], or leukemoid reaction following an infection or a surgical procedure [18]. Cases of splenic rupture caused by peliosis have also been reported [19]. An inflammatory disease such as systemic vasculitis, connective tissue diseases, polychondritis, seronegative arthritis, or immune cytopenias, notably immune thrombocytopenia, is found in 10–30% of CMML patients and can be diagnosed before CMML or concomitantly [8, 20, 21].

32.2.2.2 Biological Presentation

Chronic peripheral monocytosis (monocytes >1 × 10^9/L and >10% of white blood cells) is the cornerstone of CMML diagnosis. Monocytosis can be associated with hyperleuko-

cytosis and an increase in the absolute neutrophil count (ANC). Immature myeloid cells (IMC) may be seen on the peripheral blood (PB) smear. Since the FAB classification, "myelodysplastic" CMML (CMML-MD; WBC < 13×10^9/L) has been distinguished from myeloproliferative CMML (MP-CMML; WBC ≥ 1×10^9/L) on the basis of a WBC cutoff of 13×10^9/L [10]. Although this cutoff is rather arbitrary, its prognostic impact has been validated, and systematic search of an optimal cutoff confirmed its relevance [22]. Anemia (normocytic or macrocytic) and thrombocytopenia can be present at diagnosis resulting from BM dysplasia but cytopenias can also be of immune origin [8, 20, 21] or worsened by splenomegaly.

Examination of the PB smear may reveal a variable percentage of immature myeloid cells. These usually represent fewer than 10% of WBCs; a higher proportion may be indicative of alternative diagnoses such as aCML. Specific parameters of the automated CBC may guide the interpretation of the PB smear [23].

Bone marrow aspiration or biopsy can reveal dysplasia, increased cellularity, reticulin fibrosis, nodules of plasmacytoid dendritic cells, ring sideroblasts, and sometimes masts cells [11]. Excess of blasts cells has a major prognostic value [12]. The enumeration of blasts and promonocytes is concordant across hemopathologists, though the distinction between normal and abnormal monocytes, which is not a diagnostic criterion, is less reproducible [24].

Cytogenetic abnormalities are seen in 30% of CMML patients, most frequently trisomy 8, loss of chromosome Y, monosomy 7, del(7q), trisomy 21, and del(20q). Molecular mutations are found in 80% of patients and mostly affect epigenetic (*TET2*, *ASXL1, EZH2, IDH, DNMT3A*), splicing (*SRSF2, U2AF1, SF3B1, ZRSR2*), and signaling (*NRAS*, K*RAS*, *CBL, JAK2*) genes [13, 14].

Hypergammaglobulinemia resulting from inflammation is common in CMML patients. Association of CMML with a monoclonal gammopathy of undetermined significance (MGUS), multiple myeloma, or lymphoid malignancy is rare but reported [15]. *TET2* mutation can indeed be found in immature myeloid progenitors of patients with lymphoid malignancies and could thus lead to the association of a myeloid and a lymphoid malignancy in the same patient [16].

32.2.2.3 Diagnostic Criteria

The diagnosis of CMML relies on three pillars, namely (1) persistent peripheral blood (PB) monocytosis >1 × 10^9/L with monocytes accounting for >10% of white blood cells (WBC), (2) evidence of clonality or dysplasia in one or several lineages, and (3) exclusion of other myeloid neoplasms.

The proportion of monocytes among WBCs is important to tease out CMML from aCML and unclassified MDS/MPN that may often present with absolute, but not relative monocytosis. Of note, bone marrow monocytosis is not a diagnostic criterion in CMML or in other MDS/MPNs. Monocyte lineage dysplasia is infrequent and difficult to assess. Persistence of peripheral monocytosis for 3 months or more has long played a key role in ruling out reactive monocytosis caused by infections or inflammatory conditions in an era when myeloid clonality could only be assessed by cytogenetics, which are normal in around 60% of CMMLs. The recurrence of a limited set of gene mutations in CMML now provides a more reliable means to assess clonality and accelerate diagnosis [22, 25].

Beyond assessment of dysplasia, a careful review of bone marrow histopathology is important to determine the percentage of blasts and exclude AML, defined by the 20% or more blasts in the PB or bone marrow. Importantly, promonocytes must be included in the blast count in CMML [26].

BCR-ABL+ CML and classical MPNs can present with monocytosis but should be distinguished from CMML and other MDS/MPNs, although there is overlap in the clonal architecture of *JAK2*-mutated MPNs with monocytosis and CMML [27]. CMML cases presenting with eosinophilia are now considered among hypereosinophilic syndromes because they are caused by distinct genetic lesions (*PDGFRA*, *PDGFRB*, *FGFR1* rearrangement or *PCM1-JAK2* gene fusion) and respond to specific tyrosine kinase inhibitors (Table 32.1). Myelodysplastic syndromes without peripheral monocytosis but with evidence of marrow monocytosis may represent early stages of CMML [28] but are not considered as MDS/MPNs by the 2016 WHO classification.

Because of the occurrence of auto-inflammatory manifestations in *bona fide* CMML, it may sometimes be difficult to tease out reactive monocytosis from CMML in some older adults with inflammatory symptoms and monocytosis. An excess of classical (CD14+/CD16−) monocytes representing >94% of monocytes in the PB as assessed by flow cytometry robustly identifies CMML from reactive benign monocytosis which is instead defined by the accumulation of intermediate (CD14+/CD16+) and nonclassical (CD14-/CD16+) monocytes [17, 18]. Disappearance of slan-positive nonclassical monocytes also marks CMML with associated inflammation [19].

32.2.3 Prognostic Assessment

CMML is a heterogeneous disease with variable prognosis. Risk assessment relying on disease- and patient- related factors is central to guide therapeutic management.

High WBC count and blast excess represent the two most widely established prognostic factors in CMML, forming the basis for subclassification of CMML within the WHO classification [1]. The 2016 edition follows on previous ones and

on the FAB proposal to distinguish myelodysplastic CMML (MD-CMML) with a WBC <13 × 10^9/L from myeloproliferative CMML (MP-CMML) with WBC × 10^9/L [10]. MP-CMML more frequently harbors RAS/MAPK mutations and is endowed with worse prognosis [22, 29, 30]. However, there is a continuum between these subtypes and MD-CMML often progresses to MP-CMML [20]. WHO experts also proposed three different CMML subgroups based on BM and PB blasts percentage: CMML-0 when blasts <2% in PB and <5% in BM; CMML-1 when blasts from 2 to 4% in PB and/or 5 to 9% in BM; and CMML-2 when blasts are from 5 to 19% in PB and/or 10 to 19% in BM and/or Auer nods are present. The prognostic relevance of this 3-tier classification has been validated [29, 31]. Other proposed CMML prognostic criteria include cytopenias (hemoglobin level and platelet count), other myeloproliferative features such as high LDH, circulating immature myeloid cells, presence of splenomegaly or extramedullary disease, cytogenetics, and more recently recurrent gene mutations. Cytogenetics is normal in two-thirds of CMML patients and there is no CMML-specific cytogenetic lesion. Two cytogenetic classifications dedicated to CMML have been proposed, which only differ on the assignment of trisomy 8 to the intermediate risk [32] or poor risk group [30]. Normal karyotype and loss of Y are considered as favorable and chromosome 7 alterations or complex karyotype as adverse in both classifications.

Over the last decade, a new generation of CMML-specific prognostic scores have been proposed, all of which have been externally validated [22, 30, 33, 34]. The CMML International Prognostic Scoring System (CPSS) accounts for red blood cell transfusion dependency (which can be substituted for by the hemoglobin level), WBC (with the FAB cutoff of 13 × 10^9/L), BM blasts, and cytogenetics [35]. The Mayo prognostic score accounts for peripheral monocyrtes and immature myeloid cells, hemoglobin, and platelets [36]. The GFM score, accounting for age, WBC, hemoglobin. and platelets, was the first to account for genetic lesions, focusing on *ASXL1* mutations [22]. CPSS has recently been refined to account for mutations in *ASXL1, RUNX1, NRAS,* and *SETBP1* in the molecular CPSS (CPSS-mol) [21, 22].

When mutational profiling is lacking CPSS [13], MD Anderson [23] and Mayo [36] scores have been shown to have a comparable prognostic performance that is slightly superior to first-generation CMML or to MDS prognostic scoring systems [33]. When molecular data are available, it is preferable to use molecular scores such as GFM [14], CPSS-mol [24], or the molecular Mayo Clinic model [25]. For the sake of simplicity and in keeping with habits inherited from the MDS IPSS era, it is tempting to use CPSS or CPSS-mol dichotomized into lower risk (CPSS low/intermediate-1) and higher risk (CPSS intermediate 2/high). Higher-risk CMMLs represent ~50% of cases and have a median survival below 3 years (and below 2 years for patients in the high-risk group) in the CPSS-mol development cohort [34] and thus require disease-modifying intervention. In contrast, patients in the lower risk categories of the CPSS-mol cohort have 5 year or more of median expected survival, and thus, in older patients, it is often considered adequate to favor management of symptoms or watchful waiting. Other than age, patients' comorbidities have also to be taken into account in treatment decisions, although no specific comorbidity study has been undertaken in CMML patients [26].

32.2.4 Treatment Algorithm

Allogeneic hematopoietic stem cell transplantation (HSCT) remains the only curative treatment for CMML [37]. Therefore, the first step of CMML management is to determine HSCT eligibility. The second step is to establish treatment goals based on risk assessment and patient will. In case of higher-risk disease, because treatments truly altering disease evolution are limited in CMML, accrual to a clinical trial should first be sought. The final step, if the emphasis is put on quality of life rather than cure or delayed progression, is to prioritize symptoms that must be alleviated (cytopenias or myeloproliferative features) and always consider watchful waiting as a reasonable option (Fig. 32.1).

32.2.5 Potentially Disease-Modifying Therapies

32.2.5.1 Allogeneic Stem Cell Transplantation

Many CMML patients are too old or frail to be eligible for HSCT. This procedure remains risky in CMML, with a 3-year non-relapse mortality ranging from 20 to 50% according to risk factors, and a ~30% cumulative risk of relapse [38–44]. HSCT is also associated with frequent cGVHD-mediated morbidity in CMML [45].

Current guidelines, inspired by MDS data, recommend upfront HSCT in CMML patients with higher-risk disease [46]. This empirical recommendation has been recently supported by results from a large retrospective analysis [36]. It is also often recommended to perform HSCT with no more than 10% BM blasts [44], although there is no prospective evidence supporting the use of pre-HSCT therapy to decrease posttransplant relapse risk. Historically, patients have been bridged with either intensive chemotherapy or HMA [47]. Low CR rate and significant hematological and thus infectious toxicity with chemotherapy have led to favor HMAs as bridging therapy in recent years [45, 46]. Progresses with HSCT in CMML will come from better bridging therapy, e.g. using HMA-based combinations, access to haploidentical donors, and posttransplant maintenance or preemptive interventions [48].

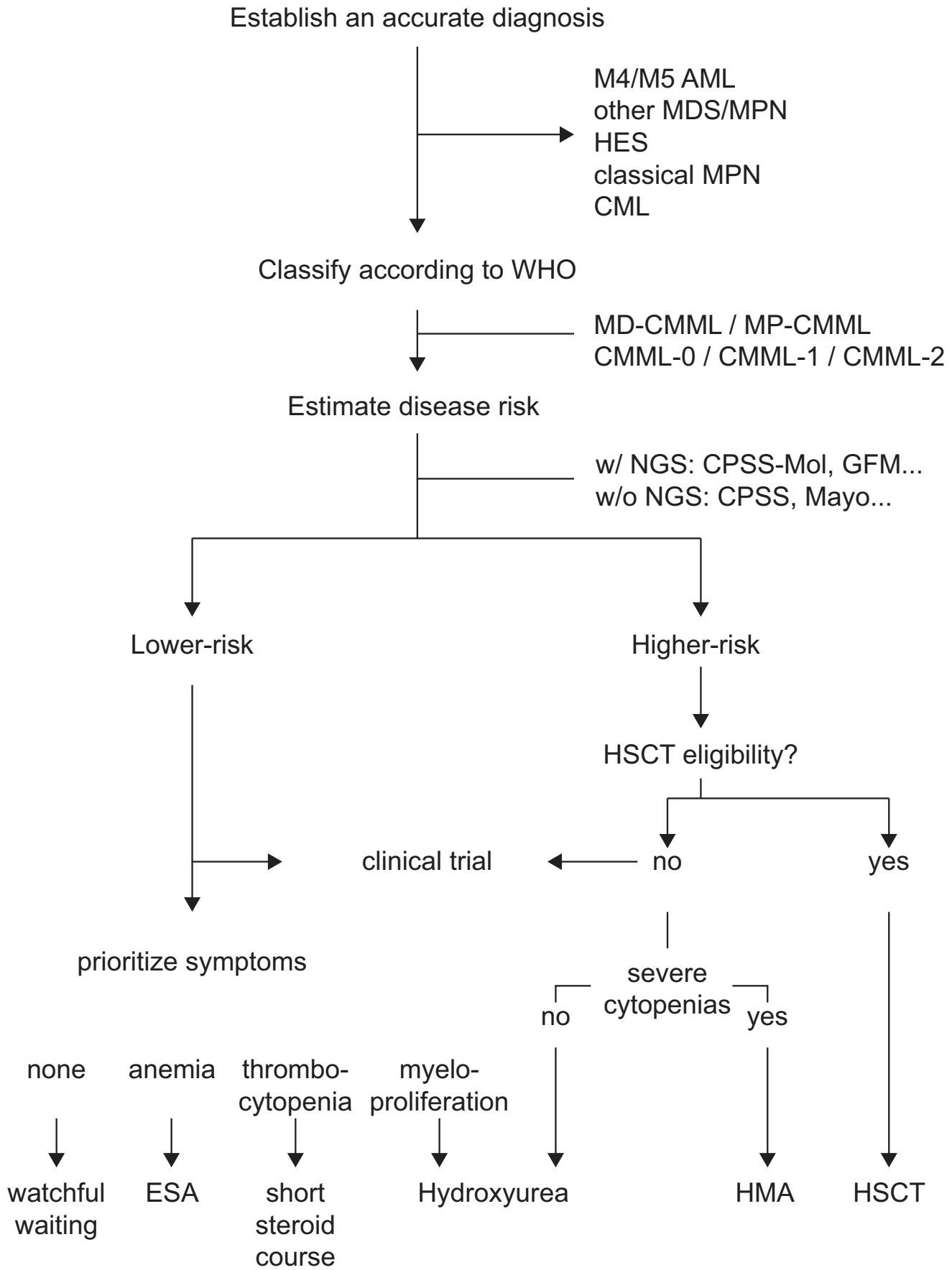

Fig. 32.1 Proposed treatment algorithm for CMML. *ESA:* Erythropoietin stimulating agent; *HSCT:* hematopoietic stem cell transplantation; *HMA:* Hypomethylating agent; *CML:* Chronic myeloid leukemia; *NGS:* next generation sequencing

32.2.5.2 Intensive Chemotherapy

CMML is often chemoresistant and data are limited to retrospective single center series reporting that short-lived CR can be achieved with anthracycline–cytarabine combinations in ~40% of patients, followed by inevitable relapse [49]. Data with liposomal chemotherapy (CPX-351) in CMML are limited, but low response rate has been reported in AML secondary to CMML [50], confirming the chemoresistance of this disease.

32.2.5.3 Hypomethylating Agents

First-generation HMAs azacitidine and decitabine have been shown to delay progression in MDS [51, 52]. In CMML, biological studies have shown that HMAs induce hypomethylation in driver genes or to lead to expression of tumor suppressive microRNAs [53], but fail to alter clonal architecture [54]. Both are approved in the United States for the treatment of CMML, while only azacitidine is approved in Europe to the specific of CMML-2 with WBC $<12 \times 10^9$/L, on the basis of MDS pivotal trials accruing less than 20 CMML cases [51, 55–57].

Until recently, data on HMA in CMML originated from retrospective series [58–64] or single-arm prospective phase II trials accruing less than 50 patients [55, 65–69]. Collectively, these series reported an average 50% overall response rate using MDS response criteria, and up to 25% complete response rate, with a median overall survival around 20 months, although no formal meta-analysis has been carried [46]. Of note, CMML-specific response criteria have recently been proposed, accounting for both dysplastic and proliferative disease features [70]. Applying these criteria to a retrospective series of CMML patients treated with single-agent HMAs yielded an overall response rate of ~70%, including ~50% in patients with CPSS intermediate-2 or high-risk disease [71].

Though HMAs can mitigate proliferative symptoms, including resolution of splenomegaly or extramedullary skin lesions [58, 65, 71, 72], several reports have shown that MP-CMML retains a poor prognostic value in the context of HMA therapy [49–51]. Importantly, two retrospective studies comparing azacitidine and decitabine have found superposable results with both HMAs, suggesting that these two drugs are interchangeable when used as single agents in CMML [61, 71]. The hematological toxicity of HMAs seems to compare favorably to data in MDS patients, but detailed information in patients with proliferative CMML is still missing [42].

A large retrospective study has suggested that HMAs do not confer a survival advantage in patients with lower-risk CMML [73]. In MP-CMML patients, this retrospective study suggested a survival benefit of HMA over hydroxyurea (HY), with previous HY reducing the response rate to HMAs [63]. However, the recently completed prospective randomized DACOTA trial (NCT02214407, EudraCT: 2014-000200-10) failed to identify a survival benefit of single-agent decitabine over HY in MP-CMML patients with high-risk features, although decitabine provided a higher response rate compared to HY [52]. Though no interaction was noted between baseline Hb and platelet levels, it is reasonable to prioritize HMAs in patients with profound cytopenias. Potential epigenetic biomarkers are yet to be validated [53]. A multicenter retrospective series reported that patients with *TET2* mutations but wildtype *ASXL1* may have superior response rate to HMAs, though not translating into a strong survival benefit [74]. This genetic biomarker can nevertheless instruct pre-HSCT therapy. Oral cedazuridine/decitabine (ASTX727) has similar pharmacokinetics to the standard IV decitabine regimen and is likely to supplement this approach in the near future [75]. Similar to MDS, the outcome of CMML patients after HMA failure is dismal, and there is no benefit in switching HMA in this difficult situation [76]. Future trials will study HMA-based combinations and inclusion in clinical trials should always be favored when possible. For instance, combinations of HMAs with the BCL-2 inhibitor venetoclax is approved in AML, and also provides encouraging results in MDS [77]. Though monocytic differentiation and *RAS*-mutated clones have been shown to lead to BCL-2 independence in AML, the limited data with venetoclax (mostly combined with HMAs) in CMML suggest that it induces responses in treatment-naïve patients, though its activity is probably more limited in patients having previously failed HMAs or other prior therapies [78]. Preclinical data suggest MCL-1 may be a relevant antiapoptotic target in CMML [79]. Other promising HMA-based combinations include addition of the Neddylation inhibitor Pevonedistat, which may also restore sensitivity to BCL-2 inhibition [80, 81].

32.2.5.4 Targeted Therapies

FLT3 mutations are exceedingly rare in CMML and should lead to reconsidering diagnosis for CMML. The minority (<5%) of CMML patients with *IDH1/2* mutations should be accrued to ongoing MDS trials of IDH inhibitors whenever possible, until the results of these trials are made available. Encouraging results have been reported with enasidenib in *IDH2*-mutated MDS [82]. Splicing mutations, especially *SRSF2,* are common in CMML. Drugs that target spliceosome components are still in early stages of development, with limited clinical activity as single agent in MDS [83]. Though targeting the MAPK pathway is appealing in the ~60% of patients with MAPK pathway mutations, the limited data available with single-agent MEK inhibition have yielded disappointing results [84]. Combination therapy may be required to provide better responses upon MEK inhibition [79], or kinase dependencies other than MEK be targeted in RAS-pathway mutated CMML. Notably, a preclinical study

has identified RAS-driven epigenetic upregulation of polo-like kinase 1, a kinase with clinical-grade inhibitors [85].

The 10% of CMMLs with *JAK2* mutations could be targeted with ruxolitinib. GM-CSF receptor-driven aberrant pSTAT5 is prominent in the ~60% of CMMLs with signaling pathway mutations [86]. Ruxolitinib is safe and reduces spleen size in 50% of patients [87]. Monoclonal antibodies targeting the GM-CSF cytokine may also represent a way to target this pathway. One such antibody, lenzilumab, is well tolerated and induces clinical benefit in 38% of patients [88].

Targeting CD123 with tagrasofusp (SL-401), the diphtheria toxin fused to the IL-3 ligand, may be particularly relevant in the subset of CMMLs with a pDC infiltrate [89]. Tagrasofusp provides a 71% spleen response rate and a 17% bone marrow blast clearance [90]. CMML stem cells also invariably express CD123 [91]. Additional targets currently in preclinical investigation include PI3K isoform delta [92].

32.2.5.5 Symptomatic Treatments

In patients that are not eligible for HSCT and with lower-risk disease, watchful waiting is the default option, with regular assessment of cytopenias, proliferative features (high WBC and related general symptoms, splenomegaly, extramedullary disease) and autoimmune or auto-inflammatory disease (AID). Clinical manifestations rather than specific figures should trigger interventions, notably for anemia, thrombocytopenia, and high WBC. Significant changes should trigger bone marrow evaluation. Management of patients with both increasing cytopenias and myeloproliferation is particularly difficult, as treatments may be antinomic, but this situation of signals disease progression, triggering disease-modifying options.

32.2.5.6 Management of Anemia

Around 40% of CMML patients present with an Hb <10 g/dl at diagnosis and up to 20% are transfusion dependent. Lower hemoglobin levels are correlated with worsening quality of life, especially in older, potentially comorbid patients. The management of anemia in CMML is largely inspired by studies carried in MDS. Erythropoietin stimulating agents (ESA) can induce a response in 60% of patients with half of them being transfusion independent, similar to an MDS population [27, 28]. However, the median duration of response of 7 months appears to be shorter than in MDS [29]. Anemia is a poor prognostic factor in CMML [22, 35]. Interestingly, achievement of erythroid response with ESA seems to be associated with better survival [29]. Red blood cell (RBC) transfusion is indicated for symptomatic anemia and the transfusion threshold should be guided by patient tolerance of anemia. Transfusion strategy can be more complex in MP-CMML because of splenomegaly, of the association of constitutional symptoms, and of cytoreductive therapy. Iron chelators are usually recommended for transfusion-dependent patients having received more than 25 red blood cell (RBC) units or with a ferritin level >1000 ng/ml, although there is no formal prospective evidence that it alters patient's outcome [30]. New agents are currently being evaluated for the treatment of anemia in lower-risk MDS patients, such as the activin receptor ligand trap luspatercept [32] or the telomerase inhibitor imetelstat [93] but data in CMML is still lacking.

32.2.5.7 Management of Thrombocytopenia

Trombocytopenia is also an adverse factor in CMML [22]. Importantly, platelet counts can be lowered by enlarged spleen and/or immune thrombocytopenia [21, 94]. Aside from patients receiving antiplatelet therapy, bleeding symptoms can be more important than anticipated based on platelet counts in some patients [21], suggestive of acquired platelet dysfunction [95].

In patients with very low (e.g. $<30 \times 10^9$/L) platelet counts or with significant bleeding, a short course of steroids or intravenous immunoglobulins may be tested, especially when the bone marrow morphology or Monoclonal Antibody Immobilized Platelet Antigen (MAIPA) test is suggestive of immune thrombocytopenia [21, 94]. Following a first report raising concerns on the risk of leukemic progression [22], the thrombopoietin receptor agonist Eltrombopag (ELT) has been explored in a phase II study on 30 CMML-0 patients with platelets $<50x10^9$/L. ELT proved safe in terms of increases in liver tests and WBC if started at 50 mg/d and progressively escalated, with 47% of patients achieving platelet response [33]. However, these were mostly short-lived, suggesting that ELT can be useful in the setting of active bleeding episode or planned hemorrhagic surgery. Some responses to danazol have also been reported in CMML patients with thrombocytopenia [96]. In most situations, however, platelet transfusion support remains the mainstay of severe thrombocytopenia management. Transfusion policy (prophylactic versus curative, threshold) must be tailored to patient medical history, co-medications, and bleeding symptoms.

32.2.5.8 Management of Neutropenia

Neutropenia is infrequent in CMML except as a result of therapies with known hematological toxicity. Significant neutropenia should trigger bone marrow assessment of disease progression. Rarely isolated neutropenia can be of immune origin or caused by a concomitant T-cell large granular lymphocytic leukemia-type clonal proliferation [97]. Treatment-related neutropenia should be managed by tapering, delaying, or interrupting cytoreductive therapy. Use of G-CSF is not recommended but can be discussed for few days in case of severe infectious complications.

32.2.5.9 Management of Auto-inflammatory Manifestations

A therapeutic intervention should be proposed to patients presenting disabling clinical auto-inflammatory symptoms, e.g. seronegative arthritis. Steroids are efficient in 85% of cases but half of the patients become steroid-dependent [6]. Following conclusive preliminary reports [43], prospective studies are ongoing to confirm the activity of HMAs as steroid-sparring strategy.

32.2.5.10 Management of Myeloproliferation

Increases in WBC are often associated with spleen enlargement, worsening of performance status, and with fatigue. General symptoms are currently poorly captured in CMML studies and should receive greater attention now that the recent consensus MDS/MPN response criteria account for them [70]. These guidelines suggest using the MPN SAF questionnaire [98] to monitor patient-reported outcomes in MDS/MPN, though this questionnaire has still to be validated in this setting. A close inspection of the CBC must be undertaken to differentiate blasts from immature myeloid cells, keeping in mind that there can be a continuum between MP-CMML and aCML [99]. Brutal but often transient increases in WBC count are frequently seen in the context of infection or inflammation. Thus, watchful waiting a few weeks is often preferable before undertaking bone marrow reevaluation or initiating therapy in this context. Rare instances of life-threatening post-procedure leukemoid reaction, where WBC increase is accompanied by organ failure reminiscent of cytokine release syndrome, may require prompt cytoreduction [18]. Otherwise, there is no consensus WBC threshold to trigger therapeutic intervention in MP-CMML. Patients with WBC 30–40 × 10^9/L can be perfectly asymptomatic, and there is to date no evidence that lowering WBC *per se* alters the disease's history.

Hydroxyurea (HY) is often considered the main option for oral cytoreduction in patients with high WBC, splenomegaly, or constitutional symptoms. HY have been shown to be superior to oral Etoposide in the only randomized trial dedicated to CMML published so far [47]. Although theoretically HY inhibits the ribonucleotide reductase required for AZA metabolism, HY can also be used in association with HMA when they are initiated. HY can lower WBC and reduce splenomegaly in more than 80% of patients, often at the expanse of anemia or thrombocytopenia [100]. HY may also reduce skin lesions. No formal evaluation of improvement in constitutional symptoms has been performed with HY. Recently, the JAK inhibitor ruxolitinib has been suggested to improve disease-related constitutional symptoms, possibly by reducing inflammatory cytokines [87, 101]. Monocytes often present an inflammatory expression profile in CMML [12], and the presence of inflammatory monocytes has been associated with worse prognosis [102]. Further studies will determine whether ruxolitinib, alone or in association with HMAs [103], can alter disease progression in CMML.

32.2.5.11 Management of Extramedullary Manifestations

Splenomegaly is the most frequent extramedullary localization and has been associated with poor prognosis. When splenomegaly is symptomatic or in the presence of other extramedullary disease, therapy initiation should be considered. Skin lesions in CMML patients must be carefully inspected and a skin biopsy undertaken, as the pathology report may reveal infiltration by myeloblasts, mature or plasmacytoid dendritic cells [104].

Hydroxyurea and single-agent HMAs are efficient strategies to reduce splenomegaly and CMML skin infiltration [65, 100]. Ruxolinitib and tagrasofusp also provide spleen responses [87, 90]. When other extramedullary sites are suspected (e.g. pleuritis, pericarditis), a biopsy should be performed to document the localization and rule out myeloid sarcoma (i.e. extramedullary AML transformation). These localizations may be life-threatening and often require prompt intervention with HMAs or HY, or with IL-6 signaling blockade by tocilizumab [105].

32.3 Atypical Chronic Myeloid Leukemia

32.3.1 Epidemiology

Atypical chronic myeloid leukemia (aCML) is a rare subtype of MDS/MPN with an overall incidence of 1 per 100,000 persons/year. As all adult MDS/MPNs, the disease is more common in elderly with a median age at diagnosis of 69 years [57], without obvious male or female preponderance [51]. Prognosis of aCML is heterogeneous but overall poorer than CMML with median OS ranging from 12 to 30 months in retrospective studies [58].

32.3.2 Presentation and Diagnosis

Presentation of aCML resembles CML [57]. Patients with aCML can be asymptomatic at diagnosis and referred because of the incidental discovery of hyperleukocytosis but constitutional symptoms, inflammatory manifestations, or tumoral symptoms can reveal the disease. Clinical presentation includes hepatomegaly, splenomegaly, or sometimes other extra medullary infiltrates in 44% of patients [106, 107].

Hyperleukocytosis and cytopenias can be present together at diagnosis. The prototypical aCML CBC displays hyperleucocytosis with an increase in ANC and immature myeloid

cells, whereas basophilia is generally absent, and monocytes fewer than 10% of WBCs [60, 61]. Platelet count can be increased (19%) resulting from myeloproliferation, but thrombocytopenia can also be present at diagnosis due to bone marrow infiltration or splenomegaly, along with anemia (90%) [61]. Lactate dehydrogenase (LDH) is frequently increased. Almost half of the patients harbor cytogenetic anomaly. They are not specific and mainly involve chromosomes 8, 12, 12, 14, 17, 19, 20, and 21. The single most frequent lesion is trisomy 8 [59]. Unlike CNL (chronic neutrophilic leukemia) which is strongly associated with *CSF3R* mutations, aCML is associated with *SETBP1* and/or *ETNK1* mutations in almost 30% of patients, although *CSF3R* mutations can be found in 0% to 40% of aCMLs [108, 109]. The *ASXL1-SETBP1* mutation combination is specifically enriched in aCML [110]. Mutations in *NRAS/KRAS*, *SRSF2*, *TET2*, *CBL,* and *CSF3R* have also been described with variable frequencies [62–64].

Per WHO criteria, aCML is defined by hyperleukocytosis (WBC $\geq 13 \times 10^9$/L) with ≥10% immature myeloid cells, minimal basophilia (<2% of leukocytes) and monocytosis (<10% of leukocytes), and <20% blasts in the bone marrow and peripheral blood [1]. BM displays hyperplastic myeloid hyperplasia and dysplastic granulopoiesis and in some cases multilineage dysplasia. Cytogenetics and molecular genetics must rule out *BCR-ABL1* CML, classical MPNs, and hypereosinophilic syndromes. Mutations in *JAK2*, *CALR,* and *MPL* are thus typically absent as are *PDGFRA*, *PDGFRB*, *FGFR1,* or *PCM1-JAK2* rearrangements (Table 32.1) [1].

32.3.3 Prognosis

Outcome of aCML is overall poor, with 40% of patients transforming to AML in a median of 18 months from diagnosis, translating in median OS ranging from 12 to 30 months at most [107, 111, 112]. Few studies have assessed patient prognostic factors [66] but age >65 years, hemoglobin level <10 g/dL, WBC $>50 \times 10^9$/L, and higher percentage of circulating immature myeloid cells have been reported to have a negative impact [51, 61]. In retrospective studies, mutations in *TET2* [67], *SETBP1,* or *ASXL1* [61] have been suggested to carry poor prognosis. A hazard ratio-weighted prognostic model taking into account age, anemia, and *TET2* mutations has been developed [67].

32.3.4 Treatment Algorithm

Treatment of aCML and other rare MDS/MPN is poorly codified, due to the lack of consensual risk stratification and paucity of prospective studies [68]. Most data emerge from small retrospective studies, where aCML management was derived from CMML or classical MPN algorithms. Recently, definition of consensus response criteria from an international clinical research consortium and identification of disease-defining lesions opens the perspective of rational clinical trial design in these rare and difficult to treat entities [70, 113, 114].

As a general rule for MDS/MPNs, HSCT remains the only putatively curative option and transplant should be systematically considered in younger patients, as is eligibility for clinical trials (Fig. 32.2).

32.3.4.1 Patients Eligible to Allogeneic Hematopoietic Stem Cell Transplantation

All patients eligible for transplantation based on age and comorbidities with a suitable donor should be referred to HSCT. In addition to younger patients, transplantation should be considered particularly for older patients who are fit and present high-risk features such as anemia, high WBC counts, and/or high percentage of circulating immature myeloid cells. It may also be discussed for patients with *SETBP1* and *ASXL1* mutations. However, there is no standard of care and no consensus recommendations to guide the

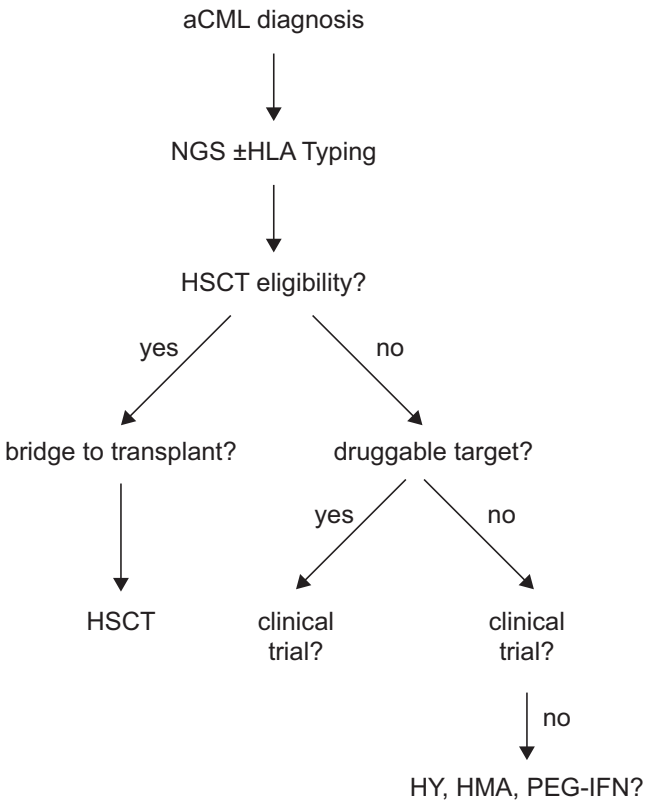

Fig. 32.2 Proposed treatment algorithm for aCML. *ESA:* Erythropoietin stimulating agent; *HSCT:* Hematopoietic stem cell transplantation; *HMA:* Hypomethylating agent; *CML:* Chronic myeloid leukemia; *NGS:* Next generation sequencing

timing of the transplantation [68]. In addition, data on aCML outcome after transplantation are scarce and heterogeneous [69, 70]. Mittal *et al.* reported a 2-year OS of 47% ($n = 7$) and Onida *et al.* a median OS of 70 months and a 5-year relapse-free survival of 36% ($n = 42$) [69, 71]. Another study reported a median survival of 47 months after transplantations [115, 116], while the EBMT registry reported a 5-year NRM of 24% and a 40% relapse risk [117]. Importantly, *ASXL1* mutations do not seem to alter the survival benefit of HSCT in MDS/MPN [118].

There is no study on the optimal timing of HSCT, but given the overall poor prognosis of aCML, it is reasonable to plan it as soon as possible, especially as no data are available on the role of bridging therapy such as hydroxyurea or HMA in patients with high WBC, very large splenomegaly, or excess of blasts. The impact of disease control on transplantation outcome remains unknown [71]. The risk to delay transplantation is to finally fail to transplant the patient because of disease evolution or toxicity of bridging therapy. Posttransplant strategies such as prophylactic HMAs could also be envisaged to mitigate the high risk of relapse, but no data has been reported so far in aCML.

32.3.4.2 Patients Not Eligible to Allogeneic Hematopoietic Stem Cell Transplantation

Non-transplant approaches are palliative and mostly aim to improve symptoms. Thus, patients not eligible for HSCT should be accrued in clinical trials whenever possible and should be screened for targetable mutations. Preclinical work, followed by case reports suggest that *CSF3R* T618I hotspot mutations may be targeted by ruxolitinib. Ruxolitinib could also be proposed to JAK2-V617F mutated patients [119]. However, presence of co-mutations such as those in *SETPB1* may limit the activity of ruxolitinib in this context [120]. In vitro, truncating mutations in *CSF3R* are dependent on Src signaling, and could be targeted by dasatinib [108], but these lesions are rare in MDS/MPN and no clinical experience is available for this drug to date. As for CMML, the activity of the MEK inhibitor trametinib as single agent is confined to case reports in *RAS*-mutated aCML [74]. Therapeutic targeting of *SETBP1* and *ETNK1* is still at an early preclinical stage [121–123].

In the absence of response or lack of druggable target, cytoreduction should be envisaged in patients with proliferative disease and without limiting cytopenias. Responses to HY are frequent but short-lived [112]. ESAs and/or transfusion support are required in case of symptomatic anemia.

Data on HMA in aCML are limited. Case reports have noted complete responses after decitabine but their duration and the hematological toxicity of HMA are still to be described [124]. A recent phase II trial investigated the combination of HMAs to ruxolitinib in MDS/MPN. Though this combination improved the efficacy of ruxolitinib, this approach benefited mostly patients with MDS/MPN-U and/or *JAK2* mutations [103]. Future trials combining HMAs with JAK inhibitors regardless of *JAK2* and *CSF3R* status are awaited in aCML and other MDS/MPNs.

Anecdotal responses to IFN have been reported [112], requiring further investigation in the era of pegylated formulations [125]. IFNα therapy may be particularly relevant in MDS/MPNs with pDC marrow infiltration (20–30% of CMMLs), as IFNα may activate cytotoxic T cells and NK cells, as clonal pDCs express lower levels of IFNα than expected [89, 126].

Splenectomy or splenic irradiation must be carefully weighed and restricted to rare palliative instances and certainly not a standard pre-HSCT bridging therapy, due to the high risk of infection, bleeding, thrombosis, or flare in WBC and platelet counts following the procedure [107, 112, 127].

32.4 Myelodysplastic/Myeloproliferative Neoplasm with Ring Sideroblasts and Thrombocytosis

32.4.1 Epidemiology

Myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T) is also a rare MDS/MPN disease accounting for less than 1% of all myelodysplastic syndromes [77]. Its existence as a distinct entity has been questioned over time [78]. The disease was previously known as refractory anemia with ring sideroblasts and thrombocytosis (RARS-T) [65] but was renamed in MDS/MPN-RS-T after the 2016 revision of the WHO classification [1]. Median age at diagnosis ranges from 68 to 75 years depending on series with a slight male predominance (M:F 1.25:1) [79]. Median survival is around 5 years [80].

32.4.2 Presentation and Diagnosis

The presentation of MDS/MPN-RS-T mixes features of essential thrombocytemia (ET) and sideroblastic anemia. Anemia and thrombocytosis can be discovered incidentally in asymptomatic patients. As in ET [81], MDS/MPN-RS-T patients can present vasomotor symptoms including headaches, palpitations, acral paresthesia, atypical chest pain, and erythromelalgia [128]. Constitutional symptoms such as asthenia, weight loss, and night sweats can also be present. Bleeding due to an acquired von Willebrand disease in case of major thrombocytosis or a thrombotic event (arterial or venous thrombosis) can also be inaugural.

The frequency of thrombotic events (3.6 per 100 patient-years) is similar to ET (3.9 per 100 patient-years) and more frequent than in MDS with ring sideroblasts (0.9 per 100 patient-years) [71]. Thrombotic events may be more frequent in patients with *SF3B1* mutations [128, 129].

MDS/MPN-RS-T associates thrombocytosis with anemia [80]. Bone marrow morphology reveals dyserythropoiesis with ring sideroblasts accounting for ≥15% of erythroid precursors, and megakaryocyte morphology reminiscent of ET or primary myelofibrosis (PMF). WHO criteria for MDS/MPN-RS-T also exclude patients with a prior history of MDS or MPN (with the exception of MDS-RS) and those with *BCR-ABL1*, *PDGFRA*, *PDGFRB*, *FGFR*1, and *PCM1-JAK2* rearrangements, t(3;3)(q21q26), inv(3)(q21q26), or del(5q). Patients with reactive thrombocytosis or with ET and <15% bone marrow ring sideroblasts should also be excluded [1] (Table 32.1).

Almost 80% of patients have normal cytogenetics. MDS/MPN-RS-T epitomize the overlap between MDS and MPN, as they are characterized by RARS-defining mutations in the spliceosome gene *SF3B1*, found in up to 85% MDS/MPN-RS-T patients, and frequent ET-defining co-mutations (*JAK2* V617F in 50% or *CALR* and *MPL* in <10%) [110, 130, 131].

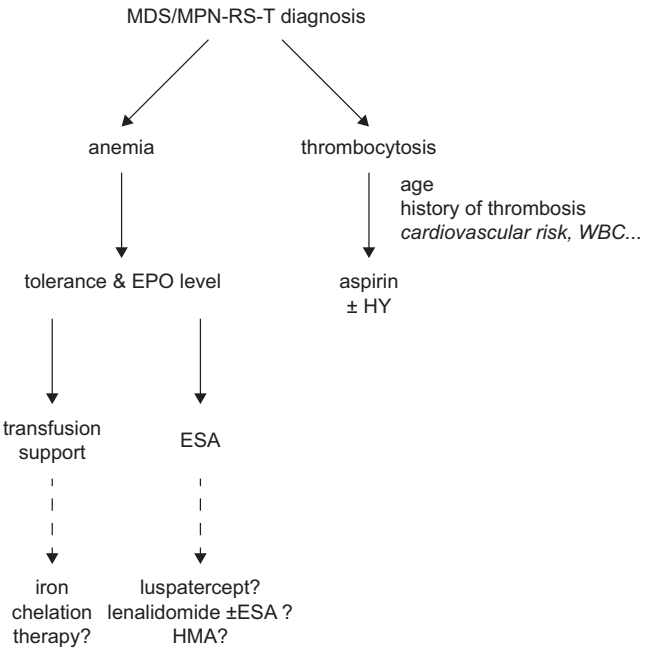

Fig. 32.3 Proposed treatment algorithm for MDS/MPN-RS-T. *ESA:* Erythropoietin stimulating agent; *HSCT:* Hematopoietic stem cell transplantation; *HMA:* Hypomethylating agent; *CML:* Chronic myeloid leukemia; *NGS:* Next generation sequencing

32.4.3 Prognosis

With a median survival of 76 months, MDS/MPN-RS-T patients have a better outcome than those with MDS-RS with single lineage dysplasia (MDS-RS-SLD, 63 months) but inferior to ET (117 months) [132]. Thrombotic events do not seem to impact OS [82].

A prognostic model has been proposed for MDS/MPN-RS-T accounting for abnormal cytogenetics, *ASXL1* and/or *SETBP1* mutations, and anemia (Hb level < 10 g/dL) to identify three risk groups with median survivals of 80, 42, and 11 months [85].

32.4.4 Treatment Algorithm

There is no formal guidelines for MDS/MPN-RS-T and current management is inspired from MDS-RS and ET therapy algorithm with the additional difficulty of balancing the treatment of anemia and thrombocytosis [86] (Fig. 32.3).

32.4.4.1 Management of Anemia

Anemia in MDS/MPN-RS-T patients can be managed with ESAs. In retrospective series, 45–55% of MDS/MPN-RS-Ts respond to ESAs, with a median response duration of 20 months, figures similar to MDS-RS. As in MDS, lower endogenous EPO levels predicted higher response rate. Some thrombotic events were noted despite aspirin prophylaxis warranting further studies on the safety of ESAs in this population [133].

Lenalidomide, HMAs, luspatercept, and imetelstat have been investigated as second-line therapies following ESA failure in lower-risk MDS. Case reports suggest that lenalidomide could be active in MDS/MPN-RS-T, but this option should be investigated in the context of a clinical trial, due to the risk of thrombosis [88, 89]. As in MDS, single-agent HMAs give disappointing results in this context [133].

Luspatercept represents a promising option in patients with *SF3B1* clones. However, because increase in platelet counts has been noted in MDS trials [134, 135], this option should also be investigated in the context of a clinical trial. Imetelstat may also represent an option for these patients [90]. In younger patients with RBC transfusion dependence, iron chelation may be envisaged according to MDS guidelines [136].

32.4.4.2 Management of Thrombocytosis

Indication for cytoreductive therapy with hydroxyurea and/or initiation of anti-aggregating agent in MDS/MPN-RS-T empirically follows ET risk stratification. This stratification is based on age and thrombotic event history [81, 91, 92]. ET patients older than 60 years and/or a history of thromboembolic event should start cytoreduction. The IPSET score (International Prognostic Score of Thrombosis in World Health Organization-essential thrombocytopenia) further refines the risk of thrombosis of ET patients by accounting

for cardiovascular risk, leukocyte count (>11 × 10^9/L), and presence of *JAK2* V617F mutation in addition to age and history of thrombosis [137]. The British committee for Standards in Hematology (BCSH) risk stratification considers age, history of thrombosis or hemorrhage, diabetes/HTA, platelet count >1500 × 10^9, and leukocytes >11 × 10^9/L for risk stratification [138]. Whatever the stratification used, patients at high risk must start cytoreductive therapy and antiplatelet aggregation therapy. A watch and wait attitude can be adopted for low-risk patients while aspirin alone has to be considered for intermediate-risk patients [93, 94]. Cytoreductive therapy can worsen anemia and must be adjusted depending on hemoglobin levels. Pegylated IFNα can also be discussed in some patients [95]. Finally, HSCT should only be considered in patients with progressive disease considering the morbimortality associated with this procedure, with very limited specific data in MDS/MPN-RS-T [96].

32.5 MDS/MPN, Unclassifiable (MDS/MPN-U)

32.5.1 Epidemiology

Unclassifiable MDS/MPNs are rare entities corresponding to MDS/MPNs not fulfilling the specific criteria of CMML, aCML, or MDS/MPN-RS-T [1]. MDS/MPN-U represent less than 5% of all myeloid disorders.

32.5.2 Presentation and Diagnosis

Patients with MDS/MPN-U often have constitutional symptoms, leukocytosis, thrombocytosis or thrombocytopenia, anemia, and splenomegaly. Organomegaly and peripheral immature myeloid cells seem less frequent than in aCML [61].

Diagnosis results mostly from exclusion of classical MPNs including PMF, and other MDS/MPNs. MDS/MPN-U must be associated with dysplastic features in ≥1 hematopoietic cell line and have less than 20% blasts in the bone marrow. Myeloproliferative features should be associated with a mixture of monocytes, neutrophils, and immature myeloid cells in proportions excluding a diagnosis of CMML or aCML. Prior history of MDS or MPN should be excluded and *BCR-ABL1, PDGFRA*, *PDGFRB*, *FGFR,1* and *PCM1-JAK2* rearrangements such as t(3;3)(q21q26), inv(3)(q21q26), or del(5q) should also be absent (Table 32.1).

The genetic landscape of MDS/MPN-U is variable, with no specific footprint, suggesting that CMML, aCML, and MDS/MPN-U represent a continuum rather than distinct entities [25, 110, 139, 140]. Activating mutations of signaling pathway and mutations in epigenetic regulators or RNA splicing machinery have been described but are not specific [119]. Aside from *JAK2* mutations that can be detected in up to 25% of cases, the mutational spectrum is comparable to that of CMML [126, 140].

32.5.3 Prognosis

Median OS ranges from 12 to 26 months in retrospective studies [106, 140–143]. MDS, but not PMF prognostic classifications, notably IPSS-R, seem to distinguish groups of MDS/MPN-U with different prognoses, although the prognosis of "lower-risk" MDS/MPN-U remains poor [143].

32.5.4 Treatment Algorithm

The rarity of the disease, its heterogeneity, and the absence of clinical trials make patients management difficult. Treatment algorithm is thus inspired from those of other MDS/MPN diseases (Fig. 32.4).

As for other MDS/MPNs, patients considered at higher risk, e.g. based on IPSS-R should be identified and referred rapidly to HSCT. There is no specific data on how to bridge patients to transplant. HMA activity in MDS/MPN-U is suboptimal, with responses in at most 26% of patients [106, 143]. Of note, MDS/MPN-U with *JAK2* mutations may par-

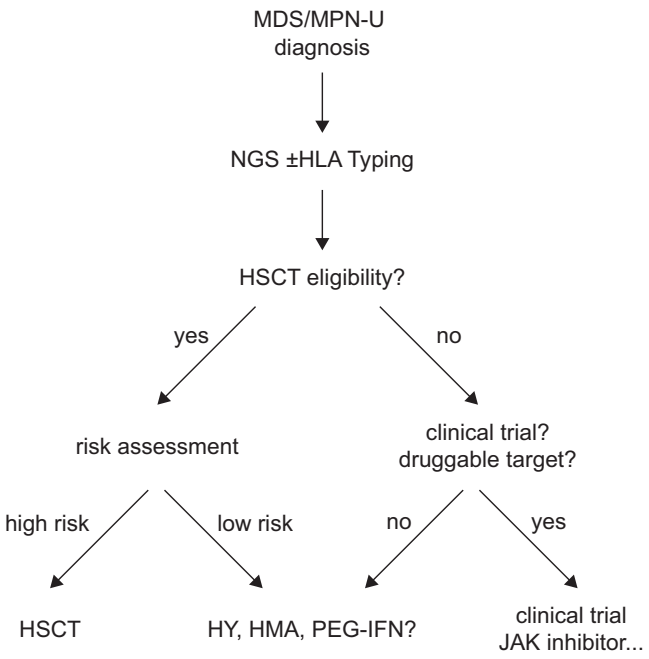

Fig. 32.4 Proposed treatment algorithm for MDS/MPN-U. *ESA:* Erythropoietin stimulating agent; *HSCT:* Hematopoietic stem cell transplantation; *HMA:* Hypomethylating agent; *CML:* chronic myeloid leukemia; *NGS:* next generation sequencing

ticularly benefit from the combination of HMAs with ruxolitinib [103]. Patients should otherwise be included in clinical trials when they are eligible or managed according to the MDS/MPN category with closest vicinity to their presentation.

32.6 Conclusion

MDS/MPN entities are rare myeloid neoplasms of the elderly with overall poor prognosis. Current treatment algorithms are defined empirically, mostly based on retrospective studies, and inspired by data accumulated in MDS and MPNs. This landscape is rapidly evolving, with an international research consortium [33], consensus response criteria [70], and dedicated international trials soon to be initiated (NCT04061421).

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Novel Strategies to Manage Cytopenia in Low-Risk MDS

33

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Abstract

Lower-risk myelodysplastic syndromes (LR MDS) constitute the majority of cases and their clinical manifestation is determined by cytopenias. Improvements in diagnosis and prognostic stratification have been obtained with the application of NGS and advanced flow cytometry. Due to this enhanced characterization, LR MDS may be treated more efficiently with several treatment options currently available. Anemia is indeed the most frequent cytopenia, present in >90% of cases, and there are effective therapies to alleviate it. Erythropoiesis-stimulating agents (ESAs) are known to be active in the majority of cases, but at present, new experimental agents are under evaluation for patients who relapse after ESAs or are refractory to them. Empirically, agents with different mode of action are investigated, like imetelstat and roxadustat. Luspatercept, a TGF-beta pathway inhibitor restoring transfusion independence in nearly half of MDS with ring sideroblasts, has been recently approved. Lenalidomide is the treatment of choice in MDS del5q; in this therapeutic setting, determination of mutant TP53 and its allele burden is crucial. Although chronic transfusions may still be a treatment of LR MDS, optimization of iron chelation therapy has been demonstrated to decrease organ damage and prolong survival.

Thrombocytopenia, although present in around 30% of LR MDS, impacts on overall survival, but there is no standard treatment for it. Thrombomimetic agents are active in decreasing bleeding episodes and increasing platelet number, but have not been approved, most probably because of some concerns linked to their stimulating activity on early hematopoietic progenitors.

Finally, isolated neutropenia is rare in LR MDS. There is no evidence in favor of the use of myeloid-stimulating factors in preventive therapy. Some LR MDS present anemia associated with thrombocytopenia or neutropenia. In these cases, the use of hypomethylating agents, especially the oral formulations, may be considered, with the caveat of inevitable myelosuppression.

Keywords

Low-risk myelodysplastic syndromes · NGS · Thrombocytopenia · Eltrombopag · Romiplostim · Luspatercept

33.1 Introduction

After consolidated morphological diagnosis of myelodysplastic syndrome (MDS), to complete patient evaluation, it is mandatory to assess several clinical variables and determine prognosis within International Prognostic Scoring System-Revised (IPSS-R) [1] that is since several years the most used prognostic tool in practice. IPSS-R distinguishes 5 risk categories, and the cases whose score makes them belong to very low, low, and intermediate categories (the latter with a score ≤3.5) are considered "lower risk" (LR MDS). The majority of patients with MDS have an IPSS-R lower-risk disease: In the Italian National MDS registry (FISiMets) as much as 75% belong to lower-risk IPSS-R categories.

Treatment of LR MDS aims at resolution of symptoms due to cytopenia and subsequently improvement of quality of life. LR MDS present with heterogenous clinical signs and symptoms. Some patients with LR MDS progress to AML quite rapidly and have a survival shorter than that predicted by IPSS-R score. It would then be relevant to detect the characteristics of worse prognosis. Depth of cytopenias determines severity of symptoms and clinical manifestations of the disease. Complications due to chronic anemia, bleeding secondary to severe thrombocytopenia and infection as a

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result of neutropenia may impact on survival and not only on quality of life.

The kinetics of blood count decrease, beyond the steady-state presence of cytopenias at diagnosis, may be an independent outcome indicator in LR MDS [2, 3]. In fact, instead of determining signs of progression by marrow examination, the simple evaluation of the relative drop >25% in platelets at 6 months after diagnosis as investigated in >800 LR MDS patients was demonstrated to predict for significantly shorter overall survival (OS). Together with red blood cell transfusion dependence, platelet drop may constitute an easy and straightforward prognostic classifier, independent of IPSS-R. Equivalent drop in neutrophils does not seem to impact on OS [2, 4].

The special prognostic relevance of thrombocytopenia in LR MDS had already been stressed by the MD Anderson prognostic score [5]. In their model, the characteristics associated with worse survival ($P < 0.01$) at multivariate analysis were low platelets, anemia, older age, higher percent of marrow blasts, and poor-risk cytogenetics. The suggested system allowed to distinguish three categories of LR MDS patients with significantly different survival: from 80.3 months OS for category 1 to category 3 with a median survival of 14.2 months (95% CI 13–18) [5].

33.2 Quality of Life

The presence of chronic anemia is the major cause of poor quality of life in MDS patients. The occurrence of fatigue, scarce concentration, worsened cognitive impairment in elderly patients, poor physical functioning, and cardiac failure is mainly linked to low levels of hemoglobin. On the other hand, thrombocytopenia and neutropenia may contribute to this condition of decreased quality of life [6]. Levels of HQoL correlate with hemoglobin levels, and Hb <10.7 g/dL is the cutoff level below which functional well-being is poorer in patients. Cardiac remodeling appears at the threshold of 10.7 g/dL hemoglobin [7].

Although LR MDS therapies are designed to resolve cytopenias, their efficacy should be monitored not only with increase of hemoglobin, platelets, or neutrophils, but also with patient-reported outcomes. Health-related quality-of-life (HQoL) measurement is increasingly included in clinical trials to evaluate the activity of novel agents, in parallel with clinical improvements [6].

To perform this evaluation, several scoring systems have been designed, only few specific for MDS. The awareness of their importance and correct application to define the effective activity of innovative agents and the real advantage of some treatments, beyond improvement of blood counts, is leading to novel approach to MDS therapy, especially for LR MDS.

Correction of anemia with red blood cell transfusions, although it may save lives, it is not always accompanied by improvement in HQoL. In a recent study, the majority of patients receiving RBC transfusions improved, but 19% declared a decrease of HQoL [8].

It is clear that therapy of LR MDS has to be chosen taking into account these aspects as well.

33.3 Therapy of Thrombocytopenia

As mentioned above, platelet counts influence prognosis of LR MDS and may account responsible for fatal bleeding. Severe hemorrhagic events represent the third cause of MDS-related death (13%), after infections and progression to AML due not only to thrombocytopenia but also to functional defect of platelets typical of MDS [9].

Overall, in the Italian MDS National Registry (FISiMets), only 39% of LR MDS patients at diagnosis had platelets $<100 \times 10^9$/L and a smaller number present with symptomatic or severe thrombocytopenia, but the ones who do have a higher risk of disease progression and complications [5, 9].

At present, there is no approved specific therapy for MDS-related thrombocytopenia [9].

The first intervention in case of severe thrombocytopenia or bleeding is constituted by platelet transfusions from multiple donors and from single-donor apheresis. This emergency therapy should be carefully evaluated before its application, as its efficacy is limited and temporary, while it may induce alloimmunization and, although rarely, transmit bacterial infections. Irradiated products are mandatory in case of LR MDS with a transplant program [10]. Moreover, frequent and chronic platelet transfusions may induce refractoriness [11].

There have been several studies to test the activity of TPO mimetics in MDS.

Eltrombopag is an oral thrombopoietin (TPO) receptor agonist which interacts with the transmembrane domain of c-Mpl, approved for therapy of chronic immune (idiopathic) thrombocytopenia resistant or intolerant to other treatments, hepatitis-C-related thrombocytopenia, and aplastic anemia.

In MDS, eltrombopag has been evaluated in the setting of LR MDS as single drug and in MDS IPSS Intermediate 1 and higher in combination with azacitidine.

In LR MDS with severe thrombocytopenia (platelets $<30 \times 10^9$/L), eltrombopag at 14-day escalating doses of 50–300 mg/daily continuous has induced a significant response compared to placebo in terms of decrease of severe bleeding events (14% vs 42%, respectively) and increase in platelet counts: platelet responses occurred in 47% of patients in the eltrombopag group versus 3% in the placebo group [12]. Consistent results of hematological improvement were obtained in another phase 2 study [13] in which predic-

tors of response to eltrombopag were identified: presence of a PNH clone, marrow hypocellularity, thrombocytopenia with or without other cytopenia, and elevated plasma thrombopoietin levels at study entry. In both studies, some multilineage responses, with increase of hemoglobin and/or neutrophils, were observed [12, 13]. Safety profile was good with apparently no difference in progression of disease and with no expansion of mutated clones [13]. Eltrombopag has been evaluated also retrospectively in "real life setting" in LR MDS and prevalently lower-risk chronic myelomonocytic leukemia (CMML) according to CMML-specific Prognostic Scoring System [14]. Doses of 50–75 mg induced HI-P in 77% of treated patients, according to IWG 2006 criteria [15].

The other specific thrombomimetic agents investigated in LR MDS is romiplostim, whose mechanism of action differs from that of eltrombopag.

Romiplostim is a fusion peptibody TPO analog that increases platelet production via binding and activation of the thrombopoietin (TPO) receptor (c-Mpl). Several study with romiplostim single drug or in association with hypomethylating agents (the latter in higher risk MDS) has been conducted [16–19]. Although efficacy was well demonstrated, the occurrence of marrow blast increase and disease progression during the randomized study somehow impaired further development of investigational trials, even if it was clear at 5-year follow-up that blast increase was transient [20]. In one of the phase 2 study, 46% of patients treated with weekly/biweekly administration subcutaneously (or IV) of 750 μg romiplostim responded, and bleeding events and platelet transfusions were diminished among patients with durable platelet response. The intravenous route of administration was not considered adequate because of adverse reactions. Responses were anyhow confirmed in the randomized trial with platelet responses seen in 36.5% treated cases vs 3.6% in placebo ones [19]. Despite these results, and because of still some concerns, none of the TPO mimetics has been granted approval for treatment of MDS and severe thrombocytopenia remains without a specific approach [21].

An investigational phase 3 randomized trial with cc486, oral azacitidine for LR MDS with transfusion-dependent anemia and thrombocytopenia with median count of 25×10^9/L, was performed. The drug was administered daily for 21 days on a 28 day-cycle at the dose of 300 mg in patient who had a median platelet count. Although the primary endpoint was RBC transfusion independence, cc498 was effective on megakaryocytopoiesis and the rate of HI-P in this population of elderly LR MDS with high-risk clinical features was 24.3% versus 6.5% in the placebo arm [22]. Because of the observation of early deaths in patients with severe baseline neutropenia, the role of cc486 in LR MDS and its application setting has to be assessed in further studies.

Finally, it has to be taken into account that, in LR MDS, thrombocytopenia, especially if particularly severe, may be caused or exacerbated by autoimmune phenomena or overt concomitant autoimmune diseases [23]. To this regard, first-line treatment with corticosteroids yielded 93% responses, equivalent to that obtained in ITP, while intravenous high-dose immunoglobulin was less effective in MDS [23, 24]. Second-line treatments were also effective as in ITP, while incidence of severe bleeding was as expected higher in MDS possibly, due to intrinsic functional defects of platelets [23, 24].

33.4 Therapy of Anemia

Anemia is the most frequent and earlier cytopenia for MDS patients and especially in LR MDS can often be the only one. Anemia is thus the main manifestation of MDS. Relieving its symptoms is the principal goal for the treating physician. Given the fact that MDS affect elderly individuals, anemia may also be multifactorial and may superimpose to the one generated by the dyserythropoiesis typical of this pathology. Severity of symptoms is driven by comorbidities and patient-reported outcomes are essential to define the moment to start therapy.

RBC transfusions may save lives, mainly preventing cardiac events, but there are several disadvantages in this that should be considered an emergency practice. First of all, even if effective in increasing hemoglobin levels, these are fluctuating in the transfusion interval periods, lowering the possible benefit; patients have to depend on hospital organization and, as we have experienced during the current Sars-Cov2 pandemic, shortage of blood may lower the threshold for transfusions.

Finally, chronic transfusions determine iron overload and require ion chelation therapy, whose optimal application, as demonstrated in the only randomized international clinical study performed, may in fact avoid organ damage and prolong survival [25].

Erythropoiesis-stimulating agents (ESAs) are extremely active in LR MDS and avoid or delay inception of chronic RBC transfusions. Their activity was well known since decades, but only recently transfusion independence and significant improvement in hemoglobin levels were confirmed in a randomized trial versus placebo [26]. ESAs have been shown to be active at doses of 40.000/80.000 U/weekly subcutaneously, overall equivalent in terms of erythroid response, with higher doses seemingly more effective in patients with transfusion dependence and higher IPSS-R, although without significant impact on OS [27]. Overall, there was no impact of doses on OS. Predictors of response are as follows: IPSS-R lower-risk categories, transfusion independence, low ferritin levels, isolated ery-

throid dysplasia [28]. The presence and number of somatic mutation impact on OS upon treatment, but there is only a trend to lower response rates in patients carrying several mutations [29]. Finally, in a real-life study significative advantage in OS by ESA treatment was observed in patients with Hb 8–10 g/dL, and with a diagnosis of RA, RARS, or del(5q) [30].

The rate of response in the registration trial was inferior to what previously observed in real life, due to the design of the study itself, with a heterogenous population of transfusion-dependent and transfusion-independent patients, early suspension of drug administration upon optimal response, weight adjusted instead of fixed high dose of erythropoietin alpha (EPO alpha). Anyway, effectiveness was confirmed and EPO alpha has been approved for LR MDS patients with hemoglobin levels <10 g/dL, with endogenous EPO levels <200 U/L [26, 31].

Notwithstanding the fact that majority of LR MDS patients respond to EPO treatment, this response is transient and almost all of them in the end will need RBC transfusions. In a category of patients with expectation of long survival, loss of response/relapse to chronic transfusions may determine a real significant poor quality of life and thus there is a need for alternative treatments relieving from transfusion dependence [32, 33].

In this sense, several agents have been tested in ESA relapsed/refractory LR MDS patients. A very interesting approach was that aimed at targeting TGF-beta pathway. TGF-beta activation inhibits maturation of late-stage erythroblast progenitors and it is upregulated in MDS cells, where SMAD7 expression, negatively regulating it, is decreased. Oral agent galunisertib is a selective inhibitor of TGF-β receptor I kinase (ALK5) that inhibits SMAD2/3 activation. Its use in transfusion-dependent ESA relapsed/refractory LR MDS yielded 24.4% erythroid response, with predictors of response were markers of stem cell differentiation block or SF3B1 mutation [34].

Treatment of ESA relapsed/refractory LR MDS with luspatercept was indeed more successful, especially in the subgroup of MDS with ring sideroblasts [35, 36]. Luspatercept is a ActRIIB/IgG1 Fc recombinant fusion protein fusion protein that traps selected TGF-β superfamily ligands (including GDF-11 and Activin B) and reduce Smad2/3 signaling [36]. Although active in all subtypes of MDS [35], in MDS-RS luspatercept, at a dose of 1–1.75 mg/kg subcutaneously every 21 days, induced 47.7% transfusion independence >8 weeks in treated patients versus 15.8% in placebo ones [36]. On the basis of these results, luspatercept, having shown a very safe profile, completely devoid of myelosuppressive effects, has been approved in 2020 as second-line treatment for ESA refractory/relapsed MDS-RS patients, both by FDA and by EMA. More studies are ongoing comparing luspatercept with ESA in front line transfusion-dependent LR MDS and in association with ESAs in different settings.

Another agent that has raised relevant interest is imetelstat. It is a competitive telomerase inhibitor with monthly IV administration that has shown activity in non-del5q LR MDS lenalidomide and HMA-naïve patients [37]. In this setting, 42% of cases became transfusion independent for >8 weeks upon treatment and such responses were of long duration, with one-third of cases still responsive after 1 year of treatment. While the randomized phase 3 clinical study versus placebo is still ongoing, it has to be stressed that imetelstat shows on target efficacy, diminishing telomerase activity and hTERT expression, but apparently has also disease-modifying activity, because treated patients significantly decrease VAF of SF3B1 mutation, both events correlated with transfusion independence [37].

Other agents with different mechanisms of action are under evaluation, like roxadustat, an oral HIF-alpha hydroxylase inhibitor approved in some countries for treatment of anemia of chronic kidney failure [38]. Preliminary results demonstrate achievement of transfusion independence >8 weeks in 38% of LR MDS patients. The limit of this agent seems to be the need of specific range of endogenous EPO levels to obtain response. More results are awaited, together with the preliminary ones related to agents with spliceosome inhibitory activity still under investigation as natural products or of specific synthesis [39]. In particular, studies with target therapy, like mutant IDH1 and IDH2 inhibitors ivosidenib and enasidenib, are ongoing also for LR MDS.

A subset of LR MDS whose response to ESAs is rather limited and of short duration is constituted by MDS del5q. These patients may have good hemoglobin increase with ESAs, but they finally lose response and become transfusion dependent in the totality of cases. Since more than a decade, lenalidomide is known to relieve anemia and determine transfusion independence in MDS del5q [40]. In the pivotal study [40], 83% of patients achieved erythroid response upon treatment with 10 mg/day of lenalidomide. Later [41] it has become clear, applying more stringent criteria, that this oral agent may induce transfusion independence >26 weeks in 43–56% of cases [41].

Lenalidomide induces transfusion independence and hematological improvement in MDS del(5q) via ubiquitination and degradation of casein kinase by the E3 ubiquitin ligase [42]. Optimal response, with achievement of cytogenetic complete response (CCyR), is accompanied by myelosuppression, while lenalidomide-CCyR is lower in TP53-mutated patients. Therefore, it has been considered that mutant TP53 predicts poor outcome and progression and likewise does the presence of multiple (>2) cytogenetic abnormalities, beyond del5q [43]. These observations have raised concern even on the opportunity to treat with lenalidomide TP53 mutated patients and patients with complex

karyotype, in fear of acceleration of clone selection and progression. Recently, it was clarified that if TP53 mutation allelic imbalances are analyzed, a difference is apparent between TP53-mutated patients with multiple hits (multi-hit) consistent with biallelic targeting (frequently associated with complex karyotype and the traditionally known poor outcome) and patients with monoallelic TP53 mutation, characterized by a better prognosis [44]. In MDS del5q treated with lenalidomide, probability of overall survival is significantly shorter in the presence of multi-hit mutations of TP53, while monoallelic mutations yield survival curves superimposable to wild-type MDS del5q [44]. This observation adds to the mandatory determination of the presence of mutated TP53, the absolute importance of evaluation of its allelic state before starting therapy with lenalidomide in LR MDS del5q patients.

Lenalidomide treatment has been evaluated as second-line approach also in non-del5q LR MDS patients who had become transfusion dependent after ESA failure or deemed ineligible for it [45]. In fact, off-label use of lenalidomide is quite frequent. In a SEER Medicare database evaluation, among 676 lenalidomide-treated patients only 21% were MDS del(5q), and only 40.7% were transfusion dependent. An international randomized phase 3 trial of lenalidomide versus placebo was thus deemed necessary and demonstrated that 27% of patients achieved transfusion independence and these advantageous results were more significant in LR MDS cases with EPO levels <100 U/L (45% of response), in those with prior ESA therapy and in those without ASXL1 mutation [46]. Although lenalidomide impacted on outcome only for a minority of LR MDS, HQoL was not worsened by this treatment [47], indicating the possibility of its use in selected cases. Anyhow, the drug was not submitted for the approval of health authorities and its development in non-del5q cases stopped.

33.5 Therapy of Neutropenia

Isolated neutropenia is a rare finding in LR MDS [48] and it is generally not associated with worsened survival. Prophylactic therapy with granulocyte colony-stimulating factors is not recommended as it does not decrease the number of infective episodes or prolongs OS [49]. Patients with severe confirmed chronic neutropenia and predisposing risk factors (i.e., diabetes) who experience recurrent infections should instead receive prophylactic antibiotics and the addition of growth factors should be considered during severe infections. Neutropenia is the cytopenia that is more difficult to alleviate, even with treatment with hypomethylating agents (HMAs). The occurrence of the concomitant component of autoimmune neutropenia [23] or the preexistence of congenital neutropenia [50] should also be taken into account. Overall, there is scarce evidence and no randomized trials are available to conclude on the clinical relevance of treatment with myeloid growth factors in neutropenic LR MDS [51].

33.6 Therapy of Pancytopenic MDS

In the great majority of cases, LR MDS present with isolated anemia, and as indicated above, even more rarely with isolated neutropenia or thrombocytopenia. There are anyhow LR MDS cases characterized by anemia and thrombocytopenia and normal or good risk karyotype that are therefore included in the lower-risk IPSS-R categories. In these cases, HMA is quite effective in normalizing counts. Treatment of LR MDS with HMAs is not approved in Europe, but early studies confirmed their efficacy [52] and are used currently in many countries. Addition of ESAs to azacytidine did not increase response rates [53]. Although not devoid of disadvantages: chronic need of treatment, myelosuppression, dependence on hospital care, sometimes HMAs are the only therapeutic alternative available. The availability and approval of oral HMAs open new horizons for total oral treatment and home management of LR MDS with pancytopenia [54]. A recently analyzed investigational study of CC486 in LR MDS cases with high-risk features indicated a bilineage response rate of 23%, but not for neutrophils. To this point, severe baseline neutropenia worsened by the first cycles of therapy and drove to sepsis and early death. These observations have imposed further evaluation of this drug, possibly with strict selection of patients, as mentioned above [22].

Lower dose and different schedule of HMAs have proven effective and well tolerated in LR MDS [55].

A therapeutical approach that is underutilized is certainly the immunosuppressive one.

There are several available immunosuppressive regimens that have been investigated in MDS, with extremely different response rates and a clear bias in interpretation of results: scattered studies, very limited number of cases in each study, patients belonging to all IPSS-R categories and not selected for LR IPSS, different endpoints taken into consideration. Agents used alone or in combination are alemtuzumab, cyclosporine, etanercept, horse ATG and rabbit ATG, sirolimus.

Overall response rate to all immunosuppressive treatments was 48.8% (30% CR) when evaluated in a retrospective study of a large cohort of patients [56] and it was confirmed (42.5%) in a recent meta-analysis including 13 clinical trials with >500 treated patients [57]. The most common regimens were anti-thymocyte globulin alone or in combination with cyclosporin with a trend toward higher response rates with combination therapy. OS was signifi-

cantly improved in responsive patients [56]. While predictors of response were not evaluable in the meta-analysis, in the retrospective study, achievement of response, especially erythroid, was associated with a hypocellular bone marrow (cellularity <20%); use of horse ATG plus cyclosporine versus rabbit ATG or ATG without cyclosporine. In this study age, transfusion dependence, presence of paroxysmal nocturnal hemoglobinuria or large granular lymphocyte clones, and HLA DR15 positivity did not predict response, not consistently with sporadic previous reports. Toxicity and myelosuppression of ATG impair their use in the elderly LR MDS population and may be the main cause underutilization of this possibly effective treatment.

Finally, the only curative option for MDS is indeed hematopoietic stem cell transplant (HSCT), especially when pancytopenia is threatening survival. The most recent recommendations for LR MDS [58] indicate for fit patients with very low, low, or intermediate IPSS-R risk score the need to program transplant strategy in the first line for those with high-risk features (poor-risk cytogenetic characteristics, persistent blast increase [>50% or with >15% BM blasts], *life-threatening cytopenias [neutrophil counts, <0.3 × 109/L; platelet counts, <30 × 109/L], high transfusion intensity ⩾2 units per months for 6 months).* For the LR MDS patients without such characteristics, transplant should be programmed only after failure of previous standard or investigational therapies [58, 59]. The choice to transplant LR MDS can in fact be supported by NGS evaluation of somatic mutations. Recurrent somatic mutation characterizes >90% of MDS, is generally less numerous in LR MDS than in higher risk MDS, and increases during the natural history and progression of the disease, but their presence, nature, and number may suggest earlier decision to transplant based also on predicted outcome [60].

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Allogeneic Hematopoietic Stem Cell Transplantation for MDS and CMML: When and How?

34

Harinder Gill, Yammy Yung, Cherry Chu, and Amber Yip

Abstract

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) remains the only curative treatment in both MDS and CMML. In MDS, there is an increasing prevalence of high-risk MDS undergoing HSCT, whereas only a selected group of patients with low-risk MDS will receive standard or low-intensity HSCT for permanent disease eradication. Other than the disease risk itself, patients' age, comorbidities, performance status, and disease risk act as limiting factors to the efficacy of HSCT in both MDS and CMML patients. Hence, not all patients are eligible for transplantation. Prognostic markers should be carefully evaluated before considering HSCT. In this chapter, we review the indications of allo-HSCT in MDS and CMML.

Keywords

Myelodysplastic syndrome · Chronic myelomonocytic leukemia · Allogeneic hematopoietic stem cell transplantation

34.1 Introduction

Myelodysplastic syndrome (MDS) and chronic myelomonocytic leukemia (CMML) are both clonal hematopoietic stem cell disorders which display heterogeneity. The former is characterized by ineffective hematopoiesis with features of cytopenia and dysplasia [1, 2], while the latter is characterized by both dysplastic and proliferative components [3–5]. The 2016 World Health Organization (WHO) classification divides MDS into seven subtypes based on the number of dysplastic lineages, cytopenia, amount of ring sideroblasts in erythroid lineage of bone marrow, the percentage of blasts in peripheral blood and bone marrow, and the cytogenetic abnormalities. Table 34.1 summarizes the diagnostic criteria and classification of CMML according to the 2016 WHO classification [6].

Allogeneic hematopoietic stem cell transplantation (Allo-HSCT) remains the only curative treatment in both MDS and CMML. In MDS, there is an increasing prevalence of high-risk MDS (HR-MDS) undergoing HSCT, whereas only a selected group of patients with low-risk MDS (LR-MDS) will receive standard or low-intensity HSCT for permanent disease eradication [7]. Other than the disease risk itself, patients' age, comorbidities, performance status, and disease risk act as limiting factors to the efficacy of HSCT in both MDS and CMML patients [8]. Hence, not all patients are eligible for transplantation. Prognostic markers should be carefully evaluated before opting for HSCT. They can be generally classified under two major categories, patient-related and disease-related factors [9, 10].

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Table 34.1 Diagnostic criteria and classification of CMML according to the 2016 WHO classification

Diagnostic criteria			Remarks
Clinical	1. Monocytosis. – Persistent monocytosis in the peripheral blood: ≥13 × 10^9/L; – monocytes accounting for ≥10% of WBC count, 2. <20% blasts in the peripheral blood and bone marrow 3. Dysplasia in ≥1 myeloid lineage(s)		If dysplasia is not absent/ minimal, diagnosis made by: – Fulfillment of other criteria – Presence of an acquired clonal cytogenetic/ molecular genetic abnormality in myeloid cells – Persistence of monocytosis for ≥3 months with exclusion of other causes
Exclusion of other disease	4. Not meeting WHO criteria for *BCR-ABL1* CML, PMF, PV, or ET 5. No evidence of *PDGFRA*, *PDGFRB*, or *FGFR1* rearrangement or *PCM1-JAK2* (should be specifically excluded in cases with eosinophilia)		
Subcategory			*Remarks*
	Blast % in PB	*Blast % in BM*	*Presence of auer rods*
CMML-0	<2	<5	No
CMML-1	2–4	5–9	No
CMML-2	5–19	10–19	Yes
Dysplastic type	– WBC in peripheral blood: <13 × 10^9/L		
Proliferative type	– WBC in peripheral blood: ≥13 × 10^9/L		

Abbreviations: *CMML*: chronic myelomonocytic leukemia; *CML*: chronic myeloid leukemia; *PMF*: primary myelofibrosis; *PV*: polycythemia vera; *ET*: essential thrombocythemia; *PB*: peripheral blood; *BM*: bone marrow

34.2 Disease-Related Factors in MDS

Revised international prognostic scoring system (IPSS-R) is the standard of risk stratification of MDS patients at diagnosis. In clinical practices, a dichotomous classification (low vs high risk) is utilized for making treatment plan. Despite a higher predictive potential of IPSS-R in assessment of overall survival (OS) and time to transformation in MDS patients (Dxy values of 0.43 vs 0.33 and 0.53 vs 0.44, respectively) in comparison with the dichotomous risk stratification [11], the limited treatment availability only allows the simplified classification for treatment selection [12, 13]. LR-MDS consists of patients of IPSS-R very low, low, and intermediate risk with score of ≤3.5, whereas HR-MDS comprises of patients of IPSS-R intermediate risk group with score >3.5, high- and very high-risk groups [12]. Both methods displayed no dynamic properties with progressive loss of prognostic power over time. Nonetheless, treatment primarily emphasizes on supportive care for LR-MDS. On the other hand, more intensive or aggressive treatments are offered to HR-MDS with the use of hypomethylating agents (HMAs) or allo-HSCT in general [14].

In addition, Della Porta et al. demonstrated a higher hazard ratio (HR) of IPSS-R lower risk in patients after transplantation in comparison with that of the non-transplanted patients owing to risk of non-relapse mortality (NRM). HRs showed otherwise in IPSS-R high-risk patients, where mortality of non-transplanted patients was higher than that of the transplanted [15]. Occasionally, HSCT may also be considered in lower risk, younger patients depending on the presence of additional poor prognostic markers, severe cytopenia, heavy transfusion burden, poor genetic risks, or upon disease progression [16]. However, no consensus on the selection of lower risk individuals for HSCT has yet been reached. In addition, karyotypic abnormalities in IPSS-R, in particular, exhibit great prognostic value following HSCT with high rate of relapses and shorter OS in "very poor" and "poor" cytogenetic risk group of IPSS-R [10, 17]. Another prognostic score, WHO classification-based prognostic scoring system (WPSS), may also help provide a dynamic risk stratification for MDS patients. When disease deteriorates and shifts from lower risk to WPSS intermediate risk, HSCT may be considered [10]. Despite the wide acceptance of IPSS-R, or even other prognostic models (e.g., WPSS) developed over the past for prognosis of OS and risk of leukemic transformation in MDS patients since 2012, these prognostic tools are not specific for HSCT. Further investigations are required to produce a better model for patient selection for HSCT.

34.3 Disease-Related Factors in CMML

Numerous prognostic systems have been designed for personalized risk stratification in CMML patients and were covered in previous chapter. With deeper understanding in the molecular landscape of CMML, high-risk molecular mutations are integrated into current prognostic models. This includes CPSS-mol, Mayo molecular model (MMM), and GroupeFrançais des Myélodysplasies (GFM) [18–21]. However, consensus has not been reached in the treatment of CMML patients. Allogeneic hematopoietic stem cell transplantation (allo-HSCT), the only potential curative treatment, remains loosely codified and decision varies with physician's discretion. In general, disease factors, patient comorbidities, and donor availability are the three cardinal factors to be considered.

Disease status is a vital factor to be considered when choosing the most appropriate allo-HSCT candidate. Among

Table 34.2 CMML-specific prognostic scoring system

	Score		
Criteria	0	1	2
WHO subtype	CMML-1	CMML-2	/
FAB subtype	Dysplastic type	Proliferative type	/
CMML-specific cytogenetic risk classification	Low	Intermediate	High
RBC transfusion dependency	No	Yes	/
Risk groups and score			
Low	0		
Intermediate-1	1		
Intermediate-2	2–3		
High	4–5		

CMML-specific cytogenetic risk classification: low: normal and isolated –Y; intermediate: other abnormalities; high: trisomy 8, complex karyotype (≥3 abnormalities), and abnormalities of chromosome 7
RBC transfusion dependency: ≥1 RBC transfusion every 8 weeks for 4 months
CMML: chronic myelomonocytic leukemia; *FAB*: French–American–British; *RBC*: red blood cells; *WHO*: World Health Organization

Table 34.3 CMML transplant score

Criteria	Points
Every 1-mark increase in the comorbidity index	1
>2% blasts in the bone marrow	4
ASXL1- and/or *NRAS*-mutated	4
Risk groups (total marks: 20)	5-year survival
0–1	81%
2–4	49%
5–7	43%
8–10	31%
>10	19%

ASXL1: additional sex combs like 1; *CMML*: chronic myelomonocytic leukemia; *NRAS*: neuroblastoma RAS viral oncogene homolog

the different prognostic systems available, the CMML-specific prognostic scoring system (CPSS) is widely espoused [10, 22] (Table 34.2). Patients grouped under intermediate-2 and high risk shall be prioritized for allo-HSCT [10, 23]. IPSS-R could also be used for CMML patients with dysplastic subtype [10].

However, CPSS is designed and is only validated in non-transplant settings. Therefore, a new prognostic model is developed recently [24] (Table 34.3). The CMML transplant score incorporated genetic information and clinical status for patient selection in transplant settings. It was shown to be superior over CPSS as it failed to demonstrate the association of non-relapse mortality (NRM) and risk stratification. Better risk stratification could also be achieved when comparing to molecular CPSS (CPSS-mol), Mayo Molecular model (MMM), and GroupeFrançais des Myélodysplasies (GFM) [24].

Disease burden could be minimized by pretransplant HMAs [10, 21, 25]. A superior transplant outcome such as longer OS, and higher engraftment rate could be achieved by receiving allo-HSCT at an earlier disease stage as comorbidities and new mutations might be acquired with time [26–29]. This was proven by the significantly longer 5-year overall survival (OS) in CMML patients in chronic phase compared to those in blast phase (51% vs 19%) [30]. Therefore, high-risk CMML patients with stable disease status should be prioritized and receive allo-HSCT as soon as possible to maximize benefits and minimize risks.

34.4 Patient Factors in Both MDS and CMML

Efficacies of HSCT in MDS and CMML are dependent on several factors. Apart from the disease risk per se, patient's characteristics such as age, comorbidity, and performance status also contribute. All fit patients should be considered for HSCT unless contraindicated by the poor disease risk and patient's intrinsic factors.

34.4.1 Age

Allo-HSCT serves as the only conventional curative treatment until the age of 65–70. Patient's age serves as an independent and crucial prognostic factor in evaluating patient's tolerability to treatment [31]. Advanced age is often associated with treatment-related mortality (TRM) [32], as elderly patients have diminished ability to withstand high-intensity treatment in HSCT. Conversely, Mcclune et al. demonstrated no significant differences in NRM, relapse rates, disease-free survival (DFS), and overall survival (OS) at 2 years were demonstrated across all 4 age groups (40–54 vs 55–59 vs 60–64 vs ≥65) with rates of 33–39%, 25–29%, 32–39%, and 35–45%, respectively [33]. However, association between age and conditioning intensity was elucidated in a study conducted by Keller et al. [34]. The ability of the patients to

withstand HSCT and higher intensity conditioning regimen decreases with growing age. Therefore, the incorporation of age to the making of HSCT decision is essential.

Besides, the choice of conditioning regimens varies with protocols in different countries. Reduced-intensity conditioning (RIC) and non-myeloablative conditioning (NMA) regimens have increasingly been used as most MDS and CMML patients present as an advanced age with comorbidities. The emergence of these regimens offers older and less fit patients options to receive HSCT with diminished and acceptable degree of TRM and NRM [35, 36]. This has led to a rising number of elderly patients with hematologic malignancies opting for HSCT [32]. However, disease relapse remains a concern and post-transplant donor lymphocyte infusion (DLI) might be helpful in these settings [36]. Therefore, it is vital to weigh the risks and benefit with cautions before deciding on the final treatment plan of patients.

34.4.2 Comorbidity

Several comorbidity indices have been developed over the past few decades. Currently available indices include the Charlson Comorbidity Index, CCI [9]; MDS-Specific Comorbidity Index, MDS-CI; and the Hematopoietic Cell Transplantation-Specific Comorbidity Index, HCT-CI.

CCI was established in 1987 with a longstanding history and was widely used for risk assessment in various medical conditions [37]. However, the CCI did not thoroughly capture the frequent comorbidities present in elderly patients. Besides, the comorbidities were not completely compatible with those of the transplant candidates. Owing to the limited sensitivity of CCI, the index was deemed unsuitable for comorbidity assessment in hematologic diseases [38]. Modification has been made based on the old CCI. This has led to the development of HCT-CI in 2005 by Sorror et al. The Pavia group has also specifically created a comorbidity score for MDS, the MDS-CI. However, no specific indices have been designed for CMML. Yet, Moreno Berggren et al. have validated the aforementioned indices in CMML, yet demonstrated no statistically significant differences between the predictive power of these indices in CMML with a C-index of 0.62, 0.61, and 0.59 in CCI, HCT-CI, MDS-CI, respectively [39]. HCT-CI in CMML has also been validated by Eissa et al. with significantly reduced survival rate of 26.6% (vs 52.7%) with higher HCT-CI score of ≥3 (vs <3) [40]. In MDS, HCT-CI is superior than CCI for predicting NRM and OS with the likelihood ratios (LRs) of 26.6 (vs 1.2) and 31.8 (vs 7.6), respectively [38]. Elsawy et al. also demonstrated higher discriminative ability of HCT-CI than CCI in both NRM and survival with a c-statistic value of 0.692 (vs 0.546) and 0.661 (vs 0.561), respectively [41]. These render HCT-CI a superior prognostic score with high sensitivity for comorbidity assessment. It also serves as strong predictors of NRM [38, 42, 43] (Table 34.4).

Table 34.4 HCT-CI risk category and impact on post-transplantation outcome [38]

HCT-CI risk group	HCT-CI score	NRM at 2 years (%)	Survival (%)	Median OS (months)
Low	0	14	71	Not reached
Intermediate	1–2	21	60	
High	≥3	41	34	14

HCT-CI: hematopoietic cell transplantation-specific comorbidity index; *NRM*: non-relapse mortality; *OS*: overall survival

The HCT-CI encompasses 17 categories of potential organ dysfunction present in HSCT patients. Comprehensive assessment of various organ dysfunction or impairment will be done prior to transplantation (Table 34.5). The inclusion of investigational criteria allows more accurate delineation of the degree of organ damage which enhances the chance of identification or capturing of patients with those comorbidities. The lower the HCT-CI score, the better the outcome post-transplantation. Apart from predicting outcome of transplantation, Sorror et al. also showed that the index can potentially provide prediction on the severity of post-transplantation graft-versus-host disease (GVHD). The higher the HCT-CI score, the higher the probability of developing grade III-IV GVDH [44]. Further investigations are paramount in establishing a better prognostic model in prediction of post-HSCT outcomes.

The choice of conditioning regimens varies with protocols in different countries. Reduced-intensity conditioning (RIC) has been increasingly used as most MDS and CMML patients present as an advanced age with comorbidities. This is important to diminish NRM and treatment-related morbidity which has been associated with myeloablative conditioning [36]. However, disease relapse remains a concern and post-transplant donor lymphocyte infusion (DLI) might be helpful in these settings [36].

34.4.3 Performance Status

The performance status or functional ability of MDS or CMML patients can be evaluated via two available assessment tools, the Karnofsky Performance Status (KPS) and Eastern Cooperative Oncology Group Performance Status (ECOG PS). It provides a gross reflection of patient's health status. Patients with KPF ≥80% or ECOG PS ≤2 should be considered for HSCT [13]. Table 34.6 summarizes the three categories of medical fitness in MDS patients that can help guide treatment decisions [13].

Table 34.5 HCT-CI scoring criteria [45]

Comorbidities	Score
Cerebrovascular	
Cerebrovascular disease (any prior diagnosis) • TIA • SAH • Cerebral thrombosis • Cerebral embolism • Cerebral hemorrhage	1
Psychiatric disorder	1
Cardiovascular	
Arrhythmia	1
Cardiovascular comorbidity (≥1) • CAD • CHF • Low EF	1
Valvular disease (≥1) • ≥ moderate/ severe valvular stenosis/ insufficiency determined by ECHO • Prosthetic mitral/ aortic valve • Symptomatic mitral valve prolapse	3 (24)
Pulmonary	
Moderate pulmonary comorbidity (≥1) • DLCO = 66–80% • FEV1 = 66%–80% • SOB on slight exertion due to pulmonary disease that cannot be corrected by blood transfusion.	2 (24)
Severe pulmonary comorbidity (≥1) • DLCO%: ≤65% • FEV1 ≤ 65% • SOB at rest due to pulmonary disease that cannot be corrected by blood transfusion • Requirement of intermittent or continuous oxygen supplementation	3 (24)
Gastrointestinal	
Inflammatory bowel disease	1
Peptic ulcer	2
Hepatobiliary	
Mild hepatic comorbidity (≥1) • TBili > ULN & ≤1.5 × ULN • ALT or AST > ULN & ≤2.5 × ULN History of HBV/HCV infection	1
Moderate/ severe hepatic comorbidity (≥1) • TBili >1.5 × ULN • ALT or AST >2.5 × ULN • History of liver cirrhosis	3 (24)
Renal	
Renal comorbidity (≥1) • Increase in serum Cr >2 mg/dL or > 176.8 μmol in ≥2 tests on 2 different days within D-24 to D-10 before HCT • CKD requiring weekly dialysis 4 weeks prior the landmark date	2 (24)
Rheumatologic	
Rheumatologic comorbidity • SLE, RA, Sjögren's syndrome, scleroderma, etc.	2
Others	
Obesity • >18 years old: BMI > 35 kg/m^2 • ≤18 years old: ≥95th percentile	1
Diabetes or steroid-induced hyperglycemia	1
Infection (≥1) • Documented infection (e.g., culture/ biopsy) • PUO. • Suspected fungal pneumonia with pulmonary nodules • +ve PPD skin test requiring anti-tuberculous prophylaxis	1
Prior malignancy	3

ALT: alanine aminotransferase; *AST*: aspartate aminotransferase; *BMI*: body mass index; *CAD*: coronary artery disease; *CDK*: chronic kidney disease; *CHF*: congestive heart failure; *Cr*: creatinine; *DLCO*: diffusing capacity of the lungs for carbon monoxide; *ECHO*: echocardiogram; *EF*: ejection fraction; *FEV1*: forced expiratory volume in 1 s; *HCT*: hematopoietic cell transplantation; *HCT-CI*: Hematopoietic Cell Transplantation-Specific Comorbidity Index; *PPD:* purified protein derivative test; *PUO*: pyrexia of unknown origin; *RA*: rheumatoid arthritis; *SAH*: subarachnoid hemorrhage; *SLE*: systemic lupus erythematosus; *SOB*: shortness of breath; *Tbili*: total bilirubin; *TIA*: transient ischemic attack; *ULN*: upper limit of normal

Table 34.6 Impact of medical fitness on treatment decision in MDS [13]

Medical fitness	Score	Recommendation	Alternative solution
Fit	ECOG PS: 0–1 HCT-CI: 0	HSCT	HMA Chemotherapy Clinical trial
Intermediate fitness	ECOG PS: 2 HCT-CI 1–2	HMA	Clinical trial
Frail	ECOG PS ≥3 HCT-CI ≥3	Supportive management	Clinical trial

ECOG PS: Eastern cooperative oncology group performance status; *HCT-CI*: hematopoietic cell transplantation-specific comorbidity index; *HMA*: hypomethylating agent; *HSCT*: hematopoietic stem cell transplantation; *MDS*: myelodysplastic syndrome

34.5 Donor Availability

Selection of patients for allo-HSCT depends on donor availability. Donor source could be divided into 4 categories: matched related donor (MRD), haploidentical donor, matched unrelated donor (MUD), and mismatched unrelated donor (MMUD) [46, 47]. Full-matched donors are most preferred as increased HLA-mismatch is associated with increased risk of non-engraftment and GVHD [8, 48]. Haploidentical transplantation could also be performed in MDS patients but its safety and efficacy remain questionable in CMML patients [47]. Interestingly, improved OS and relapse-free survival (RFS) was displayed using 10/10 MUD in CMML patients [46]. This might be contributed by the younger MUD age and in vivo T-cell depletion in the study [46]. Yet, other researches showed similar outcomes with related and unrelated donors in CMML patients [26].

Peripheral blood stem cell (PBSC) mobilization, bone marrow aspiration, and umbilical cord blood cells are stem cell sources for allo-HSCT [8]. PBSC mobilization has been most frequently used in all transplant patients including MDS and CMML as it holds a significantly superior overall and quality-adjusted life expectancy [8, 49, 50]. The use of umbilical cord blood is least preferred as it is associated with a raised TRM within 100 days of transplantation, reduced engraftment rate, and poorer OS [28].

34.6 Conclusion

All in all, existing prognostic systems for HSCT in MDS and CMML still await optimization with higher specificity and sensitivity in predicting post-transplantation outcomes in patients. Before opting for HSCT as final treatment decision, risks and benefits should be carefully assessed.

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In the Pipeline: Emerging Therapy for MDS and MDS/MPN

35

Harinder Gill, Emily Lee, and Pinky Mo

Abstract

Novel agents in combination with hypomethylating agents are an emerging strategy for clinical trials in involving higher-risk MDS or MDS/MPN and in patients harbouring high-risk mutations such as those involving *TP53*. In this chapter, we highlight the important pathogenetic pathways in MDS and MDS/MPN and their targeting.

Keywords

Myelodysplastic syndrome · Myelodysplastic syndrome/myeloproliferative neoplasm · Targeted therapy · Novel therapy

35.1 Introduction

35.1.1 Myelodysplastic Syndrome

Myelodysplastic syndrome (MDS) is a clonal haematopoietic stem cell disorder leading to the bone marrow (BM) producing dysplastic cells and cytopenia in more than one lineage, due to ineffective haematopoiesis [1]. Clinically, it is an indolent disease, which presents with variable degrees and complications of neutropenia, anaemia, and thrombocytopenia. MDS has a 2:1 male preponderance, median age of 70 years and is characterized by the presence of ≤20% blasts in the BM or peripheral blood (PB) [2–4]. However, patients may be asymptomatic and diagnosed via incidental findings for other diseases, or on routine health check-ups [2, 5].

The major complication is a 20% risk of clonal progression and transformation into secondary acute myeloid leukaemia (sAML) [6], where AML is defined by the presence of ≥20% abnormal blasts in the PB or BM [6]. As a result of the variable disease outcomes, prognostic tools have been developed for individual patient risk stratification, for example the revised International Prognostic Scoring System (IPSS-R) for determining the appropriate treatment regimen for patient management [7]. Treatment aims for symptomatic control, improvements in quality of life (QoL), alteration of the disease's natural history, inducing complete response (CR), and prolonging the duration of progression-free and overall survival (OS) [8–11]. Allogeneic-haematopoietic stem cell transplantation (allo-HSCT) is the single curative therapy for patients with MDS [12], and the IPSS-R is an important tool used when determining candidate suitability for undergoing allo-HSCT [13].

35.1.2 Myelodysplastic Syndrome/Myeloproliferative Neoplasm

Myelodysplastic syndrome/myeloproliferative neoplasm (MDS/MPN) is a recently established paradoxical disease entity comprising of overlapping features of MDS and MPN in relation to pathogenesis, clinical manifestations, therapeutic management, cytogenetics, and molecular genetics [14, 15]. This group of clonal haematologic disorders is classified into five distinct disease entities, namely chronic myelomonocytic leukaemia (CMML), BCR-ABL-negative atypical chronic myeloid leukaemia (aCML), juvenile myelomonocytic leukaemia (JMML), MPN/MDS with ring sideroblasts and thrombocytosis (RS-T), and MDS/MPN-unclassifiable (MDS/MPN-U) [14, 15]. Characterized by 1 lineage of proliferation, ≥ 1 lineage of dysplasia, and ≥ 1 lineage of dysplasia, patient presentation depends on the

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extent of abnormal cellular proliferation and dysplasia ranging from constitutional symptoms (e.g., fatigue, fever, weight loss), organomegaly, thrombocytosis, leukaemic infiltration to various organs (e.g. lungs, liver, spleen, skin), and complications of cytopenia(s) [14, 15].

MDS/MPNs are extremely rare with male predominance [16, 17]. CMML is the most common entity with an incidence of 1/100,000 and median age of 70 years [16–19]. The incidence of JMML is 0.12/100,000, with a median age of 2 years [20, 21]. aCML occurs in <1/100,000 while MDS/MPN-U accounts for around 2% and MDS/MPN-RS-T for 1% of MDS patients [22–24].

The diagnostic work-up of MDS/MPN is similar to that of MDS [14, 25]. However, given the extent of similarities between MDS and MPN, the ability of classical MPNs to evolve and exhibit dysplastic features during disease progression and the lack of disease-defining aberrations, accurate diagnoses, and therapy remains exceedingly challenging for patients with MDS/MPN [14, 15].

Clinical courses can range from indolent to rapidly progressive, and the risk of transformation into secondary acute myeloid leukaemia (sAML) remains unknown and highly variable, with reported incidences of 15–20% for CMML, 1–2% for MDS-RS-T and up to 40% for aCML [15, 16, 26–32].

35.2 Pathogenesis

35.2.1 Pathogenesis of MDS

DNA methylation is one of many common epigenetic modifications for transcriptional regulation [33]. DNA methyltransferases (DNMT) catalyse the addition of a single methyl group at the 5-carbon position of cytosine residues into 5-methyl-cytosine, inducing a structural change in DNA to alter its binding affinity to transcriptional factors for transcription inhibition [33, 34]. Various mutations involved in epigenetic maintenance such as HDAC, TET1/2, IDH1/2, EZH2, STAG2, SF3B1, SRSF2, U2AF1, DNMT3A, and ASXL1 have been reported to occur and contribute to the development of MDS [33, 35–37].

Aberrant DNA methylation resulting in epigenomic dysregulation dominates the pathogenic mechanisms involved in the development of MDS [38]. Genes involved in the control of cell-cycle progression (p14, p15 $^{INK-4B}$, p16, p51), tumour suppression (e.g. TP53, FHIT), apoptosis (e.g. DAPK, p73, survivin), cellular differentiation (WT1, RAB), and DNA repair (hMLH1) in an in vitro MDS model were found to be hypermethylated, leading to an increased expression of DNMTs and gene silencing [39].

35.2.2 Pathogenesis of MDS/MPN

The pathogenesis of MDS/MPN is a complex, multi-step process with or without the involvement of cytogenetic abnormalities, and passenger and/or driver mutations [15, 40–53]. Recurring cytogenetic abnormalities are found in 70% MDS/MPN patients such as monosomy 7, trisomy 9, trisomy 8, and deletions (e.g. del13q and del7q) which may involve genes encoding for tyrosine kinases [15, 25, 54]. Mutations are often found in genes responsible for the regulation of epigenetics (e.g. DNMT3A, IDH1/2, ASXL1, TET2), RNA spliceosome mechanism (e.g. SRSF2, SF3B1, U2AF3), signal transduction pathways (e.g. Ras, JAK, MPL, CBL, Kit, and FLT3), transcription (e.g. RUNX1, CEBPA), and DNA repair (e.g. NPM1, TP53, and WT1) [15, 40–53].

35.3 Current Treatment and Limitations

35.3.1 Allogenic Haematopoietic Stem Cell Transplantation

Allogeneic-haematopoietic stem cell transplantation (allo-HSCT) is the only cure for MDS and MDS/MPN aiming to restore normal haematopoiesis via ablating leukaemic cells for successful engraftment of healthy donor stem cells into the patient's BM [15, 16, 55–59]. However, most patients are ineligible due to factors such as presence of comorbidities and advanced age [16, 59–61]. In MDS, merely 40–50% transplant patients achieve long-term disease-free survival and 35.1% die from treatment-related mortality (TRM) in the first 100 days [12, 62–65]. In MDS/MPN, the 5-year OS and TRM is 20–30% [16, 59–61, 66–68].

35.3.2 Hypomethylating Agents

For allo-HSCT ineligible patients, hypomethylating agents (HMA) such as azacitidine (AZA) and decitabine (DEC) have been developed to reactivate and restore dysregulated haematopoiesis in the BM [69].

AZA is a first-generation cytidine ribonucleoside-analogue, where an additional nitrogen atom is incorporated into the 5-carbon of the pyrimidine ring [39, 70–72]. It is an S-phase restricted pro-drug acting via two distinct mechanisms of action to eliminate leukaemic cells: (A) phosphorylation of azacytidine into its triphosphate form and RNA incorporation induces disassembly of ribosomal compartments and prevents oncogenic protein translation, (B) formation of irreversible adducts that are capable of being

incorporated into DNA-methyltransferase 1 (DNMT1), restoring normal transcription and inducing DNA hypomethylation [70, 72].

DEC, a second-generation HMA with a deoxyribose sugar, permits direct incorporation and disruption of the DNA structure without the need of ribonucleotide reductase for conversion [70, 73–76]. This makes DEC 10% more potent than AZA [70, 73–75]. It forms covalent bonds with DNA and DNMT to allow itself to be trapped in dysplastic cells leading to proteasome-mediated degradation and irreversible cytotoxicity [70, 73–75]. Moreover, DEC targets the dysfunctional $p15^{INK4B}$, allowing re-establishment and normalization of its protein expression for cell-cycle regulation [77, 78].

For MDS, AZA yields an overall response rate (ORR) and disease improvement in 50–60% ± 10% patients [8–10, 79–82] and 30–60% [82–84] for DEC. In spite of the well-established effectiveness of HMAs in the treatment of MDS, resistance and/or suboptimal responses occur in 40–50% patients [85–87], with the median survival being ~4–6 months and ~12–14 months for higher-risk and lower-risk patients, respectively [87–89]. Exact molecular mechanisms remain unknown [90–92]. Clinical biomarkers may act as prognostic indicators, such as poor patient fitness and performance status, BM blasts >20%, advanced age, transfusion dependency (TD), low platelet (PLT) count, and complex cytogenetics (>4 abnormalities), suggest unfavourable disease outcome. Furthermore, AZA and DEC have low oral bioavailability and short half-lives (~30 mins) [93–95].

For MDS/MPN, HMAs yield an ORR of 20–50% and remission rates of <20% [16, 59, 96, 97]. Moreover, cytoreductive agents such as hydroxyurea (HU) and interferon-α (IFN-α) are used during symptomatic hyperleukocytosis for immunomodulation and lowering of white blood cell (WBC) counts to prevent complications such as retinal swelling and/or haemorrhage, focal neurological deficits, seizures, and respiratory failure [59, 98, 99].

In view of the suboptimal responses to pharmacological intervention, highly selective inclusion criteria for allo-HSCT and absence of treatment guidelines for management of HMA-refractory/resistant patients, the risks of disease complications, progression, and/or mortality remain a critical and pressing issue calling for the development of novel agents. In Figs. 35.1 and 35.2, some promising novel targets and agents are depicted for MDS and MDS/MPN, respectively. A summary of the prospective agents currently in clinical trials for the treatment of MDS and MDS/MPN is described in Tables 35.1 and 35.2, respectively.

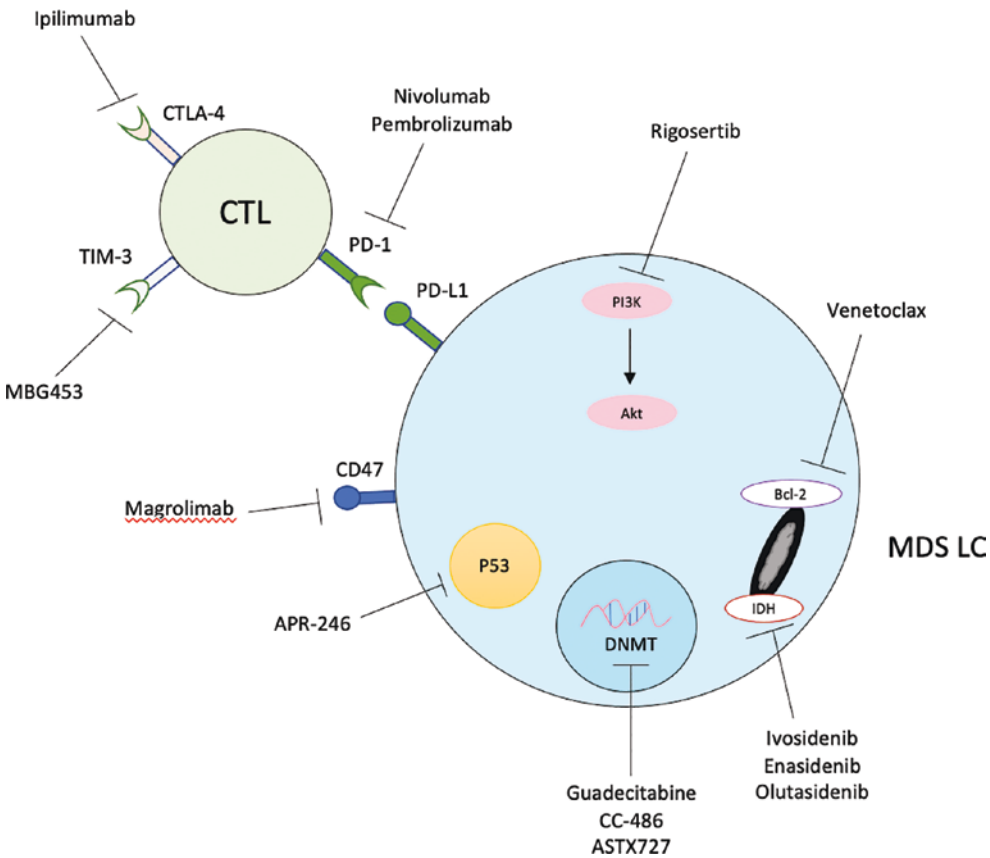

Fig. 35.1 Novel targets and agents in MDS

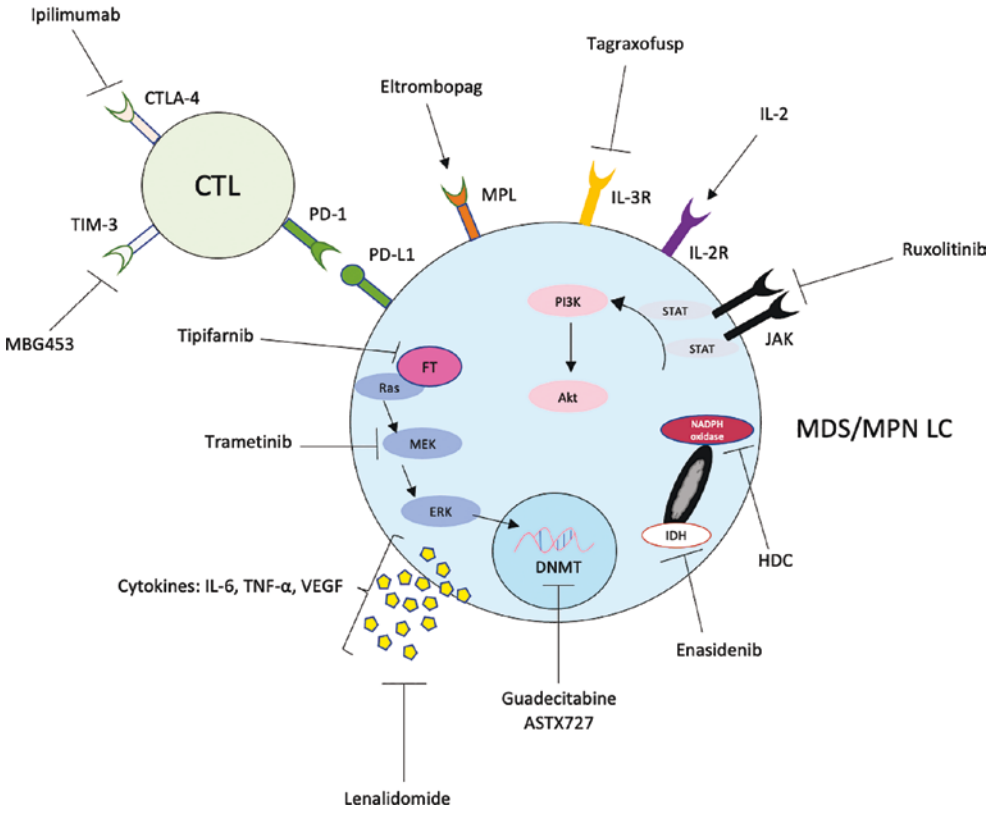

Fig. 35.2 Novel targets and agents in MDS/MPN

Table 35.1 Prospective agents in trial for the treatment of MDS

Agent	Target	Phase	NCT identifier
Guadecitabine (SGI-110)	DNMT	III	02907359
CC-486	DNMT	II	02281084
ASTX727	DNMT	I/II	03502668
Venetoclax	Bcl-2	Ib	02966782 03613532
Venetoclax + Azacitidine	DNMT + Bcl-2	Ib	02942290
Ivosidenib	IDH1	II	03503409
Olutasidenib (FT-2102) ± Cytarabine ± Azacitidine	IDH1	I/II	02719574
Enasidenib + Azacitidine	IDH2	II	03383575
APR-246 + Azacitidine	p53	III	03745716
Rigosertib	Ras	III	02562443
Magrolimab (Hu5F9-G4) ± AZA	CD47	III	04313881
MBG453 + Azacitidine	TIM-3 and DNMT	II	03946670
MBG453 + Azacitidine	TIM-3 and DNMT	III	04266301
Ipilimumab	CTLA-4	I	01757639
Ipilimumab/Nivolumab ± Azacitidine	CTLA-4, PD-1 and DNMT		02530463

Table 35.2 Prospective agents in trial for the treatment of MDS/MPN

Agent	Target	Disease(s)	Phase	NCT identifier
Guadecitabine	DNMT	CMML	III	02907359
ASTX727	DNMT	MDS/MPN	I	03306264
Lenalidomide	VEGF, TNF-α, and IL-6	MDS/MPN-RS-T	N/A	N/A
Eltrombopag	MPL	CMML	I/II	2,323,178
Tipifarnib	Farnesyltransferase	CMML	II	02807272
Trametinib	MEK	JMML	II	03190915
Ruxolitinib	JAK2	CMML aCML MDS/MPN CMML	I/II II I II	01776723 02092324 03878524 03722407
Enasidenib + Azacitidine	IDH2	CMML	II	03383575
MBG453 + Azacitidine	TIM-3 and DNMT	CMML	III	04266301
Ipilimumab	CTLA-4	CMML	I	01757639
HDC + IL-2	NADPH oxidase and IL-2	CMML	I/II	03040401
Tagraxofusp (SL-401)	IL-3	CMML	I/II	02265253

35.4 Novel Hypomethylating Agents

Guadecitabine (SGI-110) is a DEC dinucleotide derivative [100, 101]. The addition of a deoxyguanosine to DEC acts via cleaving the phosphodiester bond between the two dinucleotide parts leading to a slow release of DEC [102]. This prevents cytidine deaminase-mediated metabolism and reduces the peak plasma exposure to extend DEC's half-life [100, 101]. A phase 1/2 trial studying guadecitabine monotherapy reported an ORR of 51% and CR of 43% in HMA-naïve and HMA-refractory MDS patients [101]. Frontline guadecitabine users had superior median OS (23 vs 11.6 months) and 2-year OS (44% vs 25) [101]. A similar French phase 2 study reported 1-year OS of 33% and median OS of 17.9 months in responders versus 7.1 months in non-responders [103]. Furthermore, they suggest that AZA failure plays a role in guadecitabine response [103]. Another phase 2 study conducted in USA reported a median OS of 15 months, 2-year OS of 25%, ORR of 43%, and median event-free survival (EFS) of 14 months [101, 104]. These studies all demonstrated that ORR was higher in guadecitabine than DEC or AZA. Notably, minimal-to-no response was observed in patients harbouring TP53 mutations [103]. An ongoing phase 3 trial is currently underway to further investigate the role of guadecitabine in frontline MDS and CMML therapy (ClinicalTrials.gov Identifier, NCT02907359).

Novel oral HMA formulations have been developed aiming to enhance patient convenience, reduce patient burden, improve QoL, promote treatment adherence, provoke a prolonged administration, and achieve more potent inhibition on DNA methylation (7 days subcutaneous AZA vs 14–21 days of oral HMAs) [94, 105, 106].

CC-486 is an oral AZA formulation. Data based on CC-486 trials in AML showing favourable outcomes (46% ORR) and a similar toxicity profile versus AZA prompted clinical investigations for use in MDS patients [107–112]. For 18 MDS patients, Savona et al. reported 32% ORR including 22% CR and 17% partial remission (PR), and 33% transfusion independence (TI) [112]. Since thrombocytopenia is one of the strongest prognostic indicators for reduced survival in MDS patients, Garcia-Manero et al. evaluated the clinical implications of pre-treatment thrombocytopenia (defined as platelet count $\leq 75 \times 10^9$/L) [111]. Amongst 81 patients, they reported a 46% and 38% ORR in patients with high and low platelet count cohorts respectively. Six per cent attained CR and 10% achieved haematologic improvement (HI). Treatment-related mortality (TRM) was 9%, including 6% patients having pre-treatment platelet counts $<25 \times 10^9$/L [111]. Preceding strong clinical results, a phase II multicentre trial, is underway for patients pretreated and failed initial parenteral DEC and AZA therapy (ClinicalTrials.gov Identifier, NCT02281084). However, more investigations are required to determine the role and ability of CC-486 to replace AZA for post-transplant maintenance therapy [113, 114].

Another oral HMA formulation is ASTX727, a combination of decitabine and cedazuridine. Cedazuridine is a cytidine deaminase inhibitor, where this combination prevents DEC metabolism by cytidine deaminase in the liver and gastrointestinal tract [94, 105, 115, 116]. It aims to prolong the half-life, reduce plasma exposure, and improve potency of decitabine in DNA hypomethylation [117]. A phase 1 open-label dose-escalation study showed clinical effectiveness showing 32% ORR, including 11% CR and 16% receiving post-treatment allo-HSCT [118]. In the 2017 annual ASH

meeting, a phase 2 dose-confirmation study consisting of 60 MDS patients reported ORR in 62% including 16% CR, 28% marrow complete response (mCR) and 18% HI [119]. Another phase 2 study by Garcia-Manero et al. reported 60% ORR with 21% CR, as well as 50% originally TD patients achieving TI [106]. Median OS of 18.3 was reported [106]. In the phase 3 ASCERTAIN study (ClinicalTrials.gov Identifier, NCT03306264), clinical efficacy of ASTX727 was further re-established as they reported 64% ORR, including 12% CR, 7% HI, and 46% mCR [120]. Coherently, all the above studies showed similar toxicity profile as intravenous (IV) DEC [118, 119]. Although promising clinical results are presented, further investigations are required for its role for salvage therapy [105]. For lower-risk MDS patients, a phase I/II study using lower doses is underway (ClinicalTrials.gov Identifier, NCT03502668).

35.5 Molecularly Targeted Agents

35.5.1 Bcl-2 Targeting in MDS

The BCL-2 family consists of pro-apoptotic (BOK, BAX, and BAK) and anti-apoptotic (BCL-2, MCL-1, BFL-1/A1, BCL-w, and BCL-xL) proteins that control mitochondrial apoptosis during cellular damage [121, 122]. Sharing a common BH3 domain, they mediate mitochondrial apoptosis by regulating the mitochondrial outer membrane permeabilization (MOMP) and subsequently releasing cytochrome C followed by caspase activation [121, 122]. In MDS patients, the anti-apoptotic protein BCL-2 is often overexpressed in leukaemic cells, especially in higher-risk MDS patients, where deregulation allows protection against oxidative stress, evasion of apoptosis, drives leukaemogenesis, disease progression, and HMA resistance [115, 122–128]. Venetoclax is a selective and orally active BCL-2 inhibitor recently approved due to a phase 1b study, for administration in combination with low-dose cytarabine or an HMA for the treatment of patients with AML contraindicated to intensive chemotherapy or naïve-AML (ClinicalTrials.gov Identifier, NCT02966782). The impressive clinical benefits observed in the treatment of AML have prompt investigations in application in MDS patients [122, 129–132]. Preclinical in vitro studies report that venetoclax depleted leukaemic stem/progenitor cells, with minimal-to-no effect on normal haematopoietic cells [124, 133, 134]. Notably, in vitro patient samples treated with dual venetoclax-AZA therapy observed an increased succinate and reduced α-ketoglutarate level, interfering with the Kreb's cycle in leukaemic cells to induce apoptosis [135]. Jehangir et al. presented a case report of a 53-year-old man with multiple comorbidities including hypertension, peptic ulcer disease, gout, coronary artery disease, and hyperlipidaemia diagnosed with MDS in 2011 [136]. He developed HMA resistance and TD [136]. Subsequently, venetoclax monotherapy was administered with minimal side effects (intermittent neutropenia), no infection complications, and achievement of transfusion independence [136]. An ongoing phase 1b study by Zeidan et al. (ClinicalTrials.gov Identifier, NCT02966782) recently submitted an abstract, evaluating venetoclax as monotherapy and combination therapy with AZA [137]. It reported that combination therapy showed superiority in ORR (50% vs 7%), where the estimated median 6-month OS for monotherapy was 57% and median 9-month OS was 83% for combination therapy [137]. For venetoclax monotherapy, they reported 75% stable disease (SD) and 3.4 months progression-free survival (PFS). In combination therapy, 17% proceeded to allo-HSCT, 31% achieved SD and the estimated median 6-month PFS was 76% [137]. Similarly, Wei et al. also newly submitted a phase 1b study investigating the efficacy and safety of venetoclax-azacitidine combination therapy (ClinicalTrials.gov Identifier, NCT02942290) [138]. From 57 evaluable patients, they reported 32% ORR, 39% mCR, 19% SD, and 50% HI [138]. The estimated 12-month PFS was 59% and ORR was 74%, while the estimated 18-month OS was 74% [138]. In both studies, most common side effects include thrombocytopenia, anaemia, and neutropenia [137, 138]. In a retrospective study using venetoclax and AZA for refractory/relapsed MDS patients, ORR was 59% where 63% of these patients subsequently underwent allo-HSCT [139]. They reported the median relapse-free survival (RFS) as 15.4 months, median OS as 11.4 months for HMA-failed patients, and 19.5 months for the cohort as a whole [139]. Furthermore, based on the principles of the delayed graft-versus-tumour effect, a study is currently investigating the effects of incorporating venetoclax into conventional reduced-intensity conditioning for MDS patients proceeding with allo-HSCT (ClinicalTrials.gov Identifier, NCT03613532) [140, 141]. Although promising preclinical and clinical studies are reported, it is vital to await full results and conduct further studies on whether venetoclax will be capable of producing robust therapeutic outcomes and a safe toxicity profile in the treatment of MDS.

35.5.2 Targeting Vascular Endothelial Growth Factor in MDS/MPN

Vascular endothelial growth factor (VEGF) is pro-angiogenic, secreted dimeric glycoprotein mainly produced by erythroid and megakaryocytic precursors [142–146]. It binds to tyrosine-kinase receptors including VEGFR-1, VEGFR-2, and VEGFR-3 commonly expressed on vascular, lymphatic and smooth muscle endothelial cells, haematopoietic stem cells (HSC), and monocytes to induce angiogenesis and vascular permeability, promote encourage haematopoietic dif-

ferential and growth, and regulate lymph-angiogenesis [144, 145, 147–149]. In normal physiologic conditions, VEGFR expression on quiescent HSCs and VEGF secretions are extremely low, but can be induced by thrombopoietin (TPO) during megakaryopoiesis and hypoxic conditions [146]. In MDS/MPN, VEGFR-1 and VEGFR-2 are often overexpressed with VEGF hypersecretion, especially in MDS/MPN-RS-T and MDS/MPN-U [145, 150–152]. This disrupts normal megakaryopoiesis and granulopoiesis to drive disease development and is associated poor prognosis and rapid disease progression [144, 145, 151, 153]. Lenalidomide, an anti-angiogenic and immunomodulatory agent, approved for treating MDS-5q deletion syndrome [154]. Its mechanism of action involves activation and rapid proliferation of NK and T cells via increased IL-2 production, reducing production of monocyte-derived proinflammatory cytokines (e.g. IL-6 and TNF-α), increasing anti-inflammatory cytokines (e.g. IL-10), and inhibiting angiogenesis via downregulating VEGF, TNF-α, and IL-6 [155, 156]. Multiple case reports using lenalidomide for treatment of MDS/MPN-RS-T have observed substantial efficacy in reducing transfusion dependence (TD) [157–166]. Although its ability to maintain a durable remission is controversial, the VEGF/VEGFR pathway remains a critical target in the treatment of MDS/MPN, paving way for development of novel agents.

35.5.3 Thrombopoietin Mimetics in MDS/MPN

Thrombopoietin (TPO) is a haematopoietic cytokine for HSC maintenance and regulation of megakaryopoiesis to drive megakaryocytic expansion and differentiation into platelets [167–172]. Its receptor MPL is predominantly expressed on megakaryocytes, haemangioblasts, platelets, and HSCs, where TPO/MPL interaction activates downstream PI3K/Akt, JAK/STAT, and Ras/Raf/MAPK/ERK signalling cascades [169, 173–177]. MDS/MPN patients are frequently complicated by thrombocytopenia as a result of ineffective haematopoiesis and deranged BM microenvironment. This is associated with a poor prognosis, especially in CMML [97, 178, 179]. Eltrombopag is a synthetic, potent, and specific TPO agonist that restores normal platelet production and HSC homeostasis when bound to MPL to prevent bleeding complications [180]. A phase 1 study of eltrombopag monotherapy in CMML reported 29% patients achieving transfusion independence (TI), a bilineage HI of 14%, median OS of 7 months, rate of transformation to sAML of 29% [181]. Interim results from a similar phase 1/2 trial (ClinicalTrials.gov, Identifier NCT2323178) are as follows: 63% of patients achieved HI in platelet counts (HI-P), including 70% of those with and 56% of those without baseline platelet transfusion dependence [182]. The 12-month progression-free survival (PFS) was 41%, 12-month cumulative incidence of AML was 19%, and 12-month OS was 65% [182]. Results also suggest that eltrombopag may provide leukaemic cell protection from DNA damage; however, more confirmatory results will be presented once the trial is completed with need of larger cohorts and longer study periods in the future [182].

35.6 Targeting Epigenetic Regulators

Epigenetic modifications such as histone acetylation and DNA methylation are important in regulating transcription [183]. In patients with MDS, changes in epigenetics including aberrant DNA methylation frequently occur and contribute to leukaemogenesis and disease progression [183, 184].

35.6.1 Isocitrate Dehydrogenase 1/2 Inhibitors

Isocitrate dehydrogenase 1 (IDH1) and 2 (IDH2) are mitochondrial enzymes responsible for epigenetic regulation and cellular regulation via catalysing the oxidative phosphorylation of isocitrate into α-ketoglutarate [42, 185–187]. Mutations in the neomorphic enzymes lead to reduction of α-ketoglutarate into R-2-hydroxyglutarate, an oncometabolite [42, 185–187]. Accumulation of R-2-hydroxyglutarate induces DNA and histone hypermethylation, disrupting gene expression and blocking cellular differentiation [42, 185–187]. IDH1/2 mutations are seen in 5–12% of MDS patients, and compared to IDH1/2 wild type, the presence of IDH1/2 mutants confers less favourable prognosis [187–193]. Ivosidenib and enasidenib are orally active and potent allosteric inhibitors of mutant IDH1 and IDH2 proteins, respectively, and are approved for the treatment of refractory/relapsed AML that harbour these mutations. In a phase 1 dose escalation and expansion study using ivosidenib as monotherapy, promising results including a ORR of 75%, CR of 42%, TI of 75% lasting >56 days, relapse-free survival (RFS) of 25%, mutation clearance of 8%, and mCR of 25% lead to the rapid Food and Administrative Department (FDA) approval for the treatment of relapsed/refractory MDS patients with IDH1 positivity [194]. A phase 2 study using ivosidenib as monotherapy is currently recruiting and underway for MDS patients of all risks (ClinicalTrials.gov Identifier, NCT03503409). For enasidenib, phase 1 trials as monotherapy in IDH2-positive MDS patients observed an ORR of 53%, where 50% of HMA-failed patients responded, as well as 6% in CR, PR, and mCR [195]. In a similar phase 1/2 study, ORR was 53% including a 46% response in HMA-failed patients, the median OS was 16.9 months, and median EFS was 11 months [196]. These impressive results lead to a

multicentre phase 2 trial on MDS and MDS/MPN patients is still recruiting (ClinicalTrials.gov Identifier, NCT03383575) studying two cohorts: (A) HMA-naïve patients with high-risk MDS receiving enasidenib and AZA (B) HMA-failed patients receiving enasidenib only [197]. Preliminary results show an ORR of 67% for the entire cohort. In cohort A, ORR was 100%, CR was 33%, mCR was 66%, and HI was 17% [197]. In cohort B, ORR was 50%, CR was 17%, mCR was 8%, TI was 38%, and 8% was able to attain IDH2 clearance [197]. Another IDH1 inhibitor olutasidenib (FT-2102) is currently under phase 1/2 trials as a single agent or in combination with cytarabine or AZA (ClinicalTrials.gov Identifier, NCT02719574). Preliminary results show that ORR was 73% and 33% for combination therapy and monotherapy, respectively [198].

35.6.2 P53 Modulation in MDS

Tumour suppressor gene P53 (TP53) is located on 17p13.1, which encodes the protein p53. P53 is a critical transcription factor is known as the "guardian of the genome," regulating cellular differentiation, apoptosis, senescence, and cell-cycle arrest via upregulating target genes, e.g. Bax, Puma, p21, and Noxa [199–203]. By controlling target genes such as GLS2 and TIGAR, it is also able to control cellular redox status and metabolism [204–206]. Around 5–10% of de novo MDS and 25–30% therapy-related MDS patients harbour TP53 mutations, where it is associated with extremely poor prognosis, complex karyotype, progression into AML, and inferior response to therapy due to uncontrolled and leukaemogenic cellular proliferation [199, 207–211]. APR-246 is a novel small molecule that spares normal cells, while it covalently binds to mutant and wild-type p53 to thermodynamically stabilize p53 mutants for reactivation of its functions and restoring the conformation of misfolded p53 wild-type proteins to ultimately eradicate leukaemic cells [212–215]. Sallman et al. conducted a phase 1b/2 trial evaluating therapeutic outcomes of AZA-APR-246 in HMA-naïve patients with TP53 mutations [216, 217]. The phase 1b portion reported an ORR of 100%, CR of 82%, and mCR of 18% [217]. The phase 2 portion reported an ORR of 87%, CR of 53%, mCR of 9%, HI of 7%, and a mCR with HI of 18%, with 11% achieving a negative measurable residual disease (MRD) [216]. The median OS was 11.6%, with responders having significantly longer OS in responders (12.8 months) compared to non-responders (3.9 months) [216]. Optimistic results have led to an ongoing phase 3 clinical study comparing AZA monotherapy versus AZA-APR-246 combination therapy in MDS patients (ClinicalTrials.gov Identifier, NCT03745716).

35.7 Multi-Kinase Inhibitors

35.7.1 Targeting the Ras Pathway

The Ras family consists of three GTPases (KRAS, NRAS, and HRAS), where they function as molecular switches in converting GDP (inactive form) to GTP (active form) [218–220]. Ras activation leads to the downstream Ras/PI3K/Akt, and Ras/Raf/MAPK/Akt signalling pathways, which are crucial in maintaining and regulating normal cell survival, proliferation, metabolism, growth, differentiation, cell-cycle entry, and cytoskeleton reorganization [218–222]. Upon ligand binding, farnesyltransferase (FTase) catalyses the addition of a farnesyl isoprenoid moiety to activate downstream Ras/Raf/MAPK/ERK and Ras/PI3K/Akt signalling pathways [220, 222–225]. In MDS, hyperactive Ras drives leukaemogenesis [226–228]. Oncogenic activation of Ras is associated with worse prognosis and rarely occurs in MDS/MPN-RS-T, but is found in 10–15% of MDS/MPN-U, 15% of aCML, up to 15–20% of CMML, 25–30% of JMML [229–237]. Considering that Ras mutations are exceedingly common, its receptors and downstream molecules are attractive candidates for development of targeted therapies.

35.7.1.1 Ras Inhibitors in MDS

In MDS, hyperactive Ras drives leukaemogenesis [226–228]. Rigosertib (ON 01910.Na) is a synthetic, orally active benzyl styryl sulfone Ras mimetic that has dual action on inhibiting Ras-mediated downstream signalling pathways [238–242]. This cytotoxic agent spares normal cells, acting as a non-competitive allosteric inhibitor to selectively induce apoptosis and elimination of leukaemic cells via binding to the Ras-binding domain, promoting disruption of centrosome localization and mitotic spindle formation, followed by G2/M phase cell-cycle arrest [242–245]. In preclinical studies, the mechanism of action of rigosertib was supported by evaluation of G2/M phase arrest markers: accumulation of cyclin B1, diminished phosphorylation of CDK1, and enhanced phosphorylation of histone H3 [246, 247]. In a phase 1 study investigating rigosertib as a single agent, 33% achieved TI with 13% obtaining HI in the erythroid and 8% HI in the platelet counts [240]. Given that rigosertib is an oral agent alongside minimal-to-no overlap in toxicity profile and synergistic mechanisms of action, a phase 2 study combining rigosertib and AZA was conducted [248]. They reported that ORR was 54% in HMA-failed and 90% in HMA-naïve patients, with CR rates of 4% and 34%, respectively, where 30% of high-risk MDS patients also attained TI [248]. In a phase 2 expansion study, ORR was 59% for HMA-refractory/relapsed and 79% for HMA-naïve patients [249]. Another study conducted by Silverman et al. observed a median OS of 35 weeks, with 17% mCR, 23% partial mar-

row response (mPR), 15% SD and an overall 69% in HI, cytogenetic response, and/or marrow blast control in higher-risk MDS patients [250]. Following promising preclinical and phase 1/2 trials, the phase 3 randomized, controlled "ONTIME" evaluated rigosertib versus best supportive care (BSC) [251]. Rigosertib had limited impact on extending median OS (rigosertib 8.2 vs. BSC 5.9 months) with no partial response (PR), CR nor significant HI. However, in very high-risk MDS patients, rigosertib significantly improved median OS (7.6 vs 3.2 months) and discovered that the proportion and Identifier of trisomy 8 in aneuploid cells were significantly reduced [251]. All the above studies showed minimal myelosuppression, which is vital to MDS patients due to their suboptimal BM function [240, 248–251]. In spite of mixed results, rigosertib remains a hopeful candidate for the treatment of MDS and an ongoing phase III international open-label, randomized, controlled trial "INSPIRE" may provide more insight into the clinical implications and applications of this novel agent (ClinicalTrials.gov Identifier, NCT02562443).

35.7.1.2 Farnesyltransferase Inhibition in MDS/ MPN

Tipifarnib, an orally active, highly selective and potent FT inhibitor that blocks post-translational farnesylation of Ras and its downstream signalling pathways to correct deregulated cellular homeostasis [252]. Preclinical studies with tipifarnib monotherapy on MDS and AML cell lines demonstrate efficacious and selective eradication of leukaemic cells, modulation of cytoskeletal organization, and inhibition of aberrant signalling pathways to reduce leukaemic burden [253–258]. In a phase 2 study using tipifarnib monotherapy in JMML patients, ORR was 51%, 5-year OS was 55% and 5-year event-free survival (EFS) was 41%; it however failed to improve long-term OS nor reduce relapse rates [259]. Preliminary results from an ongoing phase 2 trial tipifarnib monotherapy study in CMML are as follows (ClinicalTrials.gov Identifier, NCCT02807272): KRAS mutation-positivity of 20%, stable disease (SD) of 57%, mCR of 14%, and symptom-reduction observed in 14% [260].

35.7.1.3 MEK1/2 Inhibition in MDS/MPN

Downstream MEK inhibition is also a promising candidate. Preclinical mouse models bearing a CMML- and JMML-like MPNs demonstrated that sustained MEK inhibition was able to suppress uncontrolled myeloproliferation, restore normal erythropoiesis and granulopoiesis, alleviate disease burden, prolong survival, and destroy leukaemic cells [261, 262]. Trametinib is an oral MEK1/2 inhibitor that represses phosphorylation and activation of MAPK to limit hyperactivated Ras signalling [263, 264]. In an in vivo mouse model of CMML, trametinib-AZA combination therapy prolonged survival, decreased spleen size, and reduced leukaemic cell engraftment [263]. A phase 1/2 study evaluating trametinib monotherapy in CMML patients with Ras mutations reported an ORR of 27%, CR of 9%, mCR or 9%, SD of 73% and partial response (PR) of 9% [264]. A similar phase 2 trial in JMML patients is underway (ClinicalTrials.gov Identifier, NCT03190915). Combination of MEK inhibitors with other agents (e.g. AZA) have only been studied in preclinical setting and have demonstrated encouraging evidence, so perhaps combination therapies could open up a new array of clinical trials.

35.7.2 Targeting JAK/STAT Pathway in MDS/ MPN

The Janus-kinase family (JAK1, JAK2, and JAK3) is a group of ubiquitously expressed, non-receptor, intracellular tyrosine kinases vital for regulating haematopoietic cellular proliferation, differentiation, survival, and function [265, 266]. It acts via the JAK/STAT pathway to regulate expression levels of proinflammatory cytokines such as nuclear factor-κB (NF-κB), IL-6, and IL-10 [265, 267]. Although MDS/MPNs lack identifying cytogenetic and/or molecular abnormalities, JAK2-V617F mutations are exceptionally common and recurrent [15]. Although rarely encountered in JMML, it is seen in approximately 4–8% of aCML, 10% of CMML, 25% of MDS/MPN-U and up to 60% in MDS/MPN-RS-T [268–273]. Mutations result in overexpression and autophosphorylation of JAK2, leading to constitutive activation of JAK2/STAT signalling and subsequent leukaemogenesis [265, 268–273]. Ruxolitinib is a highly selective and potent JAK1/2-ATP-competitive inhibitor approved for the treatment of myelofibrosis [266, 274]. By inhibiting JAK/STAT pathway, it induces cytotoxicity and reduces levels of proinflammatory cytokines to promote elimination of leukaemic cells [266, 274, 275]. In a phase 1 trial using ruxolitinib monotherapy in patients with JMML, two-third patients achieved stable disease (SD) by 1 month while one patient experienced disease relapse after receiving five cycles [276]. Another phase 1 trial investigating ruxolitinib monotherapy in CMML patients achieved an ORR of 35%, haematological improvement (HI) of 20%, spleen size reduction of 56% in patients with baseline splenomegaly, as well as symptom resolution of 91% in patients reporting pre-treatment disease-related symptoms [277]. In a similar phase 2 trial conducted on patients with CMML, aCML, and MDS/MPN-U, ORR was 57% including 45% responding after adding AZA [278]. 64% patients with baseline splenomegaly achieved >50% reduction in palpable splenomegaly and the median (OS) was 26.5 months for MDS/MPN-U, 15.1 months for CMML, and 8 months for aCML [278]. In another phase 2 study on aCML patients, ORR was 8%, supporting previous studies that aCML is a more aggressive

disease with higher resistance to therapy [279]. In view of strong preliminary results, multiple phase 1 and 2 trials evaluating involving ruxolitinib are ongoing at present (ClinicalTrials.gov Identifier, NCT01776723), (ClinicalTrials.gov Identifier, NCT02092324), (ClinicalTrials.gov Identifier, NCT03878524), (ClinicalTrials.gov Identifier, NCT03722407).

35.8 Immunotherapy

35.8.1 Targeting CD47 in MDS

CD47 is a supramolecular, integrin-associated protein receptor ubiquitously expressed on the plasma membrane on cells and its major ligand SIRP-α is highly abundant in myeloid cells and DCs [280–284]. Its functions include promoting axonal elongation and regulating the cytoskeleton of actin through the MEK/MAPK pathway for cellular adhesion, transendothelial, and transepithelial migration [280–282, 285–292]. Furthermore, it is important in self-tolerance as it is capable of inactivating macrophages and subsequent phagocytosis to prevent autoimmunity [281, 285, 286, 293, 294]. Thrombospondin-1 is another CD47 ligand regulating T-regulatory (Treg) cells in response to inflammation [282, 295]. In MDS patients, CD47 is predominantly overexpressed in leukaemic stem cells (LSCs), conferring a poor prognosis due to immune-evasion, enhanced ability to migrate into ectopic niches such as the peripheral blood, liver, and spleen, and selective engraftment into the BM microenvironment [280, 296]. Studies showed an increased expression in LSCs, especially in higher-risk MDS patients [280, 297]. A potential mechanism for anaemia and ineffective erythropoiesis was also proposed as erythroblasts were found to have reduced CD47 expression, which would promote phagocytosis [280, 297]. It also inhibits the recruitment and activation of T cells, NK cells, and DCs by downregulating the expression of IL-12, IFN-y, and TNF-β [284]. In response to strong preclinical evidence of anti-CD47 therapy showing immune restoration and selective eradication to oncogenic cells with limited toxicity to normal cells, a targeted macrophage immune checkpoint inhibitor and anti-CD47 monoclonal IgG4 antibody magrolimab (Hu5F9-G4) were developed [113, 283, 298–304]. In a phase 1b trial evaluating magrolimab-AZA combination therapy in HMA-naïve MDS patients, impressive results were reported: ORR was 100%, CR was 54%, HI was 85%, mCR was 39%, 17% with mCR or CR attained MRD negativity, 40% with baseline cytogenetic abnormalities achieved complete cytogenetic response (CCyR), 63% showed complete CD47 clearance on LSCs, and all patients (2/2) harbouring TP53 mutations were responsive to treatment [299]. Based on the positive results, a breakthrough therapy designation was granted by the FDA and the phase 3 randomized, double-blinded, placebo-controlled "ENHANCE" trial with magrolimab monotherapy or combination therapy is ongoing (ClinicalTrials.gov Identifier, NCT04313881).

35.8.2 Targeting T-Cell Immunoglobulin and Mucin Domain-Containing Protein 3

T-cell immunoglobulin and mucin domain-containing protein 3 (TIM-3) is an immune checkpoint receptor vastly expressed on FoxP3$^+$ Treg cells, dendritic cells (DC), NK cells, macrophages (MQ), CD4+ type-1 helper T (Th1) cells, CD8+ cytotoxic T cells (CTL), and Th17 cells that produce IL-17 [305–308]. The four identified TIM-3 ligands include galectin-9, phosphatidylserine, high mobility group box (HMGB)-1, and ceacam-1 [305–308]. TIM-3 signalling plays a major role in suppressing innate and active immunity by exhausting T cells, inducing cellular apoptosis and inhibiting cellular proliferation by suppression of CTLs via the TIM3/PD1/CTL pathway, inhibition of Th1 responses via suppressing IFN-y and TNF expression, downregulation of DCs for tumour-recognition and enhancement of FoxP3$^+$ Treg cellular activity [305–307, 309–316]. In the BM of MDS patients, preclinical studies have found that TIM3 expression is significantly increased via the upregulation of cytokines such as IL-10, transforming growth factor (TGF)-β1, vascular endothelial growth factor (VEGF), IL-8, IL1-1β, granulocyte-colony stimulating factor (G-CSF), and macrophage inflammatory protein (MIP)-1α, especially on leukaemic blasts and monocytes with even higher expression in patients with BM blasts ≥5% or those that have transformed into sAML [317–323]. BM secretion of galectin-9 is also markedly increased [305, 324], and through the deregulated galactin-9/TIM-3 axis, β-catenin- and NF-κβ-mediated auto-stimulatory signalling loops are induced to amplify expression of anti-apoptotic (e.g. CCL2, WNT11, and IL-2R) and pro-proliferative genes (e.g. IL-6R, CXCL8, and CXCR4) for facilitating migration and sustaining self-renewal capacities of leukaemic cells and directly suppressing adaptive and innate immunity to drive leukaemogenesis [307, 316, 317, 324]. TIM-3 is preferentially expressed on leukaemic cells, and even in lower-risk patients, deregulated TIM-3 and galectin-9 expression is associated with an extremely poor prognosis deeming TIM-3 as a potential therapeutic target [317]. MBG453 is a human anti-TIM3 IgG4 antibody with high specificity and selectivity [307, 324–326]. Preclinical studies report that MBG453 induces TNF and IFN-y expression to reverse T-cell dysfunction, re-sensitizes DCs to tumour-derived stress factors via the transport nucleic acids into endosomal vesicles for tumour-recognition, and promotes anti-tumour activity of

CTLs for the elimination of leukaemic cells [307, 324–326]. An abstract was recently submitted for an open-label, multicentre phase 1b dose-escalation study evaluating MBG453 with AZA or DEC, where no treatment-related Grade 4 adverse effects nor deaths were observed [327]. For MBG453-AZA, ORR was 70% in high-risk MDS patients with HI of 30%, mCR of 60%, and PR of 10% [327]. For MBG453-DEC, ORR was 58% with HI of 16%, mCR of 21%, and CR of 26% [327]. Building on propitious preclinical and clinical results, the phase 2 "STIMULUS-MDS1" (ClinicalTrials.gov Identifier, NCT03946670) and phase 3 "STIMULUS-MDS2" trial (ClinicalTrials.gov Identifier, NCT04266301) in MDS and CMML are currently underway.

35.8.3 Targeting PD-1/PD-L1 and CTLA4

Complex processes are involved in T-cell activation: T-cell receptors (TCR) bind to specific antigen (Ag)-major histocompatibility (MHC) complexes with aid of costimulatory signals. Surface CD28 molecules on T cells bind with CD80 or CD86 on antigen-presenting cells (APC) to stimulate IL-2 production, cellular proliferation, and activation [328, 329]. Immune checkpoint pathways involving cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed death 1(PD-1) are crucial in regulating this process to prevent potential generation of autoreactive T cells [328–330]. CTLA-4 is another ligand of CD28 showing greater affinity than CD80 and CD86 [331, 332]. It is expressed exclusively on T cells and competitively binds to CD28 and inhibits CD28/CD80 and CD28/CD86 costimulatory signals during early phases of naïve T-cell activation in the lymph nodes to induce anergy [328–333]. Moreover, constitutive expression on Tregs suppresses CD4+- and CD8+-mediated immunity [328, 334–338]. Contrastingly, the PD-1 pathway primarily inhibits activated T cells in the peripheral tissues at later stages [328, 339, 340]. PD-1 binds to its ligands programmed death ligand 1 (PD-L1) and PD-L2 to suppress IL-2, TNF-α and IFN-y expression for T-cell inactivation [328, 333, 341–343]. Tregs express high levels of PD-1 with auto-proliferative effects and suppression of cellular immunity [344]. Furthermore, PD-1 expression on myeloid cells, NK cells, and B cells regulates antibody production and cell lysis [329, 333, 345]. Studies have shown that patients with MDS have aberrant PD-1, PD-L1, PD-L2, and CTLA-4 expression mediated by IFN-y and TNF-α and are associated with an unfavourable prognosis via promoting LSC dormancy and persistence, driving disease progression, and aggravating immunosuppression [320, 346–351]. Phagocytic capacities of tumour-associated macrophages (TAM) are also disrupted by PD-1 overexpression [352]. Additionally, studies reveal that first-line HMAs promote expression of receptors and ligands contributing to further impairment of immune responses and potential resistance to therapy [320, 350, 351, 353–355]. This calls for the development of novel agents targeting these pathways.

Ipilimumab is a humanized anti-CTLA-4 IgG1 monoclonal antibody reversing T-cell inhibition and re-activating CTL-mediated anti-tumour activity [356, 357]. In a phase 1b study evaluating ipilimumab monotherapy, all patients were previously treated with ≥1 agents and results are as follows: SD >6 months was 27.3% with no overall objective response achieved [358]. However, 27.3% proceeded to post-ipilimumab allo-HSCT and remain in remission, with median OS of patients ineligible for allo-HSCT to be 1 year [358]. Another similar phase 1 study in MDS and CMML has completed but results have not been announced (ClinicalTrials.gov Identifier, NCT01757639).

Pembrolizumab is a human anti-PD-1 IgG4 monoclonal antibody inhibiting the PD-1/PD-L1 and PD-1/PD-L1 axis [359]. In the phase 1b "KEYNOTE-013" trial investigating pembrolizumab as a single agent, results are as follows: mCR was 11%, SD was 52%, HI was 11%, PR was 3%, median 24-week OS was 49%, and no CR was achieved [360]. For intermediate-risk MDS patients, median 1-year OS was 89% and median 2-year OS was 57% [360].

Based on these phase 1 results, an ongoing phase 2 study evaluating ipilimumab and/or nivolumab (another human anti-PD-1 IgG4 monoclonal antibody) with or without azacitidine is underway and still recruiting (ClinicalTrials.gov Identifier, NCT02530463). Preliminary results report an ORR of 50% and 29% in newly diagnosed MDS and HMA-failed patients, respectively [361].

35.8.4 Interleukin 2 Inhibitors in MDS/MPN

Interleukin 2 (IL-2) is a pleiotropic cytokine produced and secreted by T cells that bind to its receptor (IL-2R) to trigger the JAK/STAT, Ras/Raf/MAPK/ERK, and PI3K/Akt signalling pathways for immunoregulation, regulation of HSCs, and cellular survival [362–376]. It supports T-regulatory cell (Treg) homeostasis and differentiation, promotes cytotoxic T-cell (CD8+) differentiation, cell growth, and proliferation, activates and enhances expansion of Th1, Th2 T-helper cells (CD4+), and NK cells, prevents Th17-CD4+ cellular production, and induces haematopoiesis [362, 375–382]. In patients with MDS/MPN, increased IL-2 secretion and IL-2R overexpression are associated with an inferior prognosis as hyperproduction of defective CD8+, CD4+, Treg, and NK cells in an altered bone marrow (BM) microenvironment leads to ineffective haematopoiesis and compromised anti-tumour activity effects to drive leukaemogenesis [350, 383–394]. Moreover, heightened production of reactive oxygen species by myeloid cells desensitizes naïve T and NK cells to

IL-2 stimulation as well as induce oxidative stress, contributing to further immunosuppression [395–399]. Preclinical evidence demonstrated that IL-2 monotherapy AML cell lines were able to restore normal haematopoiesis, re-sensitize and reactive T and NK-cell-mediated immunity, normalize IL-2 levels, and induce apoptosis in autologous leukaemic cell lines [400–402]. However, subsequent trials investigating IL-2 monotherapy yielded disappointing results [403–408]. However, the addition of histamine dichloride (HDC), a histamine derivative, acts as an oxygen radical scavenger by targeting NADPH oxidase to promote the efficiency of IL-2-mediated immune stimulation and provide protection from myeloid-derived oxidative stress [408–413]. Preclinical studies suggested that HDC-IL-2 combination therapy produced synergistic effects on the activation and cytotoxic responses mediated by NK and T cells in elimination of AML- and CML-derived leukaemic cell lines [401, 408–410, 412–415]. A phase 3 trial in AML patients for post-consolidation maintenance therapy reported ≥3-year leukaemia free survival (LFS) of 75% [408]. Based on strong preclinical and clinical data, it is hypothesized that this combination may benefit patients with MDS/MPN and we are currently awaiting results from a phase 1/2 trial in CMML patients (ClinicalTrials.gov Identifier, NCT03040401).

35.8.5 Interleukin 3 Inhibition in MDS/MPN

Interleukin 3 (IL-3) is a T-cell-derived multi-lineage-colony-stimulating factor (M-CSF) that induces differential, growth, and anti-apoptotic effects, especially during granulopoiesis and monocytopoiesis [416–419]. It binds to its receptor IL-3R and mediates downstream signalling via the JAK/STAT, PI3K/Akt, and Ras/Raf/MAPK/ERK [418–422]. In MDS/MPN, spontaneous IL-3 secretion and IL-3R expression on leukaemic cells are correlated with shorter OS and increased risk of transformation due to uncontrolled haematopoiesis, enhanced ability to evade apoptosis and immunity, and selective engraftment and quiescence in the BM microenvironment, especially in CMML [423–427]. Preclinical studies investigating anti-IL3 therapy implied efficacy in JMML therapy, as well as demonstrated effectiveness in significantly diminishing leukaemic cell load, prolonging OS, and reducing symptom burden in CMML-bearing mice [428]. Tagraxofusp (SL-401), a novel and selective diphtheria toxin-derived IL-3 recombinant protein, acts via cellular endocytosis and toxin release to halt protein synthesis and induce apoptosis [429]. Preclinical studies on AML-bearing mice demonstrated remarkable results in prolonging median disease-free survival (>72 vs. 31 days) with 33% achieving complete eradication of leukaemic cells and another 33% attaining progressively enhanced leukaemic cell kill [430]. Encouraging preliminary results were recently reported from an active phase 1/2 trial in relapsed/refractory CMML patients (ClinicalTrials.gov Identifier, NCT02268253): toxicity profile was manageable and all of patients with baseline splenomegaly achieved reduction in spleen size, including 75% patients attaining ≥50% reduction [431]. Marrow complete response (mCR) was 14% and treatment duration for >6 months was 43%, but more conclusive results are being anticipated [431]. Given its high expression on leukaemic myeloid cells, IL-3 is a promising candidate for targeted immunotherapy and should be considered for other MDS/MPN subtypes.

35.9 Conclusion

MDS and MDS/MPN are highly complex, under-diagnosed, indolent, and progressive clonal haematological malignancies [15, 432]. Despite the emergence and therapeutic efficacy of HMAs and allo-HSCT therapies, an effective treatment or definitive cure yielding high disease-free survival rates is yet to be found [16, 59–61, 66–68, 96, 97]. This urges for the development of novel therapies hoping to reduce disease burden, improve QoL, and achieve disease eradication.

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Molecular Landscape and Personalized Prognostic Prediction of MPNs

36

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Abstract

The past two decades have seen a plethora of studies and observations on driver and non-molecular alterations of prognostic significance in myeloproliferative neoplasm. In this chapter, we describe the molecular landscape of classical Philadelphia chromosome-negative myeloproliferative neoplasms (MPNs). Their relevance in the classification and prognostic assessment of MPN will be discussed.

Keywords

Myeloproliferative neoplasm · Gene mutations · Prognosis

36.1 Introduction

Myeloproliferative neoplasms (MPNs) are a group of clonal haematologic stem cell disorder characterized by proliferation of ≥1 haematopoietic cell lineage(s) with transitional forms between one another and a propensity of transforming to secondary acute myeloid leukaemia (sAML) [1–4]. According to the 2016 World Health Organization (WHO) classification, MPN can be categorized into three classical Philadelphia-chromosome negative (Ph-negative) subgroups: polycythaemia vera (PV), essential thrombocythaemia (ET), primary myelofibrosis (PMF), as well as four other clinicopathologic entities: chronic myeloid leukaemia (CML), chronic neutrophilic leukaemia (CNL), chronic eosinophilic leukaemia, not otherwise specified (CEL-NOS) and MPN, unclassifiable (MPN-U) [1, 3, 5, 6].

This chapter focuses on the molecular landscape and its prognostication in classical Ph-negative MPNs, which typically harbours *Janus kinase 2* (*JAK2*)/*Calreticulin* (*CALR*)/*Myeloproliferative leukaemia virus oncogene* (*MPL*) mutations.

36.2 Overview of Classical Ph-Negative MPNs

36.2.1 Polycythaemia Vera (PV) and Essential Thrombocythaemia (ET)

PV comprises of clonal proliferation of all three haematopoietic cell lineages (primarily proliferation of erythroid progenitors), resulting in a hypercellular panmyeloid bone marrow [1]. A raised haemoglobin concentration or haematocrit is typically seen [5, 7], signifying an elevated thrombotic risk due to increased rheology. Such presentation is mainly contributed by its unique molecular landscape, which PV is inevitably driven by *JAK2* mutations. Over 95% PV patients harbour *JAK2V617F* mutation, while the remaining small proportion carries *JAK2 exon 12* mutation [4, 5, 8–12]. The diagnosis of PV according to the WHO classification is summarized in Table 36.1 [3, 6, 12–14].

ET is characterized by a sustained increase in the platelet count due to clonal megakaryocyte proliferation [7, 15, 16]. Hence, arterial and venous thromboembolic events such as Budd-Chiari Syndrome, cerebral venous sinus thrombosis,

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Table 36.1 Diagnosis of PV and ET according to 2016 WHO classification

	PV	ET
Major criteria		
1. CBC/clinical feature	– Hb >16.5 g/dL in men/>16 g/dL in women; or – Hct >49% in men/>48% in women; or – Increased red cell mass	– Platelet count ≥450 × 10^9/L
2. Morphology in BM biopsy	– Trilineage proliferation with pleomorphic mature megakaryocytes	– Proliferation mainly of the megakaryocyte lineage with increased numbers of enlarged, mature megakaryocytes with hyperlobulated nuclei – No significant left-shift of neutrophil granulopoiesis or erythropoiesis – Very rarely minor (grade 1) increase in reticulin fibres
3. Molecular genetics	– Presence of *JAK2V617F*/*JAK2 exon 12* mutation	– Presence of *JAK2*, *CALR* or *MPL* mutation
4. Exclusion of other diseases	/	– Not meeting WHO criteria for *BCR-ABL1*$^+$ CML, PV, PMF, MDS, or other myeloid neoplasms
Minor criteria		
	– Subnormal serum erythropoietin level	– Presence of a clonal marker (e.g. abnormal karyotype); or – Absence of evidence for reactive thrombocytosis
Diagnosis made by fulfilling:		
	– All three major criteria; or – Two major criteria + one minor criterion	– All four major criteria; or – Three major criteria + one minor criterion

CBC, complete blood count; *CML,* chronic myeloid leukaemia; *ET,* essential thrombocythaemia; *Hb,* haemoglobin; *Hct,* haematocrit, *MDS,* myelodysplastic syndrome; *PMF,* primary myelofibrosis; *PV,* polycythaemia vera

pulmonary embolism are not uncommon [15]. Microvascular events such as erythromelalgia, transient ischemic attack and ocular migraine could also occur [15]. In some patients with extreme thrombocytosis, haemorrhagic complications might be developed due to consumption of von Willebrand factor (vWF) and development of acquired von Willebrand's Disease (vWD) [15]. *JAK2V617F* mutation is the most prevalent mutation and accounts for 50–60% in ET patients, followed by *CALR* (20–25%) and *MPL* (3–5%) [16, 17]. Table 36.1 summarized the diagnostic criteria of ET.

Both PV and ET share a more indolent clinical course compared to PMF. A vast majority of patients enjoy a prolonged median survival of more than 15 years with good symptom and disease control [18]. Yet, approximately 10% patients might transform to secondary myelofibrosis (SMF) with time. Less than 5% PV and ET patients might undergo leukaemic transformation, but most of them undergo SMF before transforming to sAML [18, 19]. Difference in driver mutations, concurrent somatic mutations, history of thrombosis/cardiovascular disease, advanced age are some of the key factors that affect disease prognosis.

36.2.2 Myelofibrosis (MF)

PMF is characterized by predominant megakaryocytic and granulocytic myeloproliferation coupled with progressive generalized fibrosis of the bone marrow, leading to impaired medullary haematopoiesis and increased extramedullary haematopoiesis (EMH). Progressive massive splenomegaly and marked constitutional symptoms are usually observed [1, 2, 20, 21]. PMF could be divided into prefibrotic (pre-PMF) and overt fibrotic stage [3]. Its diagnostic criteria are summarized in Table 36.2.

Similar to ET, around 55–60% MF patients carry *JAK2V617F* mutation, whilst others harbour *CALR* (25–30%) and *MPL* (5–10%) [5, 8, 13, 17]. MF patients. As MF has a more dismal outcome, several prognostic systems have been designed for better risk stratification and treatment intervention. Molecular genetics have been incorporated into various prognostic models for better disease assessment.

Table 36.3 outlines the epidemiology and clinical features of Ph-negative MPNs.

Table 36.2 Diagnosis of PMF according to 2016 WHO classification

	Prefibrotic PMF	Overt PMF
Major criteria		
1. Morphology	– Megakaryocytic proliferation and atypia, without reticulin fibrosis > grade 1, accompanied by increased age-adjusted BM cellularity, granulocytic proliferation and often decreased erythropoiesis	– Megakaryocyte proliferation and atypia accompanied by either reticulin and/or collagen fibrosis (grade 2 or 3)
2. Exclusion of other diseases	– Not meeting WHO criteria for *BCR-ABL1*$^+$ CML, PV, ET, MDS, or other myeloid neoplasms	
3. Molecular genetics	– Presence of *JAK2*, *CALR*, or *MPL* mutation; or – In the absence of these mutations, presence of another clonal marker or absence of minor reactive BM reticulin fibrosis	
Minor criteria (presence of ≥ 1 of the following confirmed in two consecutive determinations)		
1. CBC count	– Anaemia not contributed to a comorbid condition	
	– Leukocytosis ≥ 11×10^9/L	
2. Biochemistry	– LDH level above the upper limit of the institutional reference range	
3. Clinical feature	– Palpable splenomegaly	
4. Morphology	/	– Leukoerythroblastosis
Diagnosis made by fulfilling: all three major criteria + at least one minor criterion		

CBC, complete blood count; *CML,* chronic myeloid leukaemia; *ET,* essential thrombocythaemia; *LDH,* lactate dehydrogenase; *MDS,* myelodysplastic syndrome; *PMF,* primary myelofibrosis; *PV,* polycythaemia vera

Table 36.3 Epidemiology and clinical features of PV, ET and PMF

	PV	ET	PMF	References
Incidence	0.4–2.8 in 10^5 individuals/year	0.59–2.3 in 10^5 individuals/year	0.3–2 in 10^5 individuals/year	[1, 12, 14, 22–25]
Median age of diagnosis	60 (10% patients present below the age of 40)	20–40	65–70	
Gender distribution	Slight male predominance	Slight female predominance	Slight male predominance	
CBC parameters				
WBC	↑	Normal	↑	[3–5]
Hb	↑↑	Normal	Normal/↓	
Plt	↑	↑↑	↑	
Common clinical features				
Arterial and venous thrombosis	✓	✓	✓	[1, 6, 12, 21, 24, 26–28]
Aquagenic pruritus	✓	×	×	
Constitutional symptoms	✓	✓	✓✓	
Haemorrhage (due to thrombocytosis)	✓	✓ (Acquisition of acquired vWD when platelet >1000×10^9/L)	Uncommon	
Hepatomegaly	✓	Rare	✓	
Microvascular/vasomotor disturbances (e.g. headache, transient neurologic or ocular disturbances, tinnitus, atypical chest discomfort, paraesthesia and erythromelalgia)	✓ (Abnormal interactions between platelet and endothelium)		Uncommon	
Splenomegaly	Mild to moderate	Mild to moderate	Massive	
Disease progression				
Median survival	~15 years	~18 years	~6 years	[6, 18, 19, 22–24, 29–31]
Progression to SMF in 15 years	5–19%	4–11%	N/A	
Transformation to sAML at 10 years	3%	<1%	10–20%	

ET, essential thrombocythaemia; *Hb,* haemoglobin; *Plt,* platelet; *PMF,* primary myelofibrosis; *PV,* polycythaemia vera; *sAML,* secondary acute myeloid leukaemia; *SMF,* secondary myelofibrosis; *vWD,* von Willebrand's Disease; *WBC,* white blood cell

36.3 Driver Mutations: *JAK2*, *CALR* and *MPL*

Driver mutations *JAK2*, *CALR* and *MPL* that lead to constitutive JAK-STAT activation are hallmarks of classical Ph-negative MPNs [4]. The three driver mutations are usually mutually exclusive, but coexistence of driver mutations could also occur in rare scenarios [2, 5]. Five-percent MPN patients might not harbour any driver mutations. Hence, they are grouped under triple-negative MPN and other somatic mutations are usually present in these patients [2, 5]. The additional somatic mutations and their prognostic impact would be discussed in subsequent sections.

36.3.1 JAK2V617F

JAK2V617F mutation is the most frequently detected gene in Ph-negative MPNs, which accounts for >95% in PV patients and approximately 60% in ET and PMF patients [2, 4, 5, 8–13, 16].

Being a cytoplasmic tyrosine protein kinase, *JAK2* is associated with a myriad of haematopoietic cytokine receptors including erythropoietin receptor (EPO-R), thrombopoietin receptor (TPO-R; MPL) and granulocyte colony-stimulating factor receptor (G-CSF-R) [4, 9]. It is located on chromosome 9p24.1, and is comprised of four major structural units: the JH1 (JAK Homology) domain in the C-terminal, the JH2 pseudokinase domain, the SH2 (Src Homology 2)-like domain and the FERM (4.1/ezrin/radixin/moesin) domain in the N-terminal [4, 5, 7, 17] (Fig. 36.1). Specific ligand binding leads to receptor dimerization, hence JAK2 autophosphorylation and JAK2-receptor transphosphorylation [4]. This prompts the activation of JAK-STAT pathway and subsequent downstream signalling pathways for gene transcription [4, 5, 7].

Somatic *JAK2V617F* mutation involves a nucleotide base change from guanine (G) to thymine (T) at codon 617 of the auto-inhibitory JH2 pseudokinase domain. Thus, the resultant amino acid is converted from valine to phenylalanine in exon 14 [1, 5, 7, 32]. This destabilizes the usual JH1–JH2 domain conformation, and consequently alters the normal auto-inhibitory functions of the JH2 domain. As a result, the JAK-STAT, mitogen-activated protein kinase (MAPK) and phosphoinositidie-3-kinase (PI3K) signalling pathways are aberrantly activated, giving rise to independent cytokine production and excessive cellular proliferation of all three lineages [1, 4, 9, 32–34].

The heterogeneity of clinical presentations could be partly explained by the difference in *JAK2V617F* allelic burden [2, 8, 17, 34]. PV and PMF patients are generally associated with homozygous mutation and a higher (>50%) mutant allele burden due to loss of chromosome 9p in mitotic recombination [5]. As for ET patients who are *JAK2V617F-positive*, heterozygous mutation is usually reported [5, 8, 34,

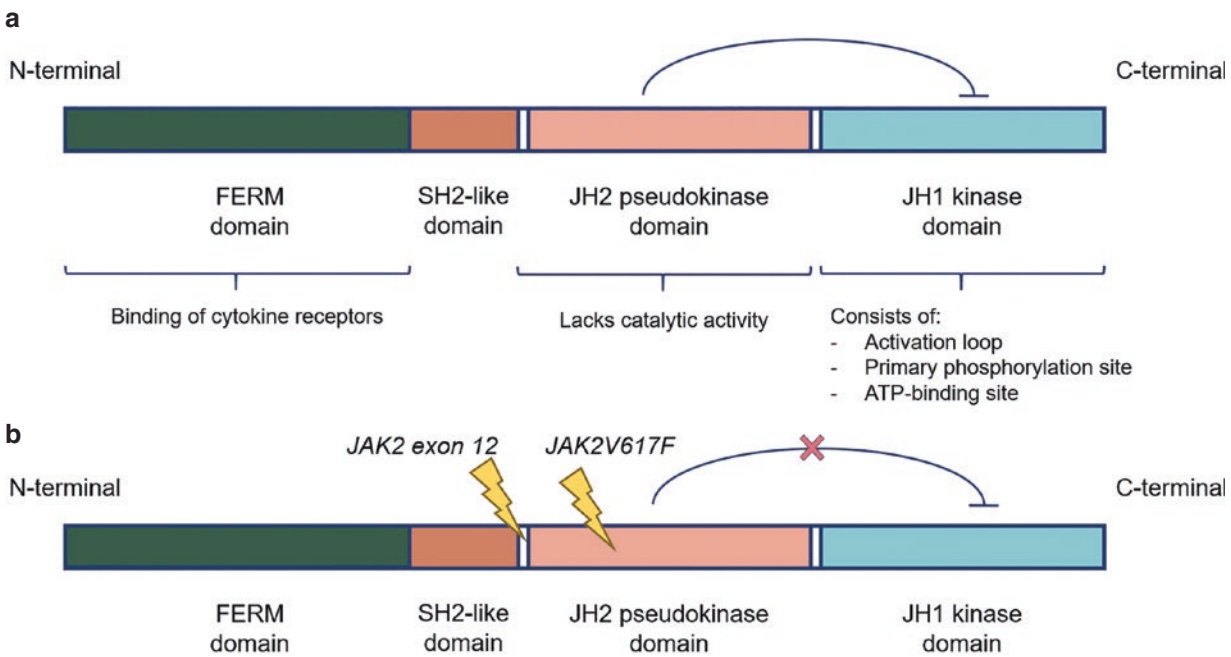

Fig. 36.1 Schematic diagram of *Janus kinase 2*. (**a**) shows the structural unit of *JAK2-wild type* which is comprised of four structural subunits. (**b**) shows the site of *JAK2V617F* and *JAK2 exon 12* mutation. The normal inhibitory effect of JH2 domain to JH1 domain is hampered, resulting in constitutive activation of JH1 kinase and downstream signal pathways for cellular proliferation

35]. A high-mutant allele burden is associated with increased thrombotic complications, more significant splenomegaly and symptoms, as well as an elevated risk of SMF transformation [7, 8, 11, 17, 34–37]. Therefore, it is not surprising that ET has a better prognosis compared to PV and PMF [38]. Some also correlate a high-mutant allele burden with a haematological picture of higher haemoglobin count, raised leukocyte count, lower platelet count in ET patients but the findings are not always consistent [7, 8, 11, 17, 34–37, 39]. Such interesting observation might be contributed by ethnic difference, in which a positive relationship of the aforementioned clinical picture with higher *JAK2V617F* allele burden is elucidated in Caucasians but not in Asian patients [34]. Yet, further investigations are warranted.

36.3.2 JAK2 Exon 12

JAK2 exon 12 is specifically identified in 2–3% of *JAK2V617F-negative* PV patients [40–42]. Therefore, in patients who resemble PV phenotypically but proven *JAK2V617F-negative*, investigation for the presence of *JAK2 exon 12* somatic mutations shall be performed [12]. Several gain-of-function somatic mutations of *JAK2 exon 12* have been identified and all of them are located within the linker region between the SH2 and JH2 domains [8, 41, 42]. The heterozygous *JAK2 exon 12* mutation subsequently results in cytokine-independent activation and downstream signalling pathways. In contrast to *JAK2V617F* mutation, most of these patients have a younger age of onset with more prominent erythrocytosis but lower white blood cell and platelet counts [7, 40, 42, 43]. Despite the discrepancies in clinical presentations, significant difference on constitutional symptoms, thrombotic risks and long-term survival are not identified [10, 40, 44]. It is also worth noted that *JAK2 exon 12* mutation is more pervasive in Chinese than that of Westerns and other East Asians [45].

36.3.3 CALR

CALR is the second most frequent mutation which is detected in 20–25% ET patients and 25–30% PMF patients [4]. Located on chromosome 19p13, *CALR* functions as a calcium-binding chaperon protein for calcium homeostasis in the endoplasmic reticulum (ER) [46, 47] (Fig. 36.2). Two

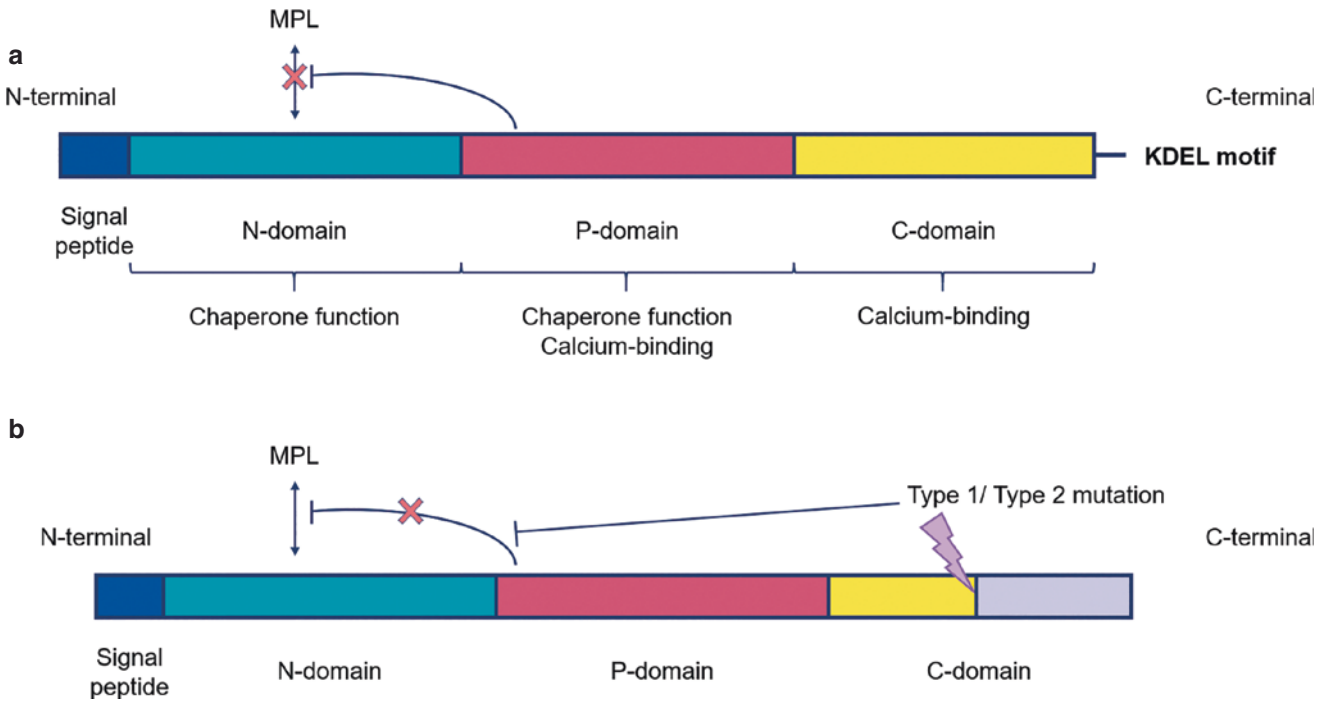

Fig. 36.2 Schematic diagram of *calreticulin*. (**a**) shows the structural unit of *wild type calreticulin*. *Calreticulin* is composed of four major subunits: signal peptide, the amino-terminal N-domain (N-domain), the proline-rich domain (P-domain) and carboxy-terminal C-domain (C-domain). KDEL (Lys-Asp-Glu-Leu) motif is located at the C-terminal that acts as an endoplasmic reticulum retention signal. (**b**) displays type 1/type 2 *CALR* mutation, which occurs at the C-domain in exon 9. This hampers the normal inhibition of P-domain towards MPL-N-domain interaction, leading to increased binding to MPL. The loss of KDEL motif in *mutant CALR* further facilitates the export of MPL towards the cell surface, hence subsequent aberrant signalling pathways

major types of mutation have been identified in *CALR exon 9* [46–48]. Type 1 mutation involves a 52-bp deletion while type 2 mutation is resulted from a 5-bp insertion [10, 48]. Both of them generate a +1-frameshift mutation, giving rise to a conformational change in the C-terminal [46–50]. The negatively charged amino acid sequence in *wild type CALR (CALR-WT)* is replaced by positively charged methionine- and arginine-rich polypeptide [50]. This facilitates the interaction between MPL and mutant CALR. As the KDEL (Lys-Asp-Glu-Leu) motif for ER retention on the C-terminal is also lost [48–50], MPL is exported to cell surface, entailing TPO-independent activation and clonal dysmegakaryopoiesis [4, 48].

Clinically, *CALR* mutation has a more favourable prognosis in comparison with *JAK2* and *MPL* mutations [51]. *CALR-positive* PMF patients, especially those with type 1 mutations, have a lower risk of thrombosis and disease progression to sAML [5, 38, 48, 51–53]. On the other hand, *CALR-positive* ET patients do not confer significant difference in overall survival (OS) when compared to *JAK2-mutated* cases [51, 54]. Yet, patients with type 2 variants are usually younger and present with a higher platelet count than its type 1 counterpart [7, 55].

36.3.4 MPL

MPL, also known as TPO-R, is encoded by *MPL* gene on chromosome 1p34 [8], and is present in 3–5% ET patients and 5–10% PMF patients [56, 57]. *MPLW515L* and *MPLW515K* are the two most common somatic gain-of-mutation genes located at the transmembrane domain in exon 10, resulting in TPO-independent activation and marked thrombocytosis [2, 57, 58]. Other rarer *MPL* substitutions include *MPLW515A, MPLW515A* and *MPLW515G* [2, 5].

Figure 36.3 depicts the signalling pathways of the three driver mutations.

36.3.5 Triple-Negative MPNs

The term "triple-negative MPNs" is used to describe the remaining 10% ET and PMF patients who are proven negative for all three driver mutations. This is exhibited in approximately 20% ET patients and 10–15% PMF patients [2, 4, 7]. Alternatively, these patients usually carry additional non-disease-specific somatic mutations. It is recommended

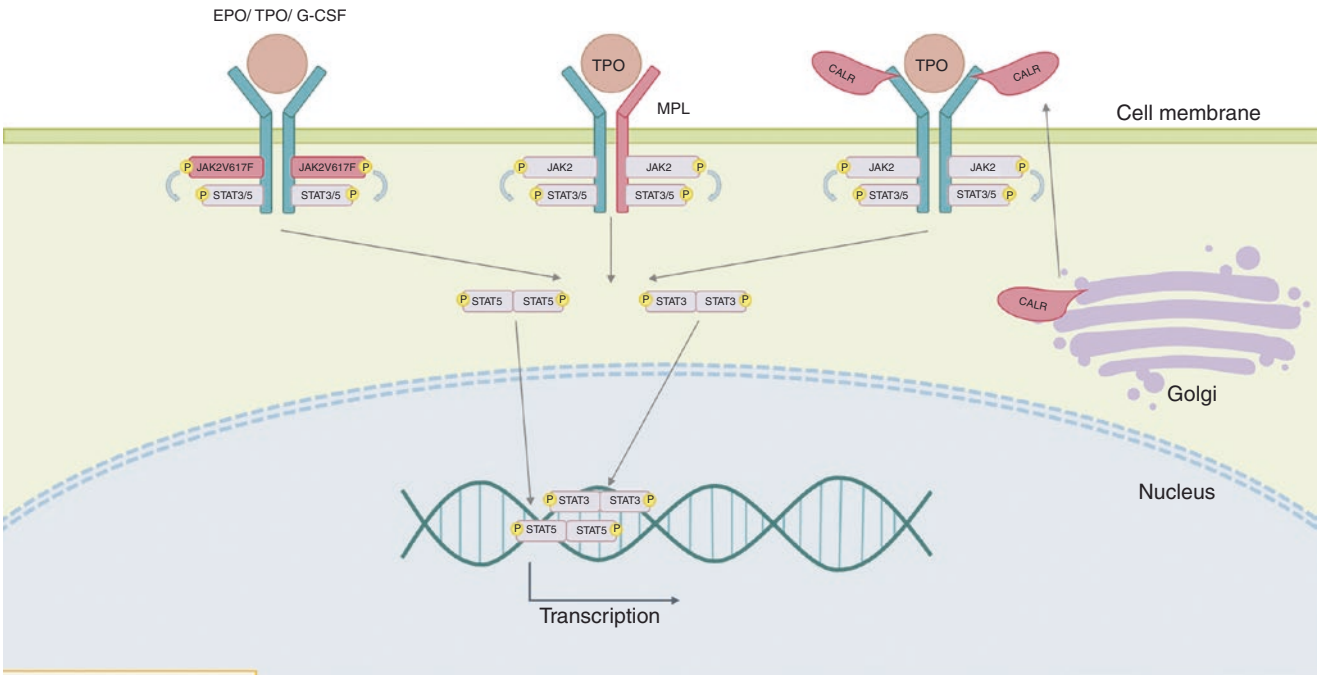

Fig. 36.3 Signalling pathways of the three MPN driver mutations. The three MPN driver mutations *JAK2, CALR* and *MPL* are highlighted in red in the diagram. All of them lead to aberrant JAK-STAT pathways and subsequent transcription. EPO, erythropoietin; G-CSF, granulocyte colony-stimulating factor; TPO, thrombopoietin

that high-molecular risks (HMR) clonal markers (i.e. *ASXL1, EZH2, IDH1/2, SRSF2*) shall be investigated in triple-negative MF patients [59].

36.4 Other Somatic Mutations

Acquisition of non-driver somatic mutations is implicated in disease progression of Ph-negative MPNs. It could be classified into five classes according to gene functions, namely DNA methylation, histone modification, messenger RNA (mRNA) splicing, signal transduction and transcription regulation (Table 36.4).

36.4.1 DNA Methylation

TET2, DNMT3A and IDH1/2 are notable DNA methylation gene mutations identified in MPNs. Interestingly, the clinical phenotype of MPNs is partially influenced by the order of mutation acquisition [5, 32, 60]. Incidence of *TET2* increases with age [7]. Thus, if *TET2* acquisition precedes *JAK2V617F,* patients are usually older at diagnosis [32, 60]. Heterozygous mutations favour an ET phenotype [5, 32, 60]. On the contrary, in "*JAK2*-first" patients, homozygous *JAK2V617F/TET2* clone dominance is promoted, giving rise to a PV phenotype. This finding is also consistent in *DNMT3A* [17]. Different from *TET2* and *DNMT3A*, *IDH1/2* plays a more pathogenic role in disease progression regardless of *JAK2V617F* mutational status [61, 62]. A poorer spleen response to ruxolitinib has also been shown [20].

36.4.2 Histone Modification

EZH2 and *ASXL1* are well-established histone modification gene mutations that confer dismal outcomes [63, 64]. Both of them are regarded as HMR mutations with a profoundly shortened OS and rapid progression to sAML [5, 63–65]. The detrimental prognosis of reduced survival is particularly imparted in patients who carry *ASXL1* but not *CALR* [59, 66]. Clinically, a more aggressive clinical course is demonstrated. Thrombotic risks are augmented in ET patients with *ASXL1* mutations [37], revealing the cardinal role of histone modification genes mutations.

36.4.3 mRNA Splicing

mRNA spliceosome mutations of *SRSF2, SF3B1, U2AF1* and *ZRSR2* are associated with an advanced clinical course. Except *ZRSR2* playing a relative minor role in disease progression, *SRSF2, SF3B1* and *U2AF1* are indications of poor prognosis. Phenotypic changes such as anaemia and thrombocytopenia are developed which reduce OS and enhance SMF/sAML transformation [5, 32, 67–69]. Recently, it is discovered that the OS of PV patients carrying *SRSF2* is diminished. Hence, *SRSF2* is included in Mutation-enhanced International Prognostic Scoring System (MIPSS-PV), a newly proposed prognostic system for PV patients [17, 70].

36.4.4 Signal Transduction

Signal transduction gene mutations are uncommon in chronic MPNs but incidence increases with leukaemic transformation [8, 32, 71]. Mutations of *RAS* and *PTPN11* are associated with progression to sAML and shortened survival in blast crisis respectively [4, 71, 72]. Being a negative regulator of JAK-STAT pathway, mutant *SH2B3* (also known as *LNK)* plays a key role in PV patients [73, 74]. Loss of gene function causes aberrant *JAK2* activation, hence erythrocytosis [17]. Therefore, the search of *SH2B3* mutation is recommended in *JAK2-negative* PV patients to support diagnosis [12, 17] although *SH2B3* and *JAK2V617F* mutations are not mutually exclusive.

36.4.5 Transcription Regulation

Mutations of transcription regulators are present as late genetic events during disease progression [5, 8, 32, 75]. Thus, the occurrence of these genetic mutations, *TP53* in particular, indicates a more grievous outcome via the development of clonal dominance and homozygous mutations [4, 76]. Treatment in MPN in accelerated/blast phase has been challenging. It was displayed that intensive therapy is limited to patients without *TP53* mutation and have <4 concomitant somatic mutations [76].

Table 36.4 Overview of non-driver somatic mutations in classical Ph-negative MPNs

Gene	Chromosome location	Type of mutation	Protein function	Frequency (%)				Prognostic impact	References
				PV	ET	PMF	sAML		
DNA methylation									
TET2	4q24	Loss of function (point mutations/ deletions)	– Oxidizes 5mC into 5-hmC, resulting in DNA demethylation – Loss of *TET2* hypermethylates DNA, as well as promotes self-renewal and acquisition of additional mutations in HSCs	15–30	10–20	10–15	25	– Disease initiation – Disease progression if acquired as secondary mutations – Clinical phenotype influenced by the order of acquisition of *TET2/DNMT3A* and *JAK2V617F*	[2, 5, 14, 33, 60, 72]
DNMT3A	2p23	Missense, hotspot	– Regulation of DNA methylation at CpG dinucleotides	5	5	5–15	20		
IDH1	2q33.3	Missense, hotspot	– Decarboxylation of isocitrate to α-KG – Mutation increases 2-HG that hampers histone demethylation and HSC differentiation	<2	<2	3–5	12	– Disease initiation – Progression to SMF, sAML	
IDH2	15q26.1			<2	<2	3–5	7		
Histone modification									
EZH2	7q35–36	Missense, indel (loss of function)	– Histone methyltransferase of PRC2 for histone modification	<5	<5	3–12	13–15	– Rapid fibrotic and leukaemic transformation – Associated with *JAK2V617F* mutation	[1, 2, 7, 14, 33, 63–66, 72]
ASXL1	20q11	Missense, indel	– Histone methylation through PRC2-mediated H3K27 trimethylation, leading to repression and silencing of chromatin-modifying genes	5–10	5–10	10–25	20–40	– Adverse prognosis, especially in the absence of *CALR* mutation – Rapid fibrotic and leukaemic transformation – Higher thrombotic risks in ET patients	

Gene	Chromosome location	Type of mutation	Protein function	Frequency (%)				Prognostic impact	References
				PV	ET	PMF	sAML		
Messenger RNA splicing									
SRSF2	17q25.1	Missense, hotspot	– Spliceosome assembly – Mutation alters the balance of pre-mRNA splicing, resulting in miss-spliced products and nonsense-mediated decay	<5	<5	10–20	10–20	– Adverse prognosis; leukaemic progression	[2, 14, 33, 67–69, 72]
SF3B1	2q33.1	Missense	– Encodes a component of the spliceosome complex and interacts with polycomb group proteins	5	3	10	4–7	– Phenotypic change (anaemia, thrombocytopenia) – Progression to SMF	
U2AF1	21q22.3	Missense	– Encodes a component of the spliceosome complex for RNA processing and splicing, hence translation of proteins	<5	<5	5–20	6	– Phenotypic change (anaemia, thrombocytopenia) – Disease progression	
ZRSR2	Xp22.2	Missense	– A component of the minor spliceosome for U12 intron splicing	<5	<3	1–10	5	– Minimal prognostic impact on thrombosis and survival	
Signal transduction									
LNK/SH2B3	12q24	Missense (loss of function)	– An adaptor protein that negatively regulates JAK-STAT pathway	1–3	0–5	0–6	10	– Synergism with *JAK2V617F/CALR* mutation for disease progression	[2, 33, 71, 72]
CBL	11q23	Missense (loss of function)	– Protein of the Cbl family of E3-ubiquitin ligases that negative regulates JAK-STAT pathway and STAT5 phosphorylation	<1	<2	<7	4	– Disease progression	
RAS	1p13.2	Missense (activation)	– Proto-oncogene (small GTPases) for cellular signal transduction (RAS/RAF/MEK; RAS/PI3K pathways)	<1	<1	3–4	7–15	– Disease progression – Associated with increased cellular proliferation	
FLT3	13q12	FLT3-ITD	– Transmembrane tyrosine kinase receptor for FLT3	<3	<3	<3	10–15	– Disease progression	
PTPN11	12q24.13	Missense	– Positive regulator of RAS signalling pathway	<1	<1	1	2–5	– Shortened survival in sAML	

(continued)

Table 36.4 (continued)

Gene	Chromosome location	Type of mutation	Protein function	Frequency (%)				Prognostic impact	References
				PV	ET	PMF	sAML		
Transcription regulation									
TP53	17p13.1	Missense, indel	– Important tumour suppressor gene that regulates cell cycle, DNA repair and cellular apoptosis	1	2–3	2–3	11–35	– Disease progression – Associated with complex karyotype – Shortened survival	[2, 14, 33, 72, 75]
CUX1	7q22	Deletion of 7q	– Transcription factor regulating TP53	<3	<3	<3	Raised	– Disease progression	
IKZF1	7p12.2	Deletion of 7p, indel	– Encodes the transcription factor Ikaros for lymphopoiesis – Mutation occurs at late stages and induces cytokine hypersensitivity	<3	<3	<3	21	– Progression to sAML	
RUNX1	21q22.3	Nonsense, missense, indel	– Transcription factor that binds to CBF-β for haematopoiesis	<2	<3	<5	4–13	– Progression to sAML	

2-HG, 2-hydroxyglutarate; *5-hmC,* 5-hydroxymethylcytosine; *5mC,* 5-methylcytosine; *α-KG,* alpha ketoglutarate; *ASXL1,* additional sex comb like-1; *CBF-β,* core-binding factor beta; *CBL,* casitas B-lineage lymphoma; *CUX1,* cut-like homeobox 1; *DNMT3A,* DNA methyltransferase 3A, *ET,* essential thrombocythaemia; *EZH2,* enhancer of zeste 2; *FLT3,* fms-related receptor tyrosine kinase 3; *H3K27,* histone H3 lysine 27; *HSCs,* haematopoietic stem cells; *IDH1/2,* isocitrate dehydrogenase 1/2; *IKZF1,* IKAROS family zinc finger 1; *LNK,* lymphocyte adapter protein; *PMF,* primary myelofibrosis; *PRC2,* polycomb repressor complex 2; *PTPN11,* protein tyrosine phosphatase non-receptor type 11; *PV,* polycythaemia vera; *RUNX1,* runt-related transcription factor 1; *sAML,* secondary acute myeloid leukaemia; *SF3B1,* splicing factor 3B, subunit 1; *SH2B3,* SH2B adapter protein 3; *SRSF2,* splicing factor, serine/arginine-rich 2; *TET2,* ten-eleven translocation 2; *U2AF1,* U2 small nuclear RNA auxiliary factor 1; *ZRSR2,* zinc finger CCCH-type, RNA binding motif and serine/arginine-rich 2

36.5 Conclusion

Classical Ph-negative MPNs are a group of heterogenous disease that arise from the haematopoietic stem cell level. They are mostly driven by three major driver mutations: *JAK2, CALR, MPL.* Non-driver somatic mutations could be acquired in early stages of the disease as well as during disease progression. The unique molecular landscape has provided insights in disease pathogenesis. These pathogenic molecular mutations are hence identified and integrated into current prognostic models for personalized risk stratification. Consequently, therapeutic strategies could be tailored and optimized, resulting in better symptom control and disease modification.

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Treatment Algorithm for Polycythemia Vera

37

Jeanne Palmer and Ruben Mesa

Abstract

Polycythemia Vera (PV) is a myeloproliferative disease characterized by a high red blood cell mass. Patients often present with an elevated hemoglobin, though may have elevated platelets and white blood cells as well. The majority of the patients who have this disease will have a mutation in the Janus Kinase (JAK) 2 gene, such as *JAK2V617F* mutations and *JAK exon 12* mutations. This article will review the biology of the disease, as well as diagnosis. We will also discuss different treatment options available. Finally, we will review the long-term risks of the disease, such as transformation to myelofibrosis and acute leukemia.

Keywords

Polycythemia vera · Myeloproliferative neoplasm · Treatment

37.1 Background and Presentation

Polycythemia Vera (PV) is a one of the chronic myeloproliferative neoplasms (MPN) which is characterized by a high red blood cell mass. This disorder was initially described in 1882 by Louis Henri Vaquez, however many other scientists have contributed to the understanding of this disorder. The most common presentation of PV is a patient with an elevated hemoglobin, though approximately a third of patients will present with a thrombotic event. Other laboratory findings include leukocytosis and thrombocytosis. The prevalence of this disease is estimated at 44–57 per 100,000 [1–3]. It appears to impact both genders equally, and the median age of diagnosis is approximately 60 years of age [4].

The biology of MPNs has been elucidated over the last 20 years with the advent of molecular testing. The majority of the patients will harbor Janus Kinase (JAK) 2 mutations, such as *JAK2V617F* mutations and *JAK exon 12* mutations. The more common mutation, *JAK2V617F,* was initially described in 2005 by several groups of investigators [5–8]. This mutation, which occurs within the autoinhibitory pseudokinase domain JAK2, leases to JAK2 kinase hyperactivity and abnormal signaling through the JAK-STAT pathway. This is felt to contribute to the underlying pathology of the disease, however notably is also present in other MPNs such as essential thrombocythemia and myelofibrosis.

In addition to laboratory abnormalities, patients with PV may have associated symptoms such as headache, visual changes, erythromelalgia, peripheral neuropathy, night sweats, aquagenic pruritis, and gastrointestinal disturbances [9]. These symptoms are as a result of both vasomotor disturbances, as well as inflammation [9]. Approximately a third of patients may present with an enlarged spleen [4].

Long-term, there is also a risk of progression to myelofibrosis (MF) and acute myelogenous leukemia (AML). The estimated annual incidence of AML ranges from 0.17 to 3.0 [10]. The risk of progression to MF is dependent on age as well as duration of time with the disease. A recent meta-analysis, the odds of MF transformation were found to increase on average 6% (95% CI 1–11%) for each year of age, while those of mortality increase by 21% (95% CI 9–33%) [11].

37.2 Work Up and Diagnosis

When a patient presents with erythrocytosis, it is important to utilize a systematic approach to identify an etiology. A detailed history is critical, and must include questions regard-

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ing a history of cardiopulmonary disease, smoking, exogenous testosterone use, sleep apnea, and any evidence of a malignancy [12]. Another setting associated with erythrocytosis is renal transplant, this phenomenon usually is short-lived and often responds to ACE inhibitors [13]. At the initial evaluation, it is important to check for Janus Kinase 2 mutations, such as *JAK2V617F* mutations and *JAK exon 12* mutations in addition to erythropoietin level (EPO). Janus Kinases are a family of protein-tyrosine kinases that are critical in signaling pathways to promote cell growth. The JAK family includes JAK1, JAK2, JAK3, and TYK2 (tyrosine kinase 2). JAK1/2 and TYK2 are found in many cells throughout the body, and JAK3 appears to be confined to hematopoietic cells. In hematopoietic cells, JAK proteins bind to the juxtamembrane region of specific cytokine receptors, and promote cell growth and differentiation through JAK-STAT signaling pathways [14]. However, in the event these mutations are negative, the likelihood of having PV is quite small. EPO is a hormone produced by fibroblasts in the kidney that promotes the growth of erythrocytes. EPO level is controlled by hypoxia-induced transcription factor-2 (HIF-2) which is regulated by oxygen tension. In settings where oxygen tension is low, due to hypoxemia, anemia, or a change in the hemoglobin results in a higher affinity for oxygen, EPO will increase.

In PV, EPO is commonly low; however, 20% of patients will have a normal EPO at diagnosis. If the EPO level is elevated, it is important to evaluate for cardiopulmonary disease, including overnight pulse oximetry, pulmonary function tests, and echocardiography. EPO may also be produced exogenously by certain tumors, including cerebellar hemangioblastoma, hepatocellular carcinoma, renal cell carcinoma, and uterine leiomyoma. A CAT scan of the brain, neck, chest, abdomen, and pelvis will help rule out evidence of a malignancy. If the patient is young, or there is a family history of erythrocytosis, one may consider evaluating for a hereditary erythrocytosis [12].

The diagnosis of PV depends on meeting major and minor criteria (see Table 37.1). The major criteria for PV include a hemoglobin of greater than 16.0 g/dL in females or 16.5 g/dL for males, abnormal bone marrow biopsy findings, and presence of JAK2 V617F mutation. Minor criteria include subnormal serum erythropoietin level [15]. The major differences between 2008 WHO criteria as compared to 2016 criteria include the bone marrow biopsy findings being considered a major rather than a minor criteria, the lower hemoglobin levels, and removal of the minor criterion of the formation of endogenous erythroid colonies (EEC). This decrease in the hemoglobin level was designed to detect masked PV [16].

Table 37.1 Diagnosis of PV

Polycythemia vera
Major criteria
Hemoglobin > 16.5 g/dL (men) > 16 g/dL (women) or hematocrit > 49% (men) > 48% (women) or increased red cell mass (RCM)
BM with hypercellularity (age-adjusted) and trilineage myeloproliferation with pleomorphic, mature megakaryocytes
Presence of JAK2 mutation
Minor criteria
Subnormal erythropoietin
PV diagnosis requires meeting either all three major criteria or the first two major criteria and one minor criterion

37.3 Risk Assessment and Treatment

The cornerstone of managing PV in the short-term is to reduce the risk of thrombosis, as well as mitigate any symptom burden; in the long-term, reduce the risk of transformation to AML or MF. To determine the optimal management for patients, initially the patient risk must be assessed. The two most important risk factors for thrombosis include age of patient, and history of thrombosis [17]. To achieve the short-term goals, it is important to start aspirin (ASA) and maintain a hematocrit of 0.45 (0.42 in women).

ASA became a mainstay of treatment for PV following the European Collaboration on Low-Dose Aspirin in Polycythemia vera (ECLAP) study. In this study, 1638 patients were enrolled; 1120 were entered into a prospective observational cohort study; and 518 were enrolled in a double-blind placebo-controlled, randomized trial to assess the efficacy and safety of low-dose ASA. The primary endpoint of this study was the cumulative rate of nonfatal myocardial infarction, nonfatal stroke, or death from cardiovascular causes and the cumulative rate of nonfatal myocardial infarction, nonfatal stroke, pulmonary embolism, major venous thrombosis, or death from cardiovascular causes. This study showed that the relative risk of nonfatal myocardial infarction, nonfatal stroke, or death from cardiovascular causes in the ASA group as compared to the placebo group was 0.41 [95% CI 0.15–1.15; $p = 0.08$] which was not significant; however, if the risk of the above events in addition to pulmonary embolism, deep venous thrombosis were considered, the ASA group as compared to the placebo group showed a relative risk of 0.41 [95% CI 0.18–0.91; $p = 0.02$].

The hematocrit goal of less than 0.45 was confirmed in the cytoreductive therapy in PV (CYTO-PV), where a more stringent control of hematocrit was associated with a reduced risk of cardiovascular events [18]. In this study, 365 patients with documented PV based on 2008 criteria were randomized to two different hemoglobin goals, 0.45 or 0.50. The primary endpoint was time until death from cardiovascular causes or thrombotic event (including stroke, acute coronary syndrome, transient ischemic attack, pulmonary embolism, abdominal thrombosis, deep vein thrombosis, peripheral

artery thrombosis). The majority of the patients were high risk based on age or history of thrombosis. After a median follow-up time of 31 months, the primary endpoint was observed in 5/182 (2.7%) in the low hematocrit group and 18/183 (9.8%) in the high hematocrit group (HR was 3.91; [95% CI 1.45–10.53]; $p = 0.007$).

This hematocrit goal can be achieved by either serial phlebotomy or cytoreductive therapy. In patients considered low risk, who are less than 60 years of age, and have no history of thromboembolic disease, they should be started on ASA and initiate therapeutic phlebotomy. In patients who are considered higher risk, based on age greater than 60 and/or a history of a thromboembolic event, it is recommended to use ASA, phlebotomy, and cytoreduction. Response is measured as hematologic response, which is normalization of the hemoglobin, as well as spleen response, is normalization of spleen size [19]. Newer concepts revolve around molecular response as well as symptom response. Molecular complete response is defined as eradication of the molecular clone, whereas partial response is a 50% reduction [19]. Symptom response is defined as a decrease in at least 10 points in the MPN-TSS [19].

Phlebotomy is always initiated at diagnosis, even if systemic therapy is also pursued (see Fig. 37.1). Options for cytoreductive therapy are reviewed in Table 37.2. Upfront therapeutic options include hydroxyurea or interferon [20]. Hydroxyurea (HU, hydroxycarbamide) is a cytoreductive therapy that has been used for many years in the treatment of PV [11, 21, 22]. Many prefer this as a first-line therapy as it is easy to administer, lower cost, and favorable toxicity profile. The major side effects people experience include mouth sores, nonhealing ulcers on the extremities, and gastrointestinal distress; however, these are rare and usually associated with higher doses.

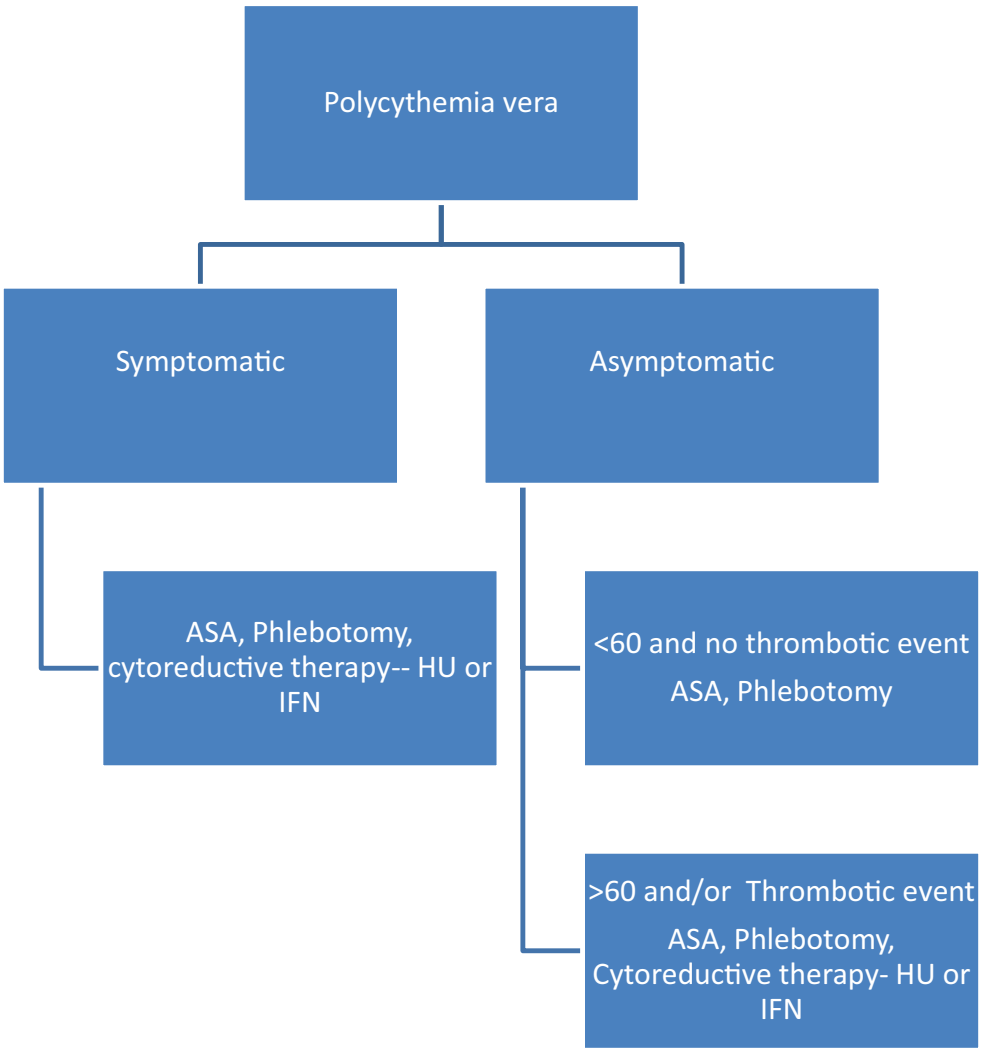

Fig. 37.1 Treatment algorithm for polycythemia vera

Table 37.2 Pros and cons of standard cytoreductive medications

Cytoreductive therapy	Pros	Cons	Who?
Hydroxyurea	• Control in myeloproliferation • Reduction in thrombosis in high-risk PV	• Mucocutaneous toxicity • Increased risk of skin cancer • Decreased blood counts	• Older patients • High-risk PV
Pegylated interferon – Pegylated IFN-2a – Pegylated IFN-2b	• Possible anticlonal activity • Control of counts	• Tolerability long-term • Impact on QoL • Minimal impact on splenomegaly	• Younger patients • Limited comorbidity • Avoid if patient has history of depression
Ruxolitinib PV	• Control of hemoglobin • Reduction in thrombotic risk • Control of symptom burden • Reduction in splenomegaly	• Cost • Infection • Weight gain	• Intolerant to HU • High symptom burden • Splenomegaly

Another treatment option is interferon (IFN). IFN has been used for decades to treat myeloproliferative neoplasms, and was a mainstay of treatment of CML prior to the introduction of imatinib [23]. Its use in non-CML MPNs has been explored [24, 25]. It is effective in reducing blood counts, but may also have a disease-modifying effect. The original form of interferon alfa, Intron or IFN-alfa 2b, was very difficult to tolerate, however with the introduction of pegylated formulations of interferon, such as pegylated interferon-2a (Peg-IFN), the side effect profile is much more manageable [25–27]. Although in the studies there are up to 37–39% who experienced grade 3 adverse events, only 12.5–13.9% of patients discontinued the medication due to these side effects [25–27]. The common side effects of Peg-IFN include flu-like symptoms, fatigue, and depression. It is also not recommended to use this therapy in those with autoimmune disease as it may exacerbate those disease.

Due to perceived intolerability of Peg-IFN, a novel monopegylated interferon ropeginterferon alfa-2b (Ropeg-IFN) was studied in a large randomized study, divided into the PROUD-PV study (first 12 months) and CONTINUATION-PV (12–36 months). In this study, 257 patients were randomized, 127 were treated in each group (three patients withdrew consent in the hydroxyurea group). Median follow-up was 182.1 weeks (IQR 166.3–201.7) in the Ropeg-IFN and 164.5 weeks (144.4–169.3) in the standard therapy group. In PROUD-PV, 26 (21%) of 122 patients in the Ropeg-IFN group and 34 (28%) of 123 patients in the HU group met the composite primary endpoint of complete hematological response with normal spleen size. In CONTINUATION-PV, the primary endpoint was met in 50 (53%) of 95 patients in the Ropeg-IFN group versus 28 (38%) of 74 patients in the hydroxyurea group, $p = 0.044$ at 36 months, suggesting ongoing response even after 12 months. Complete hematological responses, without meeting spleen criterion, in the Ropeg-IFN group versus standard therapy group were: 53 (43%) of 123 patients versus 57 (46%) of 125 patients, $p = 0.63$ at 12 months (PROUD-PV), and 67 (71%) of 95 patients versus 38 (51%) of 74 patients, $p = 0.012$ at 36 months (CONTINUATION-PV). As a result of these studies, Ropeg-IFN was approved by the European Commission in February of 2019. At the time of this writing, Ropeg-IFN is awaiting approval in the USA.

The PROUD-PV was mainly studying high-risk patients, to better understand the potential benefit in upfront therapy for low-risk patients. Low-PV was a multicenter randomized study that evaluated phlebotomy alone (63) versus phlebotomy plus Ropeg-IFN (64). The primary endpoint in this study was maintenance of hct <45% and lack of progression of disease, as defined by in progressive symptomatic thrombocytosis and progressive leukocytosis, as well as the occurrence of any vascular or major bleeding complication at 12 months [28]. They found a higher response rate in the experimental group was seen (42 [84%] of 50 patients) than in the standard group (30 [60%] of 50 patients; absolute difference 24%, 95% CI 7–41%; $p = 0.0075$). The study was ended early due to overwhelming efficacy of the treatment, and now provides another therapeutic option for patients with PV [28].

In patients who are not effectively controlled or intolerant of HU, or have a significant symptom burden, ruxolitinib is an option. The RESPONSE study was a randomized study comparing ruxolitinib with BAT (which was HU in over half the patients) in patients who were intolerant or resistant to HU. The primary endpoint was hematocrit control as well as spleen response (35% reduction in spleen volume reduction). The primary endpoint was met in 28% of patients in the ruxolitinib arm, and 1% in BAT arm. Hematologic response was observed in 60% of patients in the ruxolitinib arm, and 20% of patients in the BAT arm. Impressively, 49% of patients in the ruxolitinib arm had 50% reduction in their symptom burden, as assessed by MPN-TSS, as compared to 5% of the patients in the BAT arm [29].

Other treatment options include busulfan, however in carefully selected patients as it may cause marrow aplasia or progression to AML. Additionally, it is important to consider clinical studies.

37.4 Symptom Burden in PV

For years it has been recognized by treating physicians and MPN patients that in addition to the hematologic abnormalities, there is a significant symptom burden associated with MPNs. These symptoms range from fatigue, early satiety, abdominal pain, inactivity, headaches, concentration problems, dizziness, numbness, insomnia, sad moods, sexuality problems, night sweats, itching, bone pain, weight loss, and fever. All of these can severely impact the quality of life of patients with MPNs. To better characterize these symptoms, as well as quantitate them, the MPN symptom Assessment Form was developed in the early 2000s to capture these symptoms in a reproducible fashion [9, 30, 31]. Approximately 50% of patients diagnosed with PV will present with PV-related symptoms including fatigue, headache, visual disturbances, and pruritus [32].

In a recent observational study that collected data on over 2000 patients with PV, they found that the symptoms in PV were not always controlled with control of blood counts [33], suggesting that there is more involved than just hematocrit. Ruxolitinib is a medication that may be very good for not only count control, but also symptom control, and should be considered in the highly symptomatic patient [29, 34].

37.5 Thrombosis

Thrombosis is one of the more serious complications of PV, and can include both venous thrombosis as well as arterial thrombosis. There is an abundance of data demonstrating the increased risk of both thromboembolic events and cardiovascular disease in patients with PV [4, 17, 35, 36].

There have been several studies that have looked at the prevalence of thrombosis in patients with PV. In a large metanalysis of studies evaluating use of HU, the risk of thrombosis is dependent on age and history of thrombotic event, and ranges from 1.9% person/year to 6.8% person/year, and the incidence appeared to be stable over time [11]. Data from the CYTO-PV study reported rates of thrombosis around 2.7% [37].

Clinical risk factors for thrombosis include age and history of thrombosis. In the ECLAP study, age > 65 (relative risk 2.08 [95% CI 1.25–3.45]) and history of thrombosis (relative risk 2.09 [95% CI 1.55–2.81]) were the most important clinical risk factors. Factors that contribute to thrombosis include WBC count, prior thrombotic events [38]. In patients who have experienced thrombosis with MPN, it is important to treat with both cytoreduction, as well as anticoagulation [39].

37.6 Transformation to AML

Transformation to AML is one of the more devastating outcomes associated with polycythemia vera. The incidence appears to be somewhere between 2% and 5% but difficult to ascertain given the duration of time between diagnosis of PV and transformation to AML [10, 40, 41]. The clinical variables associated with progression to acute leukemia include treatment with alkylating agents such as chlorambucil, as well as use of pipobroman, busulfan, and radioactive phosphorus (P^{32}) [4, 10, 40]. Higher age is also associated with an increased risk of transformation.

Somatic mutations may also help predict patients who are going to progress to acute leukemia. Poor outcomes, such as leukemia transformation, progression to MF, and inferior survival, have been associated with *ASXL1*, *SRSF2*, and *IDH2* [42]. In a deep analysis, there have been mutations specifically associated with acute leukemia in a time-dependent fashion. Short-term transformations are associated with a complex molecular landscape including mutations in *IDH1/2*, *RUNX1*, and *U2AF1*; long-term transformations are associated with mutations in *TP53*, *NRAS*, and *BCORL1* [43].

Once a patient has developed acute leukemia, it is generally associated with a poor prognosis. Treatment presently is similar to that of de novo AML including standard induction chemotherapy, as well as hypomethylating agents [44, 45] and generally allogeneic stem cell transplant [44].

37.7 Conclusions

Polycythemia vera is a disease characterized by elevated red blood cell mass, hypercoagulable state, and a complex symptom burden. When treating polycythemia vera, it is critical to consider the risk of the patient, as well as the symptom burden present. Maintaining hematocrit values of <0.45 is critical, as well as use of ASA. Choosing a cytoreduction agent should take into consideration both patient risk as well as burden of potential side effects.

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Treatment Algorithm of Essential Thrombocythemia

38

Jennifer O'Sullivan, Anna Green, and Claire Harrison

Abstract

Essential thrombocythemia (ET), one of the BCR-ABL-negative myeloproliferative neoplasms (MPNs), is a hematopoietic malignancy characterized by overproduction of platelets due to clonal expansion of megakaryocytes. Enhanced constitutive JAK-STAT (janus kinase 2—signal transducer and activator of transcription) signaling is central to disease pathophysiology. ET, presenting with persistent thrombocytosis, may represent a myriad of conditions and careful establishing of an accurate diagnosis is key to subsequent optimal management. This chapter reviews in detail goals of therapy in ET and treatment strategies incorporating cytoreductive, noncytoreductive, and novel therapies. Finally, approaches to specific scenarios and their management are discussed including young patients, triple-negative for classical driver mutation ET patients, pregnancy, and splanchnic vein thrombosis.

Keywords

Essential thrombocythemia · Myeloproliferative neoplasm · JAK-STAT signaling

38.1 Background

Essential thrombocythemia (ET) is a rare hematopoietic malignancy typified by overproduction of platelets due to clonal expansion of megakaryocytes. It is a member of the BCR-ABL-negative hematopoietic malignancy family known as myeloproliferative neoplasms (MPNs), which share a common pathophysiology underpinned by acquisition of a JAK-STAT signaling mutation in a hematopoietic stem cell [1] causing excessive proliferation of one or more of mature myeloid cells. The majority of cases can be explained by mutations acquired in three genes; janus kinase 2 (JAK2), calreticulin (CALR) and myeloproliferative leukemia virus oncogene (MPL) genes. The first identified and most common disease-causing mutation in all MPNs is a single nucleotide change in the JAK2 gene, JAK2V617F [2, 3], causing constitutive JAK-STAT signaling and thus increased gene transcription and protein expression. Amplified JAK-STAT signaling is central to all MPNs including those without an identifiable driver mutation [4]. Although JAK2V617F accounts for >95% mutations in the related MPN, polycythemia rubra vera (PV), it is present in 50% of those with ET. One-third of ET patients have a mutation in the CALR gene which encodes for calreticulin, a protein with multiple functions including regulating calcium homeostasis [5, 6]. Patients most often have type 1 CALR mutations, a 52-base pair deletion (bpd) and approximately 20% of CALR-mutant cases comprised of type 2 mutations (5-bp insertion) [7]. CALR mutations all cause a 1-bp frameshift mutation converting the negatively charged C-terminus to a mutant positively charged terminus with aberrant activity that upregulates JAK-STAT signaling by binding specifically to the TPO receptor [8, 9] but not the erythropoietin receptor. This explicates the clinical consequences of an ET or MF but never a PV phenotype. The MPL gene encodes for the TPO receptor. Gain-of-function missense mutations in this gene [10] are reported in <5% of ET cases [11] and more often in older patients. No JAK-STAT driver mutation is observed in the remaining ~10% of ET patients; these are denoted "triple-negative" cases.

The estimated incidence of ET is ~1 per 100,000 [12] with a median age at presentation of 68 years [13] and is more prevalent in women [14, 15]. For the majority, ET has an indolent disease trajectory with many diagnosed incidentally. Nevertheless, it is associated with a number of significant clinical consequences; a heightened risk of vascular

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thrombosis throughout its course, both arterial and venous, and in the longer-term, although rare, a risk of disease progression to myelofibrosis or acute leukemia. Increasingly, disease-related symptoms and their impact upon quality of life separate from these clinical events have also been recognized [16] as discussed below.

38.2 Diagnosis

Establishing an accurate diagnosis is key to facilitating appropriate management. Thrombocytosis, defined by a persistently elevated platelet count ≥450 × 10^9/L, may be due to a myriad of conditions with divergent management strategies, expected clinical course and prognosis.

Diagnosis and management of ET require an integrated approach (Fig. 38.1a, b), to address the major and minor criteria as set out in the WHO diagnostic criteria [17] and exclude secondary causes of thrombocytosis. The components of diagnosis are a detailed history (inherited, smoking, infection, inflammation, symptomatology), full blood count, blood film, iron status, C-reactive protein, and genetic tests, including detection of *JAK2V617F*, *CALR*, *MPL,* and *BCR-ABL1* rearrangement. If a secondary cause of thrombocytosis (thus excluding ET) cannot be confirmed based on these initial investigations, bone marrow aspiration and bone marrow trephine biopsy should then be performed. Consideration should be given to the need for a myeloid gene panel to assess for clonal markers in non-JAK-STAT genes, if *JAK2V617F*, *CALR* or *MPL* mutations not present.

Clonal thrombocytosis can be surmised by the presence of a JAK-STAT signaling mutation (Major Criteria four), or the presence of another clonal marker or absence of evidence for a reactive cause of thrombocytosis. Investigation of reactive causes for thrombocytosis requires combined assessment of the full blood count, blood film, iron status, C-reactive protein, and bone marrow trephine, which may show features such as increased macrophage activity and no or few megakaryocytes with morphology typical of ET (Fig. 38.2a, b).

Next, ET should be differentiated from other myeloid neoplasms that may mimic ET, in particular polycythemia rubra vera (PV), primary myelofibrosis (PMF), *BCR-ABL1*-positive chronic myeloid leukemia (CML), myelodysplastic (MDS)/MPN overlap syndromes (notably MDS/MPN-with ring sideroblasts and thrombocytosis, MDS/MPN-RARS-T), myelodysplastic syndrome (MDS) with isolated del(5q) or MDS with chromosome 3 abnormalities, all of which may present with thrombocytosis. The full blood count, aspirate,

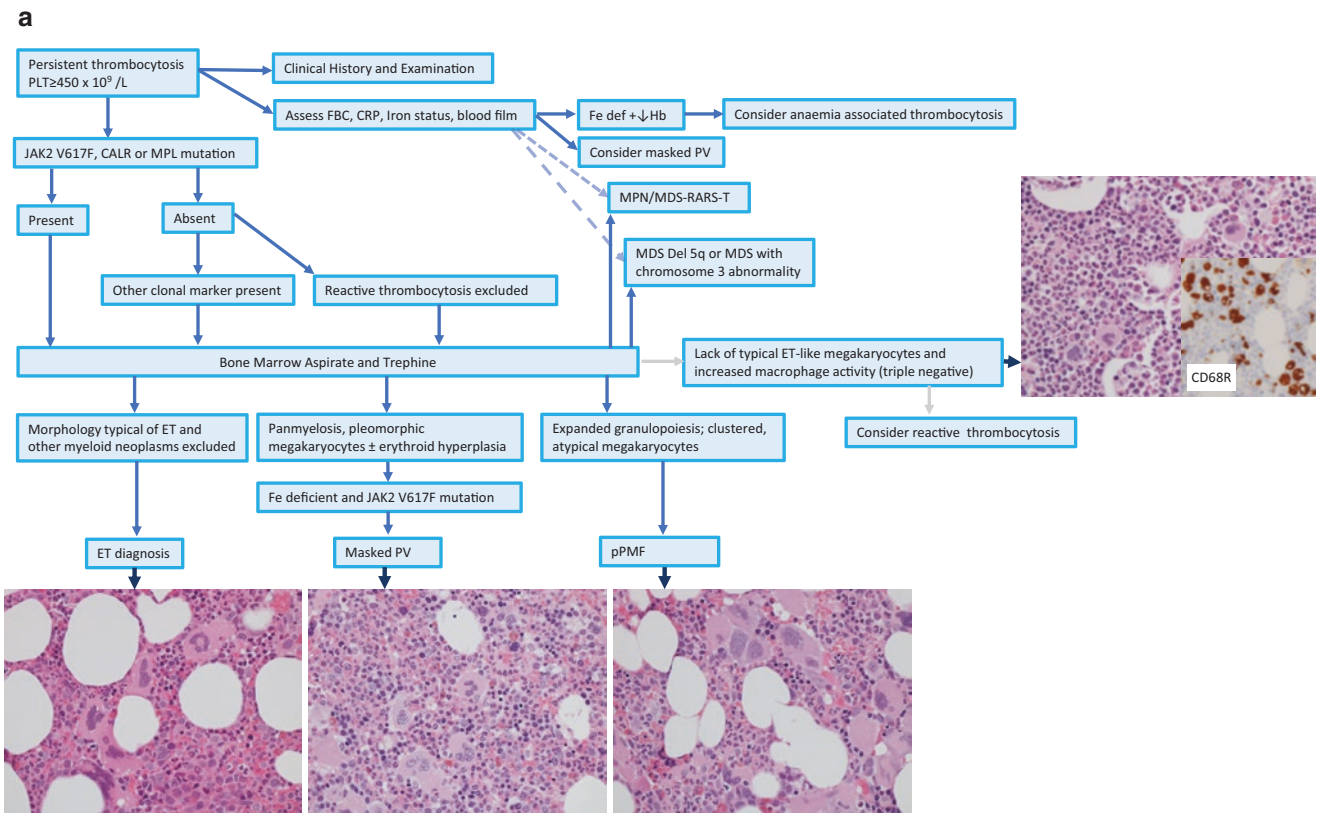

Fig. 38.1 Approach to ET diagnosis (**a**) and management (**b**). *HC* hydroxycarbamide; *r-IFN* recombinant interferon alpha

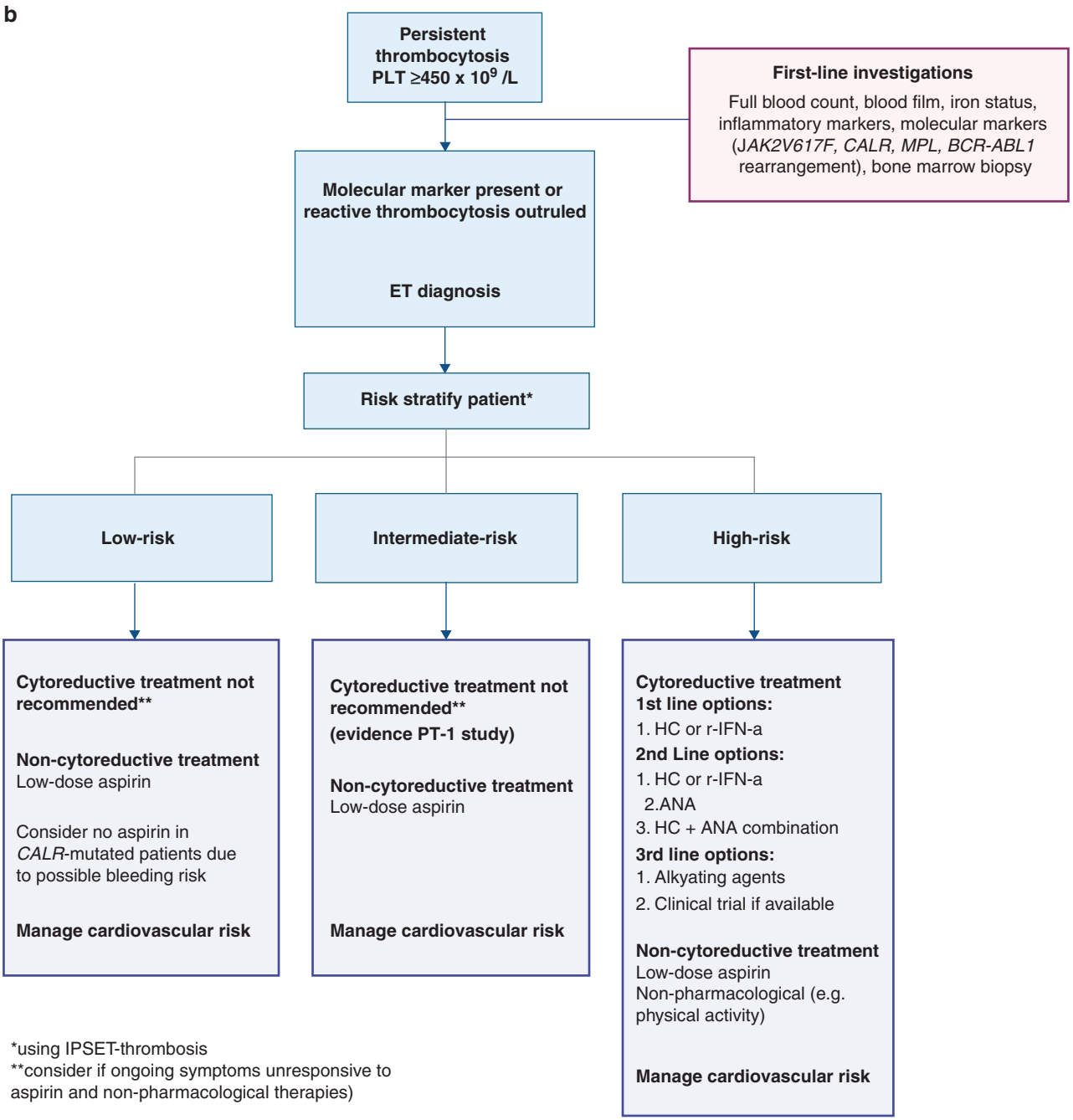

Fig. 38.1 (continued)

bone marrow trephine, and molecular results in combination enable exclusion of these other myeloid neoplasms in cases of ET [17].

The bone marrow trephine biopsy is an integral part of establishing a diagnosis of ET [17] The bone marrow shows a proliferation of megakaryocytes, which show infrequent clustering. The megakaryocytes are large, with hyperlobated nuclei (typical "staghorn" appearance) (Fig. 38.2c). Other than cases of post-ET myelofibrosis, there is usually no or very little increase in stromal reticulin fibers.

Iron deficiency, a feature of PV, may obscure the normally elevated hemoglobin and hematocrit levels of PV in a patient presenting with normal red cell indices in the presence of a raised platelet count and thus mimic ET. This MPN subtype is denoted "masked PV" and has been reported to have a higher risk of MF progression than classical PV and importantly has a higher risk of thrombosis than ET, with management including hematocrit control to <45% [18]. The WHO 2016 updated criteria adjusted hemoglobin range down to capture these cases when making a diagnosis of PV [17]. The

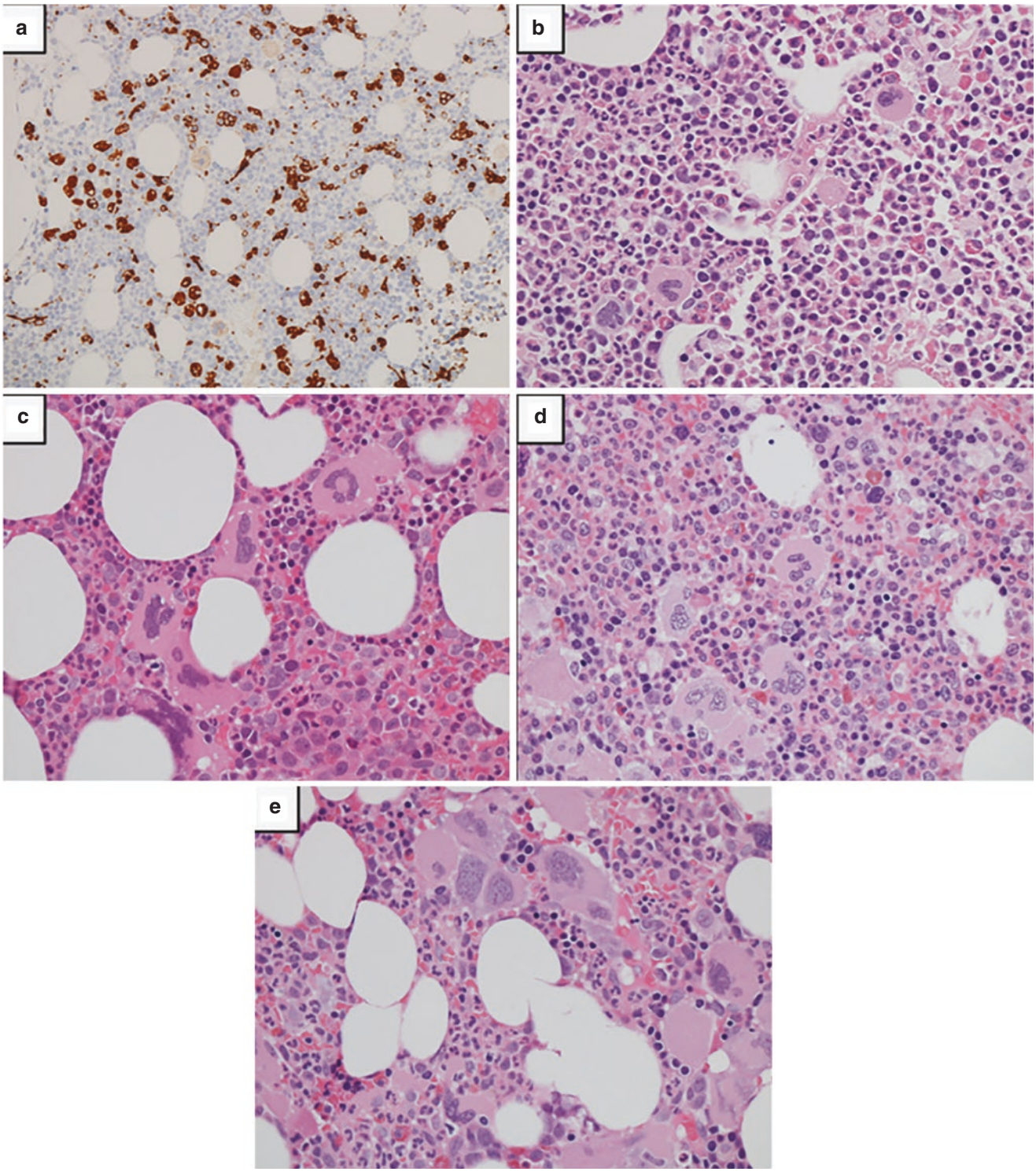

Fig. 38.2 Bone marrow biopsy images of diseases presenting with thrombocytosis**.** (**a** and **b**) Reactive thrombocytosis, with normal megakaryocyte morphology and increased macrophage activity, as highlighted by CD68R immunohistochemistry (**a**). (**c**) Typical morphology of ET. (**d**) Masked PV. (**e**) Prefibrotic PMF, with expanded granulopoiesis and clusters of atypical megakaryocytes

bone marrow trephine biopsy in masked PV shows morphological features consistent with PV, comprising panmyelosis, with increased, pleomorphic megakaryocytes, which form loose clusters (Fig. 38.2d**).**

Moreover, an additional category within MPNs has been defined in recent years; prefibrotic myelofibrosis (pPMF) based on morphological characteristics on bone marrow biopsy and clinicopathological features [17]. Although, mak-

ing this diagnosis can be challenging due to inconsistently reproducible histological findings [19], discerning pPMF from ET is important for accurate prognostication. pPMF is associated with a higher risk of leukemic and myelofibrotic transformation at 15 years of almost ~12% and ~17% respectively as compared with ~2% and ~9% respectively in ET [20] and a shorter overall survival; 76–86% in pPMF and 89–96% [20, 21]. Strict adherence to the WHO criteria [17] allows distinction between pPMF and ET in most cases, with pPMF typically showing expanded granulopoiesis and clusters of markedly atypical megakaryocytes with often bulbous or cloud-like nuclei (Fig. 38.2e).

38.3 Management of ET

38.3.1 Therapeutic Goals

To formulate a management plan for the patient, the objectives of treatment should be considered carefully. Dissonance between patients and their treating clinicians regarding treatment goals has been reported; patients assigning most importance on prevention of disease progression as compared with treating clinicians who placed more emphasis on symptom improvement and thrombosis prevention [16, 22]. Herein, these treatment goals are explored in more detail.

38.3.1.1 Vascular Sequelae

Vascular events are the major causes of morbidity and mortality in ET; thrombotic and hemorrhagic, with the former being the most prevalent. Paradoxically, an increased bleeding risk most particularly in patients with extreme thrombocytosis due to adsorption of von Willebrand factor to the surface of ET platelets resulting in an acquired von Willebrand's disease [23].

Thrombotic events are reported at a rate of 1.9 per 100 patient-years [24] increasing with age [25] compared to the general population which is 0.1–0.2% per year [26]. Venous events occur more commonly. In the UK Primary Thrombocythemia 1 (PT-1) study, major venous thromboembolism occurred at 0.6% per year [27]. The aim of pharmacological treatments in ET is to normalize platelet counts to minimize the risk of developing a thrombotic event. At present, few treatments available can modify the natural course of the disease.

Patients should be examined for the presence of cardiovascular risk factors as these increase the risk of subsequent thrombotic event [28]. *JAK2*V617F-mutated ET have a high risk of thrombosis than non-JAK2-mutated patients [28]. Although, *CALR*-mutant ET appeared to have a marginally lower risk of thrombosis with a large cohort with an incidence of 1.3% patient-year as compared with 1.95% for JAK2V617F-mutated ET, this difference was not statistically significant ($p = 0.09$) and multivariable analysis showed no impact of *CALR* mutation status on risk of thrombosis [29].

38.3.1.2 Symptom Burden

Patients with ET and all MPN subtypes experience a significant burden of symptoms (reported in 57–74%) which substantially impact their daily lives; affecting their quality of life, ability to work, and their relationships [16, 30, 31]. ET may be incidentally diagnosed through a routine blood test and the patient considered asymptomatic. However, many of these patients report nonspecific symptoms not recognizing as relating to MPN [30] that predated diagnosis by more than a year and did not prompt presentation for a medical review. An additional important consideration is the psychological impact of experiencing symptoms and adjusting to a cancer diagnosis and a projected reduced life expectancy. Many patients report symptoms of anxiety and depression [30, 32, 33] which further affect quality of life. Although in MF, patients with the highest risk disease reported greatest degree of symptom burden, ET patients with low-risk disease can often have a high degree of symptom [30, 34]. Women overall report a greater symptom burden [15, 16].

Symptoms are broad ranging from fatigue, vasomotor symptoms (including pruritus, erythromelalgia, night sweats, bone pain, headaches), constitutional symptoms of weight loss. Fatigue is widely reported in over 70% of ET patients [31]. It is often the most debilitating and challenging symptom to manage due to multifactorial etiology. Vasomotor symptoms are caused by abnormal activated platelet and interactions with the endothelium which can result in microthrombi. Low-dose aspirin can be effective in improving these symptoms [35].

In recent years, this understanding of the importance of symptom burden has led to the development of standardized and validated tools to objectively evaluate symptoms. These have been instrumental in capturing symptom burden in MPNs and to allow clinician to track alterations in the patient clinical status and can provide an early surrogate marker of disease progression. They have enabled better study of symptoms in the context of MPN research and crucially, are incorporated into clinical trials as meaningful and key endpoints. The Myeloproliferative Neoplasm Symptom Assessment Form total symptom score MPN-SAF [36] is 27-item questionnaire that can be applied in ET as well as PV and MF and shortly after an abridged questionnaire was developed, MPN-SAF total symptom score (MPN-SAF TSS or also called MPN-10 score) [37]. The latter is widely used in the field to monitor clinical status and response to therapies. The most recent NCCN guidelines in the US now recommend incorporating symptom assessment formally in monitoring response to treatments for MPNs [38].

38.3.1.3 Disease Progression and Survival

As aforementioned, the life expectancy in a patient with ET approaches a median survival of 33 years in patients below 60 years [39]. Disease progression to myelofibrosis or leukemia in ET patients rarely occurs; a 15-year incidence of 9% and 2% respectively [20]. Leukemic transformation has a particularly poor outcome with a median survival of less than 6 months [40] and limited treatment options.

The influence of driver mutation status on the risk of developing myelofibrosis in ET varies in the literature. Survival in ET is equivalent across the mutation groups [39, 41] and for *JAK2*V617F+ and *CALR*-mutated ET, previous studies have been found to have similar rates of MF transformation [39, 41]. However, more recent studies have reported an increased risk in *CALR*-mutated patients [42, 43] and specifically with a type 1 mutation [7]. *MPL* mutations have been linked with higher risk of MF transformation than the other driver mutation groups [44]. Triple-negative ET without additional clonal markers has been associated with the lowest risk of disease progression (0.5% MF and 1% AML transformation at median follow-up of 8 years) [42]. This study included over 2000 patients with MPN and examined the factors that contribute to disease progression and survival and developed a model to predict prognosis finding that the presence of mutations influenced the likelihood to progression to MF and to an even greater extent leukemia in which mutations attributed to one-third of the risk [42]. Mutations in non-JAK-STAT signaling genes have been detected in 29–72% of patients [42, 45–47]. Overlapping mutations are associated with risk of progression to MF and leukemia including mutations in epigenetic genes, splicing factor genes, signaling genes (*NRAS, GNAS*) and some mutations were more specific for leukemia risk; RUNX1, TP53, *IDH2*. *TP53* mutations are associated with particularly high risk of leukemia [42, 48] with a hazard ratio 15.5 (95% CI 7.5–31.4, $P < 0.001$) when compared with *JAK2* heterozygous group [42]. Interestingly, loss of the *JAK2*V617F mutation at leukemic transformation has been described with emergence of mutations in *TP53, TET2* suggestion clonal evolution.

Increasing age is the most significant contributor to MF risk and survival in ET [40, 42, 49, 50] and to a lesser extent leukemic risk for which the balance of risk shifts to acquired genetic factors [42]. Male gender has been associated with increased risk of myelofibrotic transformation in ET independent of mutation status [47, 51].

Various groups have harnessed this information by integrating clinical and genomic information to develop prognostic models to predict risk for disease progression and survival [42, 49, 52]. These will need independent validation, in particular to determine their utility in the clinical setting. However, identifying subgroups of patients at higher risk of disease progression will be important going forward with development of newer targeted therapies.

38.4 Therapeutic Strategies

An individualized approach is warranted for management of ET. These patients live with a chronic condition and often with a significant symptom burden. Contemporaneous therapies such as antiplatelet agents and cytoreductive therapies to lower the platelet count target thrombotic risk, but, for the most part do not mitigate the risk of disease progression. These therapies once commenced are usually required indefinitely for efficacy but over time patients may accrue side effects or their disease becomes resistant, newer targeted treatments are being evaluated generally in the second-line setting. As such, treatment decisions require careful consideration; mutation profile, prognosis, patient preferences, drug toxicity profiles, mode of administration, effect on quality of life, and fertility in younger patients are all factors requiring consideration here.

Prognostic scoring systems have been developed in MPNs to risk stratify patients and help determine the optimal management plan. Current ET risk stratification tools estimate a patient's risk of a thrombotic event. Classically, the main determinants of thrombosis risk have been based on age and prior thrombosis. A subsequent model, IPSET-thrombosis [28] was developed to incorporate additional factors influencing thrombosis; cardiovascular risks and *JAK2*V617F-mutated status (Table 38.1). This three-tiered model assigns a patient as low-risk (<2 points), intermediate-risk (2 points), and high-risk (≥2 points). It has since been revised [53] and independently validated [54] to include four risk categories (very low, low, intermediate, and high). It includes age (threshold of 60 years), *JAK2*V617F mutation status, and thrombosis history and excludes cardiovascular factors. *CALR* mutation status has not been incorporated into the model as it did not alter the predicted thrombosis risk in analysis of a large ET cohort [29]. Scoring all patients at diagnosis using the IPSET-thrombosis is recommended [38, 55].

Response to treatment is assessed most often by a combination of clinical and hematological parameters. Formal response criteria were developed in 2009 [56] and revised more recently [57] by the European LeukemiaNet (ELN) and International Working Group-Myeloproliferative Neoplasms Research and Treatment (IWG-MPN) to incorporate clinical, blood count parameters, molecular and histological parameters. These have most relevance for application in the setting of clinical trials, but they are yet to be prospectively validated and their utility in day-to-day practice is low; gener-

Table 38.1 Risk stratification

Scoring system	Classical [35]	IPSET-thrombosis[a] [28]	Revised IPSET-thrombosis [53]
Factors (point)	Age ≥ 60 years [1] Thrombosis history or major bleeding [1] Platelet count ≥1500 × 10^9/L [1]	Age ≥ 60 years [1] Prior thrombosis [2] CV risks [1] JAK2*V617F* mutation [2]	Age ≥ 60 years [2] Prior thrombosis [3] JAK2*V617F* mutation [1]
Risk groups (annual thrombosis rate) *Very-low* *Low* *Intermediate* *High*	NA 0 (~1.5–2.5%) NA ≥1 (~6–8%)	NA (1.03%) 2 (2.35%) 3–6 (3.56%)	0 (0.44%) 1(1.59–2.57%) 2 (1.44%) 3 (2.36–4.17%)

CV risks cardiovascular risk factors (one or more of: hypertension, diabetes mellitus, active smoking)
[a]Recommended in standard practice

ally, response of blood counts, symptoms, and toxicity are assessed in the clinic.

38.4.1 First-Line Cytoreductive Treatments

Cytoreductive treatment is recommended for ET patients classified as high-risk for thrombosis development due to any one of the following age ≥ 60 years, prior thrombotic/hemorrhagic event, platelet count ≥1500 × 10^9/L [55]. Although, cytoreduction is not required for low-risk patients whose thrombotic risk is similar to that of the general population [58], it may also be appropriate for a subset of low-risk patients with uncontrolled ET-driven symptomatology (Fig. 38.1b).

First-line treatment may be hydroxycarbamide (HC) or interferon (IFN), and rarely in some situations anagrelide (Table 38.2). HC, a ribonucleotide reductase inhibitor, was the first trialed therapy in ET for thrombosis prevention and demonstrated efficacy over control in high-risk ET patients [60]. Moreover, the PT-1 Trial showed that treatment with HC in combination with low-dose aspirin is superior to anagrelide plus low-dose aspirin in reducing risk of a thrombotic event [27]. Anagrelide-treated patients achieved similar control of their platelet counts but these patients had higher rates of MF transformation, serious hemorrhage and were more likely to stop treatment than those treated with HC. These findings were corroborated in a large European-wide study of 3649 ET patients; anagrelide-treated patients had similar thrombosis rates to those on other cytoreductive agents but had higher rates of hemorrhage and MF transformation [66]. Anagrelide is therefore more frequently and appropriately used in the second-line setting.

Intermediate-risk ET can be defined in many ways and there is a lack of evidence to guide the management of these patients. Cytoreduction for so-called "intermediate-risk" patients who are aged 40–59 years, has been investigated within the context of a randomized clinical trial—PT-1 Intermediate-risk; 382 patients were randomized 1:1 to HC with aspirin or aspirin alone [71]. There was no difference between the two arms showing that the addition of HC did no reduce vascular events or MF/leukemia transformation. This study suggests the majority of this group of patients would not benefit from cytoreductive treatment.

There has been historical concern regarding the possible leukemogenic effect with HC use [72] and potential teratogenicity. However, HC-related leukemogenicity has not been reported in larger MPN studies. For younger patients (<40 years), consideration of an alternative first-line agent is recommended with interferon-alpha, although it is not licensed for use in ET. Excellent responses have been shown in patients [64] treated with pegylated-interferon-alpha-2a (rIFN-α); a large systemic review and meta-analysis inclusive of 730 ET patients, the overall response rate (complete and partial hematological responses) was 80.6%. IFN shows disease-modifying effects, with 6–30% of ET patients demonstrating normalization of bone marrow histology [64]. Driver mutation molecular responses are observed with IFN therapy; ranging 9–33% for *JAK2*V617F-mutated patients [64] and in 42% of *CALR*-mutated patients studied [65], although noting for CALR this was a small study of 31 patients. Interestingly, patients failing to achieve a molecular response have tended to a higher proportion of non-JAK-STAT signaling mutations suggesting a role in resistance to treatment [73]. Tolerability was an issue with older preparations of interferon but the advent of pegylated formulations has improved this, though rIFN-α still has a higher rate of side effects than HC [74]. The discontinuation rate in a meta-analysis of IFN use in ET was acceptable reported 8.8% per year [64].

Until recently, IFN was the only agent regarded as possessing disease-modifying potential. Interestingly, The Myeloproliferative Disorders Research Consortium (MPD-RC)-112, a study comparing HC with IFN upfront in high-risk ET and PV, showed similar response rates including improvement in histological parameters but molecular responses were not evaluated [74]. In summary, for first-line

Table 38.2 Cytoreductive therapies in ET

Drug	Therapy line	Responses	Toxicities
Hydroxycarbamide (HC)	First-line	1. CHR 25% and partial HR in 58% [59] 2. Thrombosis rate after 2 years on treatment (PT-1 study) ~4% [27, 60] 3. Molecular responses 4. *JAK2*V617F+ PMR ~50% (small study, $n = 21$) [61]	Well-tolerated, ~5% side effects reported in large retrospective study (97/1912 ET pts) [62] Hematological AEs reported in ~5% [27] Nonhematological • Dermatological ~10% (rashes, ulcers, nonmelanoma skin cancers • Mild GI disturbances in ~5–10% No evidence of increased leukemogenicity with HC treatment alone [63]
Interferon-alpha	First-line Or Second-line after HC	1. Complete and partial HR ~80% [64] 2. Thrombosis rate per patient year low at 1.2% [64] 3. Molecular responses; • *JAK2*V617F+ CMR 9–27% [64] • *JAK2*V617F+ PMR 17–33% [64] • *CALR*+ PMR 42% [65]	Overall AEs reported in studies are variable but ≥ grade 3 range 0–64.7% [64] Hematological AEs: Leukopenia, anemia, thrombocytopenia Nonhematological AEs. • Flu-like illness early in treatment • Autoimmune conditions later in treatment; thyroiditis, hepatitis, vascul • Neuropsychiatric 10–15% Discontinuation rate per patient year ~9% [64]
Anagrelide	Second-line	1. Equivalent platelet control to HC [27, 66, 67] 2. Increased rates of arterial and hemorrhagic events [27] 3. Reduced venous thrombosis compared with HC (PT-1 [27], ANAHYDRET [67], and EXELS [66] cohorts) 4. Molecular responses not reported but not widely studied 5. Increased risk of myelofibrotic transformation	Hematological AEs in 8–15% [27, 67] Nonhematological AEs • Cardiac symptoms (57/122 vs. 18/137, $p = 0.01$ [67]), most frequently palpitations • GI disturbances in 15–22% [27, 67] Discontinuation rate higher ~21% versus 10% for HC, $p < 0.001$ [27] in the PT-1 trial
Ruxolitinib	Third-line (dependent on availability)	1. In HC-resistant/intolerant ET, equivalent CHR to BAT [68] 2. Thrombotic events similar with ruxolitinib versus BAT 3. Molecular responses rare	Hematological AEs • ≥ grade 3 anemia in 19% and thrombocytopenia in 5.2% [68] Nonhematological AEs • Nonmelanoma skin cancers equivalent in ruxolitinib and BAT arm
Busulfan	Third-line (patients >60 years)	1. CHR in 83–90% [69, 70] 2. Thrombotic events 11–19% 3. Leukemic transformation ~8%	Hematological AEs 15–30% [69, 70]

AE adverse event, *BAT* best available therapy, *CHR* complete hematological response, *pts* patients

treatment in ET, HC or rIFN-α is usually used for first-line therapy [55, 75].

38.4.2 Therapies for Nonresponders/ Intolerant of First-Line Treatments

Resistance or intolerance to HC (HC-RES/INT) in first line has been observed in approximately 20% of high-risk patients [76]. Those who were resistant had a significantly poorer outcome with higher rates of MF transformation and shorter survival of 26% at 10-years [76]. ELN guidelines recommend consideration of rIFN-α or anagrelide for second-line therapy in this instance. Data concerning anagrelide are discussed above, this drug is interestingly often used in combination with HC in the second-line setting. The MPD-RC-111 study was a phase 2 trial in a small cohort of HC-RES/INT ET and PV patients examining the efficacy of rIFN-α. It demonstrated good overall hematological response rates; 69.2% at 12 months with complete response rates greater in *CALR*-mutated patients [77].

The first clinically approved JAK1/JAK2 inhibitor, Ruxolitinib (RUX), is approved for patients with symptomatic MF [78] and with polycythemia vera (PV) [79], with excellent responses seen in terms of a reduction in spleen size (in MF), achievement of hematocrit control (in PV), and improvements in patients' symptoms and quality of life (across both MF and PV). In view of this relative success, the role of these drug patients with ET who are HC-RES/INT was investigated in a UK phase 2 trial (MAJIC-ET) randomizing patients 1:1 to receive RUX or best available therapy (BAT). The primary outcome of complete hematological response (CHR) within 1 year of treatment was similar in both arms [68]. Disease transformation was not mitigated by

RUX treatment. RUX is not currently recommended for treatment of ET. Other agents for consideration in older patients after first- and second-line options are alkylating agents such as busulfan [69], melphalan or pipobroman [72] but these are associated with heightened leukemogenicity and caution is required when considering these treatments.

38.4.3 Noncytoreductive Treatments

Aspirin is an irreversible COX1 inhibitor which has proven to reduce the risk of thrombosis development (evidence extrapolated from efficacy in PV from the ECLAP study [80]) and vasomotor symptoms in ET. Management of all ET patients with low-dose aspirin is recommended for the vast majority of ET risk groups [35, 55] with some exceptions. A retrospective study showed an increased bleeding risk in low-risk CALR-mutated patient without reduction in thrombotic risk [81]; 12.9 episodes versus 1.8 per 1000 patient-years in CALR versus JAK2V6127F-mutated patients respectively, $p = 0.03$. Extreme thrombocytosis (defined as a platelet count $\geq 1500 \times 10^9$/L) may be associated with acquired von vWS [23]. Whether aspirin should be used at all in low-risk ET patients lacking a JAK2 mutation has been debated, in our practice we would usually use aspirin unless it enhanced bleeding or was contraindicated.

Nonpharmacological interventions such as physical activity have been shown to improve symptoms such as fatigue and quality of life in hematological malignancies [82]. Although few studies have specifically investigated the effect of physical activity in MPNs, a recent study of 858 MPN patients found self-reported physical activity including aerobic activity, yoga, and strength training was associated with reduction in symptom burden [83]. In addition, a prospective study of a 12-week online course of yoga in MPN patients showed small effects on symptom burden [84] but this was a small cohort positive effect may be underappreciated.

38.5 Role of Novel Agents in ET

Beyond the use of the JAK inhibitor, ruxolitinib, in MPNs, which predominantly has shown success in PV and MF, there are no other approved targeted therapies addressing the continued unmet need for agents which are disease altering. Many targeted agents, alone or in combination with ruxolitinib, have been investigated in MPNs but to date this is principally in MF. In ET, various targeted approaches are under investigation in the second-line setting with promising signals emerging (Table 38.3). Telomerase activity is enhanced in cancers and in MPN patients [89] with a specific sensitivity of ET megakaryo-

Table 38.3 Novel therapies in ET

Drug	Study	Mechanism of action	Outcomes	Toxicities
Imetelstat	Phase 2 [2015] [85]	Competitive telomerase inhibitor	Complete hematologic response in 89% (16/18) Molecular responses in 7/8 *JAK2*V61F+	Grade 3/4 neutropenia in 22% (4/18) & thrombocytopenia 19% (19 pts) Grade 1/2 abnormal liver function tests in 100%
Vorinostat	Phase 2	HDACi: removes acetyl groups from histones regulating gene expression	Overall 35% response rate [86] In patients discontinuing vorinostat; clinicohematological responses were present in 63% [86] Modest molecular responses (5.6% after 3 months) [86]	Hematological AEs rare Nonhematological AEs common; fatigue, renal impairment, diarrhea, nausea, weight loss, headache, leg ulcers 52% discontinuation (33/63) during the study period due to AE in 28 pts and lack of response in 5 pts
Bomedemstat	Phase 2 (NCT04254978)	LSD1i: epigenetic modifier regulating gene transcription by removing mono- and dimethyl groups from histone H3	Clinical data in ET pending as trial enrolling In MF, preliminary results show 86% reduction in spleen size with ~30% reporting >50% reduction at 24 weeks [87]	Toxicity data in MF as ET trial enrolling In MF, grade 3 AEs related to Bomedemstat were reported in 10.5% (4/38); painful splenomegaly, rectal bleeding, heart failure, and headache [87]
CPI-0610 (in combination with ruxolitinib)	Phase 2 (planned)	BETi: epigenetic modifier with attenuation of NF-kB signaling in murine models [88]	Clinical data in ET pending as trial planned In MF, spleen size reduction in 94% and symptom improvement in 93% Bone marrow fibrosis improved in 58%	Toxicity data in MF as ET trial planned Hematological AEs; grade ≥ 3 anemia (8.3%) and thrombocytopenia (8.3%) Most common AEs (≥20%) any grade; diarrhea, nausea, cough, and URTI

AE adverse event, *BETi* bromodomain and extra-terminal protein inhibitor, *HDACi* Histone-deacetylases inhibitors, *LSD1i* Lysine-specific demethylase 1 inhibitor, *URTI* upper respiratory tract infection

cytes to telomerase inhibition [90]. Hematologic (89% complete responses) and driver mutation molecular responses were seen with use of imetelstat, a competitive telomerase inhibitor, albeit in a small cohort of ET patients refractory/intolerant to previous therapies [85]. Half of these patients had mutations in additional genes which were also responsive to imetelstat [91, 92]. However, this agent has not moved forward to incorporation into approved treatments for ET, perhaps because of the mode of administration and also the risk of neutropenia.

Histone-deacetylases inhibitors (HDACi) target HDAC enzymes which broadly remove acetyl groups from histones regulating gene expression. Panobinostat in combination with ruxolitinib has elicited moderate responses in advanced MF [93] but in ET, this class of drugs have proven less successful. Vorinostat, a pan-HDAC inhibitor, has demonstrated utility at reducing platelet counts in ET but the toxicity profile (discontinuation in 52% due to adverse events) [86] has limited considerations for application in ET.

Lysine-specific demethylase 1 (LSD1) is an enzyme and epigenetic modifier-regulating gene transcription by removing mono- and dimethyl groups from histone H3. It is indispensable for normal hematopoiesis [94] and this has been harnessed as a therapeutic for MPNs. LSD1 levels are increased in MPNs [95] and irreversible inhibition of LSD1 in a *JAK2*V61F+ mutant MPN murine model showed disease-modifying effects of improvement of the MF phenotype, reduction in bone marrow fibrosis, and *JAK2*V61F+ allele burden and crucially, improved survival [96]. These promising findings have been followed by phase 2 clinical trials of IMG-7289 (Bomedemstat) monotherapy, a small molecule LSD1 inhibitor, in MF (NCT03136185) and ET (NCT04254978) which are ongoing. Preliminary data in advanced MF are encouraging with reduced spleen volumes, symptom scores, mutant allele burdens, fibrosis scores with no safety signals [87].

Other therapies targeting the epigenome in MPN are small molecular inhibitors of bromodomain and extra-terminal (BET) proteins. In murine MPN models, combination therapy with BET inhibitor and ruxolitinib showed attenuated NF-kB signaling, reduction in cytokine production, and amelioration of the disease phenotype with reversal of bone marrow fibrosis [88]. MANIFEST is a phase 2 study of a BET inhibitor, CPI-0610, as monotherapy or in combination with ruxolitinib in advanced MF patients is underway and interim results have shown good tolerability with anemia and spleen responses and bone marrow fibrosis intimating possible disease modification [97]. An expansion of this study to incorporate a small arm of HC-RES/INT ET is under consideration.

CALR-mutated MPN patients have been shown to exhibit immune responses against epitopes in the *CALR* C-terminus [98]; the mutant *CALR* could be considered a neoantigen and may be a target for immunotherapy (cell- or antibody-mediated [99, 100]) but these approaches are still at a pre-clinical research stage.

38.6 Approach to Specific Scenarios

38.6.1 Young Patients

Although commoner with aging, ET is frequently reported in younger (variably defined) patients. Current guidelines are tailored to older patients and may not be directly applicable to management of younger patients. Selecting the age threshold for defining this group has ranged from studies including patients <40 years [101] to a recent review inclusive of patients aged less than 20 years by Ianotto et al. in 2019 [102]. In this analysis, they identified studies which collectively included 471 patients with MPN, in which 396 were patients with ET. Interestingly, lower JAK2V617F mutation proportion across the studies of 31.7% as compared with ET overall. Furthermore, the driver mutation profile otherwise in this cohort was distinct from that found in older ET patients consisting of 10% CALR-mutated, 2% MPL, and a much higher prevalence of triple-negative cases (57%). This enrichment for triple-negative cases raises the question of an alternative diagnosis or pathogenesis in these patients, potentially inherited or reactive etiologies. The incidence of thrombosis is lower in younger patients [102] and the pattern of thrombosis differs to that of older patients; splanchnic vein thrombotic events are more often identified in younger patients [101].

Thus, the best approach to managing younger patients to prevent thrombotic complications is uncertain. The majority of patients would fall into a low-risk category and under current guidelines would not require cytoreductive treatment. Even though most patients included in the comprehensive recent review were not in the high-risk category, strikingly over 60% were treated with cytoreductive therapies [102]. The treatment reason was not indicated in the studies. This is an area of unmet need.

38.6.2 Triple-Negative Thrombocytosis

This subgroup is worth specific discussion due to distinct clinicopathological features and probable underlying pathophysiology. They are defined by younger age and prevalence of female gender. They usually have the most favorable prognosis [42]. This is in striking contrast to triple-negative cases of PMF where the prognosis is poor with shortened survival as compared with driver-mutated disease [39], certainly suggesting triple-negative ET and PMF have a divergent underlying disease biology.

Prior to assigning triple-negative status to a patient; it is important to consider an inherited or reactive etiology. Hereditary thrombocytosis is rare but families with mutations in JAK-STAT signaling genes have been described. These included mutations affect *MPL*, for example a *MPL* S505N with an autosomal-dominant mode of inheritance was described in families in Italy presenting often in childhood associated with thrombotic risk, splenomegaly, and progression to fibrosis. Germline mutations in *JAK2* pseudokinase domain have been identified; heterozygous *JAK2*V617I was reported in a family with a clinical phenotype of thrombocytosis and vascular events in those >40 years [103]. Mutations have also been identified in the kinase domains presenting with hereditary thrombocytosis; *JAK2* R564Q [104], *JAK2* S755R [105], *JAK2* R938Q [105], and *JAK2* R867Q [105].

It is important to consider investigating more closely for a low-level driver mutation/noncanonical JAK-STAT signaling mutation or a non-JAK-STAT driver clonal marker. Indeed, detection of a low-level or noncanonical JAK-STAT driver mutation, would have implications for the patient as this may alter the management and prognosis. A recent study of 35 triple-negative ET patients was rescreening for canonical JAK-STAT signaling mutations finding that 23% (8/35) had a mutation detected at repeat testing. Although the numbers in this study are small, it suggests a role for considering rescreening triple-negative patients at a later interval. However, this is not at present recommended in routine clinical practice. The sensitivity of detecting JAK-STAT mutations may be improved by analysis of granulocytic or platelet RNA [106], though this is not performed routinely.

A further study of 17 triple-negative ET patients targeted *JAK2* and *MPL* with whole-exome sequencing and next-generation sequencing finding several mutations; a *JAK2*V617F at a very low level, *SH2B3* mutation, and atypical *MPL* mutations [107]. No clonal marker was identified in seven cases finding these were cases of polyclonal thrombocytosis. In a recent large study of MPNs, noncanonical mutations in *JAK2* and MPL were identified in 16 triple-negative ET patients [42]. Patients may have a clonal marker in non-JAK-STAT genes such as *TET2* [42, 45, 47], *DNMT3A* [42, 45], *TP53* [42, 47], *PPM1D* [42], splicing factors (*ZRSR2* [47], *SF3B1* [45], *U2AF1* [45]). However, it is worth considering the possibility of concomitant clonal hematopoiesis of indeterminate potential [108]. To determine the relevance of these mutations, it is necessary to consider the context of clinical presentation, laboratory, and histological findings.

Long-term evaluation of these triple-negative patients will be interesting to determine outcome and whether an intervention is ever required at any point in a patient with a modest thrombocytosis and no complications.

38.6.3 Pregnancy

Since ET is more prevalent in women including those of reproductive age, understanding the potential complications of ET during pregnancy and appropriate clinical management is warranted. The incidence of an MPN pregnancy in a UK prospective study of 58 women with MPN was 3.2/100,000 maternities per year [109], noting that the majority were women with ET (81%). Maternal morbidity and pregnancy complications are increased in ET; maternal thrombosis and hemorrhage, miscarriage, stillbirth, intrauterine growth restriction, preeclampsia, and premature labor [109, 110]. The live birth rate in a systematic review and meta-analysis of MPN pregnancies (which included the UK prospective cohort study [109]), was 71.3% which is lower than the expected live birth rate of approximately 80% in the general population [110]. Successful pregnancies occurred more often in ET patients than in PV [110]. Of note, JAK2V617F mutation status has not been associated with pregnancy complications or outcomes [109, 110].

Low-dose aspirin is considered safe in pregnant women and reduces rates of preeclampsia and fetal mortality [111]. In ET, aspirin use is associated with improved maternal and fetal outcomes without a risk of increased bleeding, alone or with heparin [110]. Practice in the UK is to recommend low-dose aspirin 75 mg to all ET patients throughout pregnancy [112] but if a patient has a platelet count >1000 × 10^9/L, a von Willebrand screen should be performed prior to commencing aspirin, this relates mainly to the risk of spinal analgesia and bleeding. Low-molecular weight heparin (LMWH) is widely used for thromboprophylaxis in pregnancy when the risk of VTE is >3% [113]. A review of absolute VTE risk in ET determined the antepartum risk associated with ET is 2.5% (95% confidence interval, CI 1.2–9.5) but postpartum this was 4.4% (95% CI 1.2–9.5) [114]; this is consistent with the standard recommendation that all ET patients should be offered LMWH prophylaxis during the 6-week postpartum period. In the UK, antepartum LMWH prophylaxis is recommended if the woman has a history of thrombosis or one additional risk factor for VTE as per the Royal College of Obstetricians and Gynecologists (RCOG) guidelines [112]. If cytoreductive therapy is needed in pregnancy, interferon is the only therapy considered safe [115] and observational data suggest use may be associated with a higher live birth rate [110].

38.6.4 Specific Thrombotic Events

Splanchnic vein thrombosis is a rare event but MPNs are the commonest cause; up to 40% of budd-chiari and one-third of portal vein occlusion events [116]. The prevalence of *JAK2*V617F mutation in a meta-analysis of patients with

SVT was 32.7% [117] suggesting a role for screening for JAK-STAT signaling mutations in patients presenting with SVT, interestingly *CALR* mutations are less prevalent in this setting. Acute treatment is similar to other VTE events with full-dose anticoagulation recommended [118]. Although no prospective clinical trials are available, data suggest benefit in continuing anticoagulation indefinitely in these patients [118]. First-line cytoreductive therapy is recommended for these patients and ideally a management approach should be multidisciplinary including hematology, gastroenterology, and radiology or surgery in the event of need for interventional procedures (e.g., angioplasty or stenting) or liver transplantation [118].

38.7 Future Perspectives

Advancements in techniques and understanding of cancer genomics have allowed integration of this information into routine management of patients. This is evidenced in ET management with incorporation of driver mutation status into risk stratification to inform treatment decisions. Furthermore, more extensive genetic testing of other genes commonly mutated in myeloid malignancies has allowed improved refinement of patient subgroups and improved prognostication for patients. Targeted mutation testing using next-generation sequencing techniques is now more widely available, more affordable, and is becoming increasingly standard in routine clinical practice but more focused and applicable to MF than ET. Beyond risk stratification, molecular testing to monitor treatment response remains an endpoint in clinical trials but we would anticipate that over time this will become commonplace to help identify at earlier patients who may be nonresponders or losing response to treatment and alternative treatments or clinical trials may be considered though the evidence base for this needs refining. More detailed molecular annotation of ET and all MPNs may also have implications for newer targeted treatments (e.g., epigenetic modifiers) that will become available; specific molecularly defined subgroups may demonstrate improved responses. Concerning day-to-day management, first-line treatment options are clear but a gap is the management of symptoms and addressing which therapies will affect long-term disease evolution will be important future targets.

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Prognostic Models for Primary and Secondary Myelofibrosis

39

Harinder Gill and Garret Leung

Abstract

Although patients with primary and secondary myelofibrosis are at risk of disease progression into acute myeloid leukemia, they are heterogenous at presentation and have a highly variable survival. In the last decade, with the rapid expansion of our knowledge in the impact of cytogenetics and molecular makers, these have been, in addition to the traditional clinical parameters, incorporated into different prognostic models. These models not only help in disease prognostication, but they also play an important role in treatment decision making.

Keywords

Primary myelofibrosis · Secondary myelofibrosis · Prognostic models

39.1 Primary Myelofibrosis

The International Prognostic Scoring System (IPSS) was published by the International Working Group for Myelofibrosis Research and Treatment (IWG-MRT) in 2009 after studying more than 1000 patients diagnosed with primary myelofibrosis (PMF) [1]. The IPSS was calculated based on five clinical variables at disease presentation: age greater than 65 years, presence of constitutional symptoms, hemoglobin level less than 10 g/dL, leukocyte count greater than 25 × 10^9/L, and circulating blasts cells greater or equal to 1%. It categorizes patient into four risk groups: low-risk (0 variable), intermediate-1 risk (1 variable), intermediate-2 (2 variables), and high-risk (more than 2 variables), with the corresponding median survivals of 11.3 years, 7.9 years, 4 years, and 2.3 years.

The Dynamic International Prognostic Scoring System (DIPSS) was published 1 year after the IPSS, providing a time-dependent risk evaluation for patients with PMF [2]. The DIPSS incorporates all the five clinical variables included in IPSS but gives a greater weight to the presence of anemia (hemoglobin less than 10 g/dL) and it can be used anytime during the disease course. It also categorizes patients into low-risk (score 0), intermediate-1 risk (score 1 or 2), intermediate-2 risk (score 3 or 4), and high-risk (score 5 or 6) with the corresponding survivals of not reached, 14.2 years, 4 years, and 1.5 years.

The IPSS and DIPSS are only based on clinical parameters. Although they are easy to use and can provide us with valuable information even in resource-restricted situations, the prognosis of PMF was further refined after the identification of IPSS-independent cytogenetic risk groups [3]. The DIPSS-plus published in 2011 incorporates prognostic information from unfavorable karyotype (complex karyotype or sole or two abnormalities that include +8, −7/7q−, i(17q), inv(3), −5/5q−, 12p− or 11q23), thrombocytopenia (platelet count <100 × 10^9/L), and anemia requiring transfusion into the original DIPSS score [4]. The corresponding median survivals of low-risk (score 0), intermediate-1 risk (score 1), intermediate-2 risk (score 2 or 3), and high-risk (score 4–6) are 15.4 years, 6.5 years, 2.9 years, and 1.3 years.

The revised cytogenetic risk stratification in PMF has identified a three-tiered risk model: very high-risk, defined as single or multiple abnormalities of −7, i(17q), inv(3)/3q21, 12p−/12p11.2, 11q−/11q23, or other autosomal trisomies not including +8/+9 (e.g., +21, +19); favorable, defined as normal karyotype or sole abnormalities of 13q−, +9, 20q−, chromosome 1 translocation/duplication or sex chromosome abnormality including -Y; and unfavorable, which includes all other abnormalities [5]. Studies also identified that

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absence of type 1/like CALR mutation, presence of ASXL1, EZH2, SRSF2, IDH 1/2, and U2AF1 mutations were associated with inferior outcome [6–8].

The Mutation-enhanced International Prognostic Score System for transplant-age patients was published in 2018 integrating clinical, genetic mutations with (MIPSS70-plus) or without (MIPSS70) cytogenetic information [9]. MIPSS70 assigns score of 1 to hemoglobin <10/dL, circulating blasts ≥2%, bone marrow fibrosis grade ≥2, presence of constitutional symptoms, absence of CALR type 1-like mutation, and high-molecular risk (HMR) mutation (ASXL1, EZH2, SRSF2 or IDH1/2), whereas a score of 2 to leukocyte count >25 × 10^9/L, platelet count <100 × 10^9/L, and two or more HMR mutations. Three-category risk model was developed: low-risk (score 0 or 1), intermediate-risk (score 2–4), and high-risk (score 5 or above) with the corresponding median survivals of not reached, 6.3 years, and 3.1 years. MIPSS70-plus includes seven variables only and it assigns a score of 1 to hemoglobin <10 g/dL, circulating blasts ≥2%, presence of constitutional symptoms and HMR mutation; a score of 2 to absence of CALR type 1/like mutation and two or more HMR mutations; and a score of 3 to unfavorable karyotype (defined as any abnormal karyotype other than normal karyotype or sole abnormalities of 20q2, 13q2, +9, chromosome 1 translocation/duplication, -Y, or sex chromosome abnormality other than -Y). Four risk categories are constructed: low-risk (score 0–2), intermediate-risk (score 3), high-risk (score 4–6), and very-risk (score 7 or above) with the corresponding of median survivals of not reached, 24.2 years, 10.4 years, and 3.9 years. When compared with IPSS, 46% of the MIPSS70 high-risk patients are upgraded from lower IPSS risk categories (3.7% from low, 25.9% from intermediate-1, and 16.7% from intermediate-2 risk) while MIPSS70-plus includes 10.2% of patients originally classified as low or intermediate risks by DIPSS-plus [9].

Although MIPSS70-plus includes cytogenetic information into the prognostication, it does not examine the difference between very high-risk (VHR) and unfavorable karyotypes [9]. MIPSS70-plus Version 2.0 addresses this issue by replacing the two-tiered cytogenetic risk with a three-tiered one according to the refined cytogenetic-risk categorization; in addition, it also adds U2AF1 Q157 as an additional HMR mutation and replaces hemoglobin <10 g/dL with the new sex- and severity-adjusted tiered anemia category [3, 10]. MIPSS70-plus V2.0 assigns four points to VHR karyotype; three points to unfavorable karyotype and ≥2 HMR mutations; two points to one HMR mutation, absence of type 1/like CALR mutation, presence of constitutional symptoms and severe anemia; and one point to moderate anemia and ≥2% circulating blasts. There are five categories in MIPSS70-plus: very low-risk (score 0), low-risk (score 1–2), intermediate-risk (score 3–4), high-risk (score 5–8), and very high-risk (score 9 or above); the corresponding median survivals are not reached, 16.4 years, 7.7 years, 4.1 years, and 1.8 years [10]. MIPSS70-plus V2.0 refined MIPSS70-plus low-risk patients into very low-risk (20%), low-risk (68%), and intermediate-risk (10%); and refined 25% of MIPSS70-plus high-risk patients to the very high-risk group [10].

Genetically inspired prognostic scoring system (GIPSS) was published in 2018 and it is exclusively based on genetic markers [11]. This scoring system assigns two points to VHR karyotype; and one point each to unfavorable karyotype, absence of type 1/like CALR mutation, and presence of ASXL1, SRSF2, and U2AF1 Q157 mutations. Four categories are developed: low-risk (score 0), intermediate-1 risk (score 1), intermediate-2 risk (score 2), and high-risk (score 3 or above); the corresponding median survivals are 26.4 years, 8.0 years, 4.2 years, and 2 years. GIPSS has been shown to outperform DIPSS in patients with myelofibrosis using an external cohort [12]. While GIPSS is simple to use and has good alignment of risk distribution with MIPSS70-plus, especially regarding the low- and high-risk patients, GIPSS intermediate-1 and intermediate-2 risk patients may be reclassified by the MIPSS70-plus to higher- or lower-risk categories [11].

In conclusion, MIPSS70-plus V2.0 provides us with a more comprehensive risk assessment for PMF patients. An alternative stepwise approach is to start with the simple-to-use GIPSS to identify low-risk and high-risk patients and to use MIPSS70-plus to further risk stratify GIPSS intermediate-1 and intermediate-2 risk patients [13].

39.2 Secondary Myelofibrosis

Patients with polycythemia vera (PV) have a reduced life expectancy compared with the general population, whereas the effect of essential thrombocythemia (ET) on survival is controversial [14, 15]. The ten-year risks of myelofibrosis and leukemic transformation are 8–9% and 1–3% [15, 16]. The overall risk of leukemic transformation in MF is around 20% [17]. Survival of PV and ET patients is prognosticated by the IPSS-stratifying patients into low-, intermediate-, and high-risk groups based on patient age, white cell count, and previous thrombosis with the corresponding median survival of 28 years, 19 years, and 11 years for PV and not reached, 25 years, and 14 years for ET [18, 19].

PMF and myelofibrosis secondary to PV/ET (SMF) are clinically and biologically different: PMF has higher transfusion dependence, lower percentage of complex karyotype and shorter median survival; the most frequent cause of death is blast phase progression in PMF and nonclonal progression in SMF; among the HMR mutations (ASXL1, EZH2, IDH1/2, SRSF2) which are associated with inferior outcome in PMF, only SRSF2 mutation has prognostic

impact in SMF [20]. However, a more recent targeted next-generation sequencing of 86 PMF and 59 SMF patients shows that PMF has more ASXL1 and SRFS2 mutations and that poor survival is associated with SRSF2 and TP53 mutations in PMF and with ASXL1 and TP53 mutations in SMF [21].

PMF prognostic models have been used in patients with SMF but whether this is adequate in discriminating different risk groups in SMF remains controversial [22–24]. The Myelofibrosis Secondary to PV and ET-Prognostic Model (MYSEC-PM) was developed in 2017 specifically for risk stratification in SMF [25]. MYSEC-PM assigns two points to hemoglobin <11 g/dL, circulating blasts ≥3%, and CALR-unmutated genotype; one point to platelet count $<150 \times 10^9$/L and the presence of constitutional symptoms; and 0.15 points to any year of age. Four categories are derived: low-risk (score < 11), intermediate-1 risk (score 11 to <14), intermediate-2 risk (score 14 to <16), and high-risk (score 16 or above); the corresponding median survivals are not reached, 9.3 years, 4.4 years, and 2 years. MYSEC-PM has been tested in external cohorts of SMF patients and it has more accurate prediction of survival than IPSS [26, 27].

Although more comprehensive and accurate tools have been developed for risk stratification in PMF and SMF patients, one must not forget that these tools are all based on historical cohorts which had limited treatment choices in the past. With the continuously emerging new therapies, the clinical course and outcome of patients with myelofibrosis may change rapidly and we need constant updates on prognostic models for better treatment decision making for our patients.

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Treatment Algorithm for Primary and Secondary Myelofibrosis

40

Harinder Gill and Garret Leung

Abstract

The management of primary myelofibrosis (PMF), postpolycythemia vera myelofibrosis (PPV-MF), and postessential thrombocythemia (PET-MF) is driven by their clinicopathologic and genomic characteristics, and their risk categories. In this chapter, the treatment algorithms of PMF, PPV-MF, and PET-MF are discussed highlighting the relevance of the personalized approach.

Keywords

Primary myelofibrosis · Postpolycythemia vera myelofibrosis · Postessential thrombocythemia myelofibrosis · Treatment algorithm

40.1 Introduction

MPN patients are at risk of cardiovascular complications, including cerebral vascular events, acute coronary syndromes, and venous thrombosis. PV and ET patients may transform into myelofibrosis whereas all MPN patients may have disease progression into acute myeloid leukemia. Additionally, MPN patients have considerable symptom burdens which are usually underappreciated by the treating physicians [1, 2]. Therefore, the treatments of MPN should focus on three aspects: preventing disease transformation, alleviating debilitating symptoms, and minimizing treatment toxicities.

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40.2 Preventing Disease Transformation

40.2.1 Disease-Modifying Agents

The leukemogenic effect of hydroxyurea has long been a matter of controversy. Although there are no large prospective randomized trials addressing this question, two large uncontrolled studies on ET and PV do not support the leukemogenic role of hydroxyurea [3, 4]. Nevertheless, the incidence of leukemic transformation increases with disease duration [5], it is therefore recommended to use hydroxyurea with cautions or to avoid its use in young patients, particularly those younger than 40 years, who may require the treatment for decades [6–8].

PEG-IFN alpha is free from leukemogenic or teratogenic effects. It induces a complete molecular response (undetectable JAK2V617F) in 10–36% of PV and ET patients and an average of 56–85% reduction in the proportion of cells with the JAK2V617F mutant gene [9–12]. The molecular response is achieved gradually over time with a median time to response of 24 months [10]. It also appears that PEG-IFN targets JAK2V617F clones specifically without effect on the TET2 mutant cells [13]. Earlier small pilot studies indicate that PEG-IFN may retard the progression of early PMF and achieve complete response, as well as transfusion independence in 39% patients [14, 15]. It is also shown to reduce marrow fibrosis, cellularity, megakaryocyte density, and naked nuclei density in both primary and secondary myelofibrosis [16]. Another study on 30 low- and intermediate-1 risk patients with PMF showed that more than 70% of the patients improved or remained stable with recombinant interferon treatment, including 37% achieving complete or partial response; it also showed improvement in bone marrow morphology in 40% of the patients [17]. The latest meta-analysis of ten studies with 141 patients with myelofibrosis confirmed an overall response rate of 49.9% [18].

Ruxolitinib reduces JAK2V617F allelic burden in PV, ET, and MF patients [19–21] and leads to a significant and durable reduction in splenomegaly [22–24]. Phase III con-

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trolled trials show a survival benefit in patients treated with ruxolitinib compared with placebo or best available therapy [22, 23]. It became the first drug approved by the US Food and Drug Administration (FDA) specifically for MF in 2011 [25].

PEG-IFN and ruxolitinib have a potential disease-modifying effect on MPN with the former showing ability in retarding disease progression, improving BM morphology, and the latter having benefit in spleen size control and survival. Although the JAK2V617F allelic burden is an indirect measure of disease activity, a higher allelic burden is associated with splenomegaly and transformation to myelofibrosis [26]. Whether a reduction in molecular burden could be readily translated into clinical benefit in disease progression remains an area of research.

The COMBI study investigated the combination of ruxolitinib and pegylated interferon in the treatment of 32 PV and 18 MF patients [27]. It showed promising results in MF patients with 44% achieving remission (28% complete remission), 41% achieving a molecular response, and a 35% reduction in median JAK2 allele burden [27].

40.3 Alleviating Debilitating Symptoms

40.3.1 Symptom Burdens

While our knowledge of the disease biology is rapidly expanding, QoL of MPN patients has not been systemically studied until this recent decade. The MPN Landmark survey assessing 813 MPN patients (PV = 380, ET = 226, MF = 207) in 2014 reported reduced QoL due to MPN-associated symptoms, even in those with low-prognostic risk scores; the symptoms were so significant that 35–56% patients canceled their planned activities and 21–40% called in sick in the preceding 30 days [28]. Around one-third of MPN patients indicate not to be able to participate in their job as the consequence of the disease [2]. PV and ET patients have similar symptom burdens and reduced QoL compared with those with MF [2, 29, 30]. Some even reported worse QoL in PV patients [31]. Interestingly, such significant symptom burdens are sometimes under-appreciated and being ascribed to other causes by the treating physicians, for example, stress, burned out or overstrained, depression or even hystery [2]. The MPN-SAF TSSs for PV, ET, and MF are 18.7, 21.8, and 25.3 in the original study [29]. A local study on Chinese population observes a corresponding means of 19.8, 24.6, and 23.9 without significant difference among MPN disease subtypes ($p = 0.509$) [32]. Similar to previous studies [29–31], fatigue is the most common and severe symptom with a mean score of 4.1 and is present in 84% of the patient.

40.3.2 Symptom Control

Randomized controlled trials show improvement in QoL in ruxolitinib-treated patients compared with placebo or best available therapy in PV and MF patients [21, 33–35]. Our prospective study investigates precisely the effect on QoL of ruxolitinib compared to PEG-IFN and conventional treatments and it showed that the former leads to significant improvement in QoL, the improvement is rapid, significant and durable—more than 50% reduction in mean MPN-SAF TSS is seen after merely 3 months of treatment, and the effect lasts over the two-year study period [32]. Effect of PEG-IFN on the QoL of MPN patients is not well-studied, only one study on PEG-IFN alpha-2b reveals clinically significant impairments in QoL at 6 but not at 24 months [36]. However, QoL of PEG-IFN-treated patients in our cohort remains static over time. There is no difference in symptom burdens between PEG-IFN and conventional treatments.

Another JAK2 inhibitor, fedratinib, was recently approved by FDA in 2019 for the treatment of adult patients with intermediate-2 or high-risk primary and secondary myelofibrosis [37]. Fedratinib showed significant spleen volume reduction (≥35%) and total symptom score reduction (≥50%) in 30% and 27% patients with ruxolitinib resistance or intolerance [38].

40.3.3 Treatment for Anemia

Low-dose thalidomide at 50 mg daily with a three-month prednisolone taper starting at 0.5 mg/kg/day induced improvement in anemia in 62% of patients with a median hemoglobin increase of 1.8 g/dL [39]. Lenalidomide at 10 mg daily (21 days of a 28-day cycle) with prednisolone also showed overall response rates of 30% for anemia and 42% for splenomegaly in MF patients [40]. A small case series showed improvement in hemoglobin, cytogenetic in all three patients with del(5q)-associated primary or post-PV myelofibrosis [41]. Pomalidomide also showed activity in MF-associated anemia, particularly in JAK2V617F-postive patients with <10 cm palpable splenomegaly and <5% circulating blasts [42]. However, high rates of discontinuing treatment were observed at 68% and 89% at 1 year and 2 years [42].

We generally seldom use androgens in the treatment of MF-associated anemia due to side effects including fluid retention, hepatotoxicity, and virilization.

40.4 Minimizing Treatment Toxicities

Approximately 13–16% patients cannot tolerate hydroxyurea due to adverse reactions with the most common one being mucocutaneous lesion [43–45]. Phase 2 trials on patients treated with PEG-IFN showed that more than 90% patients experience adverse events—most commonly fatigue, muscle pain, gastrointestinal upset, and depression; 6–20% developed grade 3 or 4 toxicities—most commonly neutropenia and elevated transaminase [10, 12]. About 20% of patients discontinue therapy due to treatment-related toxicity [10]. Nevertheless, a recent retrospective study shows that PEG-IFN is well-tolerated in MPN patients treated off clinical trials and less than 20% report adverse events [46]. Ruxolitinib is generally well-tolerated with majority adverse events being grade 1 or 2; the most frequent hematologic ones are anemia and thrombocytopenia whereas the most common nonhematologic ones are fatigue, diarrhea, and peripheral edema [33, 34]. In the five-year final analysis, infections are higher in patients receiving ruxolitinib, including urinary tract infection, pneumonia, herpes zoster infection, sepsis and septic shock, and tuberculosis [23].

In ruxolitinib-treated patients, tuberculosis infection occurred in 6% of patients in a prospective study on Chinese [32], in contrast to 1% reported in the original study [23]. This reflects the increased prevalence of tuberculosis in the Asian population [47]. For this reason, tuberculosis prophylaxis should be given to patients with evidence of prior infection. In addition, patients positive for hepatitis B core antibodies (anti-HBc) have estimated HBV reactivation rates of 8% and 31% at 6 months and 12 months. This observation is unique in our population due to a high seroprevalence of anti-HBc in East Asia [48]. Hence, patients with positive anti-HBc should regularly be monitored for hepatitis B reactivation.

In summary, hydroxyurea and ruxolitinib are generally well-tolerated. However, one needs to watch out for tuberculosis infection and hepatitis B reactivation in treatment with ruxolitinib, particularly in Asian population. PEG-IFN has more adverse events reported by patients; although most are grade 1 or 2, various autoimmune diseases can occur and should be excluded before and be monitored during treatment.

40.5 Proposed Treatment Algorithm

Patients with myelofibrosis should have full clinical, pathologic, and molecular workup with proper risk assessment with GIPSS and MIPSS70-plus V2.0 as described in the previous chapter (Fig. 40.1).

Lower risk (very-low, low, and intermediate-risk) patients can be put on observation or treated with PEG-IFN for potential disease-modifying effects; ruxolitinib should be considered in the presence of debilitating symptoms.

Although the idea of being the only potentially curative therapy for myelofibrosis is attractive, one must not forget about the treatment-related morbidity and mortality associated with allogeneic hematopoietic stem cell transplant. A recent review on survival outcomes of 1928 MF patients showed ten-year OS of 47% and 35% HSCT (551 patients) and non-HSCT (1377 patients) [49]. The benefits were

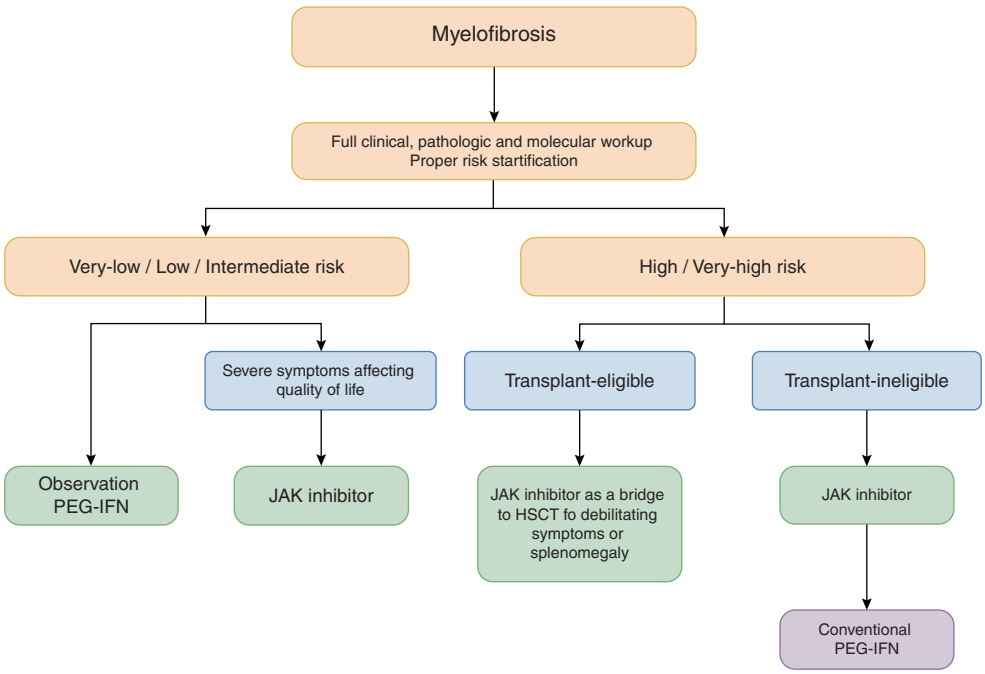

Fig. 40.1 Treatment algorithm for primary and secondary myelofibrosis

mostly seen in patients with higher DIPSS and were only observed beyond 1 year of treatment arm assignment, which was explained by the high risk of transplant-related mortality (TRM) in the first year after HSCT [49]. While higher-risk patients may benefit from HSCT in the long-term, the five-year TRM rates could reach up to 40% in DIPSS high-risk patients [50]. Hence, allogeneic HSCT should only be considered in the high- and very high-risk situations. JAK2 inhibitor should be considered in eligible patients as a bridge to HSCT for controlling symptoms and splenomegaly. In HSCT-ineligible patients, JAK2 inhibitor should be considered for symptoms and spleen control. However, the use of JAK2 inhibitor may be limited by different degrees of cytopenia in advanced myelofibrosis.

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Diagnosis and Management of Prefibrotic Primary Myelofibrosis (Pre-PMF)

41

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Abstract

This review is focused on prefibrotic primary myelofibrosis (pre-PMF) and evaluates clinical impact in relation to World Health Organization-defined essential thrombocythemia (ET). Compared to ET, patients with pre-PMF present with higher leukocyte counts, lower hemoglobin levels, higher lactate dehydrogenase values, and more frequently palpable splenomegaly. Incidences of *JAK2*V617F and *CALR*-mutated pre-PMF patients are superimposable to ET. Nondriver high-risk mutations in *ASXL*1, *EZH2*, *SRSF*2, and *IDH* 1/2 are increased in pre-PMF compared to ET. Vascular complications are not significantly different while hemorrhagic events are increased in pre-PMF. Median survival (around 13 vs. 19 years), 10-year cumulative rates progression to overt myelofibrosis, and transformation to blast phase are significantly different for pre-PMF versus ET. Primary objective of therapy is to prevent major thrombohemorrhagic complications. Because most pre-PMF patients fall in the lower prognostic IPSS group, observation alone or aspirin is recommended, while patients at intermediate risk may require a symptom-driven treatment and high-risk patients need cytostatic drugs.

Keywords

Prefibrotic primary myelofibrosis · Clinical presentation
Essential thrombocythemia · Differential diagnosis
Outcome · Prognosis · WHO criteria

41.1 Diagnosis of Pre-PMF

41.1.1 General Aspects

Although early/prefibrotic primary myelofibrosis (pre-PMF) was already included in the 2001 myeloid classification system of the World Health Organization (WHO) as "chronic idiopathic myelofibrosis prefibrotic stage" [1], until recently a conflict of opinion has been expressed about its existence. In this context, clinical experts from reference institutions applied different diagnostic criteria for myeloproliferative neoplasms (MPNs) like (primary) myelofibrosis (PMF/MF) or essential thrombocythemia (ET) that were not appropriate to recognize pre-PMF. Following an update in 2008 with a more detailed description of clinical and morphological features [2] and relevant clinical-pathological studies [3–6], interest and acceptance of pre-PMF increased significantly. After a renewed revision in 2016 [7], including a further refinement of the diagnostic criteria especially the differentiation between ET and pre-PMF [8], a number of papers with supporting results even from former critical authors were published [9–15, 16, 17]. The salient question still arises in which compartment were the pre-PMF cases hidden when applying other than WHO diagnostic criteria? Considering the experience of other groups, particularly the compartments of ET or early PMF/MF may contain pre-PMF cases [4, 10, 12, 18, 19]. Regarding incidences of pre-PMF, relative frequencies may be grossly calculated by referring to results obtained during a reclassification of representative bone marrow (BM) biopsies and corresponding clinical data from treatment-naive so-called ET patients to differentiate "true" ET cases. By following this procedure, ranges between 14% and 18% were reported mostly after centralized evaluations derived from centers of excellence [4, 9, 12, 14]. In clinical practice, a rate of "false" ET, i.e., pre-PMF, is supposed to range at least between 20% and 30% and may be closer to reality [20].

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41.1.2 Morphology

A key issue of the WHO classification of MPNs is the stringent postulate of an integrated approach that includes hematologic, morphologic, cytogenetic, and molecular genetic findings [7, 16, 17] which was explicitly emphasized to distinguish ET from pre-PMF, based on an accurate morphologic assessment of BM biopsies [17, 21]. In this regard, the WHO puts a special weight on morphology as a cornerstone of diagnosis (Table 41.1). Since the diagnostic impact of an adequate BM specimen cannot be overstated, the WHO is very strict and mandates trephine biopsies to be ≥1.5 cm in length, obtained at right angles to the cortical bone in treatment-naïve patients at diagnosis [16, 22]. Evaluation of trephine biopsies to differentiate subtypes of MPNs generally implies the recognition of standardized patters including a variety of features as hematopoietic cellularity, alterations of megakaryocytes, erythro- and granulopoiesis, and finally, amount of fibrosis and osteosclerosis [5, 16, 17, 22]. Contrasting BM biopsy histology of ET, usually in pre-PMF age-adjusted hematopoietic cellularity is increased mostly by granulocytic and megakaryocytic proliferation while erythropoiesis is often decreased. Most conspicuous are megakaryocytes showing endosteal translocation, dense clusters, hypolobulated (cloud-like), and naked nuclei with maturation defects. Additionally, WHO-defined pre-PMF exhibits a reticulin fibrosis not greater than grade 1 [23]. Regarding degree of BM fibrosis, the WHO adopted a three-graded system and distinguishes reticulin from collagen fibers [23, 24], while other groups prefer a four-graded scoring [25]. A critical comparison of both scoring schemes reveals that only the two last grades of each, i.e., 2/3 of the three-graded and 3/4 of the four-graded system are more or less identical and consistent with advanced myelofibrosis (MF) including reticulin and different densities of collagen ending up with osteosclerosis.

Reproducibility of WHO-defined morphological features [5, 16, 17, 22] for the differentiation of pre-PMF from ET has been evaluated by studying large cohorts of patients with varying numbers of involved panelists with or without prior knowl-

Table 41.1 World Health Organization and International Consensus Classification diagnostic criteria for prefibrotic primary myelofibrosis (pre-PMF)[a] versus essential thrombocythemia (ET)[b]. *Table adapted and rearranged from Barbui T et al.* [8], *Arber D et al.* [7], and *Thiele et al.* [16]

	Diagnosis	
	Pre-PMF	ET
Major criteria		
1.	Bone marrow biopsy showing Megakaryocytic proliferation and atypia[c], without reticulin fibrosis > grade 1[d], accompanied by increased age-adjusted BM cellularity, granulocytic proliferation, and often decreased erythropoiesis	Platelet count ≥450 × *10⁹/L*
2.	Not meeting diagnostic criteria for *BCR*::*ABL1* positive chronic myeloid leukemia, polycythemia vera, essential thrombocythemia, myelodysplastic syndromes or other myeloid neoplasms	Bone marrow biopsy showing proliferation mainly of the megakaryocyte lineage with increased numbers of enlarged, mature megakaryocytes with hyperlobulated staghorn-like nuclei, infrequently dense clusters nuclei. No significant left-shift of neutrophil granulopoiesis or erythropoiesis and very rarely minor (grade 1) increase in reticulin fibers[e]
3.	Presence of *JAK2, CALR or MPL* mutation or in the absence of these mutations, presence of another clonal marker[f] or absence of minor reactive BM reticulin fibrosis[f]	Not meeting WHO criteria for *BCR-ABL1* :: positive chronic myeloid leukemia, polycythemia vera, essential thrombocythemia, myelodysplastic syndromes or other myeloid neoplasms
		Presence of JAK2, CALR or MPL mutation
Minor criteria		
	Not meeting WHO criteria for *BCR;:ABL1* CML, PV, ET, MDS or other myeloid neoplasms: • Anemia not attributed to a comorbid condition • Leukocytosis ≥11 × *10⁹/L* • Palpable splenomegaly • LDH level above the upper limit of the institutional reference range	• Presence of a clonal marker (e.g., abnormal karyotype) or absence of evidence for reactive thrombocytosis

BM bone marrow, *CML* chronic myeloid leukemia, *LDH* serum lactate dehydrogenase, *MDS* myelodysplastic syndrome, *PV* polycythemia vera

[a] Diagnosis of prefibrotic PMF requires all three major criteria and at least one minor criterion

[b] Diagnosis of ET requires meeting all four major criteria or first three major criteria and one minor criterion

[c] Small-to-large megakaryocytes with aberrant nuclear/cytoplasmic ratio and hyperchromatic and irregularly folded nuclei and dense clustering

[d] In cases with grade 1 reticulin fibrosis grading of BM fibers (Thiele J et al. [23])

[e] The megakaryocyte changes must be accompanied by increased BM cellularity, granulocytic proliferation, and often decreased erythropoiesis (i.e., pre-PMF)

[f] In the absence of any of the three major clonal mutations, the search for the most frequent accompanying mutations *(ASXL1, EZH2, TET2, IDH1/IDH2, SRSF2, SF3B1)* is of help in determining the clonal nature of the disease

[g] Minor (grade 1) reticulin fibrosis[d] secondary to infection, autoimmune disorder or other chronic inflammatory conditions, hairy cell leukemia or other lymphoid neoplasm, metastatic malignancy, or toxic (chronic) myelopathies

edge of clinical data. As further detailed, more than 80% (range 76–88%) diagnostic consensus with formal assessment of interobserver variability was reached in 2033 patients derived from several independent study groups [8, 17].

Unfortunately, a comparison between 2008/2016 WHO-confirmed ET [2, 7] and other relevant classification systems to retrieve possible pre-PMF patients is prone to cause a controversial discussion and contradictory results. Following the diagnostic guidelines proposed by the Committee of Standards in Hematology (BCSH) for ET [26, 27], two groups reported a broader range of patients encompassing a significant number of cases who would otherwise be diagnosed as pre-PMF, polycythemia vera (PV) or MPN-unclassifiable [18, 19]. The latter findings caused a vivid debate about the equivalence of BCSH and WHO diagnostic criteria for ET [28]. Another source to recruit possible pre-PMF patients may be trials following the Polycythemia Vera Study Group (PVSG) criteria [29]. In this regard, the UK-PT1 Study is a multicenter international trial in ET where both newly diagnosed and previously treated adult patients of which the high-risk arm of the study has been published [30]. This multicenter trial included 809 ET patients with a time between diagnosis and enrollment of three to more than 5 years in about 40% and with a prior cytoreductive treatment in more than 30% of cases. These latter features are not compatible with the corresponding WHO diagnostic criteria for MPNs [2, 7, 16]. On the other hand, BM trephines selected from newly diagnosed and previously treated UK-PT1 patients were evaluated showing wide ranges of so-called pre-PMF (9–28%) and fiber grading (37–76%) between the three panelists and included higher levels of MF [31], consistent with grades 3 and 4 on a four-graded scale [23] together with new bone formation (osteosclerosis). In another study of the same group [32] on ET with previously treated patients mostly derived from the UK-PT1 trial, nearly 20% were presenting with a median reticulin MF grade 3 and about 5% even with grade 4 with osteosclerosis [25]. Following a central review of ET patients recruited from the UK-PT1 cohort at trial entry, Godfrey et al. [33] found that 94% met the criteria of the BCSH [26, 27] and 81% those of the WHO [2] but without relating to pre-PMF. There seems to be a problem concerning differentiation of pre-PMF clinically often mimicking ET ("false" ET) in PVSG-defined cohorts [29]. In a large multicenter international study of 2000 patients with MPNs recruited from three different sources, ET was diagnosed in a total of 1321 (66%) patients; 1098 (83%) of these cases were derived from the UK-PT1 study and 223 patients (17%) from two other cohorts [34]. Diagnostic criteria recommended by the BCSH [26, 27] were applied for the UK-PT1 patients and either the WHO 2008 [2] or 2016 [7] criteria for the other two cohorts. Despite criteria having been revised multiple times over the course of the trial, central pathology review demonstrated high levels of concordance with current diagnostic guidelines [34]. Regarding this mix-up of ET patients and classification systems representing a "real world of clinical practice," pre-PMF was not diagnosed.

41.2 Clinical and Laboratory Aspects

41.2.1 Clinical Data

Although BM morphology remains the cornerstone of diagnosis, the WHO classification envisions a strictly integrated approach, i.e., a multidisciplinary process including clinical and molecular genetic findings [2, 7, 16, 17, 22]. Personal data of 1684 pre-PMF patients derived from six studies showed no significant difference of gender distribution at diagnosis (807 males vs. 877 females) and a median age ranging in the different cohorts between 55 years and 66 years [4, 5, 10–12, 35]. In comparison with ET, main hematological characteristics of pre-PMF patients are reported in Table 41.2 to demonstrate the significant differences between both entities. Contrasting ET patients, at diagnosis treatment-naive pre-PMF patients usually present with higher counts for leukocytes and LDH values, lower hemoglobin levels, and more often palpable splenomegaly [4, 5, 10–12, 16, 20]. Constitutional symptoms (night sweats, fatigue, weight loss) have been described in about 21% of pre-PMF patients [10] compared to 6–15% in ET patients [11, 16, 20, 36]. Moreover, increased circulating CD 34-positive progenitor cells may be found [4]. In pre-PMF, rarely (2–4%) a very few erythroblasts and/or myeloblasts (<1–5%) may be observed in peripheral blood smears (left-shifting) but no leukoerythroblastosis [37]. It is of note that an elevated platelet count (≥450 × 10^9/L) at diagnosis as postulated for ET was found in most pre-PMF cases (85.4%) and 9.4% of patients failed initially to show one of the selected

Table 41.2 Frequency of defined borderline clinical criteria in prefibrotic primary myelofibrosis (pre-PMF) compared with essential thrombocythemia (ET) at presentation according to the WHO diagnostic criteria. *Table adapted and rearranged from Jeryczynski et al.* [11]

	Pre-PMF $n = 170$	%	ET $n = 225$	%	p-value
Criteria					
Anemia[a]	42	24.7	14	6.2	<0.001
WBC ≥ 11 × 10^9/L	86	50.6	55	24.4	<0.001
Elevated LDH[b]	124	72.9	50	22.2	<0.001
Splenomegaly[c]	76	44.7	25	11.1	<0.001

LDH serum lactate dehydrogenase, *WBC* white blood cell count
[a] Anemia: hemoglobin: males ≤13 g/dL, females ≤12 g/dL
[b] Elevated LDH: reference value ranges between 240 (U/L) and 250 (U/L) in different institutions
[c] Splenomegaly: palpable or ≥ 12 cm in any imaging

clinical features [11], so-called minor clinical parameters [2, 7, 22].

There is an active interest to evaluate whether laboratory or clinical parameters would help to distinguish WHO-defined ET and pre-PMF in patients presenting with thrombocythemia. Laboratory parameters listed in Table 41.2 were used in a dichotomized fashion, resulting in a step-by-step algorithm. The cut-off values at each step in this algorithm were optimized to produce the desired specificity and sensitivity. The result was that nearly 50% of all patients mimicking an ET-like phenotype could correctly be attributed to the pre-PMF group [38]. To optimize the discriminatory ability, alternative approaches were applied to classify patients, including those previously undetermined. Both calculations included a logistic regression model which weights the information contained in the laboratory parameters and in the dichotomous variables in an optimal, data-driven way improving the result to 85% [39].

The rate of arterial and venous thrombosis revealed no significant differences in pre-PMF compared to WHO-defined ET [16, 35, 36, 40–43]. In one study that considered 180 patients with pre-PMF, the cumulative risk of thrombotic complications was 25.4% compared with 21.5% in 891 ET patients, accounting for a rate of overall major thrombosis of 1.9% and 1.7% patients/year, respectively [4]. In another study on 109 pre-PMF patients, the 10-year cumulative incidence of thrombosis was 18.5% compared with 18% in 269 patients with ET [12]. Of note is that among the other predictors of arterial thrombosis like age more than 60 years, history of thrombosis or cardiovascular risk factors [41], in multivariate analysis leukocytosis was found to be important for ET and pre-PMF as well [40–44].

Arterial vascular events in pre-PMF patients included predominantly acute myocardial infarction and stroke (23%), transient ischemic attacks and peripheral arterial (20%), and abdominal thrombosis (11%) [16, 35]. Venous thrombosis was mostly consistent with splanchnic vein thrombosis (68%), deep venous thrombosis/pulmonary embolism (16%), and Budd-Chiari syndrome (10%) [35].

Frequency of major bleeding (mostly gastrointestinal) previous or at diagnosis was increased but not significantly different in pre-PMF versus ET (7% vs. 4%). However, major hemorrhage during follow-up occurred in 12% of the pre-PMF patients but in only 6% of ET patients ($p = 0.009$), implying a rate of 1.39% and 0.79% patient-years respectively ($p = 0.039$) [16, 45].

41.2.2 Molecular and Cytogenetic Data

Mutation analysis is particularly rewarding if the 2016 revised WHO criteria are taken into account [46]. A recent study evaluating a large cohort of pre-PMF patients [35] including driver and nondriver mutations is shown in Table 41.3. Altogether incidences of *JAK2* V617F mutations are very similar in pre-PMF compared to ET ranging between 52% and 67% [4, 10–12, 35] and 54–66% [4, 11, 12, 21, 36, 47, 48], respectively. Calreticulin (*CALR*) mutations have been reported in about 20% in ET patients [11, 36, 47, 48] and compared to pre-PMF reveal a tendency to increase in frequency to about 30% [11, 12]. Noteworthy is that *CALR* mutations in ET indicate a good predictability of survival in pre-PMF but not ET, with *CALR* being a more favorable mutation than *JAK2* V617F [11]. This finding confirms data from a multicenter study on overt PMF [46] and extends results that *CALR* type 1 [49] was the most favorable driver mutation regarding survival [10]. In this context, in pre-PMF, the impact of ≥2 mutated genes (see Table 41.3) was consistent with a high-mutation risk status (HMR) and also associated with significantly shortened survival [10]. Pre-PMF patients may be stratified in different risk categories: possible variables for refinement may be the HMR status and/or other molecular variables [10, 35], as has been already applied for ET and PV [50].

Table 41.3 Molecular characteristics of prefibrotic primary myelofibrosis (pre-PMF) at presentation following the revised 2016 WHO diagnostic criteria *Barbui T et al.* [46]. *Table adapted and slightly modified from Guglielmelli P et al.* [35]

Variables	Pre-PMF, *n* (%)	Mutations, *n* (%)
Driver mutation		
*JAK2*V617F	378	246 (65)
CALR type 1	288	63 (22)
CALR type 2	134	24 (18)
MPL W515x	336	17 (5)
Triple-negatives	377	31 (8)
Nondriver mutations		
ASXL1	133	28 (21)
EZH2	132	5 (4)
SRSF2	132	14 (11)
IDH1/2	132	1 (1)
HMR, *n* (%)	132	37 (28)
HMR ≥ 2	132	11 (8)

HMR high-molecular risk, points to the presence of at least one mutation in any one of ASXL1, EZH2, SRSF2, IDH1/2. HMR ≥ 2 means the presence of two or more mutated genes among the above. Two or more mutations in the same gene are counted as one

Observations suggesting that *JAK2* V617F/*MPL* mutations were associated with a higher risk of fibrotic progression in ET [51] may imply the possibility that a number of *MPL*-mutated ET might actually represent pre-PMF [52]. It has to be noted that *MPL* mutations are rare and occur in about 3–4% of ET patients [12, 21, 36, 46, 53] but in about 6% in pre-PMF [10, 12] and 6–8% of overtly fibrotic PMF patients [10, 47]. It is remarkable that M*PL*-mutated ET cohorts present in some series with higher rates of fibrotic progression than their wild-type counterparts with 33.3% (vs. 7.5% in *MPL*-wild-type) [47, 53]. ET patients from the

UK-PT1 Study showed a relatively high *MPL* mutation rate of 8.5% [54]. This rate could be expected keeping in mind that this trial was diagnosed according to the PVSG criteria [29] allowing, in contrast to the WHO guidelines [2, 7, 16], a certain degree of overt MF, suggesting rather pre-PMF than "true" ET [20]. Following reclassification experience, it should be discussed whether the majority of routinely assigned cases of MPL-mutated ET may probably represent pre-PMF when morphologically scrutinized [52].

In pre-PMF, abnormal cytogenetics were found in 15–18% [10, 16, 35] and unfavorable karyotypes [16, 55] in 4% [10] of cases. The corresponding incidence of abnormal karyotypes in ET was only 9% [16, 36].

In aggregate, patients with pre-PMF may be accurately stratified in different risk categories and possible refinements may be additionally achieved by molecular and cytogenetic variables and finally, clonality established [10] On the other hand, until now a distinction between ET and pre-PMF is not possible without BM morphology and clinical data, required for prognosis and outcome and the meaningful interpretation of clinical trials using novel drugs [16, 17].

41.3 Management

41.3.1 General Aspects

There is overall agreement that clinical strategies to manage thrombocythemic pre-PMF are based not only on the accurate distinction from "true" ET [4, 6, 8, 16, 17], but also on emerging complications (vascular events, progression to overt PMF) and outcome (blast phase, survival). Generally, the international prognostic scoring system (IPSS) may serve as the "golden yard stick" to handle pre-PMF in daily practice concerning treatment modalities.

Risk groups consisted of only three categories since the authors failed to detect a significant difference between intermediate-1 and intermediate-2 patients—whereas the high-risk group was clearly distinguishable [10, 56]:

1. Low-risk: observation only;
2. Intermediate-risk (cumulated intermediate-1 and -2 risk groups): symptomatic-driven therapy (consider inclusion in clinical trials);
3. High-risk: intensive management (consider inclusion in clinical trials).

Taking into account that the majority of patients lie within the lower prognostic IPSS group, initially observation alone or aspirin can be recommended. On the other hand, patients at intermediate-risk may require a symptom-driven treatment for anemia, splenomegaly or constitutional symptoms, whereas high-risk patients should be treated as overt PMF including cytostatic drugs [16, 56–58].

Concerning the natural disease evolution in pre-PMF using a multistate model taking into account the different phases, probabilities, and possible interconnections revealed a direct transition to overt PMF, blast phase (BP—formerly termed acute leukemia-AML), and death of initial cases besides a number of interesting interconnections important for therapy decision making [59].

41.3.2 Vascular Events

Thrombosis and hemorrhage represent two of the main causes of morbidity and mortality in patients with MPNs. Consequently, the main goal of therapy in both pre-PMF and ET is to prevent thrombohemorrhagic complications. In thrombocythemic pre-PMF compared with WHO-defined ET, thrombotic complications are similar during the follow-up, while hemorrhagic events are increased [4, 12, 16, 35, 36, 40, 45]. In a recent study on pre-PMF, 15% of the patients developed a major thrombotic event consistent with an incidence rate of 8% arterial and 7% venous thrombosis (1.99% patients/year) accounting for a 1.00% and 0.95% patients/year, respectively [16, 35]. In studies including also a portion of ET patients diagnosed according to the PVSG criteria [29], the corresponding rates ranged from 1.5% to 2.5% patients/years [16, 40, 56, 57].

In a study with ET patients diagnosed according to the PVSG criteria [29] recruited from the UK-PT1 trial [30], a conspicuous range of reticulin fiber scores (four-graded system) [25] was found [32]. Although grades 1 and 2 were particularly frequent, nearly 20% of patients had a median reticulin grade of 3 and a small fraction even of grade 4 [32]. Increased BM reticulin fibrosis at presentation positively correlated with platelet and leukocyte counts and predicted higher rates of major bleedings during follow-up. There was also a strong association between presenting reticulin grade and transformation to overt MF [32]. In this context, it is tempting to speculate whether those patients are more likely consistent with thrombocythemic pre-PMF than ET [40–44].

Regarding WHO-defined ET, the IPSET score for thrombosis (WHO ET IPSET-thrombosis) [60, 61] was reanalyzed and validated [62]. Thus, *JAK2* V617F mutation and cardiovascular risk factors allowed the definition of distinct classes of thrombotic risk, including "very low" and "low" risk classes, and between them "intermediate" and "high" risk patients [61, 62]. For pre-PMF, the risk for total thrombotic events was accurately predictable by applying the IPSET thrombosis score, formerly developed for WHO-defined ET, that corresponded to 0.67%, 2.05%, and 2.95% patients/year

in the low-, intermediate-, and high-risk categories, respectively [35].

Treatment algorithms for pre-PMF using different drugs are mostly based on previous history of thrombosis and bleeding, age > 60 years, general cardiovascular risk factors, leukocytosis, and *JAK2* 617F mutations [16, 56]. A pragmatic approach to address the risk of bleeding and thrombosis includes: no treatment or low-dose aspirin in asymptomatic patients; aspirin or oral anticoagulation if previous arterial or venous thrombosis, and hydroxyurea as first-line cytoreduction in case of thrombocytosis or leukocytosis [16, 56].

41.3.3 Progression to Myelofibrosis (Overt PMF)

Progression to overt PMF/MF (myelofibrosis with myeloid metaplasia) has been reported in pre-PMF to occur at a 10-year cumulative incidence ranging between 9.7% and 12.3% [4, 12, 16], thus exceeding significantly calculated frequencies in WHO-confirmed ET patients (cumulative risk at 10 years ranging between 0.8% and 4.5%) [63]. Incidences may vary in different studies probably dependent of recruitment of the pre-PMF patients (ET-like vs. PMF-like clinical phenotype) and consequently, the corresponding data, e.g., 1.0% patients/year [4] compared with 2.05% patients/year [35]. However, the striking difference to WHO-defined ET (0.5% patients/year) regarding progression to overt MF is always significant [4, 16, 17].

Two cohorts of WHO-confirmed ET patients ($n = 292$ and $n = 284$) described an overall frequency of developing MF being 9.2% versus 10.3%, respectively [47]. Moreover, it has been shown that in young patients (age ≤ 40 years), fibrotic progression was expectedly higher in ET (16%) for their longer survival [64].

Discrepancies may arise when applying diagnostic guidelines in use at the time of first observation without reclassification, then the 14-year cumulative incidence of progression to overt MF may increase to 3.9% [43]. As already outlined before, this high incidence in comparison to strictly WHO-defined ET can be expected because of inclusion with cases diagnosed according to PVSG criteria that do not recognize pre-PMF [29]. As previously more detailed, serious concern is expressed regarding a comparison of the PVSG diagnostic guidelines [29] with the BCSH criteria [26, 27] and the WHO classifications [2, 7, 16, 17] for ET not only as a very challenging venture, alone for the differences in the threshold values for platelets ($>600 \times 10^9$/L vs. $>450 \times 10^9$/L). Multivariate analysis showed that in WHO-defined ET, risk factors for progression to overt MF include pre-PMF morphology, advanced age (>60 years), male sex, and anemia (<12 g/dL) [4]. On the other hand, in pre-PMF, risk factors for progression to overt PMF included anemia (hemoglobin <12 g/dL for females, <13 g/dL for males) and grade 1 [23] BM fibrosis [59].

Concerning treatment, progression to overt PMF is compatible with high risk and therefore requires cytoreductive therapy or curative stem cell transplantation if possible [56]. First-line drug of choice for cytoreductive therapy is hydroxyurea. Busulfan can be used in elderly individuals who are intolerant to hydroxyurea. Ruxolitinib may be considered in pre-PMF patients with intermediate- or high-risk disease when splenomegaly or systemic symptoms if need of treatment is present [16, 56, 58].

41.3.4 Transformation to Blast Phase (BP) and Survival

In MPNs, transformation to BP (formerly termed acute leukemia-AML) is defined as occurrence of ≥20% blasts in the peripheral blood or BM [2, 7]. Concerning pre-PMF, the overall incidence of BP ranged between 5% and about 8% [10, 16, 22, 35], while the cumulative risk at 10 years was between 2.3% and 5.8% in phenotypically ET-like patients [12, 16, 22], but reached 12% in a more PMF-like cohort [10]. Independently from these differences regarding the selection of pre-PMF patients, the corresponding "true" ET cases showed a significant lowering of the cumulative risk for BP at 10 years ranging between 0.7% and 1.9% [10, 12, 22]. A risk rate for BP of about 1% at 10 years has been proposed in WHO-diagnosed ET [48]. However, similar to fibrotic progression, incidence of BP was as high as 2% in younger patients due to their longer survival [64].

Overall median survival in larger cohorts of pre-PMF patients was found to range between 10.5 years and 14.7 years [4, 5, 10, 11, 16]. and a cumulative risk at 10 years at about 24% [4]. These incidences are significantly lower than those reported for WHO-defined "true" ET ranging from 15.7 years to about 21.8 years [4, 5, 11, 16, 17, 47, 65] and a cumulative risk at 10 years at about 15% [4]. An important point is to exclude the influence of age in this context because median survival was 35 years for younger ET patients contrasting 11 years for age groups greater than 60 years [64]. To neutralize possible effects on mortality from age-related causes and comorbidity other than the underlying ET, special methods of calculation on survival seem to be preferable [5, 65, 66]. Based on the inclusion of pre-PMF (or IMF in 2001) [1, 65] in the PVSG classification [29], a comparison between PVSG- and WHO-defined ET shows a significant loss of life expectancy of 16.5% and 8.9% [66]. Moreover, survival of 891 patients derived from a multicenter trial with strictly WHO-defined ET [2, 7] was similar to the 2008 Eurostat age- and sex-standardized incidence rates for all causes of death [4]. On the other hand, when applying a similar calculation on a sex-and age-

matched population of 292 ET patients derived from a single center in the USA, a slightly inferior survival rate was revealed [47]. Remarkable is that risk factors for overall survival were prefibrotic PMF, advanced age, history of thrombosis, leukocytosis, and anemia [4, 67].

41.4 Conclusion

The aim of this review was to discuss critically previous and novel data regarding pre-PMF and to evaluate in this context their clinical impact in relation to WHO-defined ET. For future research projects and clinical decision-making, we need: (1) an in-depth reevaluation of old ET and thrombocythemic PMF cases comparing PVSG and BCSH criteria with the 2016 revised WHO classification including explicitly treatment-naive biopsy samples taken directly at diagnosis; (2) following this centralized experience, it is necessary to validate former conclusions, particularly regarding therapy and outcome; (3) the final goal would be to launch a multicenter prospective study on ET versus thrombocythemic PMF based on the results of the above studies.

Conflict of Interest The authors declare no conflict of interest.

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Interferons in Myeloproliferative Neoplasms

42

Lucia Masarova and Srdan Verstovsek

Abstract

It took more than 30 years of clinical use of interferon (INF) in patients with myeloproliferative neoplasms (MPNs) for the official approval of the first ever interferon (ropeginterferon alpha-2b) for patients with polycythemia vera (PV), one of the classical MPNs. INF possesses broad range of biological properties, including enhancement of immune response and direct effects on malignant cells with a potential of their ultimate elimination. Despite its long-known antiproliferative, anti-inflammatory, and auspicious disease-modifying effects, side effects hampered widespread INF use and complicated its approval path for MPN patients. Notwithstanding, INF has been so far used in numerous, smaller, earlier phase studies encompassing almost 1000 patients with MPN where it constantly showed high rates of hematological, and less commonly molecular and histopathological remissions. The later responses, which in some patients persisted even after therapy discontinuation, highlighted the agent's potential to alter the disease course. Approval of ropeginterferon for PV and ongoing use of other INF forms in an off-label setting for MPN patients, will continue to further define the true role of INF within MPN treatment armamentarium. In this chapter, we provide overview of INF mechanisms and summary of clinical data of INF in patients with classical MPNs.

Keywords

Interferon · Ropeginterferon · Polycythemia vera
Essential thrombocythemia · Molecular response

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42.1 Interferons

The interferons (INF) are naturally occurring cytokines with vast immunomodulatory, antiproliferative, proapoptotic, antiangiogenic, and cell-differentiating properties. Interferon alfa (INF-α) belongs to the type I interferons, that exert its effects on cells throu direct interaction with the human interferon alfa receptor chains, and subsequent activation of signaling via the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway [1].

Interferon α-2 has been the predominant INF used as a therapeutic agent in MPN in various subcutaneous forms, e.g., r-INF-α-2b (Intron A); pegylated INF-α-2a (Pegasys); pegylated INF-α-2b (PegIntron), and the latest ropeginterferon α-2b (Besremi). Since the initial report by Essers et al. [2] on the INF action on normal hematopoietic cells and suggested mechanism to eradicate malignant clone by inducting cells exhaustion, other authors confirmed the ability of INF to directly enhance malignant cell cycling and promote their own proliferation and apoptosis [3], as particularly reported in the JAK2V617F (+) MPN malignant clone [4–6]. These findings were clinically reflected in numerous reports showing reduction of JAK2V617F allele burden (called molecular remission) by INF, an observation not previously seen to this extend with other therapies [reviewed by Kiladjian [7]].

Broad immunostimulatory effects of INF, including its ability to alter circulation and enhance activity of cytotoxic T cells, NK cells, and macrophages [8], suppress inhibitory capacity of T regulatory cells and improve immune-mediated destruction of target cells [9]; enhance maturation of dendritic cells [10] and upregulate the presentation of tumor antigens with enhanced cross-priming [11]; are believed to be able to restore, at least partly, MPNs defective immune surveillance that contributes to disease persistence and progression.

Although there is enough evidence that INF might deplete JAK2 mutant clones, studies showed that certain factors, such as germline status of interferon lambda 4 receptor [12], non-JAK2 driver mutations [e.g., CALR [13–15]], presence

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of nondriver mutations [e.g., TET2, DNMT3A, ASXL1, IDH, TP53 [16, 17]], and chronic inflammation [18] negatively impact the agent effectiveness. These abnormalities might confer advantage to the progenitor cells and drive disease resistance due to increased proinflammatory signaling and enhanced cells self-renewal [19, 20]. Recent study from 48 patients with MPNs treated with INF over a 5-year period concluded that progenitor cells harboring homozygous JAK2V617F mutation were more susceptible to INF than heterozygous cells, especially to lower doses; and that CALR type 2 mutant progenitors were more effectively targeted than type 1 CALR mutant cells, but required higher doses of INF with increased toxicity [21].

The presence of chronic perpetuated inflammation, released from the malignant clone (as inflammatory cytokines) as well as the bone marrow microenvironment itself, is considered to be one of the major driving forces of clonal evolution [22] and accurately earned MPNs the label of "the human inflammation models for cancer development" [18, 23]. In this context, the restoration of effective immune system and reduction of inflammation appeal as intriguing treatment concept in which INF might play a crucial role [24]. To prevent disease refractoriness, early treatment interventions and combinational approaches with other agents are likely to be necessary.

42.2 Interferon Use in MPN

Several INF-α compounds listed earlier have been used, almost exclusively off-label, in patients with MPNs over the past 30+ years. The clinical effectiveness of the standard recombinant INF-α (r-INF-α) in MPN patients was repeatedly reported since the 80s in studies showing effective control of blood counts with elimination of phlebotomies, improvement in MPN-related symptoms and partly splenomegaly, and even reversal of marrow fibrosis [25–27]. However, the widespread use of this INF form was limited by adverse events (AEs), such as flu-like symptoms, fever, malaise, nausea, and vomiting, reported in up to 35% of patients, and inconveniently frequent use (3 times weekly).

Following the development of pegylated forms (PEG-INF), INF became more widely used in MPNs as once-a-week dosing which offered better tolerability. Numerous small phase 2 clinical trials enrolling over 800 MPN patients confirmed the ability of PEG-INF to induce overall response rate > 80% (complete hematologic responses in ~60% of patients), reduction of JAK2V617F allele burden or molecular response in up to 65% of patients (complete molecular remission [CMR] in ~18%), and improvement in bone marrow histopathology in about 50% of patients [complete marrow remission in 20% [28–32]]. The most appealing aspects of these responses appear their durability. In a long-term follow-up of 83 ET and PV patients treated with PEG-INF, median duration of hematologic and molecular response was 66 months and 53 months, respectively, and the deepest responses were the most durable [e.g., CMR was sustained in 90% of patients] [30]. Furthermore, five of six patients who achieved CMR and discontinued therapy, remained disease-free for a median of 4 years since the last treatment. Whilst these results indicate the agent's ability to eradicate the malignant clone, relapses still occurred after therapy discontinuation, even in patients who achieved complete molecular and histopathological remission [27, 33–35]. Recently, authors from France (293 patients) predicted that patients who achieved decrease in JAK2V617F < 10% and were in CHR for >24 months prior to INF discontinuation, are the ones to remain in relapse-free and treatment-free remission [36]. Although there is no solid evidence that such a deep remission would ultimately translate into improved patient's outcome, recent study on 470 PV patients treated with INF (n = 93), hydroxyurea (HU, n = 189), or phlebotomies (n = 133) who were followed in a single institution for almost four decades, showed that INF might positively impact overall survival and slow progression to MF. According to this study, patients treated with INF (vs. those treated with HU or only phlebotomies) were more likely to be alive at 20 years (66% vs. 40% vs. 14%), had the longest MF-free survival (89% vs. 41% vs. 36% at 20 years), and the slowest rate to develop progressive grade 2+ reticulin fibrosis over time (16% vs. 49% vs. 62% at 8–14 years from diagnosis, respectively). Time on treatment analysis demonstrated that every year on INF reduced the risk of progression to MF and annual mortality by 9% and 6%, respectively [37].

Table 42.1 summarizes selection of the most relevant trials of INF in patients with MPN. Two trials of The Myeloproliferative Disorders Research Consortium (MPN-RC); MPN-RC 112 (phase III study in therapy-naïve patients using comparison to HU) and MPN-RC 111 (phase II trial in patients resistant or intolerant to HU) that evaluated PEG-INF in PV and ET patients, were recently reported. In MPN-RC 112, PEG-INF provided comparable CHR to HU, with slightly higher molecular but lower histopathological responses [38]. In MPN-RC 111, the agent provided higher responses for patients with ET (CHR at 12 months: 43% vs. 22% for PV), especially those carrying CALR mutation (56% vs. 28% for non-CALR mutant patients, respectively) [39]. Both studies showed low incidence of thrombotic events on PEG-INF (5% up to 2 years) and only one patient with PV progressed to MF. Despite a significant rate of AEs, PEG-INF discontinuation only occurred in 14% of patients. Subsequent post hoc analysis of both MPN-RC trials [MPN-RC 111, n = 114; MPN-RC 112, n = 166] focused on symptoms control, and showed that only patients with high total symptom burden [TSS ≥20 per MPN-SAF [40]] achieved improvement in symptoms between 3 months and

Table 42.1 Summary of relevant studies of interferon in myeloproliferative neoplasms

Study phase, type, ref.	Interferon treatment	Patients (number)	Outcome [primary endpoint]	Additional comments
Phase 3, PEG-INF vs. HU (1:1)—*MPN 112 trial* [38]	PEG-INF 45 μg/week	Essential thrombocythemia [81], polycythemia vera [87]; newly diagnosed	[CHR at 12 mo] PEG-INF vs. HU: 35% vs. 37% (ORR 78% vs. 70%). CHR at 36 mo: 33% vs. 17%	*Phase 3 PEG-INF in new ET/PV (vs. HU).* Median RX duration 81 weeks. Comparable CHR rates. PEG-INF > HU molecular response, but HU > PEG-INF marrow response. 2% rate of vascular complications on both agents. Grade ≥ 3 AEs PEG-INF > HU: 46% vs. 28%
Phase 2, PEG-INF—*MPN 111 trial* [39]	PEG-INF α-2a 45–180 μg/week	Essential thrombocythemia [65], polycythemia vera [50]; refractory/intolerant of HU	[ORR at 12 mo] ET ORR: 69% (CHR: 43%). PV ORR: 60% (CHR: 22%)	*PEG-INF for R/R ET and PV.* Median RX duration 80 weeks. Molecular response ~ clinical response. Grade ≥ 3 AEs in >5% < 10% were only hematologic. Disc. RX due to PEG-INF in 14%
Phase 2, PEG-INF [14]	PEG-INF mean starting dose 496 μg/mo	Essential thrombocythemia with CALR mutation [31]	[CHR] CHR 67%	*PEG-INF for CALR mutated ET.* Mean RX duration 35 months. Maintenance dose reduced by 50%. CALR declined in 65%, including two CMRs. Additional mutations ~ poor response
Phase 3, recombinant INFα-2a, r-INFα-2b, HU (1:1:1); *DALIAH trial* [57]	INFα-2a 45 μg/week, r-INFα-2b 35 μg/week, HU (≤ 60 years pt. on INF)	Essential thrombocythemia [100], polycythemia vera [136], myelofibrosis (62)	[ORR] INFα-2a or 2b > 60 years vs. ≤ 60 years vs. HU: 58% vs. 58% vs. 66%	*Phase 3 of r-INF in MPN, including MF.* Similar ORR between MPN groups. HU with comparable efficacy. Similar rate of molecular responses (~20% on HU and r-INF). AEs related to RX disc. 34% (>60 years)—45% (≤ 60 years) for r-INF, resp.—13% for HU
Phase 2, PEG-INF [30]	Peg-INF-α-2a 90–450 μg/week	Essential thrombocythemia [40], polycythemia vera [43]; newly diagnosed	[CHR] ET 73% CHR [80% ORR] PV 77% CHR [84% ORR]	*Long follow-up of PEG-INF in ET and PV.* Median follow-up 83 months. Molecular response[a]: 63% of JAK2 V617V (+): 37% ET, 63% PV. CMR 9% ET, 20% PV. RX disc. Due to PEG-INF 22%. The most common AEs: fatigue, muscle pain, nausea, diarrhea, depression
Phase 1/2, ropeginterferon (RoPEG-INF)—*PEGINVERA* [44]	RoPEG-INF α-2b 50–540 μg every 2 weeks	Polycythemia vera; new & treated [51]	Phase 1 [MTD]: No DLT & MTD = 540 mcg. Phase 2 [ORR]: 90%, CHR 47%, CMR 21%	*Dose findings study of RoPEG-INF.* Median follow-up 80 weeks. AEs-related RX disc. 20%. AEs in >20%: pruritus, fatigue, headache, arthralgia, diarrhea, flu-like, vertigo

(continued)

Table 42.1 (continued)

Study phase, type, ref.	Interferon treatment	Patients (number)	Outcome [primary endpoint]	Additional comments
Phase 3, RoPEG-INF vs. SOC (1:1); *PROUD-PV* and *CONTINUATION-PV* [46]	RoPEG-INF α-2b 50–500 μg every 2 weeks	Polycythemia vera, new or < 3 years on cytoreductive therapy [127 PROUD-PV and 95 CONT-PV]	PROUD-PV [CHR + normal spleen size]: RoPEG-INF vs. SOC 21% vs. 28%. CONT-PV: RoPEG-INF vs. SOC at 36 months: 53% vs. 38%	*Approval phase 3 study for RoPEG-INF.* Molecular response[b]: RoPEG-INF vs. SOC 44% vs. 51% at 12 months; 66% vs. 27% at 36 months. RX disc. due to RoPEG-INF 8%. AEs in >20% on RoPEG-INF: thrombocytopenia, leukopenia, increased liver enzymes, anemia, headache, arthralgia, dizziness
Phase 2, RoPEG-INF vs. phlebotomy [49]	RoPEG-INF α-2b 100 μg every 2 weeks	Polycythemia vera, low-risk [100]	[Hematocrit <45% × 12 mo & (−) progressive disease]: RoPEG-INF vs. phlebotomy 84% vs. 60%	*RoPEG-INF in low-risk PV.* Disease progression in 8% on phlebotomy and none on RoPEG-INF. AEs-related RX disc. RoPEG-INF 6%. The only grade 3 AE >5% was neutropenia on RoPEG-INF (8%). None AEs type occurred >20% of pt.

AEs, adverse events; *CHR,* complete hematologic response; *disc*., discontinuation; *DLT,* dose-limiting toxicity; *HU,* hydroxyurea; *mo,* months; *MTD,* maximum tolerated dose; *ORR,* overall response rate; *R/R,* relapsed refractory *RX,* therapy; *SOC,* standard of care

[a] Molecular response = at least 20% decline in JAK2 V617F

[b] CMR, complete molecular response (undetectable JAK2 V617F)

12 months on both agents, whereas patients with low symptoms actually felt worse [41]. As highlighted by the authors, these results raise a question of early toxicities of PEG-INF, need for a longer follow-up and poor correlation between achievement of hematologic control (CHR) and clinically meaningful benefit in symptom burden.

Further efforts to improve INF's tolerability have resulted in the development of monopegylated, ultra-long-acting ropeginterferon α-2b (RoPEG-INF) which allowed for even less frequent administration (once every 2–4 weeks) and improved patient's compliance. RoPEG-INF has been evaluated in PV patients in the phase I/II PEGINVERA and Phase III PROUD-PV and CONTINUATION-PV studies (Table 42.1), which ultimately led to its approval in Europe in 2019 for patients with PV without symptomatic splenomegaly [42] and in USA in 2021 for PV patients without any specification [43]. Phase I/II study PEGINVERA-PV established the maximum tolerated dose at 540 μg with every 2 weeks up-titration to prevent early discontinuation, showed similar spectrum of AEs as PEG-INF, and promising cumulative ORR of 90% [44]. Complete and partial MR (JAK2V617F+) were reported in 28.6% and 45.2% of patients, respectively [45]. Subsequent phase III study, PROUD-PV and its extension CONTINUATION-PV, was the first randomized trial comparing RoPEG-INF to HU in a frontline setting in patients with early stage PV with prior cytoreductive therapy up to 3 years [46]. The PROUD-PV study randomized 257 patients (1:1) to RoPEG-INF or HU. After 12 months, patients could opt to enter the extension part of the trial, CONTINUATION-PV (95 patients treated with RoPEG-INF, 76 patients with HU). Results of PROUD-PV and an interim analysis at 36 months of the CONTINUATION-PV were published in 2020 [46]. While RoPEG-INF failed to demonstrate the noninferiority for the coprimary endpoint of CHR and spleen size normalization in PROUD-PV at 12 months (possible due to only few patients with splenomegaly at enrollment), it showed superior results at 36 months in the CONTINUATION PV (CHR 71% vs. 51% for RoPEG-INF vs. HU). Similar kinetics with continuing improvement of response rate to RoPEG-INF over time was noticed on molecular level; comparable MR at 12 months (34% vs. 42%) with significantly superior MR rate with RoPEG-INF at 36 months (68% vs. 33%). Both agents had rarely grade ≥ 3 AEs and similar discontinuation rate at 8%. Favorable, deeper, and more durable responses with RoPEG-INF in CONTINUATION-PV were confirmed during the latest analysis at 60 months [47]. At this time point, 54% and 44% of responders on RoPEG-INF and HU had ongoing CHR. The dynamics of MR showed ongoing decline in JAK2 V617F allele burden on RoPEG-INF but not on HU (9.8% vs. 45% allele burden at 60 months). Patients with >50% pretreatment JAK2V617F allele burden had higher rate of MR with RoPEG-INF at 84.4% (vs. ≤ 50% JAK2 of 61.3%), but presence of nondriver mutations and/or

chromosomal aberrations had no apparent influence on MR rates (64.5% vs. 70.7% in patients without these genetic abnormalities). This analysis confirmed stable safety profile with no new emerging AEs over time and also showed that pretreatment with HU had no impact on RoPEG-INF response or incidence of AEs.

Another interesting report from CONTINUATION-PV trial tried to identify predictive factors of deep responses to RoPEG-INF. The analysis included 66 patients who were treated for 5 years with RoPEG-INF [48]. The study identified "an operational cure" in 41% of patients who achieved CHR, <10% JAK2 allele burden, and had no disease progression or thromboembolic events in the 5-year period. Lower age and lower JAK2V617F allele burden at therapy initiation were identified as predictive baseline factors for achieving a JAK2V617F allele burden <10% at 5 years and long-term disease control.

RoPEG-INF is currently being evaluated in the low-risk PV in a phase II trial in comparison to phlebotomy. Preliminary results from 100 patients showed 84% of RoPEG-INF treated patients met the composite endpoint of maintaining a hematocrit 45% for 12 months in the absence of progressive disease, which was significantly higher than 60% of patients in the comparator arm [49]. The incidence of AEs on RoPEG-INF remained low at 6%. Following this interim analysis, the study stopped patient's accrual as the study stopping rules showing higher efficacy of RoPEG-INF were met. Longer follow-up data are to be reported.

Only few studies evaluated INF in patients with MF (total 141 patients treated) and generated consensus that INF is (1) primarily effective in the earlier disease phases where it could halt the progression of marrow fibrosis [27]; it is (2) rarely effective at the advanced fibrotic stage, in patients with high-risk mutations or those with large organomegalies [29, 50]; and (3) toxicity considerably limits its use in this setting. The overall response rate was at around 50% (range, 9–100%) using different response criteria [51], including at least some control of spleen or symptoms.

As alluded to above, combinational therapy might be required to achieve deeper disease control and prevent disease resistance. The most appealing agents in combination with INF seem those targeting cytokines and further decreasing inflammation, such as inhibitors of JAK-STAT pathway (e.g., ruxolitinib) or MDM2 [52]. Preliminary results from two trials of INF and ruxolitinib showed possible synergistic effect and acceptable tolerability. The Phase II COMBI clinical trial evaluated ruxolitinib and PEG-INF in 32 PV and 18 early phase MF patients, of which 94% were intolerant of or refractory to PEG-INF. ORR was 31% and 44% for PV and MF, respectively. The combination also showed improvement in MPN symptoms and molecular responses, including CMR in few patients [53]. The most frequent AEs included anemia and thrombocytopenia with higher discontinuation rate in MF patients (32% vs. 6% for PV). However, discontinuation due to AEs was low even in patients with MF (9%) which is a favorable clinical observation considering that almost all these patients were previously intolerant or refractory to PEG-INF. Another phase I/II clinical trial, RUXOPEG, evaluated ruxolitinib and PEG-INF combination in patients with treatment-naïve higher-risk MF. This study showed decrease in spleen size and JAK2V617F allele burden in 75% and 46% patients, respectively, with hematologic control attained in almost all patients. Importantly, there were no dose-limiting toxicities and showed good tolerance with ruxolitinib 15 mg twice daily and PEG-INF up to 135 μg per week [54]. Longer follow-up of these studies might shed more light on bone marrow fibrosis changes that tend to occur later over time.

In summary, despite the dispute about INF ability to truly and ultimately eradicate the MPNs, its unique antiproliferative, proapoptotic, and immunomodulatory properties have been broadly demonstrated and place this agent among preferred treatment choices in certain MPN patients. Current consensus guidelines recommend INF for initial or salvage cytoreduction for ET and PV patients, especially of younger age or in pregnancy [55, 56] due to lack of teratogenic potential and possible achievement of treatment-free remission. We are positive that INF story will continue to develop, as recently seen with the official approval of the first-ever-INF (RoPEG-INF) for PV patients, and further research will hopefully define its true "disease-modifying abilities." The future of MPN therapy, aiming to eradicate the disease by earlier interventions and multiagent regimens, might gain INF even in more eminent place within MPN treatment options.

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Conflict of Interest No relevant COI to disclose.

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JAK Inhibitors for the Management of Myeloproliferative Neoplasms

43

Prithviraj Bose and Srdan Verstovsek

Abstract

The classic, Philadelphia chromosome-negative (Ph^-) myeloproliferative neoplasms (MPN) are characterized by universal activation of Janus kinase (JAK)-signal transducer and activator of transcription (STAT) signaling, and the JAK1/2 inhibitor ruxolitinib has shown broad clinical activity across myelofibrosis (MF), polycythemia vera (PV), and essential thrombocythemia (ET). Ruxolitinib is currently approved by regulatory authorities for the treatment of MF and hydroxyurea (HU)-resistant/intolerant PV. Although the cornerstone of management of MF, ruxolitinib has some limitations, which has led to interest in developing other JAK inhibitors, as well as ruxolitinib-based rational combinations. Problems with ruxolitinib include on-target anemia and thrombocytopenia, as well as eventual loss of clinical efficacy. Additionally, while ruxolitinib improves survival of patients with MF, its disease-modifying effects, as evidenced by improvement in bone marrow fibrosis and reduction of the mutant allele burden, appear modest; indeed, this could be a feature of all JAK inhibitors currently in the clinic. Furthermore, there is no evidence that JAK inhibitors prevent or delay disease progression and leukemic transformation. Fedratinib, a JAK2 inhibitor with an efficacy profile similar to that of ruxolitinib, is also approved for the treatment of MF. Currently, fedratinib is primarily used second-line, after ruxolitinib failure, a challenging clinical scenario. Momelotinib, a JAK1/2 and activin receptor type 1 (ACVR1) inhibitor, is being developed in the second-line setting in symptomatic, anemic patients with MF, while development efforts for pacritinib, a relatively nonmyelosuppressive JAK2 inhibitor, are focused on MF patients with severe thrombocytopenia. In HU-resistant/intolerant PV, the benefits of ruxolitinib in terms of hematocrit control, spleen shrinkage, and symptom improvement are durable, and the drug may decrease the incidence of thromboembolic events. Finally, activating mutations in *CSF3R* and molecular rearrangements that activate JAK-STAT, such as *PCM1-JAK2*, render certain rare, atypical MPN, e.g., chronic neutrophilic leukemia (CNL) and myeloid/lymphoid neoplasms with eosinophilia (MLNEo) sensitive to ruxolitinib.

Keywords

JAK inhibitors · Ruxolitinib · Fedratinib · Momelotinib
Pacritinib · Myeloproliferative neoplasms · Myelofibrosis
Polycythemia vera · Essential thrombocythemia
Chronic neutrophilic leukemia

43.1 Introduction

The discovery in 2005 that the JAK2 V617F mutation underlies the vast majority of cases of polycythemia vera (PV) and over half the cases of essential thrombocythemia (ET) and primary myelofibrosis (PMF) [1–4] spurred the development of Janus kinase (JAK) inhibitors, culminating in the approval of ruxolitinib in 2011. First approved for the treatment of MF based on dramatic benefits in terms of spleen shrinkage and symptom improvement observed in the COMFORT trials [5, 6], ruxolitinib was later shown also to improve survival of these patients [7]. Ruxolitinib was known to be effective regardless of the presence or absence of the *JAK2* mutation [8], a finding supported by the later discovery of *CALR* mutations in the majority of *JAK2/MPL*-wild type patients with ET or PMF that also activate JAK-signal transducer and activator of transcription (STAT) signaling [9, 10]. Indeed, integrated genomic profiling has demonstrated universal activation of the JAK-STAT pathway across the spectrum of classic, Ph^- MPN [11, 12]. Approval for the treatment of HU-resistant/intolerant patients with PV followed in 2014 based on the RESPONSE trials [13, 14], 5-year updates of

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which have recently been published/presented, showing durable efficacy and maintained safety [15, 16]. Although clearly active [17], ruxolitinib is not currently approved for ET. Fedratinib, a relatively selective inhibitor of JAK2 over JAK1, was approved by the US Food and Drug Administration (FDA) in 2019 for patients with MF based on data from the JAKARTA trial [18], and has also now been approved by the European Medicines Agency (EMA). While regulatory approval for momelotinib (JAK1/2 inhibitor) and pacritinib (JAK2 inhibitor) had been thwarted by mixed results in the phase 3 SIMPLIFY [19, 20] and PERSIST [21, 22] trials and, in the case of pacritinib, safety concerns, both drugs are now following new paths to registration via the phase 3 MOMENTUM and PACIFICA trials, respectively.

43.2 Ruxolitinib for Myelofibrosis

The JAK1/2 inhibitor ruxolitinib has, over the last decade, become the mainstay of drug therapy for patients with intermediate- and high-risk MF, and consensus guidelines from the US National Comprehensive Cancer Network (NCCN) also endorse its use in symptomatic, low-risk patients [23]. The COMFORT trials enrolled patients with intermediate-2 or high-risk MF according to the International Prognostic Scoring System (IPSS) with baseline platelets counts $\geq 100 \times 10^9$/L. In COMFORT-1, 41.9% of patients randomized to ruxolitinib achieved ≥35% spleen volume reduction (SVR35) at 24 weeks, compared to 0.7% of those randomized to placebo [5]. For ≥50% reduction in total symptom score (TSS50) at 24 weeks, these proportions were 45.9% and 5.3%, respectively. SVR35 was attained by 28% of ruxolitinib-randomized patients in COMFORT-2 at 48 weeks (primary endpoint) and by 32% at 24 weeks (key secondary endpoint), compared with 0% of patients receiving best available therapy (BAT) at both time points [6]. While COMFORT-2 did not use TSS50, ruxolitinib treatment resulted in marked improvements in other patient-reported quality of life (PRQOL) measures. Both trials allowed crossover after 24 weeks, and neither trial was powered to show differences in overall survival (OS). With this caveat, long-term follow-up of both trials demonstrated a survival advantage for ruxolitinib treatment [24, 25] that became even more apparent after correction for the effects of crossover by rank preserving structural failure time (RPSFT) analysis. In an exploratory, pooled analysis of data from 528 patients from both trials, median OS was 5.3 years among patients randomized to ruxolitinib, versus 3.8 years in patients randomized to placebo (2.3 years with the RPSFT correction) [7]. Rates of spleen response improved over time and the median duration of spleen response was >3 years in both trials [24, 25]. The effects of ruxolitinib on bone marrow fibrosis and the allele burden of mutant *JAK2* were relatively modest. Specifically, among ruxolitinib-randomized patients in COMFORT-2, 15.8% had improved bone marrow fibrosis and 32.2% had stable bone marrow fibrosis, while 18.5% had worse bone marrow fibrosis at their last assessment (median treatment duration, 2.2 years for ruxolitinib and < 1 year for BAT) [25]. Approximately 1/3 of evaluable *JAK2* V617F+ patients had >20% reductions in absolute allele burden at weeks 168 and 192. Of 236 evaluable *JAK2* V617F+ patients in COMFORT-1, 20 achieved partial and six achieved complete molecular responses (CMR), with median times to response of 22.2 months and 27.5 months, respectively [26]. Ruxolitinib potently suppresses an array of inflammatory cytokines in MF patients [27], and it is possible that its survival benefit is indirect, via improvements in appetite, weight, activity level [28], and renal function [29].

Although the pivotal trials of ruxolitinib were conducted in patients with IPSS intermediate-2 or high-risk disease, there has been extensive experience gained in intermediate-1 risk patients via open-label and "real-world" studies. In general, efficacy is higher and toxicity is lower in these patients who are earlier in the disease course [30–32]. In COMFORT-1 as well, *JAK2* V617F allele burden reductions were greater in patients with shorter disease duration, suggesting a benefit for earlier treatment [26]. In an Italian multicenter, retrospective study involving 408 patients with MF, intermediate-2/high-risk disease, large splenomegaly, transfusion dependence, and a platelet count $<200 \times 10^9$/L at baseline and a > 2 year-interval from diagnosis to initiation of ruxolitinib all correlated negatively with spleen response [33]. In this study, as well as in clinical trials, spleen responses to ruxolitinib were found to be dose-dependent and to correlate with survival [34, 35]. Starting doses of ruxolitinib are based on the platelet count. Although the ruxolitinib prescribing information suggests a starting dose of 5 mg twice daily in patients with baseline platelets between 50 and 99×10^9/L, data support using the more effective dose of 10 mg twice daily when the baseline platelet count is in this range [36]. From a molecular perspective, a mutant *JAK2* allele burden ≥50% was found in one study to predict a higher likelihood of response to ruxolitinib, [37] while in another study, the presence of ≥3 nondriver mutations was associated with lower odds of spleen response, shorter time to treatment discontinuation, and worse OS [38]. The presence of "high-molecular risk" mutations (*ASXL1, EZH2, SRSF2, IDH1/2*) did not, however, affect response to ruxolitinib and its survival benefit in COMFORT-2 [39].

Ruxolitinib is generally very well-tolerated, with headaches, bruising, and dizziness being the most commonly reported nonhematologic toxicities. Weight gain can be a problem in some patients. Ruxolitinib can increase lipid levels, and these should be monitored routinely. On-target ane-

mia and thrombocytopenia from JAK2 inhibition are the most common adverse effects overall and anemia is, in fact, the most frequent reason for discontinuation of ruxolitinib in clinical practice [40]. Anemia from ruxolitinib is most severe in the first 12–24 weeks of treatment, after which hemoglobin levels plateau at a new, lower baseline. Importantly, anemia due to ruxolitinib is not prognostically adverse and, in fact, ruxolitinib overcomes the negative prognostic impact of MF-associated anemia [41, 42]. Although it is generally important to optimize the dose of ruxolitinib early so as to achieve the best possible spleen response, a strategy of using 10 mg twice daily for the first 12 weeks in anemic patients (baseline hemoglobin <10 g/dL) before escalating has been shown to be safe and effective [43, 44]. Ruxolitinib is immunosuppressive, and while not common, a host of opportunistic infections have been reported [45]. It is our practice to routinely offer (inactivated) shingles vaccination to patients on ruxolitinib [46]. While ruxolitinib does not increase lymphoma risk [47–49], it does elevate the risk of nonmelanoma skin cancer (NMSC), particularly in patients who have a history of the same [49, 50]. Prognosis after ruxolitinib discontinuation tends to be poor, with median OS in the range of 13–14 months across multiple studies [40, 51, 52]. In one study, clonal evolution and declining platelet counts while on ruxolitinib were associated with worse outcomes after discontinuation [51]. Abrupt discontinuation of ruxolitinib should be avoided in order to prevent a "cytokine storm," particularly in patients with large spleens (≥10 cm) [53]. In patients proceeding to allogeneic hematopoietic cell transplantation (allo-HCT), the dose of ruxolitinib should be tapered over 5–7 days and the drug stopped the day before conditioning begins [54].

Given the central role of ruxolitinib in the pharmacologic management of MF, there is much interest in developing ruxolitinib-based rational combinations, although none has been approved yet. Some combinations aim to ameliorate/counteract disease- and/or ruxolitinib-induced cytopenias. In an open-label, phase 2 study of the activin receptor ligand trap luspatercept in 76 patients with MF, the highest response rates were seen in the cohort of patients who were on a stable dose of ruxolitinib for ≥16 weeks but red blood cell transfusion-dependent [55]. Luspatercept will be evaluated in a pivotal, phase 3 trial (INDEPENDENCE, NCT04717414) in this patient population. Low-dose thalidomide (50 mg/d) in combination with a stable dose of ruxolitinib yielded promising platelet responses in a small trial [56]. Other combination strategies have been to pair ruxolitinib with agents from different classes in efforts to increase response rates and demonstrate greater disease modification than observed with ruxolitinib alone. A popular approach in clinical trials has been to add a novel agent shown to be synergistic with ruxolitinib in the laboratory in patients exhibiting a (variously defined) "suboptimal response" to ruxolitinib monotherapy. Examples include the BH3-mimetic navitoclax [57], the phosphatidylinositol-3-kinase (delta isoform) inhibitor parsaclisib [58], the murine double minute 2 (MDM2) inhibitor KRT-232 (NCT04485260), and the bromodomain and extra-terminal (BET) protein inhibitor pelabresib [59]. The combination of ruxolitinib and pelabresib looks particularly promising, with potential for benefit in all three major aspects of the disease: splenomegaly, symptoms, and anemia. This combination has also been evaluated in the upfront setting (JAK inhibitor-naïve), and led to encouraging rates of SVR35 and TSS50 at 24 weeks of 67% and 57%, respectively [60], suggesting synergism, as demonstrated preclinically between JAK and BET inhibitors, both in vitro and in vivo [61]. This combination is now being compared to ruxolitinib plus placebo in a randomized, phase 3 trial (MANIFEST-2, NCT04603495).

43.3 Ruxolitinib for Polycythemia Vera

As noted above, ruxolitinib is approved for the second-line treatment of PV after HU failure (resistance or intolerance) [62]. Although not very common, HU resistance in PV has been associated with inferior OS and a higher incidence of leukemic transformation [63], as has development of cytopenia at lowest dose of HU needed to maintain response [64]. Approval was based on the phase 3 RESPONSE trial, which randomized 222 phlebotomy-dependent patients with splenomegaly and HU resistance or intolerance to receive ruxolitinib or standard therapy [13]. The primary endpoint, a composite of hematocrit control and SVR35, was achieved (at week 32) by 21% of patients in the ruxolitinib group and 1% in the standard therapy group. Standard therapy ended up being HU in a majority of the patients, reflecting the paucity of effective treatments for this patient population. Hematocrit control was achieved by 60% versus 20%, SVR35 by 38% versus 1%, complete hematologic response (CHR) by 24% versus 9%, and TSS50 by 49% versus 5%, of patients in the ruxolitinib and standard therapy groups, respectively, at week 32 (all differences statistically significant). Additionally, ruxolitinib provided superior control of leukocytosis and thrombocytosis compared to standard therapy [65]. Crossover was permitted after week 32 and no patient remained on standard therapy after week 80. Subsequent analyses of data from the RESPONSE trial showed that the mean change from baseline through week 208 in *JAK2* V617F allele burden ranged from −12.2% to −40% in ruxolitinib-randomized patients [66], and that markers of iron deficiency improved or normalized in ruxolitinib-treated patients [67]. Final, 5-year results from the RESPONSE trial were recently published [15]. Among 25 primary responders in the ruxolitinib

group, six had experienced disease progression at the time of the 5-year analysis. At 5 years, the probability of maintaining the primary composite endpoint (hematocrit control plus SVR35) was 74%, and that of maintaining CHR was 55%; the probability of maintaining overall clinicohematologic response was 67%. No new safety signals emerged. The RESPONSE-2 trial (n = 149) was designed very similarly, except that it enrolled only patients without palpable splenomegaly, and the primary endpoint (hematocrit control) was assessed at 28 weeks [14]. Results were similar to those of RESPONSE, with 62% of patients in the ruxolitinib group achieving the primary endpoint, compared to 19% in the BAT group. CHR and TSS50 were achieved by 23% and 5%, and 45% and 23%, of patients in the ruxolitinib and BAT groups, respectively, at week 28. Recently presented results after 5 years of follow-up of this trial showed durable hematocrit control, CHR, and symptom improvement in ruxolitinib-treated patients, as well as fewer thromboembolic events than in the BAT arm [16]. An ad hoc analysis of data from both RESPONSE and RESPONSE-2 demonstrated that ruxolitinib was superior to standard therapy/BAT in patients previously treated with interferon and also when the comparison was restricted to patients receiving interferon as standard therapy/BAT [68]. Patients on the control arms who received interferon and crossed over to receive ruxolitinib had improved hematologic and spleen responses. Of note, the incidences of herpes zoster reactivation and NMSC appear higher in PV than MF patients receiving ruxolitinib, the latter likely due to prior HU exposure.

The primary goal of therapy in PV is the prevention of thrombotic events [69]. To this end, while hard evidence is lacking that ruxolitinib decreases the incidence of thromboembolic events [70], there are several reasons to believe that it does. The RESPONSE trials were not powered to show reductions in the risk of thromboembolic events as efficacy endpoints. However, the safety analysis of RESPONSE showed that the rate of thromboembolic events was 1.8 per 100 patient-years of exposure in the ruxolitinib arm and 8.2 per 100 patient-years of exposure in the standard therapy arm [65]. Hematocrit control to <45% is the best established treatment goal in PV as far as prevention of cardiovascular events is concerned [71]. In addition, some studies have shown a correlation between leukocytosis and the occurrence of thromboembolic events [72, 73]. Thus, sustained control of the hematocrit and of leukocytosis, as achieved by ruxolitinib, should lead to a reduction in the risk of thromboembolic events. Finally, JAK inhibition impairs "neutrophil extracellular trap (NET)" formation, believed to play an important part in the pathophysiology of thrombosis in the MPN [74]. There is no evidence that ruxolitinib treatment affects the risks of progression to post-PV MF or transformation to acute myeloid leukemia (AML).

43.4 Ruxolitinib for Essential Thrombocythemia

Ruxolitinib is not currently approved for the treatment of ET, and while there is considerable off-label use, its continued development for this indication is uncertain. A randomized, phase 2 trial intended to serve as the basis for registration in the US, RESET-272 (NCT03123588), comparing ruxolitinib to anagrelide in HU-resistant/intolerant patients with ET [75] was terminated owing to poor accrual. The results of a French phase 2/3 trial in the second-line in high-risk patients (NCT02962388) and the phase 2 RUXO-BEAT trial in treatment-naïve and previously treated, high-risk patients in Germany (NCT02577926) are awaited. In the investigator-initiated, randomized, phase 2 MAJIC-ET trial conducted in the UK in which 110 patients with HU-resistant/intolerant ET were enrolled, there was no difference in CHR rates at 1 year between the ruxolitinib and BAT groups [76]. Furthermore, the rates of thrombosis, bleeding, and disease progression/transformation were not significantly different, either, at 2 years. There were very few molecular responses. However, a symptom benefit for ruxolitinib over BAT was observed. Long-term follow-up of an open-label, single-arm, phase 2 trial in 39 patients with HU-resistant/intolerant ET showed rapid and sustained declines in platelet and white blood cell counts, as well as improvement in symptoms, particularly at the 25 mg twice daily dose [17]. Most patients (56.4%) remained on therapy for ≥312 weeks, and the median change in *JAK2* V617F allele burden was −60% at week 312 (n = 12).

43.5 Ruxolitinib for Rare, Atypical MPN

Gain-of-function "membrane proximal" mutations in *CSF3R*, the gene encoding the granulocyte colony-stimulating factor 3 receptor, activate the JAK-STAT pathway and are sensitive to ruxolitinib [77]. Activating *CSF3R* mutations, e.g., T618I, T640N, T615A, occur in >80% of cases of chronic neutrophilic leukemia (CNL), a rare MPN [78]. These mutations are also found in a small percentage of patients with atypical chronic myeloid leukemia (aCML), a myelodysplastic/myeloproliferative neoplasm (MDS/MPN) [79]. Ruxolitinib was studied in a phase 2 trial in these two disease entities (n = 44, 21 CNL, and 23 aCML) [80]. Oncogenic CSF3R mutations were detected in 22 patients (50%), 16 of 21 with CNL and six of 23 with aCML. The overall response rate (ORR) was 35%. There were 11 partial responses (PR), nine in CNL patients and two in aCML patients. All four complete responses (CR) occurred in CNL patients. Ruxolitinib has also been reported to be effective in patients with the rare MPN resulting from *PCM1-JAK2* or *BCR-JAK2* gene fusions, although these responses are often transient [81].

43.6 Fedratinib

The JAK2 inhibitor fedratinib was approved by the FDA in 2019 for the treatment of patients with intermediate-2/high-risk MF and more recently also by the EMA for patients with MF with disease-related splenomegaly or symptoms who are JAK inhibitor-naïve or have been treated with ruxolitinib. In the placebo-controlled JAKARTA trial, two doses of fedratinib, 400 mg/d and 500 mg/d, were studied in 289 JAK inhibitor-naïve patients with intermediate-2 or high-risk MF and baseline platelets $\geq 50 \times 10^9$/L [18]. The primary endpoint, SVR35 at 24 weeks that had to be confirmed 4 weeks later, was achieved by 36%, 40%, and 1% of patients in the fedratinib 400 mg/d, fedratinib 500 mg/d, and placebo groups, respectively. TSS50 at 24 weeks was achieved by 36%, 34%, and 7%, respectively. JAKARTA-2 was a single-arm, open-label study of fedratinib, 400 mg daily, in patients with MF with platelets $\geq 50 \times 10^9$/L who had previously been treated with ruxolitinib [82]. A minimum washout from prior ruxolitinib therapy of ≥14 days was required, and the minimum duration of prior ruxolitinib was also 14 days, although the median duration was 10.7 months. Ninety seven patients were enrolled and by intention-to-treat (ITT) analysis, the rate of SVR35 was 31% and that of TSS50 was 27%. Determination of ruxolitinib failure for eligibility was left up to the treating physician. Because of this potential source of heterogeneity in the patient population enrolled, a reanalysis of the JAKARTA-2 data using "stringent criteria" (Table 43.1) for ruxolitinib failure was carried out [83]. Seventy nine of the 97 patients (81%) met these criteria; 65 were considered to have disease that had relapsed after or was refractory to ruxolitinib, while 14 were deemed intolerant to ruxolitinib. The rates of SVR35 and TSS50 at 24 weeks were virtually identical, at 30% and 27%, in this reanalysis. The ongoing phase 3 FREEDOM studies (NCT03755518, NCT03952039) are employing these same stringent criteria and will hopefully better define the role of fedratinib in patients with MF who have failed therapy with ruxolitinib.

A full clinical hold was placed on clinical trials of fedratinib by the FDA on November 15, 2013 owing to concerns regarding Wernicke's encephalopathy (WE), leading to all patients coming off and the development program for the drug being halted until it was resurrected much more recently. The eight potential cases of WE that occurred in 670 patients receiving fedratinib across the clinical development program were subsequently analyzed retrospectively [84]. Six of the eight patients had MF, one had PV, and one had metastatic head and neck carcinoma. Only one case was confirmed to be WE, and two others were felt to likely represent WE. The diagnosis was inconclusive in two cases and was either inconclusive or not supportive of WE in the three others. All the potential WE patients had previously experienced protracted nausea and vomiting, likely predisposing them to malnutrition and thiamine deficiency. Whether fedratinib inhibits neuronal thiamine uptake is controversial [85, 86]. However, the two patients with likely WE recovered from their neurodeficits despite continuing fedratinib. The US label for fedratinib contains a black box warning regarding encephalopathy, including WE. Thiamine levels must be checked before initiating fedratinib and periodically during therapy, and supplemented if deficient prior to fedratinib initiation. Besides anemia and thrombocytopenia from on-target JAK2 inhibition, nausea, vomiting, and diarrhea are major toxicity concerns with fedratinib, and may stem from its inhibition of *fms*-like tyrosine kinase 3 (FLT3). Antiemetic prophylaxis is recommended. Unlike ruxolitinib, the starting dose of fedratinib is 400 mg daily regardless of the platelet count, as long as the same is $\geq 50 \times 10^9$/L. An analysis of patients from JAKARTA ($n = 14$) and JAKARTA-2 ($n = 33$) with baseline platelets in the 50–99 $\times 10^9$/L range found that their rates of SVR35 and TSS50, though numerically lower, were not statistically significantly different from those of patients with $\geq 100 \times 10^9$/L platelets at baseline [87].

Table 43.1 Stringent criteria for ruxolitinib (RUX) failure used in the reanalysis of JAKARTA-2 and in the PAC203 and FREEDOM trials

Relapsed	RUX ≥ 3 mos with regrowth (defined as <10% SVR or < 30% decrease in spleen size from baseline following an initial response)
Refractory	RUX ≥ 3 mos with <10% SVR or < 30% decrease in spleen size from baseline
Intolerant	RUX ≥ 28 days complicated by development of RBC transfusion requirement (≥ 2 units/mos for 2 mos); or grade ≥ 3 thrombocytopenia, anemia, hematoma/hemorrhage while on RUX

43.7 Momelotinib

Momelotinib is a JAK1/2 inhibitor that also improves anemia in patients with MF, possibly via suppressing hepatic production of hepcidin by inhibiting the type 1 activin receptor (ACVR1, also known as ALK2) [88]. Momelotinib was studied in two phase 3, randomized controlled trials in patients with MF. SIMPLIFY-1 was a head-to-head trial that compared momelotinib, 200 mg once daily, to ruxolitinib (20 mg twice daily or dosed per label) in 432 patients with intermediate-2/high-risk MF or symptomatic, intermediate-1 risk MF, and baseline platelets $\geq 50 \times 10^9$/L [19]. SIMPLIFY-1 was designed as a noninferiority study; noninferiority was met for SVR35 at 24 weeks (26.5% of patients in the momelotinib group and 29% of patients in the ruxolitinib group) but not for TSS50 (28.4% of patients in the momelotinib group and 42.2% in the ruxolitinib group). Red blood cell transfusion rates and the rates of transfusion independence and dependence were all improved in the momelotinib group, but statistical significance could not be

claimed because of the hierarchical design. In SIMPLIFY-2, 156 ruxolitinib-exposed (≥28 days) patients with MF who had either required red blood cell transfusions on ruxolitinib or ruxolitinib dose reduction to <20 mg twice daily due to grade ≥ 3 anemia, thrombocytopenia or bleeding were randomized 2:1 to receive momelotinib, 200 mg daily, or BAT, which ended up being ruxolitinib in 89% of the patients [20]. The primary endpoint of this study, the rate of SVR35 at 24 weeks, was not met (7% of patients in the momelotinib group and 6% of patients in the BAT group). Because of this, although 26% of the momelotinib patients achieved TSS50 at week 24 (vs. 6% of patients in the BAT group) and like in SIMPLIFY-1, the anemia endpoints favored momelotinib, formal statistical testing could not be performed for these endpoints. Peripheral neuropathy occurred in 10% and 11% of patients receiving momelotinib in SIMPLIFY-1 and -2, respectively. In a phase 2, translational biology study in 41 red blood cell transfusion-dependent patients with MF, 17 (41%) achieved transfusion independence for ≥12 weeks at any time on study [89]. Of those who did not, 78% achieved a ≥ 50% reduction in their transfusion requirements for ≥8 weeks. There was an acute and sustained decline in blood hepcidin levels, associated with increased iron availability for erythropoiesis and markers of the same. Momelotinib is now being studied in a pivotal, phase 3 trial (MOMENTUM, NCT04173494) versus danazol (2:1 randomization) in 180 symptomatic (TSS ≥10) and anemic (hemoglobin <10 g/dL) patients with intermediate- or high-risk MF and baseline platelets ≥25 × 10^9/L who have previously received a JAK inhibitor, with the rate of TSS50 at 24 weeks being the primary endpoint [90]. Momelotinib was also studied in patients with PV and ET, but the study was terminated due to limited efficacy [91].

43.8 Pacritinib

Pacritinib is an inhibitor of JAK2, FLT3, IRAK1, and CSF1R that has the advantage of being relatively nonmyelosuppressive [92]. Pacritinib was also studied in two phase 3 randomized controlled trials in patients with MF and like fedratinib, had a full clinical hold placed on its development by the FDA due to safety concerns that was subsequently lifted. Pacritinib, 400 mg daily, was compared 2:1 against BAT (not including JAK inhibitors) in 327 JAK inhibitor-naïve patients with intermediate-2 or high MF in the PERSIST-1 trial [21]. There was no lower limit on the hemoglobin level or platelet count for eligibility. The primary endpoint of this trial, the rate of SVR35 at 24 weeks, was met (19% of patients in the pacritinib group vs. 5% in the BAT group). Gastrointestinal adverse effects, particularly diarrhea, were common in the pacritinib group, especially early on in therapy. In the ITT population, the rate of TSS50 was not statistically significantly superior for pacritinib versus BAT at week 24, although it was at week 48. In the evaluable population, however, the rate of TSS50 at 24 weeks was significantly higher in the pacritinib arm than in the BAT arm. Importantly, pacritinib's superiority over BAT was maintained in patients with baseline platelets below 100 × 10^9/L and 50 × 10^9/L, known poor-prognosis subgroups [93, 94] that were excluded from the COMFORT trials. PERSIST-2 compared two doses of pacritinib, 200 mg twice daily and 400 mg once daily, to BAT (which could include JAK inhibitors and was ruxolitinib in 45% of patients) in 311 patients with MF and baseline thrombocytopenia (platelets ≤100 × 10^9/L) [22]. Furthermore, prior ruxolitinib was permitted; 48% of patients had received ruxolitinib in the past. This trial was impacted by the placement of a full clinical hold by the FDA on the pacritinib clinical development program, leading to all patients having to come off, and the ITT efficacy population eventually included 75 patients randomized to pacritinib 400 mg/d, 74 to pacritinib 200 mg twice daily, and 72 to BAT. SVR35 and TSS50 at week 24 were coprimary endpoints in this trial. When considering both dosing arms together, pacritinib was statistically significantly superior to BAT for SVR35 at week 24 (18% vs. 3%) but not for TSS50 (25% vs. 14%). However, pacritinib 200 mg twice daily was statistically significantly superior to BAT for both coprimary endpoints (SVR35 at week 24, 22% vs. 3% and TSS50 at week 24, 32% vs. 14%).

Given the safety concerns that led to the full clinical hold (increased mortality from cardiovascular events and bleeding), a dose-finding phase 2 randomized clinical trial, PAC203, was subsequently conducted. In PAC203, 161 patients with MF and resistance/intolerance to ruxolitinib (as defined in Table 43.1) were randomly assigned 1:1:1 to receive 100 mg daily, 100 mg twice daily or 200 mg twice daily of pacritinib [95]. Forty four percent of patients had severe thrombocytopenia (platelets <50 × 10^9/L) at baseline. With the implementation of a range of risk mitigation strategies, no excess grade ≥ 3 cardiac or hemorrhagic events were observed. Rates of 24-week SVR35 differed by dose, being highest (9.3%) at the 200 mg twice daily dose. Rates of TSS50 through week 24 were similar across doses (7.4% at the 200 mg twice daily dose). Of note, the rate of SVR35 through week 24 at the 200 mg twice daily dose was 17% among 24 patients with baseline platelets <50 × 10^9/L. Pacritinib at this dose is now being compared (2:1 randomization) to physician's choice of low-dose ruxolitinib (5 mg once or twice daily), steroids, androgens or HU in a pivotal, phase 3 trial (PACIFICA, NCT03165734) in 348 patients with intermediate- or high-risk MF and baseline severe thrombocytopenia (platelets <50 × 10^9/L) who are JAK inhibitor-naïve or have had limited prior JAK inhibitor exposure. Severe thrombocytopenia portends a particularly poor prognosis in patients with MF [93], and data are lacking

to inform the use of both ruxolitinib and fedratinib in this patient population. It has been speculated that the constellation of cytopenias, lower mutant *JAK2* allele burdens, and smaller spleen sizes may characterize a biologically distinct subset of PMF with a "myelodepletive" phenotype and that pacritinib, perhaps because of its inhibition of IRAK1 and selectivity for JAK2 over JAK1, may be particularly beneficial in this subgroup [96].

43.9 Conclusions

As discussed in this chapter, JAK inhibitors have broad applicability in MPN, given the ubiquitous activation of JAK-STAT signaling in these diseases, particularly in the classic MPN. A number of JAK inhibitors that had entered the clinic had to be discontinued due to toxicity, while further development of some others, e.g., the JAK1 inhibitor itacitinib [97] and the JAK2 inhibitor NS-018 [98], in MPN is uncertain. In MF, there is now considerable interest in exploring drugs with other mechanisms of action, e.g., telomerase inhibitors [99], antifibrotic agents [100], MDM2 [101], lysine demethylase-1 inhibitors [102], CD123-directed therapies [103], both as single agents after JAK inhibitor failure and in combination with JAK inhibitors earlier in therapy, as discussed previously. However, it is likely that JAK inhibitors will remain the cornerstone of MF therapy far into the future. In PV, the therapeutic landscape could be altered somewhat with the advent of ropeginterferon alfa-2b [104], while other agents, e.g., the hepcidin-mimetic PTG-300 [105] and the histone deacetylase inhibitor givinostat [106], remain in clinical development. However, these agents are likely to find a role primarily in the frontline setting or as adjunctive therapy, with ruxolitinib's robust efficacy after HU failure likely continuing to ensure its place in the second-line setting.

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44 Allogeneic Hematopoietic Stem Cell Transplantation for Myelofibrosis: When and How?

Nicolaus Kröger

Abstract

Primary or post-ET/PV myelofibrosis is a heterogenous disease and the clinical course as well as life expectancies vary substantially. The median survival of myelofibrosis patients is around 6 years but about 20% will survive 20 years and longer and also 10–20% will survive less than 2 years after diagnosis. Allogeneic stem cell transplantation is considered to be the only curative treatment for myelofibrosis patients but due to its inherent therapy-related morbidity and mortality, a proper timing and selection are needed for optimal balance between cure and therapy-related complications. In the current chapter, we will focus on optimal timing ("when") and performing ("how") of allogeneic stem cell transplantation in patients with myelofibrosis.

Keywords

Allogeneic stem cell transplantation · Myelofibrosis Reduced intensity conditioning · JAK inhibitor · Minimal residual disease monitoring · Molecular mutation

44.1 When?

44.1.1 Disease-Specific Risk Score Models

To determine median survival in patients with primary or post-ET/PV myelofibrosis, different risk models have been developed and validated in recent years (see Chap. 39). At time of diagnosis of primary myelofibrosis, the International Prognostic Scoring System (IPSS) included age > 65 years, constitutional symptoms, hemoglobin level less than 10 g/dL, leukocyte count >25 × 10^9/l, and circulating blasts ≥1% [1]. The Dynamic IPSS which includes the same risk factors with a higher rating of anemia can be used at any time point in the course of the disease [2]. This scoring permits risk stratification into four groups: low-risk: median survival 135 months, intermediate-1 risk: median survival 95 months, intermediate-2 risk: median survival 48 months, and high-risk with median survival of 27 months. Additional risk factors such as transfusion dependency, platelet count <100 × 10^9/l, and unfavorable cytogenetics are included in the DIPSS plus scoring system [3]. More recently molecular genetics were included in the mutation-enhanced IPSS (MIPSS70) for potential transplant candidate patients with PMF ≤ 70 years of age [4]. In this risk score beside leukocyte ≥25 × 10^9/l, platelet count <100 × 10^9/l, constitutional symptoms, peripheral blasts ≥2%, bone marrow fibrosis grade ≥ 2, also molecular genetics were included such as non-CALR type-1 mutation and high-risk mutations such as EZH2, ASXL1, IDH1/2, and SRSF2. This scoring system enabled three risk categories with different 5-year survival rates: low-risk 95%, intermediate 70%, and high-risk 29%, respectively. A MIPSS70-plus scoring system included cytogenetics and a MIPSS70-plus version 2.0 included U2AF1 as molecular genetic risk factor and uses sex- and severity-adjusted hemoglobin thresholds [5]. The stated scoring systems were developed and validated only for primary myelofibrosis and data suggested that these systems were less useful in post-ET or post-PV myelofibrosis [6]. Thus, for post-ET/PV myelofibrosis, a different prognostic model (MYSEC-PM) was developed which includes hemoglobin ≤11 g/dL, CALR unmutated phenotype, circulating blasts ≥3%, platelet count ≤150 × 10^9/l, constitutional symptoms, and age (0.15 points per year) which allows to categorize post-ET/PV myelofibrosis patients into four prognostic risk categories [7].

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44.1.2 Comparison Between Hematopoietic Stem Cell Transplant and Conventional Therapy According to Disease-Risk Models

In order to define optimal timing for allogeneic stem cell transplantation according to the proposed disease-risk models and lack of prospective randomized studies, larger retrospective comparisons between transplanted and nontransplanted patients with PMF or post-ET/PV MF were done using statistical methods such as left truncation or COX proportional hazard models including time-dependent effects [8, 9].

A European retrospective analysis included 438 patients with PMF and age less than 65 years who received either conventional therapy prior to JAK inhibitor approval (n = 248) or allogeneic hematopoietic stem cell transplantation (n = 190). According to DIPPS score, intermediate-2 and high-risk patients benefit significantly from an early transplantation while low-risk patients benefit from nontransplant approaches, while for intermediate-1 patients, no benefit could be seen from both treatment approaches [8].

The Center for International Blood and Marrow Transplant Research (CIBMTR) compared 1928 MF patients who received either conventional (n = 1377) or allogeneic hematopoietic stem cell transplantation (n = 551) according to DIPSS. In the first year, overall survival was in all risk groups significantly worse in the transplant cohort due to higher therapy-related mortality. However, after 1-year, OS was significantly better for patients with intermediate-1, -2, and high-risk who received allogeneic stem cell transplantation. The OS benefit increased over time and became apparent [9]. Thus the European Society for Blood and Marrow Transplantation (EBMT) and the European LeukemiaNet (ELN) recommend that myelofibrosis patients age less than 70 years and risk profile intermediate-2 or high-risk according to IPSS, DIPSS, or DIPSS plus should be considered as candidates for allogeneic stem cell transplantation while intermediate-1 risk patients should be considered if other risk factors such as refractory transfusion-dependent anemia or peripheral blasts ≥2% or adverse cytogenetics according to DIPSS plus are present [10] while CIBMTR also recommends allogeneic stem cell transplantation for intermediate-2 or high-risk patients and considers allogeneic stem cell transplantation for patients with intermediate-1 risk [9]. A clear indication for allogeneic stem cell transplantation is given for into acute leukemia transformed myelofibrosis [11].

44.1.3 Transplant-Specific Risk Factors and Transplant-Specific Risk Score

Several groups tried to validate disease-specific risk models such as IPSS [12], DIPSS [13–15], DIPSS plus [16], and MYSEC [17], MIPSS70 [18], or MIPSS70+ version 2.0 [19] in the setting of allogeneic stem cell transplantation with contradictory results because beside disease-specific risk factors also patient- or transplant-specific risk factors are influencing outcome after allogeneic stem cell transplantation. Major risk factors in large transplant studies on outcome in myelofibrosis were performance status, advanced disease, age, HLA-compatibility adverse molecular genetics, low platelet count, spleen size, and high transfusion dependency with iron overload [20–29].

Despite the fact that age has been becoming a major risk factor in most of the studies, older patients should not be excluded from potentially curative allogeneic stem cell transplantation option. Encouraging series of older myelofibrosis patients (>65 y) have been reported by several groups [30, 31]. In contrast to chronological age, performance status and comorbidities should be more taking into account by selection of older myelofibrosis patients. Considering the different risk factors of transplantation and to counsel patients with myelofibrosis properly regarding the option of potentially curative allogeneic stem cell transplantation, a transplant-specific risk factor was developed to predict outcome after allogeneic stem cell transplantation for patients with primary or post-ET/PV myelofibrosis [18].

In this study, clinical, molecular, and transplant-specific factors of 361 patients with PMF or post-ET/PV MF who received allogeneic stem cell transplantation were analyzed. In a multivariate analysis, age > 57 years, Karnofsky performance status <90%, platelet count <150 × 10^9/l, leukocytes ≥25 × 10^9/l, HLA-mismatched unrelated donor, ASXL1 mutation, and nondriver mutation genotype in CALR/MPL were independent predictors for worse outcome. These risk factors permit differentiation into four risk groups with different 5-year survival: low-risk 83%, intermediate-risk 64%, high-risk 37%, and very high-risk 22% (Fig. 44.1a, b).

The score was also predictive for nonrelapse mortality and in comparison to current disease-specific scores such as DIPSS, MIPSS70, or MYSEC, this Myelofibrosis Transplant Scoring System (MTSS) predicts outcome after transplantation best with the highest concordance index (0.723). This MTSS system allows a better selection of intermediate-1 patients for allogeneic stem cell transplantation (see Fig. 44.2).

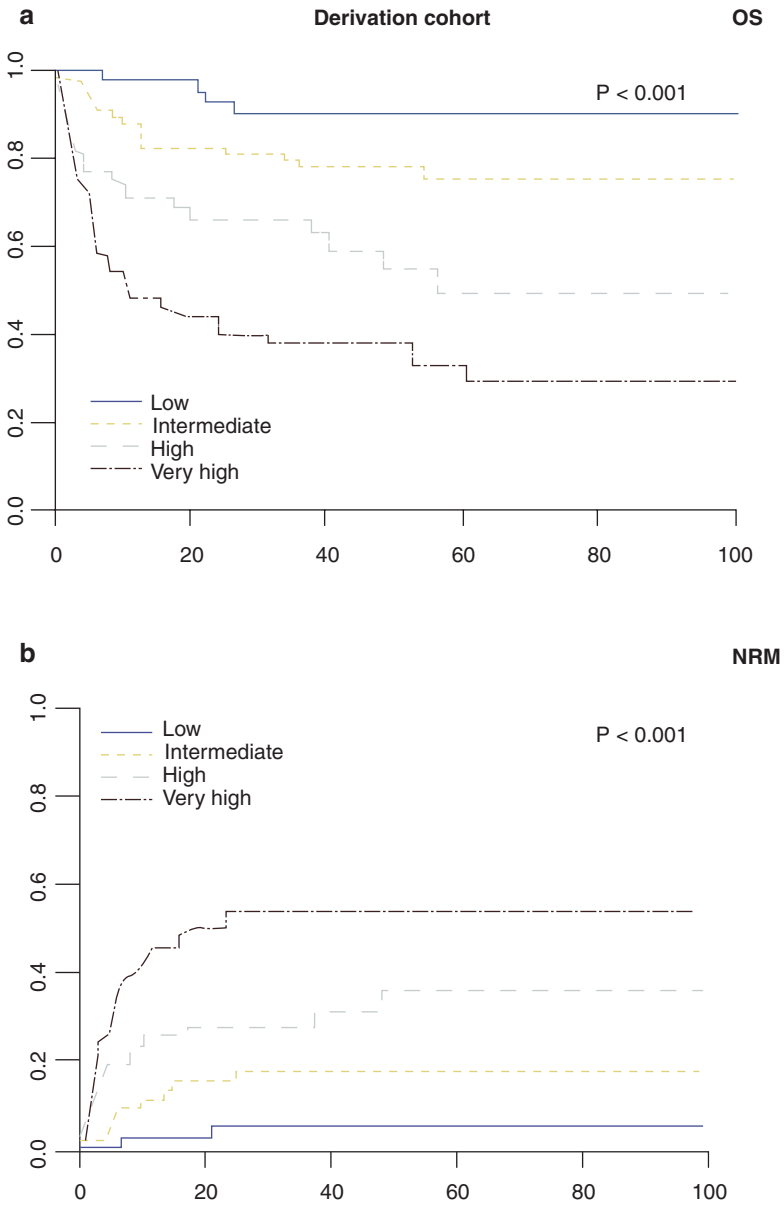

Fig. 44.1 (**a**/**b**): Overall survival (**a**) and nonrelapse mortality (**b**) of low-, intermediate-, high-, and very high-risk according myelofibrosis transplant scoring system (MTSS)

Summarizing regarding "when" primary or post-ET/PV myelofibrosis patients up to the age of 70 years with median survival of less than 5 years (DIPSS intermediate-2 or high-risk, MYSEC intermediate- or high-risk) are candidates for allogeneic stem cell transplantation. The indication for intermediate-1 patients should be considered individually and take other risk factors into account. The Myelofibrosis Transplant Scoring System is a helpful tool in decision making regarding allogeneic stem cell transplantation.

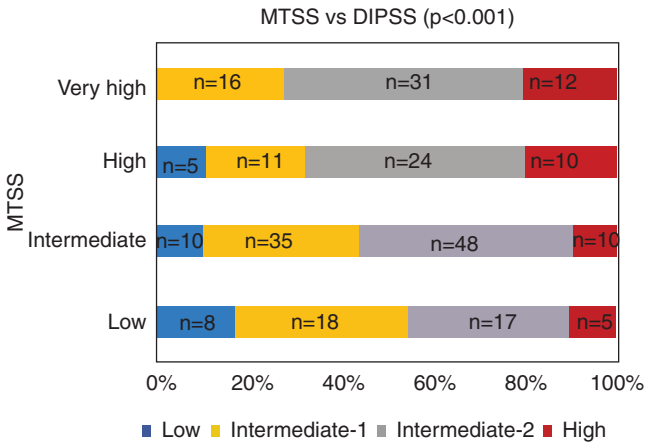

Fig. 44.2 Categorization of patients with different risk score according DIPSS into risk score according myelofibrosis transplant scoring system (MTSS)

44.2 How?

44.2.1 Donor Selection and Stem Cell Source

Results from prospective studies in myelofibrosis in Europe and US demonstrated a worse outcome of unrelated donors in comparison to HLA-identical siblings because of a higher risk of nonrelapse mortality [25, 27]. Outcome of mismatch unrelated was worse in comparison to HLA-matched donors in several studies [18, 27, 32, 33]. Smaller sets from EBMT for mismatch-related stem cell transplantation resulted in primary graft failure rate of 9% and a 2-year NRM of 38%.

A high graft failure rate and a 2-year event-free survival of only 30% have been reported in a small series of myelofibrosis patients who received cord blood as stem cell source [34]. In a Japanese study, cord blood transplantation was associated with the highest NRM (48%) [35]. Available data strongly support the use of HLA-matched donors in myelofibrosis patients undergoing allogeneic stem cell transplantation. Even if no benefit in survival could be shown for PBSC in comparison to bone marrow [14, 26, 36] because of the more ensured and faster engraftment PBSC is the preferred stem cell source in myelofibrosis [36].

44.2.2 Intensity of Conditioning Regimen

No prospective study exists comparing either reduced intensity (RIC) to myeloablative conditioning (MAC) regimen or different RIC regimens.

Smaller retrospective studies did not find significant difference in survival between RIC and MAC [26, 37–39]. In addition, a large registry study from EBMT including more than 2000 patients did not find significant differences regarding NRM, relapse, or OS between MAC and RIC [32]. Only in a long-term outcome study for 2-year survivors, a higher relapse incidence and lower disease-free survival at 10 years were observed for RIC patients [40]. Within the reduced conditioning regimen, the most commonly used regimens are busulfan/fludarabine and melphalan/fludarabine. In a retrospective comparison, a higher NRM but lower risk of relapse was seen for melphalan/fludarabine but overall survival was identical [41]. Other RIC regimens including carmustine or thiotepa have not shown superiority to busulfan-based regimen [42, 43].

In the EBMT registry, increasing use of reduced intensity regimen is observed over time and busulfan-based RIC accounts for 2/3 of the RIC in the recent time period (2015–2018) [44]. Excellent results after RIC for younger patients (<5 years) with 82% 5-year OS have been reported as well as a rapid reduction of bone marrow fibrosis and high incidence of molecular remission after RIC [33, 45, 46].

44.2.3 Splenectomy and Spleen Irradiation

Splenomegaly is a hallmark of myelofibrosis and enlarged spleen may result in poor outcome after stem cell transplantation or can cause transplant-related problems like delayed engraftment or poor graft function [28, 47, 48]. The role of splenectomy prior to transplantation remains controversial. Some studies reported a faster engraftment after splenectomy but also a higher risk of relapse for splenectomized patients has been reported [27, 49–51]. But no difference in outcome between splenectomized and nonsplenectomized patients [14, 21, 51–53]. Taken the high morbidity and mortality due to splenectomy into account and the alternative option with JAK inhibitors [54] routinely, splenectomy prior to transplantation is not recommended. An alternative to splenectomy in patients with extensive enlarged spleen who failed to respond to JAK inhibitor therapy is low-dose spleen irradiation which has been reported in smaller case series but larger studies or systematic investigation are lacking so far [55, 56].

44.2.4 JAK Inhibitor Prior to Transplantation

JAK1/2 inhibitor ruxolitinib is the first JAK inhibitor approved by FDA and EMA for patients with intermediate- or high-risk myelofibrosis (FDA) or symptomatic myelofibrosis with splenomegaly (EMA) based on results of COMFORT-I and -II studies which have shown reduction of spleen size and improvement of constitutional symptoms [57, 58]. Because reduction of spleen size and improvement of constitutional symptoms may positively influence outcome after stem cell transplantation, JAK inhibitor treatment prior to stem cell transplantation has been studied by several investigators. Most of the studies reported feasibility of this approach [59–64]. Potential side effects are withdrawal syndrome and increase of infectious risks. Preliminary results from a prospective French study reported also tumor lysis syndrome, cardiogenic shock, and sepsis [65]. Large studies from CIBMTR and EBMT suggest that outcome after transplantation is improved if transplantation is performed in responding patients in contrast to nonresponding patients or patients who have lost response to JAK inhibition [60, 66].

The current EBMT/ELN consensus guidelines recommend to start JAK inhibition at least 2 months before transplantation and to start tapering 5–7 days prior to conditioning and to stop ruxolitinib the day before conditioning [10]. Because of its anti-inflammatory properties, JAK1/2 inhibitors are highly active in steroid resistant graft-versus-host disease [67] and might be useful in preventing GVHD. In a small study in myelofibrosis patients, ruxolitinib has been continued during the transplant period until day+28 with a low incidence of GVHD [68].

44.2.5 Minimal Residual Disease and Relapse Prevention

Relapse is the major cause of treatment failure after allogeneic stem cell transplantation which is about 20–25% without any improvement in the last 20 years [44]. Donor lymphocyte infusion or second allogeneic stem cell transplantation are reasonable treatment options with curative potential [69–71]. Ruxolitinib given in relapsed patients improves constitutional symptoms and reduces spleen size but does not improve donor cell chimerism or mutation level [72].

Because donor lymphocyte infusion seems to be more effective and less toxic in patients with minimal measurable disease rather than in clinical relapse [73], detection of MRD and early intervention have become of clinical importance. It could be convincingly shown that clearance of JAK2 mutation in peripheral blood was associated with a reduced risk of relapse after stem cell transplantation [46, 74]. In addition, detection of one of the driver mutations CALR, JAK2, or MPL by qPCR in peripheral blood at day 180 post allograft resulted in a significant higher relapse rate [75–78]. If DLI fails to induce remission in relapsed patients, second allograft can result in up to 50% long-term survival [69–71].

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Thrombosis and Myeloproliferative Neoplasms

45

Alexandre Guy and Chloé James

Abstract

BCR-ABL-negative myeloproliferative neoplasms (MPNs) are acquired hematological diseases characterized by the proliferation of fully mature and functional blood cells including polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). The occurrence of arterial or venous thrombosis is a major risk during these diseases. The currently accepted risk factors are an age over 60 years and a history of thrombosis. However, many complex mechanisms contribute to this increased prothrombotic risk, involving all blood cells, plasma factors, and the endothelial compartment. In recent years, new pathophysiological mechanisms have been revealed.

Keywords

Myeloproliferative neoplasms · Thrombosis · Neutrophil extracellular traps

45.1 MPNs and Thrombotic Risk

The so-called classical myeloproliferative neoplasms (MNPs) comprise four clinical entities: chronic myeloid leukemia (CML) which is characterized by the presence of the *BCR-ABL* fusion gene (or Philadelphia chromosome), and negative *BCR-ABL* neoplasms which include polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). These neoplasms are characterized by the excessive production of differentiated, mature, and functional blood cells. They result from a mutation acquired in a hematopoietic stem cell (HSC) that proliferates clonally and causes hyperplasia of one or more blood cell lines. The discovery in 2005 of the JAK2V617F mutation led to a better understanding of the pathophysiology of the disease and was a major contribution to the diagnosis. This mutation in the gene encoding Janus kinase 2 (JAK2), with the modification of the valine at position 617 of the protein to phenylalanine, is responsible for the activation of various signaling pathways leading to an increase in the proliferation and survival of affected cells, thus explaining the phenotype found in patients [1–4]. The prevalence of this mutation is high in PV (95%), and lower in ET and PMF (50–60%). Since its discovery, other transformation-initiating mutations have been identified: the mutation of *JAK2* exon 12 in PV [5], mutations in the calreticulin gene (CALR) [6, 7], and in the gene encoding the thrombopoietin receptor (*myeloproliferative leukemia protein*, MPL) [8] in ET and PMF.

Responsible for high morbidity and mortality, arterial and venous thrombosis are major complications of negative *BCR-ABL* myeloproliferative neoplasms. A recent meta-analysis with more than 10,000 patients reported a prevalence of overall thrombosis of 20.7%, 28.6%, and 9.5% in newly diagnosed ET, PV, and PMF patients respectively [9]. Thrombosis occurs more frequently in arterial than venous settings, with a prevalence of 70% of arterial thrombosis in PV, for example [10]. In PV, cardiovascular events are the main cause of death with the occurrence of myocardial infarction, congestive heart failure or pulmonary embolism [11]. Thromboses may occur in so-called unusual territories, such as cerebral venous thromboses and venous thromboses of the splanchnic system. Finally, microcirculation disturbance is also common, leading to headaches, dizziness, tinnitus or erythermalgia.

Despite progresses in diagnosis and treatment these recent years, thrombosis is still a major problem in these diseases. This is partly due to their complex pathophysiology, involving multiple cellular and molecular partners, with mechanisms that are not yet fully understood (Table 45.1).

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Table 45.1 Main abnormalities leading to high thrombotic risk in myeloproliferative neoplasms

Cells	Characteristic	All patients	JAK2V617F+ patients	Patients with history of thrombosis
Platelets	Adenosine nucleotides content	↓		
	Serotonin content	↓		
	Platelets-monocytes aggregates	↑		↑
	Platelets-neutrophils aggregates	↑	↑	↑
	CD62P expression	↑	↑	↑
	Fibrinogen binding	↑		
	Tissue factor expression	↑	↑	↑
	Plasmatic TXA2	↑		
	Plasmatic CD40-ligand	↑		↑
	Plasmatic sCD62P	↑	↑	
	Immature platelets	↑	↑	↑
	Phosphatidyl-serine expression	↑	↑	
Neutrophils	CD11b expression	↑	↑	
	LAP expression	↑	↑	
	CD14 expression	↑	↑	
	Plasmatic MPO	↑		
	Plasmatic neutrophil elastase	↑		
	Neutrophil elastase activity	↑		
	ROS production	↑		
	Ex vivo NETs formation	↑		
	Plasmatic nucleosome levels	↑		
	Plasmatic DNA level	↑		
	Plasmatic MPO-DNA complex level	↑		↑
	Beta-1 and Beta-2 integrins expression	↑		
	Adhesion to VCAM-1	↑		
Monocytes	CD11b expression	↑	↑	↑
	Tissue factor expression	↑		
Erythrocytes	Hematocrit	↑		
	Lu/BCAM expression	↑		
Microvesicles	Blood cells-derived microvesicles levels	↑	↑	↑
	Tissue factor positive microvesicles	↑		
	Phosphatidyl-serine positive microvesicles	↑		
	MV-associated procoagulant activity	↑	↑	↑
	MV-associated thrombin generation		↑	
Coagulation factors	PAI-1 antigen level	↓		
	t-PA activity	↑		↓
	Protein C antigen	↓		
	Protein C activity	↓		
	Activated Protein C resistance	↑	↑	↑
	Protein S cleavage	↑		
	Free protein S	↓	↓	
	Protein S antigen	↓		↓
	Prothrombin F1+2 fragments	↑	↑	↑
	Thrombin-anti-thrombin complex	↑		
	D-Dimers	↑	↑	
	Factor V expression	↑		↑
	Factor VIII expression	↑		↑
	vWF antigen level	↑	↑	↑
	Thrombin generation	↑	↑	↑
Endothelial cells	Circulating endothelial cells level	↑		
	Thrombomodulin expression	↑	↑	
	Plasmatic VEGF level	↑	↑	
	Plasmatic soluble E-Selectin	↑		

↓ Decrease

↑ Increase

45.1.1 Clinical Risk Factors for Thrombosis

The first recognized risk factor for thrombosis during MPNs is an age greater than or equal to 60 years, with a 1.5-fold to 5-fold greater risk of developing thrombosis in ET and PV if the patient is older than 60 years [12, 13]. The second risk factor for thrombosis is the presence of a history of thrombosis [11, 14]. The presence of at least one of these two risk factors places the patient in the category of patients at "high risk" of thrombosis and therefore justifies the implementation of a cytoreductive treatment.

Thrombosis prevention is based on the prescription of antiplatelet or anticoagulant therapy. Aspirin is often prescribed in these at-risk patients. While its benefit has been clearly established in patients with PV [15], no studies have formally demonstrated its usefulness in ET or myelofibrosis. Some recommendations use the *international prognostic score for thrombosis in essential thrombocythemia (IPSET)—thrombosis,* established for ET [16]. This score takes into account age, history of thrombosis, presence of cardiovascular risk factors, and presence of the JAK2V617F mutation. Curative anticoagulation is indicated in the presence of a venous thrombotic episode. It sometimes needs to be prolonged over the long term, especially if the thrombosis occurs in an unusual site (such as the splanchnic territory) [17].

45.1.2 Impact of Mutational Status

As evidences increased about the different initiatory mutations that cause myeloproliferative neoplasms, the impact of mutation status on patients' thrombotic risk was questioned. In PV, given the very high prevalence of the JAK2V617F mutation, studies mainly focused on the impact of allelic load, without producing definitive results. Some teams have shown an increased risk of venous thrombosis when the allelic burden was greater than 20% (more than 20% of the alleles are then mutated) [18]. Others observed an increased risk of arterial and venous thrombosis when the allelic burden was greater than 75% [19]. However, these results have not been confirmed by other studies [20]. Therefore, the allelic burden does not seem to be an important marker in assessing the risk of thrombosis in PV, especially since it may change over time. In ET, the presence of the JAK2V617F mutation clearly leads to an increased risk of thrombosis [13], which is now taken into account in algorithms for the therapeutic management of the disease. The value of the allelic burden JAK2V617F seems to have a greater influence than in PV [18]. The risk of thrombosis is therefore lower in the presence of mutations affecting the thrombopoietin receptor (MPL) and calreticulin, with a 10-year cumulative incidence of thrombosis of 11% in patients with calreticulin mutation, 9.3% in those with MPL mutations, and 21% in patients with the JAK2V617F mutation [7].

45.2 Factors Involved in the Pathophysiology of Thrombosis Associated with MPNs

The pathophysiology of thrombosis during MPNs is complex and involves many factors: blood cells, plasma factors, endothelial compartment (Table 45.1). Many data report an important role of the JAK2V617F mutation in several cell types (Fig. 45.1).

45.2.1 Platelets

Platelets play a great role in hemostasis and thrombosis. Thus, one may have thought that increased platelets counts are associated with a higher risk of thrombosis. But this correlation has not been proven [21], and at the opposite, platelets counts higher than 1500G/L are associated with an increased risk of bleeding, probably due to the presence of an acquired von Willebrand syndrome (avWS).

Much work in patients and murine models has focused on defining platelet function during MPNs. In patients, results are contradictory. Some studies have shown a decrease in platelet function with phospholipid abnormalities, decreased levels of adhesion molecules (GPIb, GPIIbIIIa or GPVI), decrease in dense granule content, defective aggregation [22–25]. In accordance with these results, a study in ET patients demonstrated that PI3K-AKT-Rap1 pathway seems perturbed whereas SFLLRN-mediated P-selectin expression, ATP secretion, PKC activation, and calcium mobilization were unaffected [26]. Other studies are suggestive of platelet activation: (1) in vitro analysis revealed increased platelet P-selectin and phosphatidylserine expression, increased platelets-leukocytes aggregates [22, 27–31], increased aggregation, and calcium mobilization due to the preactivation of the kinase Src [32]; (2) in vivo dosage demonstrated increased expression of CD40 ligand, soluble P-selectin, and thromboxane A2 (TXA2) [30, 33]. A higher level of immature platelets [31], which have increased hemostatic power, have also been demonstrated. The presence of the JAK2V617F mutation seems associated with a greater platelet activation, with increased levels of platelets activation markers (soluble P-selectin and TXA2), increased membrane P-selectin expression [29, 34], increased platelets-neutrophils aggregates [30].

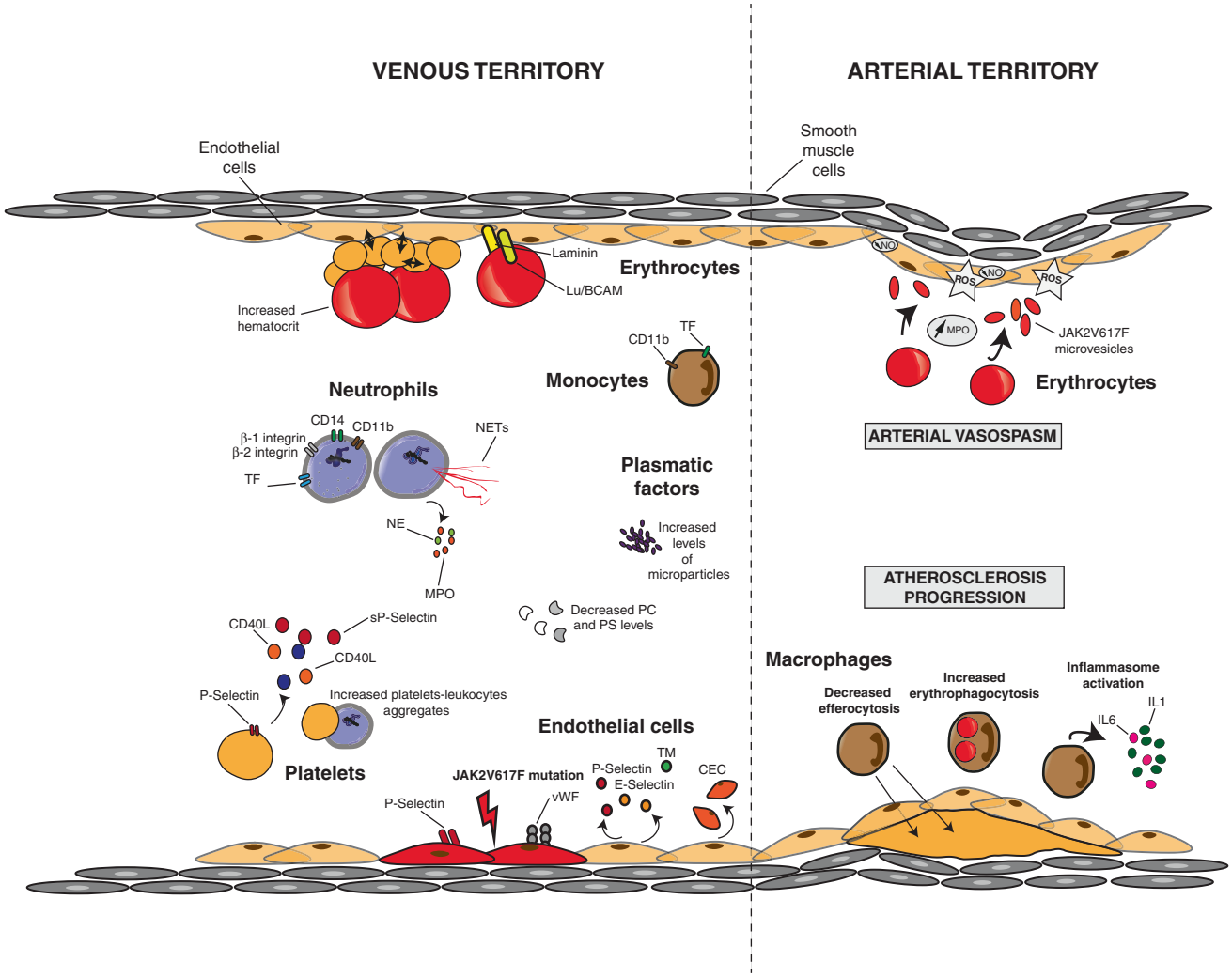

Fig. 45.1 Pathophysiology of thrombosis in myeloproliferative neoplasms (MPN) with JAK2V617F mutation. Several actors are involved in the pathophysiology of thrombosis in these diseases. Platelets are activated with increased expression on their surface of tissue factor (FT) and P-selectin, and there are increased levels of plasma activation markers such as thromboxane A2 (TXA2), CD40 ligand, and soluble P-selectin. Red blood cells also play a role: intrinsic activation (overexpression of Lu/BCAM) and, in the event of an increase in hematocrit, displacement of platelets in contact with the endothelium. Neutrophils and monocytes have been shown to be activated: increased levels of tissue factor, CD11b, increased plasma levels of neutrophil elastase (NE) and myeloperoxidase (MPO), increased formation of extracellular neutrophil traps (NETs) on their expression surface. Endothelial cells are activated with increased levels of plasma markers of endothelial activation: thrombomodulin (TM), von Willebrand factor (vWF), P- and E-selectin, and increased circulating endothelial cell levels (CEC). Endothelial cells are activated in the presence of the JAK2V617F mutation with increased expression of P-selectin and von Willebrand factor. Plasma factors play a role in this pathophysiology with increased microparticle levels, decreased protein C and protein S levels, and more frequent resistance to activated protein C

However, the heterogeneity of patient cohorts (in terms of cytoreductive treatments, types of driver mutations, allelic burden variations, antiplatelet or anticoagulant treatments) makes it difficult to interpret the results. Several teams have therefore developed murine models of MPNs to study platelet function. Depending on the mouse model used, and on the technique used to perform thrombosis, the results are contradictory. Using VavCre;JAK2^{V617F} and SclCreERT2;JAK2^{V617F} models, a French team observed platelet hyporeactivity (P-selectin and GpIIbIIIa expression) in response to different agonists, associated with a decrease in the expression of glycoprotein VI (GpVI), and an increase in bleeding time. In a model of vascular damage induced by ferric chloride, they observed increased thrombus formation, with however an increased instability of the thrombus [35]. Another study confirmed the existence of hemostasis abnormalities (increased bleeding time and decreased thrombus formation) associated with the presence of an acquired von Willebrand syndrome, without highlighting platelet abnormalities: no defects in integrins expression (β3, αIIb, β1, and α2) or platelet aggregation [36]. Another group analyzed various mouse models of thrombocytosis and did not report platelet func-

tion defect but an acquired von Willebrand syndrome, with a decrease in von Willebrand factor high-molecular weight multimers and increased bleeding time [37]. A recent study in a mouse model of myelofibrosis has showed a reduced aggregation in response to collagen, as a decreased thrombus formation, and an increased bleeding time [38]. However, contradictory results were found in a murine model of ET, providing arguments in favor of platelet activation in this setting: increased response to different agonists (thrombin, collagen), increased thrombus formation in vitro, and decreased bleeding time in vivo [39].

Therefore, giving these conflicting results, it is difficult to draw definitive conclusions from these studies. They also highlight the difficulties of interpreting results obtained with different murine models. Platelet functions, and more generally hemostatic functions, seem to also depend on the phenotype of the disease with strong arguments for the presence of acquired von Willebrand syndrome when the platelet count is very high, as is the case in patients.

45.2.2 Leukocytes

As for platelets, a lot of studies have investigated the role of leukocyte counts in the thrombotic risk during MPNs with however contradictory results [13, 21, 40], in part due to the cutoff of leukocytosis chosen and the heterogeneity in the statistical methods used for its assessment. Recently, two important studies have been published. First, a meta-analysis including more than 30,000 patients has demonstrated an association between leukocytosis and thrombotic risk mainly in ET patients and arterial thrombosis subgroups [41]. On the contrary, Ronner et al. recently observed that leukocytosis was not associated with an increased thrombotic risk in PV patients [42].

Several studies have investigated the leukocyte activation during MPNs. Monocytes are activated [27, 43], particularly in patients with a history of thrombosis [29]. In mice, a recent study demonstrated the impact of JAK2V617F-mutated macrophages in the production of proinflammatory cytokines, leading to inflammasome activation and atherosclerosis progression [44]. Neutrophils activation has also been studied extensively in MPN. In patients, numerous studies showed higher levels of CD11b, CD14, Tissue Factor (TF), phosphatidyl-serine and leukocyte alkaline phosphatase (LAP) [22, 28, 29, 43, 45–47], increased platelet-neutrophils aggregates, and increased neutrophils activation markers (MPO, elastase) [22, 28–30, 43, 45, 46]. Patients with JAK2V617F mutation showed increased expression of CD11b, CD14, TF, and LAP [22, 29, 43]. Two recent articles demonstrated a crucial role of integrins in neutrophil activation. First, JAK2V617F neutrophils from patients showed increased activation of integrin α4β1 and increased adhesion to VCAM1 (*vascular cell adhesion molecule 1)* [48]. These results in patients were confirmed using a murine model (*Vav;Cre;JAK2V617F*) showing increased expression of integrins β1 and β2 on the surface of neutrophils, thus increasing their adhesion capacity and promoting their involvement in the pathophysiology of thrombosis [49].

Activated neutrophils can release neutrophil extracellular traps (NETs), which are made of decondensed DNA associated with histones that are catapulted out of the cell (*NETosis* process). NETs play a role in the pathophysiology of hemostasis and thrombosis through various mechanisms: binding and activation of platelets [50], inhibition of anticoagulant molecules such as tissue factor pathway inhibitor (TFPI) [51] or thrombomodulin [52], activation of the extrinsic pathway via TF activation, or activation of the intrinsic pathway via the activation of factor XII [53]. Histones activate endothelial cells, inducing surface expression of TF [54]. More, histones are also able to substitute factor Va to generate an alternative prothrombinase that generates thrombin without phospholipids [55]. In parallel, myeloperoxidase, also produced by activated neutrophils, can inhibit thrombomodulin [56].

The implication of NETs in the pathophysiology of thrombosis during MPNs has been investigated. A first study did not find differences in ex vivo NETs formation from MPN patients' neutrophils. Nevertheless, they reported increased levels of circulating nucleosomes which were in favor of increased NETosis [57]. A second study published in 2018 showed increased ex vivo NETosis in MPN patients and in a mouse model of MPN, but also increased thrombosis formation with NETs in the thrombi. The role of NETosis in the occurrence of thrombosis was confirmed by the deletion of an enzyme crucial for the formation of NET, PAD4 (*protein arginine deiminase 4*), with a dramatic decrease of thrombus formation in PAD4-deficient mice [58]. Finally, the authors have showed that the administration of ruxolitinib, a JAK inhibitor, was able to diminish the occurrence of thrombosis, suggesting that JAK2 is important for NET formation. Increased NETs formation in MPN patients was confirmed by our team. We reported higher NET plasmatic marker, MPO-DNA, in patients with a history of thrombosis compared with patients without a history of thrombosis, reinforcing the hypothesis that NETs are involved in the pathogenesis of thrombosis [47]. As oxidative metabolism is important in NET formation, one group has studied the impact of N-acetyl-cysteine (NAC) on thrombosis in a mouse model of MPN. They reported increased survival associated with decreased thrombus formation in mice receiving this drug. More, the authors showed that NAC treatment of neutrophils isolated from MPN patients diminished NETs formation, suggesting that NAC acts on thrombosis occurrence

via NETs formation [59]. Altogether, neutrophils and NETs formation should be considered as important actors of the pathophysiology of thrombosis during MPNs.

45.2.3 Red Blood Cells

Hematocrit plays a major role in the risk of thrombosis. In PV, an association between the risk of thrombosis and hematocrit was already reported 40 years ago [60]. The CYTO-PV (*cytoreductive therapy in PV*) clinical trial confirmed this observation, showing an increased risk of cardiovascular events in patients with a hematocrit greater than 45%, and a greater risk of death due to a cardiovascular or thrombotic event [12]. The rheological consequences of a high hematocrit vary according to the venous or arterial setting. In veins where the blood flow is slow, a high hematocrit induces hyperviscosity, slowed blood flow, and relative hypoxia in endothelial cells. In arteries, where blood flow is high, it promotes the movement of platelets to the endothelium, leading to increased interaction between the two cell types [61]. In PV mice, a high percentage of capillaries with stalled flow were reported when the hematocrit value exceeded 55% with a majority of stationary red blood cells in the stalled vessels [62]. The importance of the hematocrit in the occurrence of thrombosis has recently been demonstrated in a mouse model deficient for a protein crucial for erythropoiesis, pleckstrin-2 (Plek2) [63]. The mice, not polycythemic any more, have a greater survival and a decrease in thrombosis rate compared to the polycythemic mice [63]. In addition to the role played by their number (evaluated by hematocrit), qualitative abnormalities of red blood cells are also observed in MPNs. An increased RBC adhesion capacity has especially been reported, due to increased interaction between Lu/BCAM (*erythroid Lutheran/Basal cell-adhesion molecule*), a membrane erythrocyte protein, and laminin, which is expressed by endothelial cells [64]. This interaction is promoted by Lu/BCAM phosphorylation via the Rap1/AKT pathway (protein kinase B) [65]. Two recent studies in mouse models of JAK2V617F MPN have highlighted the role of RBCs in arterial events during MPNs. One reported reduction in red blood cells erythrophagocytosis leading to an increase in atherosclerosis, due to decrease expression of CD47 (or IAP, for *integrin-associated protein*), a ligand of SIRPα (*signal regulatory protein alpha*) at the surface of monocytes and neutrophils [44]. Another study highlighted a novel mechanism of vasospasm during MPN. The authors demonstrated that JAK2V617F erythrocyte-derived microvesicles overexpress myeloperoxidase, thus increasing endothelial oxidative stress and inhibiting NO pathway. This led to increased arterial contraction in presence of a vasoactive agent. Finally, they have observed that treatment with antioxidants agents, such as N-acetyl-cysteine or simvastatine, could improve abnormal arterial contraction [66]. This study is in accordance with clinical studies having demonstrated dysregulated NO levels in MPN patients [67] as well as impairment of endothelial-dependent flow-mediated vasodilatation in PV patients [68].

45.2.4 Endothelial Cells

The endothelium plays an antithrombotic role under physiological conditions by helping to inhibit platelet adherence and the activation of coagulation. Several observations point to the existence of endothelial compartment activation during MPNs: increased circulating levels of thrombomodulin [45], von Willebrand factor (vWF) [45], selectins (E- and P-selectin) [69], and circulating endothelial cells [70]. As discussed above, nitric oxide (NO), a major factor in vasorelaxation, is produced in smaller quantities by endothelial cells in patients with MPNs [67]. Attention has also been focused on the role of heparanase, an enzyme that cleaves the side chains of heparan sulfates present on the surface of endothelial cells. Its action leads to the dissociation of the tissue factor pathway inhibitor (TFPI) on the cell surface, and thus to an increase in the procoagulant activity of tissue factor. Interestingly, a study has demonstrated that heparanase and TFPI levels are increased in bone marrow samples from MPNs patients, highlighting a possible new prothrombotic mechanism promoted by endothelial cells during MPNs [71].

The JAK2V617F mutation, which affects hematopoietic cells, can also be found in endothelial cells of MPNs patients, especially those with a history of thrombosis [72, 73]. The issue was therefore to unravel the role of these altered endothelial cells in thrombosis. We have shown that they express more P-selectin and von Willebrand factor on their surface. Using a murine model in which the *Jak2* gene was mutated specifically in endothelial cells, we also demonstrated that increased P-selectin expression was associated with a greater occurrence of thrombosis [74]. The increased expression of P-selectin by JAK2V617F-mutated cells was confirmed in another approach based on a model of endothelial cells derived from IPS (*induced pluripotent stem cells*) from patients with the JAK2V617F mutation. In this case, an overexpression of genes involved in inflammatory responses was observed, as well as an amplification of the proadhesive and prothrombotic properties of the cells [75]. In a mouse model allowing hematopoietic and endothelial cells' JAK2V617F expression, it has been demonstrated that JAK2V617F ECs were required for the

development of the prothrombotic and vasculopathy phenotype observed. In this study, the authors have also showed that JAK2V617F ECs possess a proadhesive and prothrombotic phenotype with notably decreased thrombomodulin and NO synthase expression, increased IL1-b expression, as well as increased E-selectin and platelet endothelial cell adhesion molecule (PECAM) [76].

45.2.5 Plasma Factors

Coagulation is activated in patients with MPN, and their levels of D-dimers [22, 45, 77], thrombin-antithrombin complex (TAT) [22, 45], as well as and F1 and F2 fibrinogen fragments [22, 30, 43, 45] are increased. Increase in FV and FVIII [30], vWF antigen [30, 45], plasmatic TF [78] has also been reported. These results are in accordance with the finding of an increased thrombin generation in MPNs patients [34, 46, 79]. Studies also report a phenotype of resistance to activated protein C (APC) [30, 46] and to thrombomodulin [80], and a decrease in coagulation inhibitors protein C (PC) and S (PS) [80–82]. Coagulation abnormalities seem more pronounced in the presence of the JAK2V617F mutation with an increase in plasmatic TF [30], vWF antigen [30], F1 + 2 prothrombin fragments [30, 43], thrombin generation [34, 80], as a lower free PS [30, 46] and a higher resistance to APC [30, 46]. Finally, an evaluation of the expression of phosphatidylserine on the surface of blood cells of MPNs patients showed that it was overexpressed in ET, resulting in shorter clotting times and increased factor X activation and thrombin and fibrin generation [77].

As the natural counterpoint to the activation of coagulation, fibrinolysis also appears to be disrupted in MPNs. However, there is disagreement on this question. For example, one study found a decrease in the concentration of PAI-1, the plasminogen activator type 1 inhibitor [81], while another found that levels of PAI-1 and tissue plasminogen activator (t-PA) were increased [22].

Microvesicles (MVs) are extracellular vesicles composed of fragments of cytoplasmic membrane released by blood cells and endothelial cells into the extracellular environment. As they express TF and phosphatidylserine, they are involved in coagulation activation. Increased microvesicles levels have been observed in MPNs patients, with no differences by type of MPNs [56], but with an increase in patients with the JAK2V167F mutation [83]. It has been shown that microvesicles isolated from MPNs patients have a greater TF expression and are able to induce increased thrombin generation [78–80, 83–86]. Finally, studies have demonstrated that patients with thrombosis have higher counts of MVs [86] but results are contradictory regarding the MVs-associated procoagulant activity [85, 87].

45.3 Conclusion

Thrombotic complications are currently the most important clinical issue in patients with MPNs. Despite cytoreductive or antiplatelet treatment, or even anticoagulant treatment following the recommendations, they can nevertheless occur. The real need for preventive treatment of these thromboses in some patients may be questioned if the risk of their onset is low. A better understanding of this complication is therefore needed so that it can be prevented and that patients can be managed appropriately. A growing body of evidence is highlighting the role of endothelial cells and neutrophils in the genesis of thrombosis. Prospective studies in patients are now required to confirm these findings.

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Eosinophilic Disorders and Systemic Mastocytosis

46

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Abstract

Eosinophilic disorders are a group of rare and highly heterogenous diseases distinguished by increased eosinophil counts and may be associated with end-organ damage. In this chapter, the diagnostic algorithm and management of eosinophilic disorders are discussed.

Keywords

Eosinophilia · Hyperoesinophilic syndrome · Chronic eosinophilic leukaemia

46.1 Introduction

Eosinophilic disorders are a group of rare and highly heterogenous diseases distinguished by increased eosinophil counts and frequent association with end-organ damage [1, 2]. Clinical presentations and prognosis of eosinophilic disorders are variable due to the myriad of underlying pathologies. Previous diagnostic difficulties regarding eosinophilic disorders heavily limited our understandings of these diseases. In spite of this, interests on these disorders have only increased during the past decades. With recent advancements in molecular strategies, pathogenesis of these disorders has been better elucidated, giving rise to improved classifications, diagnosis, and treatments. Nevertheless, these entities still prove to exert diagnostic and therapeutic challenges, with classification systems and treatment options being constantly updated. In this chapter, the causes, diagnosis, prognosis, and treatments of eosinophilic disorders will be discussed.

46.2 Eosinophilia and Hypereosinophilic Syndrome (HES)

Eosinophils account for 3–5% of circulating white blood cells (WBCs) and the upper limit of normal eosinophil count in adults varies from 0.35 to 0.5 × 10^9/L [1]. Eosinophilia is defined by an increase in levels of circulating eosinophils and can be further classified according to severity (Table 46.1). Hypereosinophilia (HE) conventionally refers to an eosinophil count of over 1.5 × 10^9/L [1] but its definition was further refined by the International Cooperative Working Group on Eosinophil Disorders (ICOG-EO) in

Table 46.1 Normal eosinophil count and various degrees of eosinophilia

Normal eosinophil count	Eosinophils (upper limit): 0.35–0.5 × 10^9/L
Mild eosinophilia	Eosinophils: 0.5–1.5 × 10^9/L
Moderate eosinophilia	Eosinophils: 1.5–5 × 10^9/L
Severe eosinophilia	Eosinophils: >5 × 10^9/L
HE (conventional)	Eosinophils: >1.5 × 10^9/L
HE (ICOG-EO)	Eosinophils > 1.5 × 10^9/L in blood on two examinations (interval > 1 month) and/or tissue HE defined by one or more of the following: 1. Percentage of eosinophils in bone marrow section exceeds 20% of all nucleated cells 2. Extensive tissue infiltration by eosinophils based on pathologist report 3. Marked deposition of eosinophil granule proteins (in the absence or presence of major tissue infiltration by eosinophils)

HE hypereosinophilia, *ICOG-EO* International cooperative Working group on eosinophil disorders

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2011 (Table 46.2) [2]. Idiopathic HE is defined by the presence of HE without associated end-organ damage.

HES is a clinical diagnosis based on patient presentation rather than a discrete entity. According to the ICOG-EO, HES is diagnosed in the presence of HE accompanied by end-organ damage, after exclusion of all alternative causes of organ damage (Table 46.3) [1, 3]. Due to diagnostic difficulties, the prevalence of HES remains unclear. Data from the Surveillance, Epidemiology and End Results (SEER) database in 2001–2005 estimated the age-adjusted incidence of HES to be 0.036 per 100,000 (coding of 9964/3 (HES including chronic eosinophilic leukaemia), International Classification of Disease for Oncology (version 3)) [4].

The causes of HES are numerous and can be broadly classified into primary, secondary, and idiopathic [3] (Table 46.4). Clinically, patients often present with weakness and fatigue, cough, dyspnoea, myalgia, angioedema, rash, fever, and rhinitis [1]. Common laboratory findings include leukocytosis and eosinophilia, anaemia, and abnormal platelet counts [1].

Table 46.2 ICOG-EO diagnostic criteria for HES

1. Criteria for peripheral blood HE fulfilled[a]
2. Organ damage and/or dysfunction attributable to tissue HE Presence of eosinophilic infiltrates accompanied by organ dysfunction, which consists of one or more of the following: (1) Fibrosis (e.g. lung, heart, digestive tract, skin, and others) (2) Thrombosis with or without thromboembolism (3) Cutaneous (including mucosal) erythema, edema/angioedema, ulceration, or eczema (4) Peripheral or central neuropathy with chronic or recurrent neurologic deficit; (5) Other less common organ manifestations of HES (liver, pancreas, kidney, etc.)
3. Exclusion of other disorders or conditions as major reason for organ damage

HE hypereosinophilia, *HES* hypereosinophilic syndrome, *ICOG-EO* International Cooperative Working Group on Eosinophil Disorders
[a] Refer to Table 46.1

Table 46.3 ICOG-EO classification of HES

Primary (neoplastic) HES (HES_N)	Underlying stem cell, myeloid, or eosinophilic neoplasm classified according to WHO guidelines and end-organ damage attributable to HE, and eosinophils are considered (or shown) neoplastic (clonal) cells
Secondary (reactive) HES (HES_R)	Underlying condition/disease in which eosinophils are considered nonclonal cells; HE is considered cytokine driven, and end-organ damage is attributable to HE Subvariant: lymphoid variant HES (clonal T cells identified as the only potential cause)
Idiopathic HES	No underlying cause of HE, no evidence of a reactive or neoplastic condition/disorder underlying HE and end-organ damage attributable to HE

HE hypereosinophilia, *HES* hypereosinophilic syndrome, *ICOG-EO* International Cooperative Working Group on Eosinophil Disorders, *WHO* World Health Organization

Table 46.4 Differential diagnosis of primary eosinophilic disorders

WHO-defined eosinophilic disorders
– myeloid/lymphoid neoplasms with eosinophilia and rearrangement of *PDGFRA*, *PDGFRB*, *FGFR1*, or *PCM1-JAK2* fusion gene (MLN-eo)
MPN[a] with eosinophilia associated with *FIP1L1-PDGFRA*
Myeloid/lymphoid neoplasms associated with *ETV6-PDGFRB* fusion gene or other rearrangement of *PDGFRB*[b]
MPN or acute leukaemia associated with *FGFR1* rearrangement
Myeloid/lymphoid neoplasms with *PCM1-JAK2*
– CEL, NOS
– Idiopathic HES
Other WHO-defined neoplasms associated with primary eosinophilia
– Systemic mastocytosis
– Chronic myeloid leukaemia
– Acute myeloid leukaemia (especially FAB M2 and M4 Eo subtypes with CBF translocations)
– MDS
– MDS/MPN overlap neoplasms (e.g. CMML)

CBF core binding factor, *CEL, NOS* chronic eosinophilic leukaemia, not otherwise specified, *CMML* chronic myelomonocytic leukaemia, *ETV6* ETS variant transcription factor 6, *FAB* French-American-British, *FGFR1* fibroblast growth factor receptor 1, *FIP1L1* FIP1-like 1, *HES* hypereosinophilic syndrome, *JAK2*, janus kinase 2, *MDS* myelodysplastic syndrome, *MDS/MPN* myelodysplastic syndrome/myeloproliferative neoplasms, *PCM1* pericentriolar material 1, *PDGFRA* platelet-derived growth factor receptor alpha, *PDGFRB* platelet-derived growth factor receptor beta, *WHO* World Health Organization
[a] Patients presenting with myeloproliferative neoplasm, AML, or lymphoblastic leukaemia/lymphoma with eosinophilia and a *FIP1L1-PDGFRA* fusion gene are also assigned to this category
[b] Cases with fusion genes typically associated only with BCR-ABL1-like B-lineage ALL are specifically excluded

End-organ damage is a prominent feature in HES and other eosinophilic disorders. Dermatological, pulmonary, gastrointestinal, as well as central and peripheral nervous involvements are frequently observed [1, 5]. Cardiac dysfunction due to eosinophilic infiltration and endocardial damage is also common [1, 5]. It is a major cause of mortality among patients due to the resultant progressive heart failure and thrombotic events [1, 5]. Hepatosplenomegaly and involvement of other organs may also occur [5]. The prognosis and treatment of HES are dependent on the underlying cause and can be substantially variable.

The term HES may sometimes be confused with idiopathic HES. The diagnosis of idiopathic HES should only be considered when all identifiable causes of HES have been exhausted [1, 3]. Owing to recent advancements in detection of clonal markers with molecular strategies and genetic sequencing, fewer patients have been classified under this entity [1].

46.3 Primary Eosinophilic Disorders

Primary, or neoplastic, eosinophilic disorders are clonal disorders arising from haematopoietic stem cells (HSCs) or other early progenitor haematopoietic cells. They are catego-

rized into a few subtypes according to the 2016 revised WHO classification. However, several other WHO-defined neoplasms can also give rise to primary eosinophilia and must not be missed (Table 46.4) [1].

Investigations for primary eosinophilic disorders should be initiated after exclusion of possible secondary causes [1]. Complete blood counts with differentials and peripheral blood smears are essential for identifying abnormal blood counts, circulating dysplastic cells, and other haematological abnormalities [1]. Serological tests for elevated Vitamin B12, tryptase, and IgE levels should also be carried out [1]. Given the prevalence of the *FIP1L1-PDGFRA* translocation, florescence in situ hybridization (FISH) should be carried out in peripheral blood as a screening test [1].

Bone marrow examinations with morphological, cytogenetic, molecular, and immunophenotypic studies are crucial for establishing definite diagnosis [1]. Morphological studies should focus on identification of dysplastic cells, blast percentage, as well as myelofibrosis [1]. Cytogenetic studies revealing abnormal karyotypes should prompt subsequent FISH analysis [1]. FISH is a molecular technique useful in identifying diagnostic translocations, including *FIP1L1-PDGFRA* and other rearrangements of *PDGFRA, PDGFRB, FGFR1*, as well as *JAK2* [1]. T-cell receptor (TCR) rearrangements can be identified by polymerase chain reaction (PCR) [1]. Myeloid next generation sequencing (NGS) panels can be used for detection of other gene mutations and rearrangements [1]. Upon detection of elevated serum tryptase level, immunohistochemistry in bone marrow aspirates for CD117, CD25, and tryptase should be performed [1]. After establishing diagnosis, appropriate treatments should be given according to the underlying entity.

46.3.1 Myeloid/Lymphoid Neoplasms with Eosinophilia and Rearrangement of *PDGFRA*, *PDGFRB*, *FGFR1*, or *PCM1-JAK2* Fusion Gene (MLN-Eo)

46.3.1.1 MPN with Eosinophilia Associated with *FIP1L1-PDGFRA*

FIP1-like 1 (*FIP1L*)-platelet-derived growth factor receptor alpha (*PDGFRA*) is the most common fusion gene within the subgroup of MLN-eo and accounts for more than 20% of patients with HES [6]. Breakpoints for *PDGFRA* is located in exon 12 while that *FIP1L* gene typically lies between introns 9 and 13, though the rare intron 16 *FIP1L* breakpoint was documented in a case report [7]. The chimeric protein possesses a dysfunctional autoinhibitory juxtamembrane domain, which renders the tyrosine kinase domain (TKD) constitutively active [8]. Other fusion partners of *PDGFRA* include *BCR*, *ETV6*, *KIF5B*, *etc.* [8]. Point mutations within the *PDGFRA* gene have also been observed [8].

Table 46.5 WHO diagnostic criteria of MPN with eosinophilia associated with *FIP1L1-PDGFRA*

1. A myeloid or lymphoid neoplasm, usually with prominent eosinophilia
2. Presence of a *FIP1L1-PDGFRA* fusion gene or a variant fusion gene with rearrangement of *PDGFRA*[a]

FIP1L1 FIP1-like 1, *MPN* myeloproliferative neoplasms, *PDGFRA* platelet-derived growth factor receptor alpha, *WHO* World Health Organization

[a] If appropriate molecular analysis is not available, this diagnosis should be suspected if there is a Ph-chromosome-negative MPN with the hematologic features of chronic eosinophilic leukaemia associated with splenomegaly, a marked elevation of serum vitamin B12, elevation of serum tryptase, and increased BM mast cells

The diagnostic criteria of MPN with eosinophilia associated with *FIP1L1-PDGFRA* are detailed in Table 46.5 [1]. Prior to the introduction of the category MLN-eo by the WHO in 2016, a clinical presentation of HES with any established eosinophilic clonality was considered as a diagnosis of CEL [7]. However, in the updated WHO guideline, *PDGFRA* rearrangements must be excluded before a diagnosis of CEL, NOS can be made [1, 7].

This entity exhibits male-dominance [6]. Apart from eosinophilia-related organ dysfunction, common features include splenomegaly, leukocytosis, eosinophilia, as well as elevated serum vitamin B12 and tryptase levels [6, 7, 9]. However, these features may not always be present in all cases [10]. While most patients present during the chronic phase of disease, acute presentations with either myeloid or lymphoid disease have been observed [11]. Reported clinical manifestations include lymphoblastic T-cell lymphoma, acute myeloid leukaemia (AML), myeloid sarcoma, chronic myelomonocytic leukaemia (CMML), and even thrombotic thrombocytopenia purpura (TPP) [10, 12–15].

The prognosis of neoplasms with *PDGFRA* rearrangement is excellent owing to their intrinsic sensitivity towards tyrosine kinase inhibitors, notably imatinib [1]. Imatinib at a dosage of 100–400 mg daily is effective for both chronic and blast phase diseases and was reported to induce complete haematologic remissions (CHR) and complete molecular remissions (CMR) in at least 90% of patients [1, 8, 9, 11]. Evidence suggested that imatinib should be continued after achieving CMR due to a high risk of relapse upon termination, which implies inability of imatinib to eradicate clonal cells [1]. Nevertheless, low rates of resistance and retained efficacy in remission induction among relapsed patients still make imatinib an ideal agent for the treatment of this entity [1, 11].

46.3.1.2 Myeloid/Lymphoid Neoplasms Associated with *ETV6-PDGFRB* Fusion Gene or Other Rearrangement of *PDGFRB*

Unlike *PDGFRA* rearrangements, platelet-derived growth factor receptor beta *(PDGFRB)* rearrangements are rare and occur in less than 1% of myeloid neoplasms [8, 16]. *ETV6-PDGFRB* was the first chimeric protein to be discovered and the most common, but more than 30 other fusion partners of *PDGFRB* have been subsequently identified, such as *HIP1, Rab5,* and *CEV14* [8, 17]. The breakpoint of *PDGFRB* occurs in exon 11 in the majority of cases, allowing preservation of the TKD, which is then constitutively activated by the dimerization motifs from fusion partners [8, 18, 19].

PDGFRB rearrangements are predominantly observed in males from 25 years to 55 years old and results in diseases of the myeloid lineage [8, 16]. Commonly observed clinical phenotypes include atypical CML, CMML, MPNs, and MDS [8, 16]. Acute phase disease (e.g. AML) was observed only in rare cases [8, 16]. Other common features include leukocytosis, eosinophilia, and splenomegaly [16]. Diagnostic criteria of this entity are described in Table 46.6 [1].

Similar to MLN-eo with *PDGFRA* rearrangements, *PDGFRB* rearrangements confer favourable prognosis and such diseases are highly treatable with imatinib at 100–400 mg daily [8, 16]. In a case series, almost all patients (96%) responded to imatinib and achieved sustained CHR and CMR, with no progression into blast phase diseases [20].

46.3.1.3 MPN or Acute Leukaemia Associated with FGFR1 Rearrangement

Myeloid or lymphoid neoplasm with fibroblast growth factor receptor 1 (*FGFR1)* rearrangements are uncommon. Translocation between FGFR1 and at least 14 other genes have been described, with the three most common being *ZMYM2, CNTRL,* and *FGFR1OP* [8]. *Myeloid or lymphoid neoplasms involving rearrangement of FGFR1 have been termed "8p11 myeloproliferative syndromes" due to the location of FGFR1 at chromosome 8p11* [21].

Table 46.6 WHO diagnostic criteria of myeloid/lymphoid neoplasms associated with *ETV6-PDGFRB* fusion gene or other rearrangement of *PDGFRB*

1. A myeloid or lymphoid neoplasm, often with prominent eosinophilia and sometimes with neutrophilia or monocytosis
2. Presence of t(5;12)(q31-q33;p12) or a variant translocation[a] or demonstration of an *ETV6-PDGFRB* fusion gene or rearrangement of *PDGFRB*

ETV6 ETS variant transcription factor 6, *PDGFRB* platelet-derived growth factor receptor beta, *WHO* World Health Organization

[a]Because t(5;l2)(q31-q33;p12) does not always lead to an ETV6-PDGFRB fusion gene, molecular confirmation is highly desirable. If molecular analysis is not available, this diagnosis should be suspected if there is a Ph-chromosome–negative MPN associated with eosinophilia and with a translocation with a 5q31-33 breakpoint

Table 46.7 WHO diagnostic criteria of MPN or acute leukaemia associated with *FGFR1* rearrangement

1. MPN or MDS/MPN with prominent eosinophilia, and sometimes with neutrophilia or monocytosisor AML or precursor T-cell or precursor B-cell lymphoblastic leukaemia/lymphoma or mixed phenotype acute leukaemia (usually associated with peripheral blood or BM eosinophilia)
2. Presence of t(8;13)(p11;q12) or a variant translocation leading to FGFR1 rearrangement demonstrated in myeloid cells, lymphoblasts, or both

AML acute myeloid leukaemia, *BM* bone marrow, *FGFR1* fibroblast growth factor receptor 1, *MDS/MPN* myelodysplastic syndrome/myeloproliferative neoplasm, *MPN* myeloproliferative neoplasm, *WHO* World Health Organization

Common clinical manifestations of this entity include MPNs and T-ALL, while AML, mixed phenotype acute leukaemia, and B-ALL are less common [21]. *The heterogenous presentations of this disease can be attributed to the distinct downstream pathways resulting from various translocations* [8, 21]. *For example, ZMYM2-FGFR1* most commonly results in T lymphoblastic lymphomas, t(8, 22) may result in a CML-like phenotype, and neoplasms with *FGFR1OP-FGFR1* may resemble CMML, or less frequently, polycythaemia vera (PV) [8, 21]. Eosinophilia is common feature but not invariable, especially in diseases with *FGFR1OP-FGFR1* or t(8, 22) [8, 21]. *Diagnostic criteria are listed in* Table 46.7 [1].

Unfortunately, this entity carries dismal prognosis and aggressive disease course, with most patients progressing into blast phase disease, usually AML or T-ALL, within 1–2 years [1, 8, 21]. Long-term survival is uncommon [21]. This entity is resistant to imatinib, dasatinib, nilotinib, and many available tyrosine kinase inhibitors [1, 21]. While midostaurin and ponatinib both induced responses in some patients, mixed results have been observed and further investigations are warranted [22–26]. Treatment typically involves induction with intensive chemotherapy followed by haematopoietic stem cell transplantation (HSCT), which is currently the only cure for this disease [1, 8]. Several novel inhibitors of FGFR have also exhibited intriguing therapeutic prospects, including pemigatinib, an FGFR1-3 inhibitor, and futibatinib, an FGFR1-4 inhibitor [1, 27, 28].

46.3.1.4 Myeloid/Lymphoid Neoplasms with PCM1-JAK2

Janus kinase 2 (*JAK2*) rearrangements are rarely observed among haematological neoplasms [8]. The most common translocation partner of *JAK2* is *PCM1*, with *ETV6* and *BCR* being much rarer [8].

The clinical behaviour of MLN-eo with *JAK2* rearrangements greatly differs from neoplasms *JAK2* point mutations but share similarities of those with tyrosine

kinase gene rearrangements [8, 29]. While it commonly presents with MPN-like pictures, aggressive diseases with rapid progression to blast phase are often observed. Other less common presentations include CML, MDS, MDS/MPN, and B-ALL [8, 29]. Frequent clinical features include eosinophilia, myelofibrosis, hepatosplenomegaly, and lymphadenopathy [29]. The diagnostic criteria of this entity can be found in Table 46.8 [1].

Despite the suboptimal prognosis of this entity, JAK2 inhibitors such as ruxolitinib are expected to be major therapeutic breakthroughs [8]. However, emerging evidence has suggested that the encouraging responses to ruxolitinib are only transient and that the goal of long-term remissions can only be achieved via HSCT [8, 30–36].

46.3.2 Chronic Eosinophilic Leukaemia, No Otherwise Specified (CEL, NOS)

The diagnostic criteria of CEL, NOS are detailed in Table 46.9 [1]. By definition, a diverse range of genetic aberrations exist in patients with CEL, NOS. Patients typically show abnormal karyotypes, with the most common being trisomy 8 [37]. Mutations in *ASXL1, IDH1*, *TP53,* and many other genes have also been documented [37]. Multiple translocations have also been observed [8]. Of importance, patients with *FLT3*, *ABL1*, and *JAK2* (except for *PCM1-JAK2*, *ETV6-JAK2*, and *BCR-JAK2*) rearrangements may present similarly to MLN-eo patients or those with other mutations in tyrosine kinase genes, which highlights the importance of careful and accurate diagnosis [8]. In addition, the presence of genetic aberrations may not be directly related to the pathogenesis of eosinophilia, further complicating the differentiation between CEL, NOS and idiopathic HES [1, 38]. One study suggested that a few prominent features may be used to distinguish CEL, NOS patients from the latter: abnormal karyotype, myeloid mutations, and inferior survival [38].

Reported cases of this entity show major resemblance with chronic myeloid neoplasms [8, 39]. Male dominance was also observed [37]. Frequent features include leukocytosis, eosinophilia, anaemia, abnormal platelet counts, constitutional symptoms, organ involvements, and hepatosplenomegaly [39].

CEL, NOS carries a poor prognosis which is accounted by high risks of disease-related organ failures, frequent blast phase progression, and limited treatment responses [8, 37, 40]. Hydroxyurea is only useful for management of leukocytosis and eosinophilia but does not control disease progression [1, 8]. Corticoseteroids may also be used for symptomatic management and control of eosinophilia [1]. Interferon-alfa (IFN-α) demonstrated efficacy in symptomatic management and in inducing CHR and CMR in patients resistant towards hydroxyurea and prednisone [1, 8]. Imatinib also induced transient partial responses in a number of patients [1]. Nevertheless, HSCT currently provides the best chance of long-term remission and survival [1, 8].

46.3.3 Idiopathic HES

The diagnostic criteria of this entity are listed in Table 46.10 [1]. As previously mentioned, identification of genetic abnormalities may not exclude a diagnosis of idiopathic HES as they may not be disease-associated [1]. Persistent eosinophilia and end-organ damage are the major features of this entity [1].

Although the prognosis of idiopathic HES is considered superior to CEL, NOS, it is still generally poor [1]. Possible poor prognostic indicators include age > 60 years, cardiac involvement, hepatosplenomegaly, eosinophil $\geq 2 \times 10^9$/L, haemoglobin (Hb) ≤10 g/dL, and presence of genetic mutations [8, 38, 41, 42]. The first-line treatment for idiopathic HES is corticosteroids, which induces rapid reductions in

Table 46.8 WHO diagnostic criteria for myeloid/lymphoid neoplasms with *PCM1-JAK2*

1. A myeloid or lymphoid neoplasm, often with prominent eosinophilia
2. Presence of t(8;9)(p22;p24.1) or a variant translocation leading to *JAK2* rearrangement[a]

BCR breakpoint cluster region, *ETV6* ETS variant transcription factor 6, *JAK2* janus kinase 2, *PCM1* pericentriolar material 1, *WHO* World Health Organization

[a]Other variants giving rise to a fusion gene between *JAK2* and an alternative partner include *ETV6-JAK2* [t(9;12)(p24.1;p13.2)] or *BCR-JAK2* [t(9;22)(p24.1;q11.2)]

Table 46.9 WHO diagnostic criteria for CEL, NOS

1. Eosinophilia (eosinophil count >1.5 × 10^9/L)
2. Not meeting WHO criteria for *BCR-ABL1*-positive CML, PV, ET, PMF, CNL, CMML, or atypical CML
3. No rearrangement of *PDGFRA*, *PDGFRB*, or *FGFR1*; no *PCM1-JAK2*, *ETV6-JAK2*, or *BCR-JAK2* fusion gene
4. The blast cell count in the peripheral blood and BM is less than 20%, and inv (16)(p13.1q22), t(16;16)(p13;q22) and other diagnostic features of AML are absent
5. Presence of clonal cytogenetic or molecular genetic abnormality, or blast cells are ≥2% in the peripheral blood or > 5% in the BM

AML acute myeloid leukaemia, *BCR* breakpoint cluster region, *BM* bone marrow, *CEL, NOS* chronic eosinophilia leukaemia, not otherwise specified, *CML* chronic myeloid leukaemia, *CMML* chronic myelomonocytic leukaemia, *CNL* chronic neutrophilic leukaemia, *ET* essential thrombocythaemia, *ETV6* ETS variant transcription factor 6, *FGFR* fibroblast growth factor receptor, *JAK2* janus kinase 2, *PCM1* pericentriolar material 1, *PDGFRA* platelet-derived growth factor receptor alpha, *PDGFRB* platelet-derived growth factor receptor beta, *PMF* primary myelofibrosis, *PV* polycythaemia vera, *WHO* World Health Organization

Table 46.10 WHO diagnostic criteria for idiopathic HES

Exclusion of the following:
1. Reactive eosinophilia
2. Lymphocyte-variant HE (cytokine-producing, immunophenotypically-aberrant T-cell population)
3. CEL, NOS
4. WHO-defined myeloid malignancies-associated eosinophilia (e.g. MDS, MPNs, MDS/MPNs, systemic mastocytosis, or AML)
5. Eosinophilia-associated MPNs or AML/ALL with rearrangements of *PDGFRA*, *PDGFRB*, or *FGFR1* or with *PCM1-JAK2*
6. The absolute eosinophil count of >1.5 × 10^9/L must persist for at least 6 months, and tissue damage must be present. If there is no tissue damage, idiopathic HE is the preferred diagnosis

ALL acute lymphoblastic leukaemia, *AML* acute myeloid leukaemia, *CEL, NOS* chronic eosinophilic leukaemia, not otherwise specified, *FGFR1* fibroblast growth factor receptor 1, *HE* hypereosinophilia, *HES* hypereosinophilic syndrome, *MDS* myelodysplastic syndrome, *MPN* myeloproliferative neoplasms, *PCM1-JAK2* pericentriolar material 1-janus kinase 2, *PDGFRA* platelet-derived growth factor receptor alpha, *PDGFRB* platelet-derived growth factor receptor beta

Table 46.11 Causes of secondary eosinophilia

Non-neoplastic
• Infections
Viral
Bacterial
Fungal
Aspergillosis
Parasitic
Helminths (e.g. *Strongyloides stercoralis)*
Toxocariasis
Scabies
• Allergic diseases
Asthma
Atopic dermatitis
• Autoimmune conditions
• Acute/chronic graft versus host disease
• Collagen vascular disease
Churg-Strauss syndrome
Wegener's granulomatosis
Systemic lupus erythematosus
• Metabolic
Adrenal insufficiency
• Organ-specific eosinophilic conditions
Pulmonary eosinophilic conditions
Allergic gastroenteritis
• Lymphocyte-variant hypereosinophilic syndrome
• Rare syndromes associated with eosinophilia
Familial eosinophilia
Hyper IgE syndrome
Omenn syndrome
Gleich's syndrome
Eosinophilia-myalgia syndrome
Drug related
Neoplastic
• Hodgkin lymphoma and other clonal T cell disorders
• Solid organ malignancies

eosinophil counts in most patients [1]. Hydroxyurea is another first-line agent which also offers therapeutic benefit in patients with steroid resistance [1]. Finally, IFN-α can be utilized as a steroid-sparing agent to prevent significant adverse effects in patients requiring higher dosage [1]. It may also be used as a combination therapy with steroids [1]. Anti-IL-5 and Anti-IL-5 receptor antibodies, such as mepolizumab and benralizumab, are currently in studied in clinical trials as novel therapy and have shown encouraging results [1].

46.4 Secondary Eosinophilia

Apart from haematological neoplasms, eosinophilia can also occur due to a myriad of secondary, or reactive, causes. The mechanism of eosinophilia in secondary causes is cytokine-related [43]. Cytokines, such as interleukin (IL)-5, IL-3, and granulocyte/macrophage colony-stimulating factor (GM-CSF), are secreted by multiple cell types, such as T cells, mast cells, and stromal cells [43]. They then bind to the surface receptors of eosinophils and their progenitors to stimulate eosinophilic differentiation, proliferation, survival, and activation [43]. Therefore, any conditions resulting in increased secretions of these growth factors can give rise to eosinophilia.

Table 46.11 describes the secondary causes of eosinophilia. Elevated IgE is a typical finding among patients with secondary eosinophilia [1]. Infections, especially helminthic infections, are common causes of eosinophilia and are particularly relevant in developing countries [1]. This illustrates the importance of a detailed travel history, which provides guidance on potential types of causative parasites. Subsequent testing for parasites and ova, serum antibody, and stool culture should be carried out [1, 5]. Appropriate anti-parasitic therapies should be initiated upon positive testing results [5]. Allergic drug reactions are another common cause of eosinophilia, which necessitates a detailed drug history [5]. If eosinophilia is associated with organ damage, clinically suspicious agents (e.g. antibiotics, anti-convulsants) should be discontinued if possible [5]. Evaluation for autoimmune conditions involves serological testing for immune markers, such as ANA and ANCA, as well as other disease-specific testing guided by patient presentation [1]. Atopic conditions also frequently cause eosinophilia and should be excluded [1]. Regarding eosinophilic pulmonary conditions, lung function tests, bronchoscopy, as well as various serological tests (e.g. IgE against aspergillus) are useful for establishing diagnosis [1]. Hormone levels should be measured to exclude metabolic conditions, such as adrenal insufficiency [1]. Risk factors of malignancies, such as smoking and previous chemotherapies, should be enquired. In addition, serological tests for tumour markers, imaging studies, as well as genetic testing for clonal markers should be carried out to exclude any solid-organ or haematological neoplasms [1]. Finally,

serological biochemistry, imaging studies (e.g. thoracic and abdominal CT scan), as well as cardiac assessment (e.g. ECG, echocardiogram, troponin levels) should be routinely carried out for patients with eosinophilia [5].

Lymphocyte-variant hypereosinophilic syndrome is a rare condition caused by excessive cytokine secretion by abnormal T cells [1]. Cutaneous manifestations and involvement of other organ systems are prominent features of this entity [1]. For patients with clonal T cells associated with an abnormal immunophenotype or cytokine production, corticosteroids are the first-line treatment with a high response rate [1]. However, patients may require subsequent addition of other steroid-sparing agents [1]. IFN-α or other steroid-sparing agents can be used for relapsed or refractory diseases [1]. Limited efficacies have been observed with hydroxyurea and imatinib therapies [1]. Novel therapies, such as anti-IL-5 or anti-IL-5 receptor antibodies, may be beneficial but warrant further investigations [1].

46.5 Systemic Mastocytosis

Systemic mastocytosis (SM) is a disease originating from proliferation and accumulation of abnormal clonal mast cells (MCs) in multiple organ systems [44]. Broadly, mastocytosis can be divided into cutaneous mastocytosis (CM) and systemic mastocytosis (SM) [44]. The diagnostic criteria of various types of mastocytosis are listed in Table 46.12. CM is characterized by mastocytic infiltration of only the skin and not other organ systems [44]. It is more common in children and can be further classified into maculopapular CM or urticaria pigmentosa (UP), diffuse CM, and localized mastocytoma of skin [44]. CM carries a good prognosis and it is common for cutaneous lesions to resolve once children reach puberty [44]. It should be noted that marrow involvement is present in all types of mastocytosis, including CM.

SM is diagnosed usually in adults and generally follows a more aggressive clinical course than CM [45]. The diagnostic criteria are listed in Table 46.13. The most prominent feature in SM is mastocytic infiltration of multiple internal organs and invariable involvement of the bone marrow [45]. Pathogenesis of SM is highly related to *KIT* TKD mutations leading to a gain of function, with *KIT*D816V being the most common aberration [45]. Clinically, patients present with constitutional symptoms, mast cells degranulation symptoms (e.g. pruitus, urticaria, angioedema, nausea, vomiting, diarrhoea, episodic anaphylactoid attacks), skin disease (e.g. UP), as well as bone diseases (e.g. osteoporosis, osteopenia, pathologic fractures) [45]. Laboratory findings may include anaemia, thrombocytopenia, and eosinophilia [45].

SM can be categorized into five subtypes: indolent SM (ISM), smouldering SM (SSM), Aggressive SM (ASM), SM with an associated haematologic neoplasm (SM-AHN), and mast cell leukaemia (MCL). While prognosis is dependent on the exact subtype, adverse prognostic indicators include old age, weight loss, hypoalbuminemia, high serum alkaline phosphatase, anaemia, thrombocytopaenia, advanced SM, high marrow burden of blast cells, and poor risk mutations (e.g. ASXL1, NRAS) [45, 46]. Two risk stratification models have been developed for SM by the Mayo Clinic (Table 46.14) [47]. The clinical risk model is based on clinical and laboratory findings, while the clinical–molecular risk model also includes the presence of poor risk mutations as an indicator for poor survival [47]. Treatment for each subtypes of SM also varies.

Almost half of all SM cases can be classified as ISM, which typically presents at a younger age (median age: 47) [45, 46]. Skin lesions mimicking appearance of UP, gastrointestinal disturbance, and MC mediator release symptoms (MCMRS) are common features [45]. Constitutional symptoms and hepatosplenomegaly are only present in less than 20% of patients with ISM [45].

Table 46.12 WHO classification of mastocytosis

Cutaneous mastocytosis (CM)
• Urticaria pigmentosa/Maculopapular cutaneous mastocytosis
• Diffuse cutaneous mastocytosis
• Solitary mastocytoma of skin
Indolent systemic mastocytosis (ISM) [a]
• Meets criteria for SM
• No "C" findings
• No evidence of associated hematological neoplasm
Isolated bone marrow mastocytosis (provisional entity) [a]
• As above (ISM), but with bone marrow involvement and no skin involvement, generally low burden of MC
Smouldering systemic mastocytosis (SSM) [a]
• As above (ISM), but with two or more "B" findings, and no "C" findings, generally high burden of MC
Systemic mastocytosis with an associated haematological neoplasm (SM-AHN) [a]
• Meets criteria for SM and criteria for AHN as a distinct entity per the WHO classification
Aggressive systemic mastocytosis (ASM)[a]
• Meets criteria for SM
• One or more "C" findings
• No evidence of mast cell leukaemia
Mast cell leukaemia (MCL)[a]
• Meets criteria for SM
• Bone marrow biopsy shows diffuse infiltration, usually dense, by atypical, immature mast cells. BM aspirate smears show ≥20% mast cells
• In classic cases, mast cells account for ≥10% of peripheral blood white cell
• Aleukemic MCL variant (<10% circulating mast cells)
Mast cell sarcoma (MCS)
• No evidence of SM
• Generally localized destructive growth pattern
• High-grade cytology

MC mast cell, *SM* systemic mastocytosis, *WHO* World Health Organization

[a] Subtypes of systemic mastocytosis

Table 46.13 WHO diagnostic criteria for systemic mastocytosis

Major criterion
1. Multifocal, dense infiltrates of mast cells (≥15 mast cells in aggregates) detected in sections of bone marrow and/or other extracutaneous organs
Minor criteria
1. In biopsy sections of bone marrow or other extracutaneous organs, >25% of the mast cells in the infiltrate are spindle-shaped or have atypical morphology or, of all mast cells in bone marrow aspirate smears, >25% are immature or atypical
2. Detection of an activating point mutation at codon 816 of KIT in bone marrow, blood, or other extracutaneous organ
3. Mast cells in bone marrow, blood, or other extracutaneous organ express CD25 with/without CD2 in addition to normal mast cell markers
4. Serum total tryptase persistently exceeds 20 ng/mL (unless there is an associated myeloid neoplasm, in which case this parameter is not valid)
Diagnosis of systemic mastocytosis
• One major criterion and one minor criterion, or
• At least three major criteria
"B" findings
1. High mast cell burden shown on BM biopsy: >30% infiltration of cellularity by mast cells (focal, dense aggregates) and serum total tryptase level > 200 ng/mL
2. Signs of dysplasia or myeloproliferation, in non-mast cell lineage(s), but insufficient criteria for definitive diagnosis of an associated hematological neoplasm (AHN), with normal or only slightly abnormal blood counts
3. Hepatomegaly without impairment of liver function, palpable splenomegaly without hypersplenism, and/or lymphadenopathy on palpation or imaging
"C" findings
1. Bone marrow dysfunction caused by neoplastic mast cell infiltration, manifested by ≥1 cytopenia(s) (ANC <1.0 × 10^9/L, Hb <10 g/dL, and/or platelet count <100 × 10^9/L)
2. Palpable hepatomegaly with impairment of liver function, ascites, and/or portal hypertension
3. Skeletal involvement with large osteolytic lesions with/without pathological fractures (pathological fractures caused by osteoporosis do not qualify as a "C" finding)
4. Palpable splenomegaly with hypersplenism
5. Malabsorption with weight loss due to gastrointestinal mast cell infiltrates

ANC absolute neutrophil count, BM bone marrow, Hb haemoglobin, KIT cluster of differentiation 177

Table 46.14 Risk stratification models for systemic mastocytosis

Risk factors	Survival outcomes (median survival)
Clinical risk model	
Age > 60 years ASM (vs ISM/SSM) Thrombocytopenia (platelet <150 × 10^9/L) Anaemia (below sex adjusted normal) Increased ALP	No risk factor: NR, (5 year survival: 98%) 1 risk factor: NR, (5 year survival: 55%) 2 risk factors: 148 months 3 risk factors: 56 months 4 risk factors: 27 months 5 risk factors: 9 months
Clinical–molecular risk model	
Age > 60 years ASM (vs ISM/SSM) Thrombocytopenia (platelet <150 × 10^9/L) Increased ALP Adverse mutations (*ASXL1, RUNX1, NRAS*)	Low risk (≤2 points): 198 months Intermediate-1 risk (3 points): 85 months Intermediate-2 risk (4 points): 36 months High risk (5 points): 12 months

ASM aggressive systemic mastocytosis, *ISM* indolent systemic mastocytosis, *NR* not reached, *SSM* smouldering systemic mastocytosis

Table 46.15 Symptomatic management in systemic mastocytosis

Mast cell (MC) degranulation
• Symptom burden assessment
• Pruritus/flushing
First line: Histamine receptor (H)-1 antagonists (cetirizine, fexofenadine, hydroxyzine)
Second line: leukotriene antagonists (montelukast, zafirlukast)
Third line: Non-steroidal anti-inflammatory drugs (aspirin), Psolaren plus ultraviolet A (PUVA) photochemotherapy
• Abdominal pain, cramping, gastrointestinal disturbances
First line: H2 antagonists (ranitidine, famotidine, cimetidine)
Second line: proton pump inhibitors (omeprazole, pantoprazole, rabeprazole)
Third line: sodium cromolyn
Fourth line: corticosteroids (prenisone)
• Headache, cognitive impairment, depression
First line: H1 and H2 antagonist
Second line: sodium cromolyn
• Recurrent hypotension
First line: epinephrine
Second line: H1 and H2 antagonist
Third line: corticosteroids (prenisone)
Fourth line: cytoreduction (interferon-alfa (IFN-α), Cladribine)
Bone disease
• Osteoporosis/osteopenia
• Bone mineral density assessment
• Calcium and Vitamin D supplement
• Pharmacologic management
First line: bisphosphonates (alendronate, risedronate, pamidronic acid, zolendronic acid)
Second line: cytokine/immunomodulatory agent (IFN-α)
Third line: purine nucleoside analogue (Cladribine)

SSM usually affects older patients [46, 48]. The less favourable outcome of SSM as compared to ISM in conjunction with increased risks of acute transformation can be attributed to the prevalence of other adverse features, such as increased marrow burden of blast cells, anaemia, and thrombocytopenia [45, 46, 48]. Hepatosplenomegaly and high tryptase level are also frequent in SSM [48].

Isolated bone marrow mastocytosis (BMM) is a provisional entity introduced by the WHO in 2019 and is characterized by a low burden of MCs [45]. BMM is associated with a favourable prognosis despite the prevalence of MCMRS [45].

For ISM and SSM, management of MC degranulation as well as skin and bone diseases are the major goals of treatment [45]. Symptom burden should be assessed and followed by appropriate therapy against specific signs and symptoms (Table 46.15) [45]. Triggers to MC degranulation should be averted, including aspirin, narcotics, anaesthetics, contrast dye, and alcohol [45]. Given the potential need of surgical

interventions which require anaesthesia, a comprehensive perioperative assessment should be performed prior to surgery for reviewing patient status, surgical history, and for selection of an optimal anaesthetic agent [45].

SM with an associated haematological neoplasm (SM-AHN) is found in 40% of SM patients and is the second most common subtype after ISM [45]. The majority of patients have concurrent myeloid malignancies while examples of other less common neoplasms include lymphomas and myelomas [45]. Interestingly, eosinophilia is frequently identified and is particularly common in patients with MPN-phenotypes but does not affect prognosis [45]. SM-AHN confers poor prognosis and reduced survival, with AML and MDS patients exhibiting worst outcomes and MPN patients with the best [45]. Leukaemic survival is most common among patients with associated MDS [45].

Given the presence of two concurrent haematological neoplasms, investigations on their clinical, histologic, and molecular characters should be commenced to determine the urgency of treatment towards each neoplasm [45]. For example, if the associated neoplasm is aggressive (e.g. AML, poor risk CMML) while SM is of low burden or an incidental finding, the AHN should first be managed according to standard of care while SM should be managed with symptomatic control (Table 46.15) [45]. However, if the AHN is indolent (e.g. PV, ET) while SM is associated with organ dysfunction, the patient should be treated urgently as ASM while AHN should be managed with observation or according to standard of care [45]. In the unfortunate case of disease progression, re-staging should be performed for determining the major neoplasm contributing to progression, with salvage treatment (e.g. targeted therapy, allo-HSCT) given accordingly [45].

Aggressive SM (ASM) is the third most common class of SM and, as its name suggests, is associated with a higher risk of leukaemic transformation and inferior outcome [45]. Adverse risk factors, such as old age, constitutional symptoms, anaemia, thrombocytopenia, and elevated tryptase level, are frequently present [45, 46].

Mast cell leukaemia (MCL) is a rare entity associated with dismal outcome. It is distinguished from ASM by a marrow blast count of at least 20% [45]. Circulating MCs are frequently identified and their percentage can be used to further classify MCL into two subtypes: classic MCL (≥10%) and aleukaemic variant of MCL (<10%) [44]. Chronic MCL is characterized by the lack of significant organ damage as indicated by C findings [44]. MCL can be primary but secondary causes such as progression from ASM and MC sarcoma are also possible [44, 45].

In patients with advanced SM, intensive treatment to improve survival outcomes is crucial. Midostaurin is an FDA-approved, first-line agent proven to induce remissions and symptomatic improvement in the majority of SM-AHN, ASM, and MCL patients [45]. It is also effective in patients with resistance to other cytoreductive therapies and is currently studied as post-transplant maintenance therapy [45]. However, due to the broad spectrum activity of this agent towards other tyrosine kinases, incidences of adverse events are high but still manageable [45].

Cladribine is another first-line agent in SM and is efficacious against all subtypes of SM [45]. It is especially useful in patients who require prompt MC cytoreduction when compared to midostaurin [45].

Interferon-alfa (IFN-α) and pegylated IFN formulations can be given in conjunction with corticosteroids (e.g. prednisone) to enhance responses [45]. It demonstrated efficacy against all subtypes of SM and is beneficial for symptomatic management as well as bone disease [45]. However, their clinical use is limited by slow rates of responses, high risks of relapse after cessation, and frequent adverse effects [45].

Avapritinib is an FDS-approved mutant *KIT* tyrosine kinase inhibitor which induced remissions and provided survival benefits for patients with advanced SM [49]. However, this agent should not be administered to patients with platelet counts of $<50 \times 10^9$/L [49].

Imatinib is FDA-approved for *KITD816V*-negative patients or those with unclear *KIT* mutational status [45]. It is also effective for patients with *FIP1l1-PDGFRA*+ eosinophilia but is of limited use in other cases of SM [45].

Hydroxyurea is a non-selective cytoreductive agent mainly used for count control and control of hepatosplenomegaly [45]. For patients with relapsed or refractory SM, allo-HSCT should be considered [45]. Finally, clinical trials with novel agents may also improve outcome of patients. For example, ripretinib is another mutant *KIT* tyrosine kinase inhibitor with promising activity in early studies [45].

46.6 Summary

In conclusion, our knowledge of the complex category of eosinophilic disorders has experienced major breakthroughs during the previous decades. Our current diagnostic and treatment algorithms have improved the identification and outcomes of a number of eosinophilic entities (Fig. 46.1) [1, 5]. However, numerous aspects on terminology, pathogenesis, and diagnosis still remain unclear, with a number of entities still having dismal prognosis. The future direction of eosinophilic disorder should involve establishment of specialized and international registries as well as multidisciplinary treatment facilities. With persistent endeavours in studying these disorders, new advancements may soon drastically improve disease outcomes.

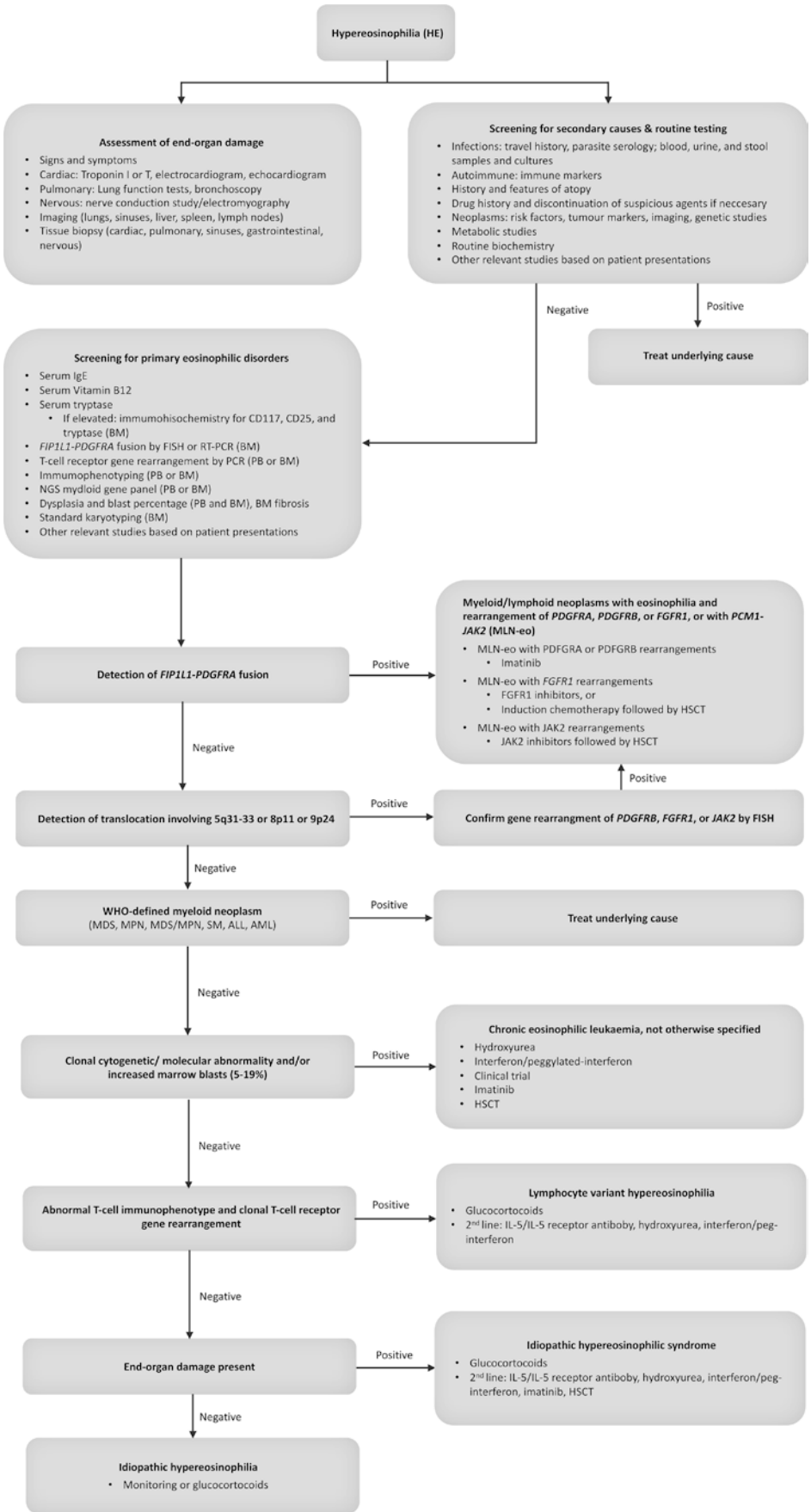

Fig. 46.1 Summary of diagnosis and treatment of eosinophilic disorders. ALL acute lymphoblastic leukaemia, AML acute myeloid leukaemia, BM bone marrow, CEL, NOS chronic eosinophilic leukaemia, not otherwise specified, *FGFR1* fibroblast growth factor receptor 1, *FIP1L1* FIP1-like 1, HSCT haematopoietic stem cell transplantation, IL-5 interleukin-5, *JAK2* janus kinase 2, MDS myelodysplastic syndrome, MPN myeloproliferative neoplasms, PB peripheral blood, *PDGFRA* platelet-derived growth factor receptor alpha, *PDGFRB* platelet-derived growth factor receptor beta, SM systemic mastocytosis

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In the Pipeline: Emerging Therapy for Classical Ph-Negative MPNs

47

Harinder Gill and Yammy Yung

Abstract

Conventional therapy for myeloproliferative neoplasm (MPN) has modest disease-modifying effect. In this chapter, we discuss the emerging novel agents and approaches that may potentially modify the underlying disease biology in MPN.

Keywords

Polycythaemia vera · Essential thrombocythemia Primary myelofibrosis · Disease modification · Targeted therapy

47.1 Introduction

The treatment of Philadelphia-negative (Ph-negative) myeloproliferative neoplasms (MPNs) emphasizes on three aspects: symptom control, prevention of vascular complications, and disease modification [1–3]. Although both polycythaemia vera (PV) and essential thrombocythemia (ET) confer excellent prognosis and survival with early treatment interventions [4, 5], standard treatment options of myelofibrosis (MF) failed to rectify disease progression effectively. Ruxolitinib, a standard and commonly used drug for intermediate- and high-risk MF patients, remains ineffective in modifying disease status despite outstanding symptom control in reducing splenomegaly and constitutional symptoms [1, 6, 7]. The use of allogeneic haematopoietic stem cell transplantation (Allo-HSCT), the only potentially curative therapy in the treatment of MPNs, is a highly selective procedure that could not benefit all patients [8, 9]. Therefore, numerous novel therapies have emerged in hope of improving disease control.

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47.2 Emerging JAK Inhibitors

JAK inhibitors are primarily used for the control of cytokine-mediated symptom complex and splenomegaly (Fig. 47.1). Ruxolitinib is a non-selective JAK1/2 inhibitor that inhibits the constitutive activation of the JAK/STAT signalling pathway, hence decreases splenomegaly and constitutional symptoms by reducing the production of proinflammatory cytokines [10]. However, its use is limited by several factors, including resistance, intolerance, and refractoriness to treatment, which can be developed during the initiation of ruxolitinib or developed overtime [10–12]. Due to disease heterogeneity of MF, a consensus definition of ruxolitinib failure has not been reached [13–15], but it is agreed that suboptimal response of spleen (<10% spleen volume reduction (SVR) or < 30% SVR from baseline), rebound of spleen size, increased constitutional symptoms, and development of transfusion dependence warrant attention [11, 14, 15]. Novel JAK inhibitors have been developed and their mechanisms of action, outcomes, and specific side effects are illustrated below and in Table 47.1.

47.2.1 Fedratinib

Fedratinib is a newly FDA-approved JAK2/FLT3 inhibitor for treating intermediate-2 and high-risk MF patients, as well as patients who are refractory or resistant to ruxolitinib [2, 8, 12]. It has a unique conformation that allows anchoring to the ATP—and peptide substrate-binding pocket of JAK2 kinase, hence inhibiting the constitutive JAK/STAT activation [8, 16]. Due to its dual inhibitory activity, genetic resis-

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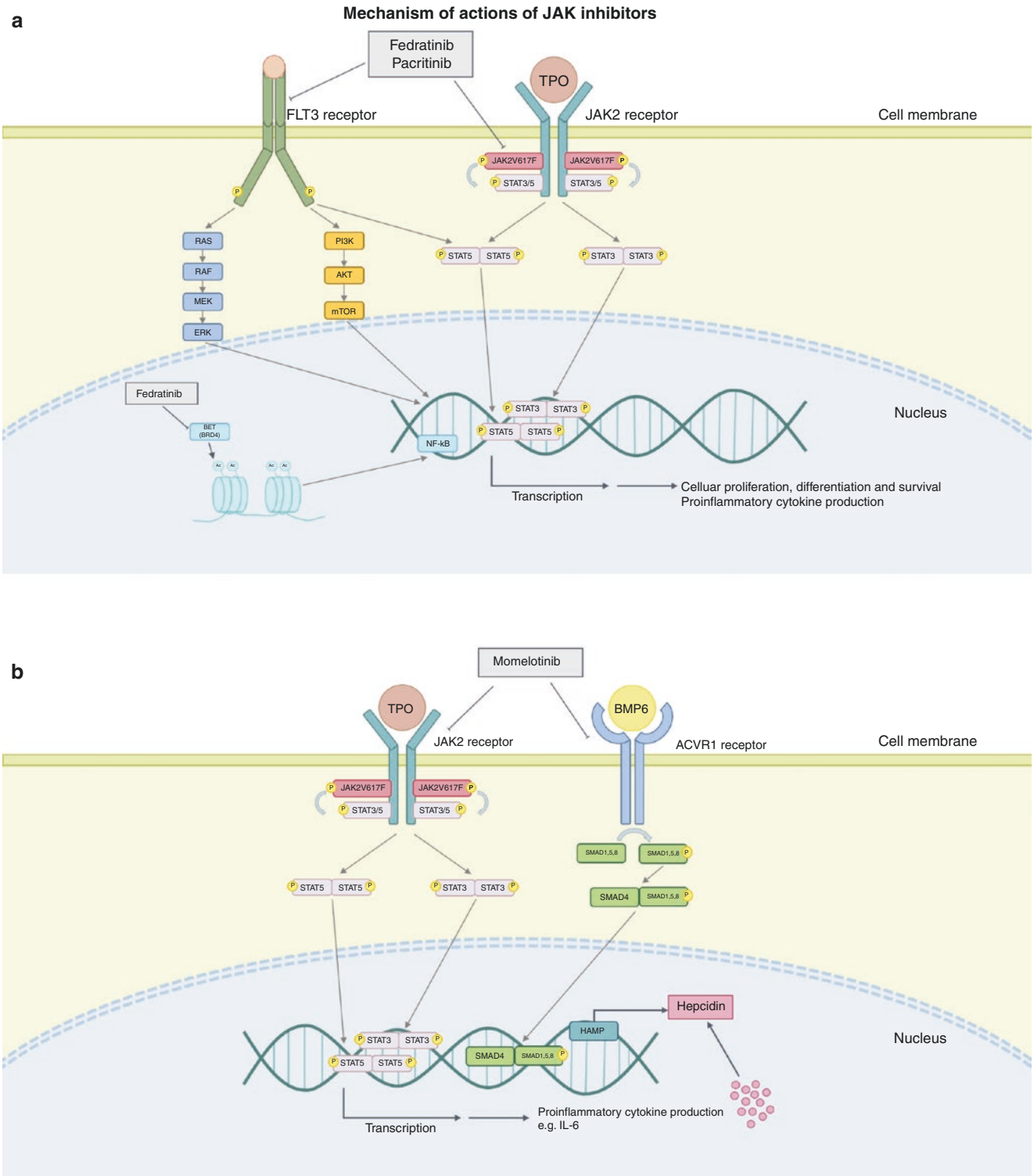

Fig. 47.1 Mechanism of actions of JAK inhibitors. (**a**) shows the mechanism of actions of fedratinib and pacritinib in inhibiting aberrant JAK2 and FLT3 activation. The off-target BRD4 inhibition of fedratinib is also demonstrated. (**b**) shows the mechanism of actions of momelotinib in inhibiting JAK2 and ACVR1 receptor. (**c**) highlights the pathway of alleviation of anaemia by momelotinib. Reduced hepcidin expression in hepatocytes restores iron sequestration by reticuloendothelial macrophages and duodenal iron absorption. Thus, serum iron level is increased to promote erythropoiesis. ACVR1, activin A receptor, type I; AKT, protein kinase B; BET, bromodomain and extraterminal domain; BMP6, bone morphogenic protein 6; BRD4, bromodomain 4; IL-6, interleukin 6; ERK, extracellular signal-regulated kinases; FLT3, FMS-like tyrosine kinase 3; HAMP, hepcidin antimicrobial peptide; JAK, janus kinase; MEK, mitogen-activated protein kinase kinase; mTOR, mechanistic target of rapamycin; NF-κB, nuclear factor kappa-light-chain enhancer of activated B cells; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; RAF, rapidly accelerated fibrosarcoma; STAT, signal transducer and activator of transcription; TPO, thrombopoietin

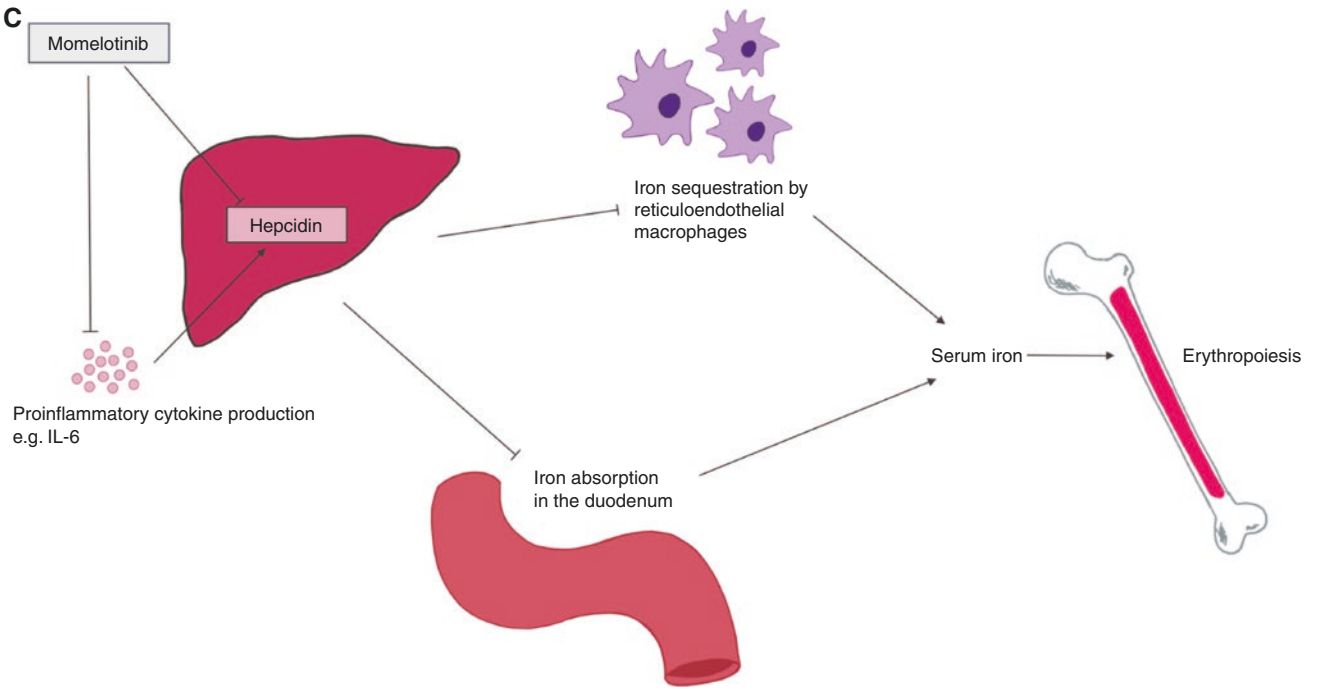

Fig. 47.1 (continued)

tance is less likely, which provides a possible solution to ruxolitinib resistance [8, 16]. In addition, it preferentially binds to JAK2 with a low binding affinity to other JAK family members such as JAK1, JAK3, and tyrosine kinase 2 (TYK2) that are involved in immune regulation [8, 12, 14, 16–18]. Hence, fedratinib has a less immunosuppressive effects than that of ruxolitinib [12].

Fedratinib also possesses off-target inhibitory effect against FMS-like tyrosine kinase 3 (FLT3) and bromodomain 4 (BRD4). It inhibits both wild-type and mutant FLT3, which are involved in the PI3K/AKT, MAPK, and STAT3 signalling pathways. The off-target inhibition of FLT3 helps reduce cellular proliferation and inflammatory dysmegakaryopoiesis, a notable feature when MF progresses to blast phase [8]. Yet, it is important to note that FLT3 mutation is mutually exclusive with JAK2V617F mutation, the most common driver gene mutation in classical Ph-negative MPNs [8, 19, 20]. Thus, this limits the usefulness of FLT3 inhibition. Besides FLT3 suppression, fedratinib acts against BRD4 epigenetically [8]. BRD4 is a member of the BET protein family that enhances proinflammatory NF-κB to increase the release of proinflammatory cytokines. As JAK/STAT pathway also activates NF-κB, blockade of BRD4 is a synergistic mechanism to attenuate NF-κB hyperactivation, resulting in diminution of cytokine production [21, 22] (Table 47.1).

It was demonstrated that fedratinib effectively reduced splenomegaly and symptom burden in both ruxolitinib-naïve and ruxolitinib-treated patients in the phase 2 and 3 JAKARTA studies [17, 23, 24]. Fedratinib possesses a longer half-life (41 h) than that of ruxolitinib (3 h), allowing a once-daily dosing with sustainable JAK/STAT inhibition [14]. Wernicke's encephalopathy (WE), a fatal neurological disorder due to thiamine (vitamin B1) deficiency, is a rare, yet severe adverse effect of fedratinib [2, 8, 10–12]. This may be contributed by malnutrition due to drug-induced gastrointestinal toxicity and impairment of thiamine uptake [10, 24]. Therefore, thiamine level should be assessed prior to drug administration and during the course of treatment. Fedratinib should be withdrawn and parenteral thiamine supplementation should be given once WE is suspected [2]. Phase 3 FREEDOM studies are currently underway to evaluate the long-term outcomes of fedratinib [25].

47.2.2 Pacritinib

Pacritinib is a JAK2/FLT3 inhibitor with off-target inhibition against non-tyrosine kinases interleukin 1 receptor-associated kinase 1 (IRAK1) and colony-stimulating factor 1 receptor (CSF1R) [26–30]. Similar to fedratinib, pacritinib suppresses JAK/STAT activation by inhibiting JAK2 wild-type (WT) and mutant JAK2V617F, as well as hampers cellular proliferation by inhibiting FLT3-WT and FLT3-ITD [27, 28]. Its additional inhibitory effect against IRAK1 and CSF1R helps suppress inflammatory pathways rapidly, resulting in early relief of constitutional symptoms [31]. Moreover, it may yield a smaller extent of myelosuppression

Table 47.1 Mechanisms and specific side effects of emerging JAK inhibitors

JAK inhibitor	Mechanism of action	Specific side effects	Latest clinical trial	References
Fedratinib	1. JAK2 inhibition – Block constitutive JAK/STAT activation – Reduce splenomegaly and constitutional symptoms 2. FLT3 inhibition – Suppress PI3K/AKT, MAPK, and STAT3 signalling pathways – Reduce inflammatory dysmegakaryopoiesis 3. BRD4 inhibition – Reduce NF-κB activation – Decrease production of proinflammatory cytokines	1. Dose-dependent GI toxicities (diarrhoea, nausea, vomiting), mostly grade 1–2 2. Blackbox warning: Wernicke's encephalopathy 3. Cytopenia (anaemia, thrombocytopenia)	FREEDOM, phase 3	[2, 8, 12]
Pacritinib	1. JAK2 inhibition – Block constitutive JAK/STAT activation – Reduce splenomegaly and constitutional symptoms 2. FLT3 inhibition – Suppress PI3K/AKT, MAPK, and STAT3 signalling pathways – Decrease cellular proliferation of MPN cells 3. IRAK1 and CSF1R inhibition – Reduce downstream inflammatory pathways – Relieve constitutional symptoms	1. GI toxicities (diarrhoea, nausea, vomiting) 2. Peripheral oedema 3. Cardiac events (prolongation of QTc, heart failure, atrial fibrillation) 4. Bleeding events (epistaxis, intracranial haemorrhage)	PERSIST-1 and 2, phase 3; PACIFICA, phase 3	[26, 27, 29–31]
Momelotinib	1. JAK2 inhibition – Block constitutive JAK/STAT activation – Reduce splenomegaly and constitutional symptoms 2. ACVR1 inhibition – Suppress hepcidin expression – Induce erythropoiesis	1. Neurological events (peripheral neuropathy, peripheral sensory neuropathy) 2. First-dose effect (hypotension, headache, dizziness, nausea) 3. Thrombocytopenia 4. Elevated lipase levels	SIMPLIFY-1 and 2, phase 3	[39, 42, 48, 49]
Ilginatinib (NS-018)	1. Preferential JAK2V617F inhibition over JAK2-WT – Block constitutive JAK/STAT activation – Reduce splenomegaly and constitutional symptoms 2. Src-family kinases inhibition – More complete inhibition of STAT3 phosphorylation by simultaneous Src and JAK2 suppression 3. Reduction of marrow fibrosis in some patients	1. Neurological events (paraesthesia, dizziness), mostly grade 1–2 2. Cytopenia (anaemia, thrombocytopenia)	Phase 1/2	[43–46]
Gandotinib (LY2784544)	1. JAK2 inhibition – Block constitutive JAK/STAT activation – Reduce splenomegaly and constitutional symptoms	1. GI toxicities (diarrhoea, nausea) 2. Nephrotoxicity 3. Hyperuricemia 4. Cytopenia (anaemia, thrombocytopenia)	Phase 2	[50, 51]

ACVR1, bone morphogenic protein receptor kinase activin A receptor, type I; *BRD4,* bromodomain 4; *CSF1R,* colony-stimulating factor 1 receptor; *FLT3,* FMS-like tyrosine kinase 3; *GI,* gastrointestinal; *IRAK1,* interleukin 1 receptor-associated kinase 1; *JAK2-WT,* JAK2-wild type; *MPN,* myeloproliferative neoplasm; *QTc,* corrected QT interval; *Src,* proto-oncogene tyrosine kinase Src

and lower the risks of opportunistic infections due to minimal JAK1 inhibition [27, 28, 30, 31] (Table 47.1).

Pacritinib is noted by its ability to achieve SVR and attenuate constitutional symptoms in patients with low haemoglobin, platelet count, and JAK2V617F allele burden [29, 30, 32]. Anaemia and thrombocytopenia are not only aggravated by ruxolitinib use, but are also observed in MF patients with myelodepletive phenotype [30, 32, 33]. Myelodepletive MF is characterized by a lower JAK2V617F allele burden, more pronounced cytopenia, and less prominent splenomegaly [32]. In general, these patients have an inferior prognosis with limited treatment options due to baseline cytopenia [32]. Pacritinib fills the above treatment void by improving transfusion dependence [29]. Promising results were shown in the phase 3 PERSIST-1

and PERSIST-2 trials [26, 30], and an ongoing phase 3 PACIFICA study is in progress [34].

47.2.3 Momelotinib

Momelotinib is a JAK1/JAK2 inhibitor that displays inhibitory effect against bone morphogenic protein (BMP) receptor kinase activin A receptor, type I (ACVR1) [35–38]. In MPNs, aberrant JAK/STAT activation increases synthesis of proinflammatory cytokines such as interleukin 6 (IL-6), leading to chronic inflammation and increased hepcidin production by hepatocytes. This impedes duodenal iron absorption and iron sequestration by splenic macrophages, resulting in anaemia in MPN patients [35, 38]. An BMP/SMAD intact signalling pathway is required for IL-6/JAK/STAT-induced hepcidin transcription, and this pathway is specifically targeted by momelotinib via the suppression of ACVR1 [35]. Inhibition of ACVR1, a member of the transforming growth factor-beta (TGF-β) family of receptors, results in decreased hepcidin expression, hence stimulating erythropoiesis and mitigating anaemia [35–38] (Table 47.1).

Improved red-cell transfusion dependence and SVR were observed in MF patients in the phase 3 SIMPLIFY studies [36, 37, 39, 40] (Table 47.1), while the efficacy of momelotinib in PV and ET patients is limited [41]. Peripheral neuropathy is a specific adverse effect of momelotinib that is not associated with any specific factors [42]. It is essential to recognize the occurrence of peripheral neuropathy to assess the need of dose reduction/discontinuation.

47.2.4 Other JAK Inhibitors

Several JAK inhibitors such as ilginatinib (NS-018), lestaurtinib (CEP-701), and gandotinib (LY2784544) are developed and evaluated in multiple phase 1/2 clinical trials [43–46]. However, their applications are discouraged by various adverse effects and toxicities [47]. Ilginatinib and gandotinib are two of the JAK inhibitors that successfully demonstrated safety and efficacy in early-phase clinical trials. Their mechanisms and side effects are summarized in Table 47.1.

47.3 Targeting Haematopoietic Stem Cells (HSCs) in MPNs

Eradication of MPN HSCs is an unmet goal in the treatment of MPNs. None of the JAK inhibitors successfully elicit meaningful results in eradicating MPN HSCs as they all lack clonal selectivity [16, 48, 52, 53]. This fosters the development of multiple novel agents, which could be used alone or as combination therapy with standard therapeutic options such as ruxolitinib and interferon-alpha (IFN-α). Details of clinical outcomes are summarized in Table 47.2.

47.3.1 Novel Interferon Preparations

IFN-α, a cornerstone in PV, ET, and lower-risk MF treatment [2], targets MPN stem cells effectively, especially in patients who harbour JAK2V617F mutations [54–56]. Complete molecular remission may even be observed with prolonged IFN-α use [57]. Ropeginterferon α-2b is a novel mono-pegylated interferon that is connected to a proline residue to produce a longer half-life and better tolerability [58–61]. Consistent with preclinical and early-phase clinical trials, durable improvements in haematological parameters, reduction in JAK2V617F allele burden, and decreased vascular complications were corroborated in the phase 3 PROUD-PV and CONTINUATION-PV studies [54, 58–60]. The promising results in PV patients have paved the way to SURPASS ET, a phase 3 study that compares the efficacy of ropeginterferon α-2b with anagrelide in HU-resistant/intolerant ET patients (ClinicalTrials.gov identifiers: NCT04285086).

47.3.2 Telomerase Inhibition

Telomerase is a negative regulator of telomere, a region of DNA sequences that is constantly lost during cell replication. Normally, telomere shortens with increased age and eventually enters replicative senescence. In MPNs, replicative senescence is inhibited despite decreased telomere length (TL), resulting in continuous proliferation of clonal stem cells [62]. Increased telomerase activity was also seen in MPN HSCs. Human telomerase reverse transcriptase (hTERT), a component of telomerase, was upregulated in rapidly dividing cells such as HSCs to increase TL [63, 64]. MPN HSCs express an exceptionally high hTERT activity, leading to uncontrolled myeloproliferation [62, 65].

Imetelstat (GRN163L) is a first-in-class 13-mer oligonucleotide telomerase inhibitor that binds to the RNA component of telomerase in MPN HSCs [63–65], hence suppressing telomerase activity. In preclinical studies, imetelstat impeded malignant colony-forming units-megakaryocytes (CFU-Meg) formation and suppressed megakaryopoiesis [64, 66]. This might explain the reason of bone marrow (BM) fibrosis reversal in MF patients as malignant megakaryocytes are the major contributors in fibrogenic cytokine production [66]. Encouraging results were seen in ET and higher-risk MF patients [67–69]. Marked reduction of mutant allele burden was observed regardless of driver mutations.

Table 47.2 Clinical trial outcomes of therapeutic options that target MPN HSCs

Novel therapy	MPN subtype	Observations and outcome	Major side effects	Clinical trial	Single/combined therapy	References
Telomerase inhibition						
Telomerase inhibitor (imetelstat)	ET	– Significant clinicohaematologic response – Decrease in mutant allele burden regardless of driver mutations due to clonal selectivity	– Myelosuppression (thrombocytopenia, anaemia, neutropenia) – Abnormalities in liver function (raised total bilirubin/ALT/AST levels)	Phase 2	Monotherapy	[67]
	Int-2, high-risk MF	– Molecular response due to selective depletion of MF HSCs/HPCs – Spliceosome mutations may interfere the efficacy of imetelstat – Reversal of BM fibrosis		Pilot study		[63, 68]
		– Better SVR, TSS reduction and increased OS in triple-negative patients		Phase 2		[69, 76]
Epigenetic regulators inhibition						
LSD1 inhibitor (bomedemstat)	Int-2, high-risk PMF and SMF	– Marked reduction in TSS – Modest improvement in splenomegaly – Mild improvement in BM fibrosis score	– No specific DLTs – No progression to sAML	Phase 2a	Monotherapy	[74]
BET inhibitor (CPI-0610)	≥Int-1 MF (JAKi-naïve)	– Improved TSS and SVR	– No specific DLTs – Anaemia, thrombocytopenia – Fatigue – GI events (diarrhoea, nausea) – Upper respiratory tract infections	Phase 2	Combined therapy with ruxolitinib	[77]
	MF (JAKi-treated)	– Improved TSS and SVR – Reduced BM fibrosis – Some anaemia response and improved transfusion dependence		Phase 2	Arm 1: Monotherapy Arm 2: Combined treatment with ruxolitinib	[78]
HDAC inhibitor (givinostat)	PV	– Decreased disease-related symptoms, e.g. pruritus – Reduced JAK2V617F mutant allele burden – Improved SVR	– Thrombocytopenia – GI events (dyspepsia, diarrhoea) – Prolongation of QTc	Phase 1b/2	Monotherapy	[81, 84]
		– High ORR (>80%), especially in the monotherapy arm – Reduced JAK2V617F mutant allele burden		Long-term study of phase 1/2 trial	Monotherapy/in combined therapy with HU	[81, 83]
HDAC inhibitor (panobinostat)	PMF, SMF	– Mild SVR – Modest reduction of JAK2V617F mutant allele burden – Prolonged use of low-dose panobinostat helps increase therapeutic effects and tolerability	– Thrombocytopenia, anaemia – GI events (diarrhoea, constipation, nausea)	Phase 2	Monotherapy	[82]
		– Modest SVR and TSS	– Anaemia – GI events (diarrhoea, nausea)	Phase 1	Combined therapy with ruxolitinib	[85]
Pro-apoptotic agents						
Inhibitor of antiapoptotic proteins of Bcl-2 family (navitoclax)	PMF, SMF	– Reduction of mutant allele burden – Improved TSS, SVR – Decreased BM fibrosis	– Cytopenia (thrombocytopenia, anaemia) – GI events (diarrhoea)	Phase 2	Combined therapy with ruxolitinib	[90, 91]

Table 47.2 (continued)

Novel therapy	MPN subtype	Observations and outcome	Major side effects	Clinical trial	Single/combined therapy	References
SMAC mimetics (LCL-161)	Int to high-risk PMF, SMF	– Haematological improvement with reduced transfusion dependence – Potential novel agent in thrombocytopenic, elderly patients who are resistant to multiple frontline agents	– Fatigue – GI events (nausea, vomiting) – Dizziness, vertigo	Phase 2	Monotherapy	[97–99]
MDM2 inhibitor (idasanutlin)	PV	– Haematological response with reduced frequency of phlebotomy – Improved TSS and SVR	– GI events (nausea, diarrhoea, constipation, abdominal pain)	Phase 1	Part A: Monotherapy Part B (patients without therapeutic response with monotherapy): Combined therapy with peg-IFN-α-2a	[101]
CD123 inhibition (tagraxofusp)	Int, high-risk r/r MF	– Improved SVR and TSS – Especially useful in targeting patients with monocytosis by suppression of CD123	– Cytopenia (thrombocytopenia, anaemia) – Abnormalities in liver function (hypoalbuminemia/raised AST levels)	Phase 1/2	Monotherapy	[112]
HSP90 inhibitor (AUY922)	r/r MPNs	– Significant improvement in SVR – Decreased JAK2V617F mutant allele burden	– Unanticipated ophthalmic events (night blindness, visual blurring, reduced visual acuity) – GI bleeding (ileocecal ulceration)	Phase 2	Monotherapy	[116, 117]
Cell signalling pathway inhibition						
Pan-class 1 PI3K inhibitor (buparlisib)	MF (JAKi-naïve, JAKi-treated)	– Lack of synergistic activity in SVR – Modest reduction of JAK2V617F mutant allele burden – Modest decrease of BM fibrosis	– Thrombocytopenia – Psychiatric events (anxiety, depression)	Phase 1b	Combined therapy with ruxolitinib	[129]

ALT, alanine aminotransferase; *AST,* aspartate aminotransferase; *Bcl-2,* B-cell lymphoma 2; *BET,* bromodomain and extraterminal domain inhibitor; *BM,* bone marrow; *DLT,* dose-limiting toxicities; *ET,* essential thrombocythemia; *GI,* gastrointestinal; *HDAC,* histone deacetylase; *HPC,* haematopoietic progenitor cell; *HSC,* haematopoietic stem cell; *HSP,* heat-shock protein; *HU,* hydroxyurea; *Int-2,* intermediate-2; *JAKi,* JAK inhibitor; *LSD1,* lysine-specific demethylase-1; *MDM2,* mouse double minute 2 homolog; *MF,* myelofibrosis; *MPN,* myeloproliferative neoplasms; *ORR,* overall response rate; *OS,* overall survival; *Peg-IFN-α-2a,* pegylated interferon-alpha-2a; *PMF,* primary myelofibrosis; *PV,* polycythaemia vera; *QTc,* corrected QT interval; *r/r,* relapsed/refractory; *sAML,* secondary acute myeloid leukaemia; *SMAC,* second mitochondria-derived activator of caspases; *SMF,* secondary myelofibrosis; *SVR,* spleen volume reduction; *TSS,* total symptom score

47.3.3 Targeting Epigenetic Regulators

47.3.3.1 Lysine-Specific Demethylase-1 (LSD1) Inhibition

LSD1 is a chromatin-modulating enzyme that plays a functional role in gene transcription via the removal of mono- or dimethyl group on histone lysine residues [70–72], hence maintaining haematopoiesis [73]. In MPN, LSD1 is overexpressed to maintain self-renewal potential of MPN HSCs [70, 73, 74]. LSD1 inhibitor, bomedemstat (IMG-7289), has exemplified outstanding results in reducing cellular proliferation, cytokine-mediated inflammation, BM fibrosis, and mutant allele frequency via apoptotic pathway and cell cycle arrest in preclinical murine models [70]. These were further elucidated in a phase 2a clinical trial in treating primary MF (PMF) and secondary MF (SMF) patients [74] (Table 47.2), and the extended phase 2b study is underway (ClinicalTrials.gov identifiers: NCT03136185). A phase 2 study for ET patients is also in progress (ClinicalTrials.gov identifiers: NCT04254978).

47.3.3.2 Bromodomain and Extraterminal (BET) Protein Inhibition

BET proteins (BRD 2, BRD3, BRD4) are a family of chromatin-reader proteins that binds to acetylated lysine residues on histones to initiate oncogene transcription and proinflammatory NF-κB activation [21, 22, 75, 76]. Preclinical studies demonstrated cross-talk between the JAK/STAT pathway and the epigenetic BET pathway, hence, suggesting BET inhibitors could act in concert with ruxolitinib to abrogate NF-κB signalling and impede downstream proinflammatory cytokine production [21, 22]. The synergistic effect was also observed in post-MPN secondary acute myeloid leukaemia (sAML) stem/progenitor cells [75]. In view of the encouraging preclinical results [22], the phase 2 MANIFEST trials were carried out [77, 78] (Table 47.2). The phase 3 MANIFEST-2 study is currently underway [79] (ClinicalTrials.gov identifiers: NCT04603495).

47.3.3.3 Histone Deacetylase (HDAC) Inhibition

Epigenetic attenuation of histone deacetylation is a possible target in MPN treatment. Overexpressed HDAC, especially in MF patients, catalyses the removal of histone acetyl groups, resulting in condensation of chromatin and silencing of tumour suppressor genes [72, 80, 81]. In MPN, besides restoring active tumour suppressor gene transcription, HDAC inhibitors increase heat-shock protein 90 (HSP90) acetylation to dampen downstream JAK/STAT signalling pathway [80]. HDAC inhibitors have been investigated in early-phase clinical trials, but a high discontinuation rate was seen due to slow onset of therapeutic effects and chronic treatment-related toxicities [80, 82]. Among the available HDAC inhibitors, givinostat was shown to be the most efficacious [81]. Both short-term and long-term safety and efficacy were manifested in the treatment of PV by givinostat [81, 83, 84]. Another pan-HDAC inhibitor, panobinostat was evaluated as monotherapy or in combined therapy with ruxolitinib in early-phase clinical trials [82, 85] (Table 47.2).

47.3.3.4 Protein Arginine Methyltransferase 5 (PRMT5) Inhibition

PRMT5 plays a functional role in post-translational protein modification by adding methyl groups to arginine residues on histones [86]. In MPN, PRMT5 is overexpressed and aberrantly phosphorylated by JAK2V617F. This hampers the histone methylation activity in PRMT5, resulting in myeloproliferation [72, 86, 87]. Early preclinical studies demonstrated C220, a PRMT5 inhibitor, is useful in abrogating erythroid progenitors, restoring splenic architecture, as well as depleting cytokine levels [86]. Synergism with ruxolitinib is manifested in both JAK2V617F and MPLW515L murine models [86]. This paved the way to the ongoing phase 1 clinical trial of PRT543 in relapsed/refractory (r/r) MF patients (ClinicalTrials.gov identifiers: NCT03886831).

47.3.4 Induction of Pro-apoptotic Pathway

47.3.4.1 B-Cell Lymphoma-Extra Large (BCL-xL) Inhibition

BCL-xL, a member of the B-cell lymphoma 2 (Bcl-2) family, possesses antiapoptotic functions and is most highly expressed in MF, followed by PV and ET regardless of JAK2 mutational status [88]. Synergism between BCL-xL inhibitors and ruxolitinib was demonstrated in preclinical trials as ruxolitinib impairs the upstream JAK/STAT pathway while BCL-xL inhibitor targets the subsequent effector phase, hence activating apoptosis [88, 89] (Fig. 47.2). This was further elucidated in the phase 2 study on PMF and SMF patients which navitoclax (ABT-263), an orally available antagonist of antiapoptotic protein of Bcl-2, worked in concert with ruxolitinib [90, 91] (Table 47.2). In the light of the favourable results, the phase 3 TRANSFORM-2 trial was commenced to compare the combined treatment with best-available therapy (BAT) in r/r MF patients [92] (ClinicalTrials.gov identifiers: NCT04468984).

47.3.4.2 Second Mitochondria-Derived Activator of Caspase (SMAC) Mimetics

SMAC ubiquitinylates and induces proteasomal degradation of the overexpressed inhibitor of apoptosis proteins (IAP) in MPN patients [93–96]. SMAC mimetics antagonize IAP and induce apoptosis of MPN HSCs (Fig. 47.2). A unique feature of SMAC mimetics is that they do not work in concert with ruxolitinib because the sensitivity of JAK2V617F clonal HSCs to SMAC mimetics depends on the aberrant JAK2 signalling [95]. LCL-161, a novel SMAC mimetic, was evaluated for MF patients in phase 2 clinical trials [97–99]. It was shown that LCL-161 could be a potential therapeutic agent for thrombocytopenic r/r elderly MF patients who have limited therapeutic options (Table 47.2).

47.3.4.3 Murine Double Minute 2 (MDM2) Inhibition

MDM2 negatively regulates and degrades TP53, a well-known tumour suppressor gene by ubiquitination [100–102]. In MPN, the overexpressed MDM2 can be targeted by MDM2 inhibitors, also termed as "nutlins," to restore TP53 activity and eradicate MPN HSCs [100] (Fig. 47.2). Apoptosis of MPN HSCs could be potentiated in combination with IFN-α. Among them, idasanutlin has demonstrated promising results in preclinical trials via selective elimination of PV and PMF JAK2V617F$^+$ HSCs. It has been evaluated in a phase 1 clinical study in PV patients. Improvements in haematological parameters and reduction of disease-related symptoms and BM fibrosis were demonstrated [101] (Table 47.2). Several clinical trials in PV, PMF, and sAML are also underway (ClinicalTrials.gov identifiers: NCT03669965, NCT03662126, and NCT04113616).

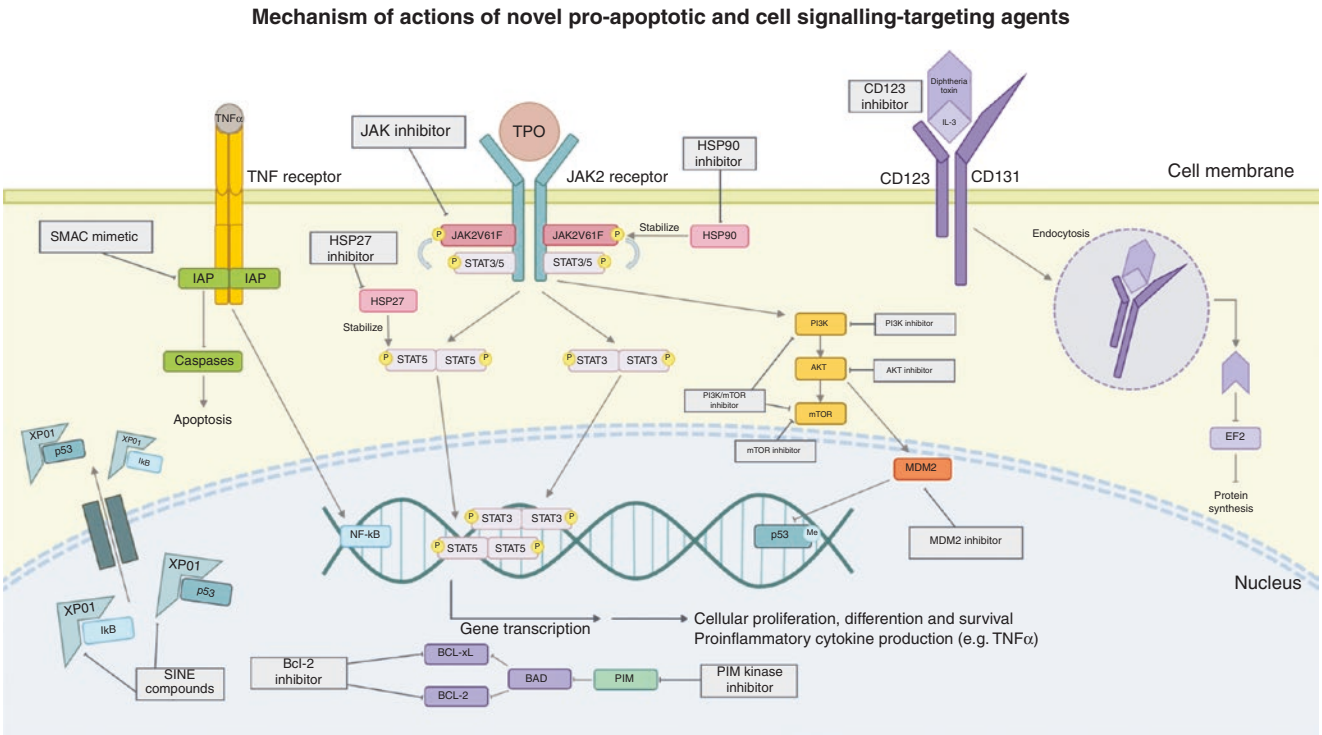

Fig. 47.2 Mechanism of actions of novel pro-apoptotic and cell signalling-targeting agents. AKT, protein kinase B; BAD, BCL2-antagonist of cell death; BCL-2, B-cell lymphoma 2; BCL-xL, B-cell lymphoma-extra large; EF2, elongation factor 2; HSP, heat-shock protein; IAP, inhibitor of apoptosis proteins; IκB, inhibitor of nuclear factor kappa B; IL-3, interleukin 3; JAK, janus kinase; MDM2, murine double minute 2; mTOR, mechanistic target of rapamycin; NF-κB, nuclear factor kappa-light-chain enhancer of activated B cells; PI3K, phosphatidylinositol-4, 5-bisphosphate 3-kinase; PIM, proviral integration site for moloney murine leukaemia virus; SINE, selective inhibitors of nuclear export; SMAC, second mitochondria-derived activator of caspase; STAT, signal transducer and activator of transcription; TNF, tumour necrosis factor; TPO, thrombopoietin; XPO1, exportin 1

However, it should be noted that persistent gastrointestinal toxicities were encountered by most patients. This contributed to early discontinuation and termination of the clinical trial [101] (ClinicalTrials.gov identifiers: NCT03287245). Moreover, idasanutlin-treated PV patients may have a temporary expansion of TP53 mutant subclones which warrants close monitoring and further validation [102].

47.3.4.4 Exportin 1 (XPO1) Inhibition

XPO1, also known as chromosomal region maintenance 1 (CRM1), is responsible for exporting essential tumour suppressor genes such as p53 and inhibitor of nuclear factor kappa B (IκB) from the nucleus to the cytoplasm [103, 104]. This interrupts the functions of tumour suppressor genes by the prohibition of nuclear localization [103] (Fig. 47.2). Selective inhibitors of nuclear export (SINE) compounds antagonize XPO1, preferentially depleting MF HSCs, as well as augmenting the activity of ruxolitinib in ruxolitinib-naïve and ruxolitinib-resistant cell lines [103]. This paved the way to the ongoing phase 2 ESSENTIAL study, which evaluates the safety and efficacy of selinexor in ruxolitinib-resistant/-intolerant PMF and SMF patients (ClinicalTrials.gov identifiers: NCT03627403).

47.3.4.5 Proviral Integration Site for Moloney Murine Leukaemia Virus (PIM) Kinase Inhibition

PIM kinase is a serine/threonine kinase that is regulated by cytokines produced in the JAK/STAT signalling pathway [105, 106]. It plays a functional role in cellular proliferation and apoptosis evasion by mediating various cellular pathways [105–107] (Fig. 47.2). In MPNs, overexpression of PIM kinase confers resistance to conventional chemotherapy, making oncogenic PIM kinase a viable therapeutic target [107]. As the activity of PIM kinase is dependent on JAK/STAT activation, single agent PIM kinase inhibitor exhibited modest efficacy in MPN cell lines [106]. Yet, PIM kinase inhibitors and JAK2 inhibitors synergized with each other to selectively eliminate MPN HSCs [105–108]. AZD1208, a PIM kinase inhibitor, was shown to sensitize MPN cells to overcome JAK2 resistance [106], while INCB053914, a pan-PIM kinase inhibitor, delayed disease progression by inhibiting the development of ruxolitinib persistence [105]. This prompts the launching of phase 1 study of TP-3654, another PIM kinase inhibitor, in patients with intermediate-2 and high-risk PMF and SMF [109] (ClinicalTrials.gov identifiers: NCT04176198).

47.3.4.6 CD123 Inhibition

CD123, the α subunit of interleukin 3 receptor (IL-3R), is normally expressed on HSCs but upregulated on clonal MPN stem cells to promote HSC differentiation and commitment into the granulocytic and monocytic lineages [110, 111]. To attenuate CD123, a truncated diphtheria toxin is conjugated with interleukin 3 (IL-3). The subsequent binding of IL-3 to IL-3R allows cleavage and release of the genetically engineered toxin, which results in the suppression of protein synthesis by antagonizing elongation factor 2 (EF2). Apoptosis is hence induced [110] (Fig. 47.2). The use of tagraxofusp (SL-401), a recombinant IL-3-diphtheria toxin conjugate, has been recently explored in MF patients in phase 1/2 clinical trials [112] (Table 47.2). Its use as monotherapy or combination therapy with ruxolitinib was further appraised in MPN-accelerated phase (AP) and high molecular risk patient samples [113]. Thus, more in-depth investigations are supported to evaluate the efficacy of tagraxofusp.

47.3.4.7 Heat-Shock Protein (HSP) Chaperone Inhibition

Elevated levels of HSPs in MPNs stabilize oncogenic client proteins (i.e. JAK2V617F), causing constant JAK/STAT activation and its downstream pathways to promote myeloproliferation [114–118]. Attenuation of HSPs, most notably HSP90 and HSP27, is a therapeutic rationale to eradicate MPN clones via cell cycle arrest and apoptosis [115] (Fig. 47.2). In preclinical studies, AUY922, a HSP90 inhibitor, cooperated with JAK1/2 inhibitor TG101209 to deplete MF HSCs via proteasomal degradation of JAK2V617F and its downstream signalling client proteins [115]. Thus, this proposes a possible mechanism to overcome JAK2 resistance. A phase 2 clinical trial was carried out to investigate the clinical significance of AUY922 in r/r MPNs [116, 117]. Yet, the presence of unanticipated toxicities has hindered its development [116, 117] (Table 47.2). HSP27 antagonist is another potential novel agent to slow down MF disease progression by impeding STAT5 stabilization. OGX-427 is a HSP27 inhibitor that was shown to mitigate fibrosis by SVR, suppression of BM fibrosis, extramedullary haematopoiesis (EMH), and megakaryocyte proliferation in murine models [119, 120]. Further studies are envisaged.

47.3.5 Targeting Cell Signalling Pathways

47.3.5.1 PI3K/AKT/mTOR Pathway Inhibition

Targeting of the PI3K/AKT/mTOR pathway in MPNs may serve as a novel therapeutic modality alongside with the attenuation of aberrant JAK/STAT pathway by JAK2 inhibitors [121–123]. In addition, it was discovered that STAT5 phosphorylation, a molecular target that was expressed in PV and PMF HSCs, was not completely abrogated even with the use of JAK2 inhibitors [124, 125]. This could be targeted by PI3K/mTOR inhibitors via the protein phosphatase 2A (PP2A)/cancerous inhibitor of PP2A (CIP2A) axis [124] (Fig. 47.2). Numerous novel agents were developed in hope of blocking the PI3K/AKT/mTOR pathway. mTOR inhibitor everolimus and dual PI3K/mTOR inhibitor BEZ-235 displayed synergy in depleting MPN HSCs preclinically when combined with JAK inhibitors ruxolitinib and TG101209, respectively [121, 126]. AKT plays a functional role in megakaryocyte specification. Hence, AKT inhibitor MK-2206 displayed activities in ameliorating proliferation of megakaryocytes. This was evidenced by reduction of EMH and BM fibrosis with limited myelosuppression in MF murine models that harbour MPLW515L and CALR mutation [127, 128]. To date, combined PI3K/AKT/mTOR inhibitors-JAK2 inhibitors therapy was shown effective in preclinical trials. Yet, only modest effects were elicited in the phase 1b HARMONY trial of buparlisib, a pan-class 1 PI3K inhibitor, in MF patients. The lower-than-expected outcomes might be attributed to the small sample size and limited duration [129] (Table 47.2). Future researches on the aforementioned individual agents are anticipated to further exploit the PI3K/AKT/mTOR signalling pathway.

47.4 Targeting of Bone Marrow Microenvironment

The self-renewal potential of MPN HSCs is maintained by the BM microenvironment [130–135]. The BM niche works in an orchestrated manner for normal haematopoiesis. This delicate balance, however, is severely disrupted in MPNs [130–135]. Classically, after a MPN HSC acquire one of the three driver mutations, it proliferates and expands in the initially unperturbed BM microenvironment [130]. Subsequent dysregulated synthesis of proinflammatory cytokines, neoangiogenesis, and local neuropathy shapes a self-reinforcing BM niche for preferential proliferation of malignant HSCs [130–136] (Fig. 47.3). In advanced stages of MPN, the neoplastic BM niche mobilizes MPN HSCs into the blood and spleen, resulting in EMH and splenomegaly [130]. Therefore, multiple novel agents are being developed to target the BM microenvironment to attenuate the vicious positive feedback loop.

47.4.1 Depleting Cytokine Production: Transforming Growth Factor-Beta (TGF-β) Inhibition

TGF-β is a major proinflammatory cytokine produced by malignant megakaryocytes to stimulate BM fibrosis [137–139]. TGF-β1, the most abundant isoform of TGF-β, binds to

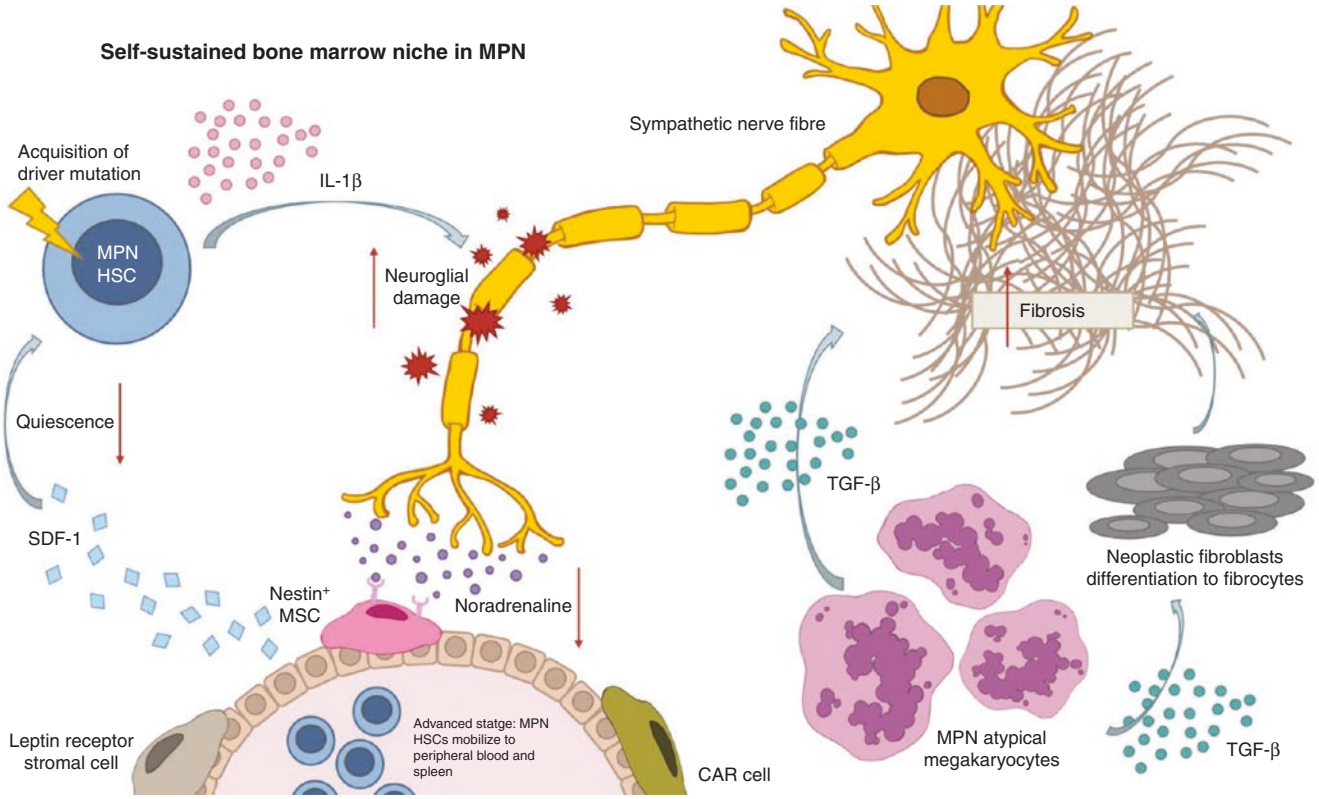

Fig. 47.3 Self-sustained bone marrow niche in MPN. The MPN bone marrow microenvironment is self-sustained by a positive feedback loop by primarily three mechanisms. Upon the acquisition of driver mutation (JAK2/CALR/MPL), the MPN HSC secretes IL-1β and damages sympathetic nerve fibres. Less noradrenaline is secreted by the damaged Schwann cells, resulting in less SDF-1 production by nestin$^+$ MSCs. Thus, quiescence of HSC could not be maintained and myeloproliferation takes place. Besides, the MPN atypical megakaryocytes secrete proinflammatory cytokines such as TGF-β to induce fibrosis via collagen deposition. The elevated levels of TGF-β also catalyse the differentiation of clonal neoplastic fibroblasts to fibrocytes, aggravating bone marrow fibrosis in MPN. In advanced stages, MPN blasts could also mobilize into the peripheral blood and spleen. HSC, haematopoietic stem cell; IL-1β, interleukin 1β; MPN, myeloproliferative neoplasm; MSC, mesenchymal stromal stem cells; SDF-1, stromal cell-derived factor 1; TGF-β, transforming growth factor-beta

its receptor to phosphorylate downstream SMAD pathway and activate transcription of TGF-β-responsive genes [138]. Thus, BM mesenchymal stromal stem cells (MSCs) are stimulated to increase collagen and glycoprotein deposition to induce BM fibrosis [137, 139, 140] (Fig. 47.4a). TGF-β1 also contributes to the differentiation of PMF neoplastic fibroblasts that aggravates BM fibrosis [139]. Galunisertib (LY2157299), a TGF-β receptor I kinase (ALK5) antagonist, attenuated fibrosis in MPLW515L murine models [141]. Activin receptor IIA ligand trap (ActRIIA) luspatercept binds to TGF-β superfamily ligands to stimulate erythroid maturation and improve anaemia [142] (Fig. 47.4b). Anaemia is a known adverse effect of conventional JAK inhibitor ruxolitinib that worsens with disease progression. Therefore, ActRIIA may play a role in overcoming the unmet need. The phase 3 INDEPENDENCE trial was commenced comparing the efficacy of luspatercept in improving transfusion dependence in MF patients (ClinicalTrials.gov identifiers: NCT04717414).

47.4.2 Inducing Megakaryocyte Differentiation: Aurora a Kinase (AURKA) Inhibition

The presence of atypical megakaryocytes is a notable BM feature of MF due to interruption of megakaryocytic differentiation and maturation [143]. In MPN, AURKA level is elevated and GATA1 expression is reduced regardless of driver mutation subtypes [143]. AURKA inhibition restores megakaryocyte differentiation and induces subsequent apoptosis. Hence, megakaryocyte-induced fibrosis is mitigated [143–146]. The promising preclinical result was evidenced in phase 1 clinical trial. MLN8237, an AURKA inhibitor, was well tolerated and demonstrated durable response in increasing GATA1 expression, normalizing megakaryocyte morphology, and reducing BM fibrosis [144, 146].

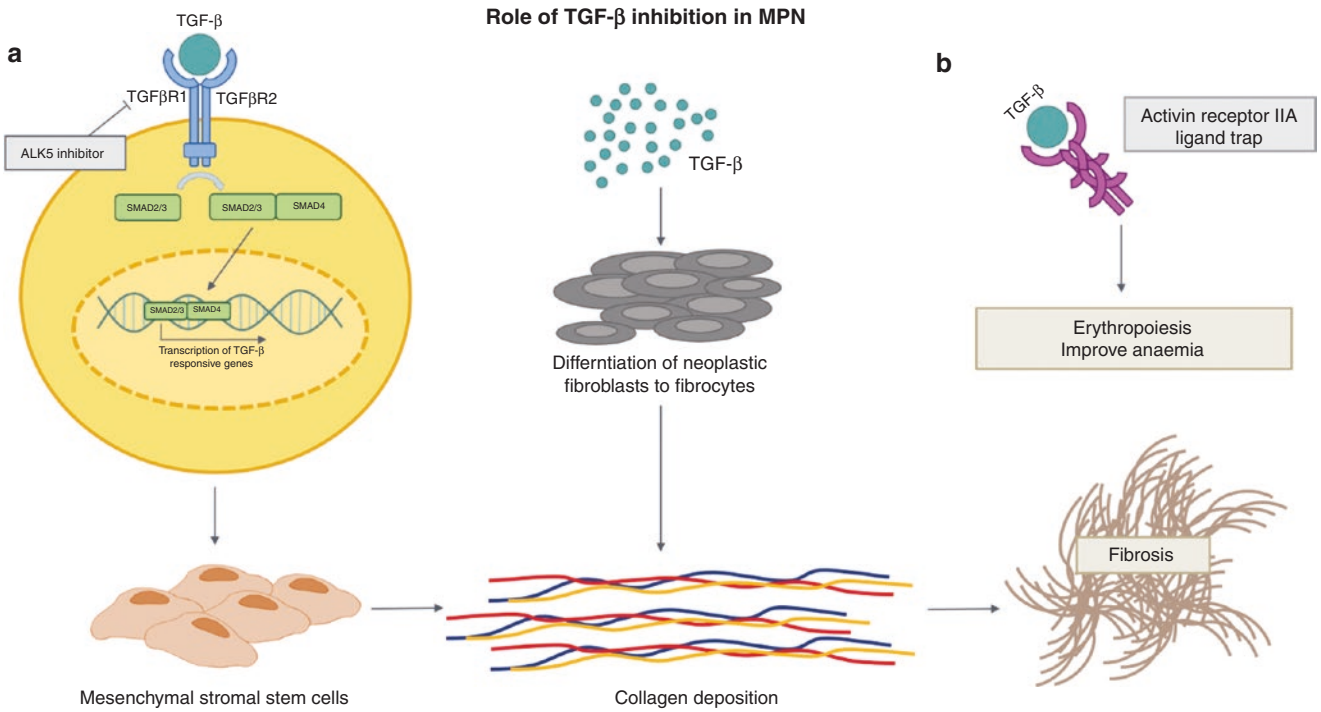

Fig. 47.4 Role of TGF-β in MPN. (**a**) shows ALK5 inhibitor antagonizes the TGFβR1 to reduce transcription of TGF-β; hence, fewer mesenchymal stromal cells are stimulated for collagen deposition and fibrosis formation. TGF-β also induces differentiation of neoplastic fibroblasts to fibrocytes to increase fibrosis, which could also be antagonized by ALK5 inhibitor. (**b**) Activin receptor IIA ligand trap binds to TGF-β to restore terminal erythroid differentiation, stimulate erythropoiesis, and improve anaemia. ALK5, TGF-β receptor I kinase; TGF-β, transforming growth factor-beta; TGFβR1/2, transforming growth factor-beta receptor I/II

47.4.3 Eliminating Neoplastic Fibroblasts: Serum Amyloid P (SAP)

Clonal neoplastic fibroblasts are plausible therapeutic targets as they are overexpressed in JAK2V617F MF patients to induce BM fibrosis [139, 147]. SAP, also known as pentraxin-2, is produced by hepatocytes to suppress fibroblast differentiation to fibrocytes [147, 148]. PRM-151 is a recombinant SAP molecule that reduces fibrosis by eliminating malignant fibroblasts, a unique pathway that is not targeted by ruxolitinib [139]. More importantly, improved anaemia was displayed via the restoration of BM cellularity and dampened production of cytokines in murine models [139]. In consistent with preclinical studies, PRM-151 ameliorated BM reticulin and collagen fibrosis, reduced splenomegaly as well as improved blood counts in a subset of MF patients in phase 2 study [149].

47.4.4 Promoting Neuroprotection: β-3 Sympathomimetic Agonist

Neuroglial damage is a recently discovered pathway that contributes to BM fibrosis [130, 131, 150–153]. JAK2V617F HSCs secrete interleukin 1β to induce local neural damage in sympathetic nerve fibres that innervate nestin$^+$ BM MSCs and Schwann cells [130, 150, 151]. As nestin$^+$ MSCs express a high level of chemokine stromal cell-derived factor 1 (SDF1, CXCL12) for the maintenance of HSC quiescence, damage of these MSCs would result in decreased SDF1 level and proliferation of HSCs [150]. On the other hand, Schwann cell death releases inflammatory cytokines and promotes BM fibrosis [130, 150]. β-3 Sympathomimetic agonist mirabegron was shown to preserve nestin$^+$ MSCs and decreased reticulin fibrosis in phase 2 clinical trial [152, 153]. Yet, the primary endpoint of reducing JAK2V617F mutant allele burden could not be reached [152, 153].

47.5 Immunotherapy

47.5.1 Immune Checkpoint Inhibitor

Similar to many other malignancies, MPNs exploit the programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) axis to escape from T-cell immunosurveillance [118, 136, 154]. PD-L1 is overexpressed in MPN patients harbouring JAK2V617F mutation, and the expression is particularly pronounced in monocytes, megakaryocytes, and myeloid-derived suppressor cells

(MDSCs) [118, 136, 154, 155]. Binding of PD-L1 to PD-1 on T lymphocytes attenuates T-cell activity, resulting in T-cell exhaustion and clonal evasion [118]. Based on this rationale, various anti-PD-1 and anti-PD-L1 antibodies have been assessed in multiple clinical trials, but to date, results were not encouraging due to inadequate efficacy, while other trials were still underway [154, 155] (ClinicalTrials.gov Identifier: NCT02421354, NCT02871323, NCT03065400).

47.5.2 Tumour-Specific Vaccination

Peptide vaccination is a recently emerged innovative therapeutic rationale in targeting MPN. PD-L1-derived JAK2V617F and CALR exon 9 mutant-epitopes could be recognized by T cells to produce immune response [155, 156]. Interestingly, T-cell response was also observed in healthy individuals, which implies effective tumour surveillance [136]. Based on the findings, vaccination of CALR exon 9-mutant epitope was examined in phase 1 trial (ClinicalTrials.gov Identifier: NCT03566446). As for JAK2V617F-directed vaccination, the target is less specific owing to the relatively small size of JAK2V617F and the close structural resemblance to JAK2-WT. This is evidenced by the smaller extent of immune response in JAK2V617F compared to CALR [156].

47.6 Conclusion

Disease status modification is an unmet medical need in the treatment of MPN due to disease heterogeneity. Besides novel JAK inhibitors which lack clonal selectivity, a myriad of plausible therapeutic agents have emerged to target MPN HSCs, modulate the BM niche, and induce immune response. Numerous clinical trials are currently underway. Their clinical outcomes are anticipated in hope of overcoming the clinical hurdles, improving patient's disease status, and minimizing risks of disease progression in MPNs.

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Current Guidelines and Treatment Algorithm of Chronic Myeloid Leukemia

48

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Abstract

Chronic myeloid leukemia is the prototype of precision medicine. With efficacious BCR::ABL1 tyrosine kinase inhibitors (TKIs), the prognosis of CML has remarkably improved. Several TKIs are approved for the treatment of newly diagnosed patients with CML in chronic phase (CP), and the choice of first-line agent is individualized and multifactorial. The importance of regular molecular monitoring by real-time quantitative PCR and pro-active management of drug-related adverse events cannot be over-emphasized. Patients who are in sustained deep molecular response might potentially be eligible for TKI discontinuation. Response to TKI should be closely monitored in high-risk patients and patients in accelerated phase. BCR::ABL1 kinase domain mutation analysis should be performed in patients who have suboptimal response to TKI. Failure of at least two TKIs should prompt referral for allogeneic hematopoietic stem cell transplantation (HSCT). Prognosis of CML blast phase remains poor; patients should be treated with a potent TKI with combination chemotherapy, followed by allogeneic HSCT once second CP is achieved.

Keywords

Chronic myeloid leukemia (CML) · *BCR*::*ABL1* Tyrosine kinase inhibitor (TKI) · Major molecular response (MMR) · European LeukemiaNet (ELN)

48.1 Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm that originates in an abnormal pluripotent bone marrow stem cell. It is characterized by the *BCR*::*ABL1* fusion gene located in the Philadelphia (Ph) chromosome, resulting from rearrangement of the long arms of chromosome 9 and 22 [1]. More commonly seen in untreated cases, CML is a triphasic disorder characterized by the chronic phase (CP), accelerated phase (AP), and blast phase (BP). Criteria for the definition of AP and BP are listed in Tables 48.1 and 48.2. With the availability of efficacious tyrosine kinase inhibitors (TKIs), the assignment of accelerated phase becomes arbitrary. It is noteworthy that AP is omitted in the latest fifth edition of the WHO Classification [2]. The worldwide incidence of CML is 1–2 cases per 100,000, and the median age at diagnosis is in the fifth and sixth decade. A younger age at diagnosis <50 years was observed in the Asian and African regions [3, 4]. There is a slight male preponderance [5]. In this TKI era, patients newly diagnosed with CML might have near-normal life expectancy with access to various TKIs, regular monitoring, and optimal management [6, 7]. Clinical management of CML will be discussed in this chapter with reference to major interna-

Table 48.1 Definition of accelerated phase (AP) (adapted from ELN [11], WHO [1] and NCCN [9])[a]

	ELN	WHO	NCCN
Blasts in blood or marrow	15–29%	10–19%	15–29%
Basophils in blood	≥20%	≥20%	≥20%
Persistent thrombocytosis unresponsive to therapy (>1000 × 10^9/L)	Not included	✓	Not included
Persistent thrombocytopenia unrelated to therapy (<100 × 10^9/L)	✓	✓	✓
Persistent or increasing high white blood cell count (>10 × 10^9/L), or splenomegaly, unresponsive to therapy	Not included	✓	Not included
CCA/Ph+ on treatment	✓	✓	✓
Additional CCA/Ph+ at diagnosis[b]	Not included	✓	✓

[a] AP is diagnosed if one or more of the above criteria are present.CCA/Ph+: clonal chromosome abnormalities in Ph+ cells

[b] WHO: includes major route abnormalities (a second Ph chromosome, trisomy 8, isochromosome 17q, trisomy 19), complex karyotype, and abnormalities of 3q26.2; NCCN: includes major route abnormalities

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Table 48.2 Definition of blast phase (BP) (adapted from ELN [11], WHO [1], and NCCN [9])[a]

	ELN	WHO	NCCN
Blasts in blood or marrow	≥30%	≥20%	≥30%
Extramedullary blast proliferation	✓	✓	✓
Large foci or clusters of blasts in the bone marrow biopsy	Not included	✓	Not included

[a] BP is diagnosed if one or more of the above criteria are present

tional guidelines, including but not limited to European LeukemiaNet recommendations [8], NCCN Clinical Practice Guidelines [9], British Society for Haematology Guideline [10], and ESMO Clinical Practice Guidelines.

48.2 Clinical Presentation

Around 95% of patients present in CP. Almost half of them are asymptomatic, and the diagnosis is made after an incidental finding of leukocytosis. Common features at presentation include weight loss, night sweats, fatigue, and splenomegaly.

For patients who are diagnosed in or transform to advance phase, they tend to develop more florid clinical features such as constitutional upset and symptoms related to severe cytopenia and/or marked leukocytosis.

48.3 Diagnostic Work-Up

A proper diagnostic work-up starts off with a physical examination, with particular reference to liver, spleen, and lymph nodes. Spleen size should always be measured (in centimeter) at diagnosis, as it is one of the key components of various prognostic scores.

In CP, the peripheral blood typically shows leukocytosis with "bimodal distribution," with peaks in the myelocytes and segmented neutrophils. Absolute basophilia is commonly observed. Atypical presentation with marked thrombocytosis without significant leukocytosis has been rarely seen.

Bone marrow aspirate is integral to the diagnosis of CML. Morphological assessment, including quantitation of blasts in the marrow, is required to ascertain the phase of disease. Apart from hypercellularity with increased numbers of eosinophils and basophils, "dwarf" megakaryocytes are typically seen in the marrow specimens. Cytogenetic analysis should also be performed with marrow sample. Marrow biopsy usually shows similar findings to that of marrow aspirate. The presence of bone marrow fibrosis in CP might carry prognostic importance [12–14]. Indeed, large clusters or sheets of small, abnormal megakaryocytes associated with marked reticulin or collagen fibrosis are considered to be presumptive evidence of AP under the WHO Classification [1]. For BP patients, flow cytometry is essential to characterize the blast lineage. A majority of BP patients have myeloid BP, while around 30% have lymphoblastic crises.

Around 90–95% of CML has the characteristic Ph chromosome, resulting from t(9;22)(q34.1;q11.2) reciprocal translocation. Variant and cryptic translocations are infrequently observed. In morphologically compatible cases which Ph chromosome cannot be identified by conventional karyotyping, fluorescence in situ hybridization (FISH) and/or reverse transcriptase polymerase chain reaction (RT-PCR) is necessary to look for the *BCR*::*ABL1* fusion gene. A qualitative RT-PCR is also mandatory to characterize the type of *BCR*::*ABL1* transcript to facilitate subsequent molecular monitoring. Quantitative RT-PCR for the baseline *BCR*::*ABL1* transcript level is not mandatory, but could be considered for assessment of the rate of early *BCR*::*ABL1* decline which might be associated with long-term response [15–17].

To complete the diagnostic work-up, a biochemical profile and hepatitis B serology should be checked. Cardiovascular risks should be adequately assessed, with documentation of fasting glucose, cholesterol, and hemoglobin A1c [18, 19]. A chest X-ray and an electrocardiogram should also be obtained at baseline. For patients who present in advance phases, HLA typing of patients and their siblings (if any) should also be arranged early.

48.4 Prognostic Scores

Four prognostic systems are available for prediction of prognosis in CP patients: Sokal, Hasford (or Euro), EUTOS, and ELTS. Table 48.3 compares the composition of the prognostic scores.

The Sokal score and Hasford score were developed before availability of tyrosine kinase inhibitors (TKIs), when the mainstay of treatment was cytoreductive agents and interferon,

Table 48.3 Prognostic scores for CML

	Sokal [20]	Hasford [21]	EUTOS [22]	ELTS [23]
Age	✓	✓		✓
Spleen size	✓	✓	✓	✓
Platelet count	✓	✓		✓
PB blast %	✓	✓		✓
PB basophil %		✓	✓	
PB eosinophil %		✓		

PB peripheral blood

respectively. On the other hand, the EUTOS score and ELTS score were developed in the TKI era; both were derived from patients who received imatinib-based treatment. EUTOS score, the simplest of the four scores using two variables, was able to distinguish high-risk patients who had lower probabilities of complete cytogenetic remission probabilities at 18 months and inferior progression-free survivals (PFS). Prognosis of CML has significantly improved with TKI, and many patients died of causes unrelated to CML. The ELTS score addressed this issue and focused on the probabilities of dying of CML. The ELTS score was recommended by the European LeukemiaNet [8, 24], but the oldest Sokal score remained a popular choice and was applied in most TKI trials.

48.5 Monitoring Treatment Response

By convention, there are three levels of response, namely hematologic response (HR), cytogenetic response (CyR), and molecular response (MolR) [9, 25].

Hematologic response is assessed based on the complete blood count (CBC) and physical examination. Complete hematologic response (CHR) is defined by all of the following:

- WBC <10 × 10^9/L.
- Basophils <5%.
- No immature cells in peripheral blood.
- Platelet count <450 × 10^9/L.
- Spleen not palpable.

Cytogenetic response (CyR) is assessed with chromosome banding analysis of marrow cell metaphases or interphase fluorescent in situ hybridization (FISH) of blood cells. Definitions of CyR according to the European LeukemiaNet are as below:

Complete cytogenetic response (CCyR)	No Ph+ metaphases.
Partial cytogenetic response (PCyR)	1–35% Ph+ metaphases.
Minor cytogenetic response (mCyR)	36–65% Ph+ metaphases.
Minimal cytogenetic response (minCyR)	66–95% Ph+ metaphases.
None (NoCyR)	>95% Ph+ metaphases.

Table 48.4 Definitions of molecular response

RQ-PCR result[*BCR*::*ABL1* (IS)]	Level of response	Meaning
≤0.1%	Major molecular response (MMR), or MR3	≥3 log reduction from the standardized baseline
≤0.01%	MR4	≥4 log reduction from the standardized baseline
≤0.0032%	MR4.5	≥4.5 log reduction from the standardized baseline
≤0.001%	MR5	≥5 log reduction from the standardized baseline

CHR and CCyR should be achieved by the vast majority of CML patients on TKI treatment. Thus, treatment response has to be assessed by a more sensitive method to accurately quantify the residual disease burden. Nowadays, molecular response is routinely assessed by real-time, quantitative PCR (RQ-PCR). RQ-PCR is also more convenient, as patients do not need to undergo invasive bone marrow aspiration [26]. Indeed, routine cytogenetic monitoring is no longer recommended by the ELN and NCCN. Molecular response (see Table 48.4) is expressed as the ratio of *BCR*::*ABL1* transcripts to *ABL1* transcripts (or to other internationally accepted control transcripts) on the international scale (IS). *BCR*::*ABL1* transcript level ≤ 1% is roughly equivalent to complete cytogenetic response [27]. *BCR*::*ABL1* transcript level ≤ 0.1% is defined as major molecular response (MMR) or MR3, which is a significant milestone as it reflects optimal response and favorable long-term outcome. Deep molecular response (DMR) is commonly referred to *BCR*::*ABL1* transcript level ≤ 0.01%, i.e., MR4 or deeper, and is one of the prerequisites for consideration of treatment-free remission (TFR). Table 48.5 shows the treatment milestones at different time points.

In general, molecular monitoring of *BCR*::*ABL1* transcripts should be performed every 3 months. Additional RQ-PCR testing may be required in case of suboptimal responses or dose interruptions/reductions. After MMR is achieved, response can be assessed every 3–6 months.

Table 48.5 Treatment milestones for CML (expressed as *BCR*::*ABL1*% on the international scale)[a]

	Optimal	Warning	Failure
Baseline	NA	High-risk ACA, High-risk ELTS score	NA
3 months	≤10%	>10%	>10% if confirmed within 1–3 months
6 months	≤1%	>1–10%	>10%
12 months	≤0.1%	>0.1–1%	>1%
Anytime	≤0.1%	>0.1–1%, loss of MMR	>1%, resistance mutations, high-risk ACA

ACA: additional chromosome abnormalities in Ph+ cells, *ELTS* score: EUTOS long-term survival score, *MMR:* major molecular response, *NA:* not applicable (*BCR*::*ABL1* ≤ 0.1% on the international scale)
[a]Adapted from European LeukemiaNet (ELN) recommendations [8]

48.6 Treatment Algorithm

48.6.1 Initial Treatment for CML

In patients with newly diagnosed CML, supportive treatment including the use of hydration and febuxostat in patients with marked leukocytosis should be started. A short course of hydroxyurea may be given to patients with marked leukocytosis and/or thrombocytosis while waiting for confirmation of diagnosis. Leukapheresis is rarely indicated nowadays and may be considered only in selected patients, e.g., those with leukostasis and pregnant women.

48.6.2 First-Line Treatment in CP

Tyrosine kinase inhibitors (TKIs) are the treatment of choice in patients with newly diagnosed CML-CP. Imatinib, nilotinib, dasatinib, and bosutinib have been approved for first-line treatment by the Food and Drug Administration (FDA) and EMA (European Medicines Agency). Radotinib was developed in South Korea and approved by Korea Food and Drug Administration (KFDA). All these five TKIs have different properties. While they have some overlapping toxicities, such as hematological toxicities and gastrointestinal upset, they also have their unique side effects as discussed below.

48.6.2.1 Imatinib

Imatinib mesylate, the first-generation TKI, was the prototype of targeted therapy. Since its debut more than two decades ago, imatinib has revolutionized the management of CML leading to a significantly better prognosis [28, 29]. The standard dose of imatinib is 400 mg once daily. It is recommended to take the drug with a meal and a large glass of water. Common side effects of imatinib include edema, gastrointestinal upset, fatigue, and electrolyte disturbance; these symptoms are usually of mild grade and tolerable even in the elderly population. A lower dose of 300 mg once daily may be considered in patients who tolerate the drug poorly if optimal response has been achieved. Long-term use of imatinib is also associated with a decline in renal function [30]. With availability of generic formulation [31], imatinib has become much more affordable and an attractive cost-effective option in front-line setting [8, 32].

48.6.2.2 2G-TKIs

Second-generation TKIs (2G-TKIs) include nilotinib, dasatinib, bosutinib, and radotinib. All of them have been compared with imatinib head to head in industry-sponsored randomized trials and shown to be superior to the first-generation TKI in terms of the rates and depths of molecular responses. However, none has been shown to result in statistically significant improvement in survival. In addition, the various 2G-TKIs have not been compared against each other in prospective clinical trials. Their unique properties and toxicity profiles have to be taken into account when treatment is planned for patients with newly diagnosed CML.

48.6.2.3 Nilotinib

Nilotinib is around 30-fold more potent than imatinib against wild-type BCR-ABL1 [33, 34]. Its clinical efficacy was well established by the pivotal ENESTnd trial [35], leading to its approval for first-line use by the U.S. FDA in 2010. The standard adult dose of nilotinib is 300 mg twice daily in newly diagnosed CML-CP patients and 400 mg twice daily in patients with advance phase. Food intake should be avoided for at least 2 h before and 1 h after drug administration. In the long-term update of ENESTnd [36], cumulative 5- and 10-year rates of MMR and MR4.5 were higher with nilotinib than imatinib (5-year rates: Nilotinib 77% and 53.5%, respectively; imatinib 60.4% and 31.4%, respectively; 10-year rates: Nilotinib 77.7% and 61.0%, respectively; imatinib 62.5% and 39.2%, respectively). Nilotinib is associated with higher risk of cardiovascular events (CVE) than imatinib, with 10-year cumulative CVE rate at 16.5% versus 3.6% in the imatinib group. Hence, cardiovascular status of all patients planned for nilotinib should be evaluated before treatment commencement. Cardiovascular risk factors should also be routinely monitored and actively optimized during nilotinib therapy. Other side effects of nilotinib include pancreatitis, hepatotoxicity, QT prolongation, and cutaneous eruption.

48.6.2.4 Dasatinib

Dasatinib is more than 300 times as potent as imatinib in vitro [34]. Its role as first-line therapy was supported by

the key DASISION trial [37]. It was approved by the U.S. FDA for treatment of newly diagnosed CML adult patients in 2010, just a few months after nilotinib. The recommended dose in patients newly diagnosed with CML in chronic phase is 100 mg once daily. Long-term data [38] showed that the cumulative 5-year MMR and MR4.5 rates were 76% and 42% for dasatinib and 64% and 33% for imatinib. Lower doses (20–50 mg once daily) of dasatinib have been suggested to yield comparable efficacy with better tolerance [39, 40]. The approved dose of dasatinib in patients with advanced phase CML is 140 mg daily. Dasatinib can be administered with or without a meal. More than 20% of patients develop pleural effusion on dasatinib [38, 41], with older age and higher dose being risk factors. Other less common but important adverse events include pulmonary arterial hypertension, colitis [42], and nephrotoxicity [43].

48.6.2.5 Bosutinib

Approval of bosutinib as a first-line agent in chronic phase CML was based on the BFORE trial [44], which showed a significantly higher MMR rate at 12 months with bosutinib than imatinib. Final update from the trial [45] also showed that the cumulative MMR and MR4.5 rates by 5 years were higher with bosutinib than imatinib (bosutinib 73.9% and 47.4%, respectively; imatinib 64.6% and 36.6%, respectively). The approved dose of bosutinib is 400 mg once daily in patients newly diagnosed with CML in chronic phase and 500 mg once daily in advance phase CML. It is recommended to take with food. Apart from the common side effects of gastrointestinal upset and transient increase in liver parenchymal enzymes, bosutinib otherwise has a favorable safety profile [45, 46].

48.6.2.6 Radotinib

Radotinib was developed in South Korea and approved for first-line treatment of chronic phase CML in South Korea. The recommended dose is 300 mg twice daily. It had been shown to be superior to imatinib in terms of MMR and DMR rates [47, 48]. Overall, it seemed to have a manageable safety profile, with liver transaminitis, rash, reduced appetite, headache, and alopecia observed more frequently in the radotinib group than the imatinib group.

48.6.3 Choice of First-Line TKI

Figure 48.1 outlines the treatment algorithm of patients with newly diagnosed CML. Imatinib, nilotinib, dasatinib, and bosutinib are approved for first-line treatment in chronic phase CML and are available in most places. Choice of the first-line TKI is individualized and multifactorial, including patient/disease factors (risk scores, co-morbidities, age, socio-financial consideration), drug factors (costs, toxicities, ease of administration), and treatment goals. Despite the presence of several 2G-TKIs in the market, imatinib remains the treatment of choice in the majority of chronic phase CML patients [8, 10] considering its reasonable efficacy and predictable toxicity profile. Upfront 2G-TKI therapy may benefit certain patients, e.g., patients with high or intermediate ELTS or Sokal scores and patients who give priority to treatment discontinuation at an early stage. Patients who achieve sustained, deep molecular response may be considered for treatment-free remission (TFR). Please refer to Chap. 49 for details on TFR.

Upon commencement of treatment, the importance of regular molecular monitoring as per international guidelines cannot be overstated. Apart from monitoring of treatment response, it is also important to prevent and/or recognize adverse effects (AE) arising from the TKIs through systematic screening and clinical vigilance [49]. Hematologic adverse events, namely neutropenia, thrombocytopenia, and anemia, are commonly observed among all TKIs, especially during the initial phase of treatment. Temporary dose interruption and/or reduction may be required. On the other hand, TKIs also have their unique toxicity profiles, and the screening strategy should be TKI-specific. For example, nilotinib and ponatinib are associated with vascular events. Cardiovascular risk factors should be screened at baseline and routinely monitored, while the patients are on the treatment. On the other hand, dasatinib is commonly associated with pulmonary AE, especially pleural effusion. Physicians should have a high index of suspicion when the patients develop respiratory symptoms and arrange a chest X-ray readily. Imatinib, the first-generation TKI, has established long-term safety data with more than two decades of clinical experience. Nevertheless, one should remain vigilant of its possible AE, such as electrolyte disturbance and renal impairment.

48.6.4 Resistance to and/or Intolerance of First-Line Treatment

Up to 40% of patients may discontinue their first-line TKIs during their course of treatment [38, 45, 50], usually due to resistance/suboptimal response, intolerance, or both. Failure to meet treatment milestones may be due to a number of possible causes, the common ones being non-compliance, disease progression, and development of BCR::ABL1 kinase domain (KD) mutation. In patients who have suboptimal response, drug compliance should be confirmed. Kinase domain mutation analysis should be arranged, and bone marrow examination with cytogenetic study should be performed in selected cases. Treatment should be intensified accordingly, e.g., increase the dose of the same TKI or switch to a

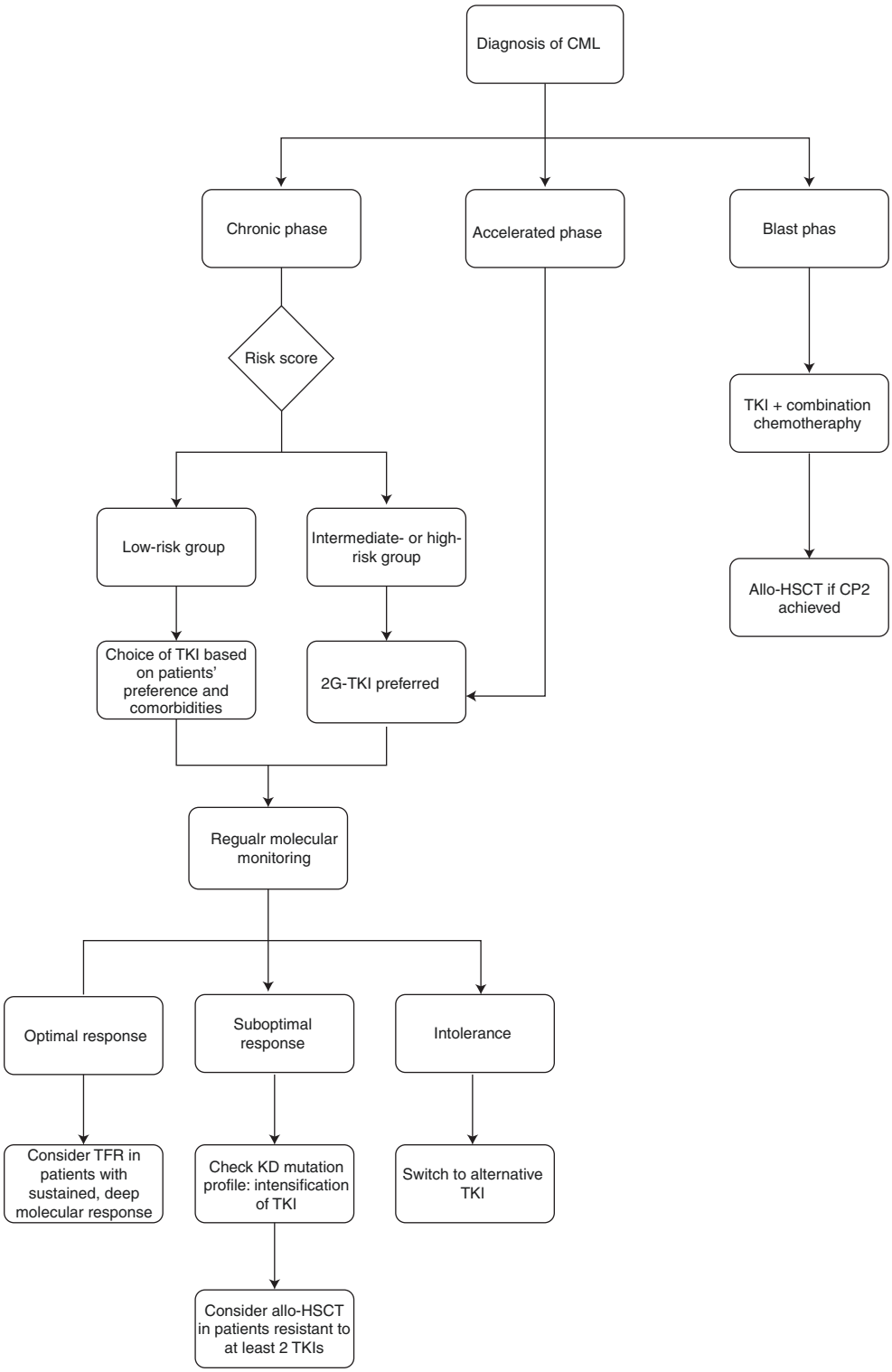

Fig. 48.1 Treatment algorithm of newly diagnosed chronic myeloid leukemia

more potent TKI. One should also start to identify potential stem cell donors in preparation for allogeneic hematopoietic stem cell transplantation (HSCT), in case the patients' responses remain unsatisfactory.

For patients who develop adverse events to their first-line TKI, symptomatic relief and supportive treatment should be provided, with or without brief dose interruption. Dose reduction could be considered, after balancing treatment efficacy and toxicity [51]. Prolonged treatment interruption is discouraged. Switching of TKI therapy should be considered in patients who fail the above measures and are deemed intolerant to the TKI.

Table 48.6 Recommended tyrosine kinase inhibitors with respect to BCR::ABL1 kinase domain mutations

BCR::ABL1 kinase domain mutations	Recommended TKI
F317L/V/I/C, T315A	Nilotinib, bosutinib, ponatinib
V299L	Nilotinib, ponatinib
Y253H, E255V/K, F359V/I/C	Dasatinib, bosutinib, ponatinib
T315I	Ponatinib

48.6.5 Second-Line Treatment

In case of intolerance, patients can be switched to any of the aforementioned TKIs based on their co-morbidities and personal preferences after a thorough discussion between the treating physicians and patients. In case of resistance, the result of BCR::ABL1 KD mutation analysis may help to guide the treatment strategy (Table 48.6). Currently, no particular TKI can be recommended as second-line treatment in the absence of BCR::ABL1 KD mutations. For patients who were on imatinib as first-line agent, they can be switched to any of the 2G-TKIs. For those who were on a 2G-TKI, other 2G-TKIs and ponatinib are possible alternatives. Treatment options in CML resistant or intolerant to 2G-TKIs will be discussed in details in Chap. 50.

48.6.6 First-Line Treatment in Advance Phases

Patients who present in AP should be started on TKI as initial treatment, preferably a 2G-TKI [52, 53]. Treatment response should be closely monitored, together with early initiation of donor search. Patients who fail to achieve optimal response should be readily referred for HSCT. BP patients should be treated with TKI plus combination chemotherapy. The type of chemotherapy regimen depends on the blast lineage.

48.7 Role of Allogeneic Hematopoietic Stem Cell Transplantation (Allo-HSCT) in CML

Allo-HSCT for CML has substantially reduced over the past two decades, as a result of effective treatment with TKIs [54, 55]. While TKIs remain the standard of care for all patients with newly diagnosed CML in chronic phase, allo-HSCT may be needed in patients who are resistant to/intolerant of multiple TKIs (see Table 48.7). Patients in AP who fail TKI treatment should be considered for HSCT. Allo-HSCT should also be offered to all transplant-eligible blast phase patients who successfully achieve second CP (CP2) [56, 57]. Allo-HSCT in CML will be further discussed in Chap. 51.

Table 48.7 Indications for allo-HSCT in CML

Chronic phase
Failure of two or more TKIs
Presence of T315I mutation and/or failure of ponatinib
Intolerant of multiple TKIs with recurrent/persistent grade 4 cytopenia despite appropriate dose reduction and supportive treatment
Accelerated phase
Suboptimal response to first-line TKI
Blast phase
In second CP

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Treatment-Free Remission in Chronic Myeloid Leukemia

49

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Abstract

Over the last decade, a medically supervised trial of tyrosine kinase inhibitor (TKI) discontinuation has become an important option for patients with chronic phase chronic myeloid leukaemia (CML). A successful attempt, termed 'treatment-free remission' (TFR), has enabled selected patients to remain off TKI therapy long-term and has now been integrated into international treatment guidelines. Approximately 40–65% of chronic phase CML patients who cease TKI while in sustained deep molecular response will be able to remain off TKI in major molecular response 12 months after drug discontinuation. Consequently, understanding the nuances and complexities of TFR is vital for physicians offering and guiding their patients through a TFR attempt. Within this chapter, we present the data underpinning the TFR strategy, including appropriate patient selection and logistics of molecular monitoring. The potential risks and benefits of a TFR attempt will also be discussed, as well as emerging predictors of a successful TFR attempt which may guide future practice.

Keywords

Treatment-free remission · Chronic myeloid leukaemia
Tyrosine kinase inhibitor · Deep molecular response
Molecular monitoring · BCR-ABL1 qPCR

49.1 Introduction

Chronic myeloid leukaemia (CML) has long been the prototype of a disease characterized by a single targetable molecular abnormality, the *BCR-ABL1* fusion oncogene which is associated with the Philadelphia (Ph) chromosome resulting from t(9;22). The introduction of tyrosine kinase inhibitors (TKIs) targeting the *BCR-ABL1* fusion kinase revolutionised the therapeutic landscape of CML, dramatically improving prognosis [1]. The first *ABL1*-directed TKI, imatinib, was introduced into clinical practice in the early 2000s after its efficacy was established in the pivotal IRIS trial [1]. The 10-year overall survival (OS) from the IRIS study was 83.3% [2]. This was closely followed by the development of more potent second-generation (2G) TKIs: nilotinib [3], dasatinib [4] and bosutinib [5]. The 5-year survival in clinical trials with these agents is similar and

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does not demonstrate a survival benefit when compared with imatinib [3–5]. Ponatinib, a third-generation TKI, was introduced into the treatment armamentarium and is used primarily after initial TKI resistance, especially in cases with the T315I kinase domain mutation that confers resistance to imatinib and 2G-TKIs [6]. More recently, the allosteric *BCR-ABL1* inhibitor, asciminib [7], has been trialled in the resistant and intolerant setting [7]. Epidemiological data indicate that the currently available TKIs give CML patients an overall survival similar to that of the age-matched general population [8].

CML treatment historically focused on preventing disease progression from chronic phase to the more aggressive stages of accelerated and blast crisis CML, with the assumption that patients would need to remain on therapy indefinitely to prevent disease progression. However, long-term TKI therapy is associated with substantial morbidity and financial costs to both patient and governmental funding bodies. While imatinib has the best safety profile of all TKIs, it is still associated with substantial quality-of-life limiting events such as gastrointestinal toxicities (e.g. nausea and diarrhea) and periorbital oedema [1]. In comparison, the 2G-TKIs are associated with a higher incidence of serious toxicities associated with prolonged exposure. For instance, ~30% of patients treated with 300 mg twice daily of nilotinib for 10 years will experience a cardiovascular event when compared to only 8% of those treated with imatinib [9]. Similarly, with dasatinib treatment, the risk of pleural effusion is 20% by 5 years, with a 5% probability of pulmonary hypertension [4], which is not always reversible on cessation of the drug [10]. From an economic perspective, TKI therapy remains costly, although the availability of generic imatinib around the world has perhaps reduced the financial impact of long-term imatinib therapy. However, the 2G-TKIs remain on patient with 1-month supply of drug costing thousands of dollars to either patients or funding bodies. For example, the estimated cost savings associated with interrupted drug therapy in the Euro-SKI study of over 750 patients was approximately €22 million [11].

The possibility of therapy interruption was conceptualized to minimize the toxicity and financial burden associated with long-term TKI therapy. Supporting evidence arose from the fact that, in the pre-TKI era, CML patients could discontinue interferon therapy and maintain cytogenetic remission. Then, in the TKI era, the French published a case series of 12 patients who discontinued TKI therapy [12]. These patients had undetectable *BCR-ABL1* transcripts for ≥2 years with only six patients requiring imatinib re-introduction following recurrence of detectable molecular disease [12]. However, the remaining patients had no evidence of molecular relapse at a median follow-up of 18 months [12], cementing the viability of long-term molecular remission in patients trialling TKI discontinuation. These preliminary observations led to the first clinical trials of treatment-free remission (TFR).

Once the safety and durability of TFR was demonstrated, this led to a paradigm shift in the goals of treatment. Molecular monitoring with the goal of attaining molecular targets to ensure the achievement of deep molecular responses not only reduces the risk of disease progression [13], but also maximizes the potential for therapy discontinuation. Identifying patients with a high priority for a TFR attempt with optimal upfront TKI selection to facilitate achieving TFR eligibility will have a long-term impact on morbidity and cost.

49.2 Deep Molecular Response (DMR)

In comparison to the historical standard-of-care treatment of interferon, TKI therapy enabled a high proportion of patients to achieve the time-specific molecular targets recommended by the various guidelines proposed by international cooperative groups such as the European LeukemiaNet (ELN) [14] and the United States National Cancer Cooperative Network (NCCN) [15]. Achievement of a complete cytogenetic remission (CCyR) by 12 months, approximately equating to a *BCR-ABL1*^{IS} of <1%, has been shown to be largely protective against disease progression to accelerated phase and blast crisis [16] with up to 90% of imatinib-treated patients meeting this milestone [2, 17]. Furthermore, achievement of deeper responses by 12 months such as a major molecular remission (MMR, *BCR-ABL1* ≤ 0.1%) predicts for a 2-year progression-free survival (PFS) of 100% compared with 85% if less than CCyR was achieved by 12 months [18] of TKI therapy. Reaching even deeper molecular responses (DMR) of MR4.5 (*BCR-ABL1*^{IS} ≤ 0.0032%) by 4 years correlates with an 8-year OS of 92% compared with 78% if the *BCR-ABL1* value remains >1% [13]. Following years of TKI therapy, a large proportion of patients will achieve a DMR (defined as either MR4, *BCR-ABL1* ≤ 0.01% or MR4.5) or in some instances, undetectable *BCR-ABL1* transcripts. By 5 years of standard dose imatinib therapy, the rate of MR4.5 approximates 30% [3, 4] whereas the rate is much higher with 2G-TKIs approaching 40–55% [3, 4]. Hence, a molecular target of MR4.5 has been an ideal therapeutic goal when initiating TKI therapy and consideration of the optimal TKI to achieve this target is imperative.

While initial monitoring in CML relied upon cytogenetic analysis to evaluate for the presence of the Ph chromosome,

once patients achieve CCyR, continuing with cytogenetic analysis alone is inadequate to evaluate for deeper responses. Instead, monitoring has hinged upon measurement of the level of *BCR-ABL1* transcripts through the *BCR-ABL1* quantitative PCR (qPCR). This highly sensitive assay has been harmonized to an International Scale (IS) across laboratories [19, 20] and can detect very low levels of *BCR-ABL1* transcripts with many institutions able to report sensitivities to an MR4.5 level or better. However, low-level *BCR-ABL1* detection is dependent upon the sensitivity of the assay with a common shortfall being the reporting of undetectable BCR-ABL1 transcripts using a relatively insensitive detection limit. It is imperative that clinicians be aware of the sensitivity of their local *BCR-ABL1* assay, especially if their patients are trying to achieve DMR or attempting TFR.

Standardized *BCR-ABL1* quantitation is only available in patients with breakpoints within the major breakpoint cluster region of *BCR*, observed in approximately 95% of patients [21, 22]. This will generate either an e13a2 or an e14a2 transcript, while a proportion of patients will co-express both transcripts, encoding a 210-kDa protein [21]. Less frequently, the breakpoint will occur in an alternative site, generating atypical transcripts such as e1a2 or e19a2, which cannot be monitored by the standardized method. Instead, a qualitative PCR method can be utilized for monitoring of these variant *BCR-ABL1* transcripts but cannot reliably assessed beyond an MMR response. In select laboratories, patient-specific quantitative PCR methods have been developed to monitor atypical transcripts, but these are neither widely available nor harmonized [23–27]. Regardless, it is critical that the *BCR-ABL1* transcript type is characterized at diagnosis of CML to ensure the appropriate monitoring method is performed; otherwise, risking false negative results if the incorrect assay is used [28, 29].

49.3 Summary of the TFR Clinical Trials

TFR may be defined as a degree of remission sustained after the medically supervised discontinuation of TKI in patients who meet strict eligibility criteria (discussed below). The first two seminal studies that were instrumental in establishing the safety and feasibility of TFR were the French STop IMatinib (STIM) [30] study and the Australian TWISTER [31] trial. Both trials involved ceasing imatinib treatment in patients with undetectable *BCR-ABL1* transcripts for ≥2 years. Almost half of the study patients ceased first-line imatinib while the remainder had interferon therapy prior to imatinib. Monthly *BCR-ABL1* testing was performed following TKI cessation, and imatinib was re-initiated either for rising *BCR-ABL1* values on two consecutive tests or loss of MMR. Similar results were obtained from both studies with the 5-year molecular relapse free survival being 38% and 45% for STIM [30] and TWISTER [31], respectively. Interestingly, the vast majority of patients who re-started imatinib experienced molecular relapse within the first 6 months of therapy (median 3–4 months) [30, 31], highlighting the importance of early *BCR-ABL1* monitoring when attempting TFR. Importantly, patients who re-started imatinib following molecular relapse remained sensitive to imatinib and most again achieved undetectable BCR-ABL1 transcripts, typically within 3–6 months of re-treatment [11].

Another pivotal trial was the interim analysis of the EURO-SKI study [11], the largest TFR study to date, which included over 750 patients across multiple sites in Europe. The TFR eligibility criteria used in this trial were less stringent than in the STIM and TWISTER studies with only a minimum of 12 months of MR4.0 mandated to qualify for a TFR attempt. The vast majority were imatinib-treated, although patients on other TKIs were enrolled, and the primary analysis was performed on the imatinib-treated population. The 6-month molecular relapse free survival (defined as remaining in MMR) was 61%, falling to 50% by 24 months. No clear plateau in molecular-relapse free survival was observed, in contrast to STIM and TWISTER, which had more conservative guidelines for TFR eligibility and molecular relapse definition.

The early TFR studies [30, 31] had stringent criteria for molecular relapse that resulted in TKI resumption while still in MR4.0 or better. Another French study explored MMR as the criterion for TKI recommencement. The A-STIM (According to STIM) clinical trial enrolled 80 imatinib treated patients utilising loss of MMR as the trigger to restart TKI. This enabled them to undertake a retrospective comparison against the original STIM relapse criteria. They found that 36% of patients lost MMR by 24 months whereas if the STIM [30] definition of molecular relapse was used, 54% would have re-started imatinib. These data suggest that 15–20% of patients who experience rising *BCR-ABL1* values may not lose MMR, at least with a median follow-up of 31 months. Studies have shown that the median *BCR-ABL1* doubling time for patients with relapse after TKI discontinuation is 9 days (range 6.9–26.5 days) [32] or ~1 log per month [30, 33]. This implies that if patients who lose MMR do not recommence TKI promptly, they are likely to exceed a *BCR-ABL1* of 1% by the following month, which puts them at a higher risk of disease progression [16, 18]. MMR is more reliably standardized around the world than deeper levels of molecular response. Consequently, loss of MMR is now widely accepted [14, 15] as the trigger to recommence TKI following a TFR attempt.

49.3.1 Ceasing Imatinib Vs. Second-Generation TKIs

The higher frequency of DMR achievement with 2G-TKIs when compared to imatinib [3, 4] means that more patients can be expected to become eligible for TKI discontinuation. However, for those who discontinue TKI, the probability of remaining in TFR has so far been similar to that observed in imatinib-treated patients. In the first-line setting, 49% of patients ceasing nilotinib in the ENESTfreedom study remained off therapy in MMR at 96 weeks [34]. Similar results were observed in patients treated with dasatinib in the Japanese first-line DADI study where the inclusion criteria were a minimum of 24 months of dasatinib and ≥ 12 months of DMR [35]. The estimated rate of TFR at both 6 months and 12 months was 55% [35] with the median duration of dasatinib treatment being 40 months. However, both the ENESTfreedom [36] and first-line DADI [37] trials show that the median duration of TKI exposure prior to TFR attempts was far shorter in 44 months and 40 months, respectively, compared with the treatment durations observed in the primarily imatinib-treated TFR studies (Table 49.1), suggesting that 2G-TKIs allow patients to become TFR eligible sooner, thereby reducing the total duration of TKI exposure.

For patients treated with second-line nilotinib, the ENESTop study demonstrated similar rates of TFR at 48 weeks and 96 weeks after TKI cessation at 58% and 53%, respectively [29]. The median duration of TKI therapy prior to the TFR attempt was 88 months with a median of 53 months of nilotinib exposure [29]. A longer duration of nilotinib exposure and of MR4.5 was associated with a higher probability of TFR. Likewise, TFR attempts in 63 patients treated with second-line dasatinib, in the original DADI trial, yielded similar results with 44% of patients remaining in TFR at 3 years [38]. The median duration of TKI treatment prior to the TFR attempt was 82 months but the median duration of dasatinib exposure was only 17 months [37]. The dasatinib exposure in this study was far shorter than duration of nilotinib treatment observed in ENESTop [29]. The DASFREE study [39], which included a combination of patients treated with either first- or ≥ second-line dasatinib, showed that a longer duration of dasatinib treatment was associated with a higher probability of TFR at 2 years.

49.3.2 TKI De-escalation Prior to TFR Attempt

The concept of TKI dose de-escalation has been controversial due to theoretical concerns regarding the emergence of resistant CML subclones exposed to a sub-therapeutic dose of TKI. The DESTINY study explored the approach of halving standard TKI dose for 12 months in patients with a *BCR-ABL1*^{IS} of ≤0.1%, followed by TKI discontinuation for those patients remaining in MMR after the period of half-dose treatment [40]. Patients were grouped according to their molecular status (MR4.0 vs. MMR) in the 12 months prior to enrolment, and the 24-month rate of molecular recurrence-free survival was 72% in the MR4 cohort as opposed to 36% in the MMR group [40]. While the 24-month TFR rate in the MR4 group is superior to that reported in most other reported TFR studies, confirmation of this finding in other clinical trials is required.

Table 49.1 Summary of the published clinical trials investigating TFR

Study	Number of patients attempting TFR	TKI ceased	Minimum specified duration of TKI exposure9.	Median duration of TKI therapy	Minimum specified duration of DMR	Median duration of DMR	Definition of molecular relapse	Percentage of patients remaining in TFR (%)	Predictors of TFR success
STIM [30, 73]	100	IM	3 years	50 months	UMRD ≥2 years	35 months	Loss of UMRD	38% at 60 months	– Longer IM treatment (≥50 months) – Low/int Sokal score – Male
TWISTER [31, 74]	40	IM	3 years	70 months	UMRD ≥2 years	36 months	Loss of UMRD	35% at 60 months	– Longer duration of interferon therapy prior to IM
STIM2 [75]	218	IM	3 years	79 months	UMRD ≥2 years	39 months	Loss of UMRD	50% at 24 months	– Longer duration of TKI therapy (≥74.8 months) – *BCR-ABL1/ABL1* < 0.0023%^{IS} on digital droplet PCR
A-STIM [32]	80	IM	3 years	79 months	UMRD ≥2 years	41 months	Loss of MMR	61% at 36 months	
KID [76]	90	IM	3 years	81 months	UMRD ≥2 years	40 months	Loss of MMR	69% at 24 months	– Withdrawal syndrome
ISAV [66]	108	IM	24 months	103 months	UMRD ≥18 months	26 months	Loss of MMR	48% at 46 months	– Older age – ddPCR results
DOMEST [77]	99	IM		100 months	MR4.0 ≥ 2 years	55 months	Loss of MR4.0	64% at 24 months	– IM duration >100 months – Low/Int Sokal score
EURO-SKI[12]	755	IM > NIL/ DAS	3 years	7.5 years	MR4.0 ≥ 1 years	4.7 years	Loss of MMR	50% at 24 months	– IM therapy ≥5.8 years – MR4.0 ≥ 3.1 years
DESTINY [40]	125	IM > NIL/ DAS	3 years	6.5 years	MR4.0 ≥ 1 years		Loss of MMR	72% at 36 months	– Longer duration of TKI exposure
First-line DADI [35]	58	DAS (first-line)	24 months	40 months	MR4.0 ≥ 1 years	23 months	Loss of MR4.0	55% at 12 months	– Lower CD4-cell count prior to discontinuation
DADI [37, 38]	63	DAS (≥second-line)		82 months	MR4.0 ≥ 1 years		Loss of MR4.0	44% at 24 months	– High NK cell counts prior to discontinuation
DASFREE [39]	84	DAS	≥2 years of DAS	69 months	MR4.5 ≥ 2 years	28 months	Loss of MMR	46% at 24 months	– Age ≥ 65 – Longer duration of DAS therapy – First-line DAS
D-STOP [78]	54	DAS		92 months	MR4.0 ≥ 2 years	51 months	Loss of MMR	63% at 12 months	– Lower levels of CD3- CD56+ NK cells at the end of DAS consolidation

(continued)

Table 49.1 (continued)

Study	Number of patients attempting TFR	TKI ceased	Minimum specified duration of TKI exposure9.	Median duration of TKI therapy	Minimum specified duration of DMR	Median duration of DMR	Definition of molecular relapse	Percentage of patients remaining in TFR (%)	Predictors of TFR success
ENESTfreedom [34, 36]	190	NIL	≥2 years	44 months	MR4.5 ≥ 1 year	30 months	Loss of MMR	49% at 96 weeks	– Low and intermediate Sokal risk
ENESTSTop [29]	126	Second line NIL	≥3 years of TKI including (≥2 years of NIL)	88 months	MR4.5 ≥ 1 year	32 months	Loss of MR4.0	53% at 96 weeks	
STOP 2G-TKI [43]	60	NIL and DAS	≥2 years	76 months	UMRD ≥2 years	29 months	Loss of MMR	54% at 48 months	– No history of prior suboptimal response or TKI resistance
STAT2 [64]	78	Second line NIL	≥2 years of consolidation NIL	99 months	MR4.5 ≥ 2 years	51 months	Loss of MR4.5	63% at 36 months	– UMRD prior to TFR attempt
LAST	172	IM > NIL/ DAS/BOS	≥3 years	83 months	MR4.0 ≥ 2 years		Loss of MMR	61% at 3 years	– Undetectable *BCR-ABL1* at TFR attempt
Adelaide data	115	IM > NIL/ DAS/BOS	≥3 years	7.0 years	MR4.5 ≥ 2 years	3.3 years	Loss of MMR	55% at 12 months	– Short halving time – e14a2 transcripts – Longer TKI treatment duration

DMR deep molecular response, *TFR* treatment-free remission, *TKI* tyrosine kinase inhibitor, *UMRD* undetectable minimal residual disease

49.4 Molecular Monitoring in Treatment-Free Remission Attempts

The safety of a TFR attempt is dependent on accessing standardized quantitative *BCR-ABL1* testing with a minimum sensitivity of MR4.0, and preferably MR4.5. As mentioned earlier, the transcript type of any patient attempting TFR should be characterized and TFR attempted only if the patient has a transcript that can measured through a standardized quantitative assay. Furthermore, the results of the *BCR-ABL1* assay should ideally be available within 2 weeks (at least for the first 6 months after TKI discontinuation when the risk of relapse is greatest) so that clinicians can promptly advise the patient in case of molecular relapse.

In clinical trials of TKI cessation, most studies have performed monthly *BCR-ABL1* testing for the first year followed by less frequent testing; thereafter [30, 31], a similar strategy was being adopted by the NCCN [15]. However, this rigorous monitoring regime is a major barrier to the widespread adoption of TFR, especially in resource-poor nations. The EURO-SKI study introduced a less stringent strategy, recommending monthly assessments for the first 6 months of the TFR attempt followed by 6-weekly testing for the next 6 months before reverting to 3-monthly monitoring indefinitely [11]. The kinetics of molecular relapse in TFR were modelled using a combination of retrospective and clinical trial data to determine the consequences of less-frequent *BCR-ABL1* testing [41]. Concentrating monthly monitoring in the first 2–6 months of the TFR attempt followed by 2 monthly *BCR-ABL1* testing between months 6 and 12 enabled a 37% reduction in molecular testing with no delay in detection of molecular relapse [41]. When less-frequent *BCR-ABL1* testing is performed, loss of DMR should prompt re-institution of monthly monitoring to ensure timely detection of molecular relapse [41]. The updated 2020 ELN guidelines [14] recommend a similar *BCR-ABL1* monitoring strategy, which may allow for TFR to become more widely accessible.

While molecular relapse generally occurs early in a TFR attempt, late relapses (defined as molecular relapse >2 years following TKI discontinuation) occur in up to 14% of patients [42]. The rate of *BCR-ABL1* rise in late relapses appears to be slower than in those experiencing relapse within the first 6 months of a TFR attempt [43, 44]. Recent follow-up of the French TFR cohorts (STIM, A-STIM) identified that patients with fluctuating *BCR-ABL1* levels below the MMR threshold during the first 24 months had a higher probability of late molecular relapse compared with those who remained in stable DMR (35% vs. 0%, respectively) [42]. The latest documented relapse after a TFR attempt was >6 years [45], but there are published cases of relapse 24–25 years after allogeneic stem cell transplantation for chronic phase CML [46, 47], highlighting the necessity of long-term regular *BCR-ABL1* monitoring of patients in TFR.

49.5 Potential Risks of TKI Discontinuation

49.5.1 TKI Withdrawal Syndrome

It was assumed that TKI cessation would result in resolution of the minor side effects commonly associated with TKI therapy, with an improvement in quality of life for patients in TFR. However, early data from the first 50 Swedish patients enrolled In the EURO-SKI study reported a 30% incidence of generalized arthralgia and joint stiffness, termed TKI withdrawal syndrome, developing within weeks of imatinib cessation [48]. These symptoms either resolved with imatinib recommencement in the event of molecular relapse or lasted for up to 12 months in the patients that remained in TFR [48]. The phenomenon of withdrawal syndrome is likely secondary to poorly defined off-target class effects of TKI withdrawal [14]. A larger dataset combining 427 patients from both STIM2 and EURO-SKI not only confirmed that withdrawal syndrome is a class effect associated with cessation of other *BCR-ABL1* inhibiting TKIs, but also showed an association with longer treatment duration and a history of prior osteoarticular symptoms [49]. No link was identified between molecular relapse and the development of withdrawal syndrome [49]. Interestingly, de-escalation of TKI therapy prior to a TFR attempt in the DESTINY trial did not reduce the frequency of TKI withdrawal syndrome [40]. Symptoms attributable to withdrawal syndrome are generally mild and responsive to simple analgesics, but occasionally may require corticosteroid therapy [49].

49.5.2 Disease Responsiveness to Retreatment

The initial TFR studies demonstrated that patients experiencing molecular relapse were responsive to retreatment with the same TKI at the same dose [30, 31]. In EURO-SKI, 86% of patients regained MMR at a median of 2.8 months following TKI recommencement and 81% re-achieved MR4.0 at a median of 3.7 months [11]. There remains a small proportion of patients that fail to reach DMR following TKI resumption, although in some cases, this is potentially due to inadequate duration of follow-up [44]. While true TKI resistance is rare following failed TFR attempts, there is a single report of detection of a new kinase domain mutation following molecular relapse in a patient enrolled in ENESTfreedom [36]. The mutation was a low-level F359V (0.5%) which confers nilotinib resistance, but due to low BCR-ABL1 transcript levels

prior to the TFR attempt, the timing of the development of F359V could not be determined [36]. Failure to re-achieve MMR within 3 months of TKI resumption should prompt evaluation for a BCR-ABL1 kinase domain mutation [15].

49.5.3 Risk of Blast Crisis

While TFR attempts are generally considered to be very safe with minimal risk of CML resistance, there have now been several reports of the development of blast crisis. These instances were all in patients who experienced molecular relapse and re-achieved MMR on TKI, but then subsequently developed blast crisis several months later. The first instance was a female patient diagnosed with chronic phase CML in the mid-1990's who achieved DMR with interferon [32]. She commenced imatinib in 2006 and was enrolled in A-STIM in 2009. MMR was lost 10 months after TKI cessation [32], and regained within 3 months of imatinib recommencement. The patient progressed to lymphoid blast crisis within 8.5 months [32]. The sudden development of myeloid blast crisis was observed in another patient enrolled in the STOP-2G TKI study 6 months after TKI resumption [50]. A third patient progressed to lymphoid blast crisis following a failed TFR attempt after second-line nilotinib treatment (switched due to intolerance) [51]. Molecular relapse occurred 20 months after nilotinib discontinuation and, despite rapid re-achievement of MR4.0, progression to lymphoid blast crisis was observed within 6 months [51]. The nilotinib-resistant mutation, Y253H, was found at blast crisis, but not at molecular relapse [51]. In fact, all three instances of blast crisis had evidence of cytogenetic evolution [32, 50, 51]. It remains unclear whether progression to blast crisis in these rare cases was part of the natural disease trajectory or somehow triggered by the period of TKI interruption.

49.6 Special Circumstances

49.6.1 Pregnancy

TKI therapy has teratogenic potential, especially if used during the first trimester of pregnancy [52]. This is suspected to be primarily related to off-target PDGFR inhibition and TKI use is associated with a higher rate of fetal malformations and spontaneous abortions. Focusing on achieving a sustained DMR in women under the age of 40 with a new diagnosis of chronic phase CML, with the aim of TFR in anticipation of future pregnancy is a valid strategy. However, the time taken to become eligible for TFR may be associated with decreasing fertility and a higher risk of fetal aneuploidy associated with maternal ageing. These relative risks will depend on maternal age at diagnosis of CML, as well as prior obstetric history. Additionally, if TFR is attempted prior to conception, a strategy for potential molecular relapse needs to be planned. Although the risk of TKI re-introduction once the first trimester is completed is largely an evidence-free zone, there have been reports of safe use of imatinib in the later stages of pregnancy [53]. Regardless, interferon would be the preferred treatment in case of molecular relapse during pregnancy. TKI therapy should also be avoided in nursing mothers until breast feeding is completed [54].

49.6.2 Paediatric CML and TFR

CML in childhood is generally a more aggressive disease [55] compared to its adult counterpart with limited evidence suggesting that there may be different biological mechanisms underlying the presentation [56]. In addition to the common TKI side effects observed in adults, there are unique toxicities that are only observed in the paediatric setting, such as slowing of growth velocity [57]. There are now limited reports [58, 59] regarding the possibility of TFR in the pediatric setting. In the largest series to date, only 4/14 patients maintained TFR [60], a proportion that seems lower than in adult studies. Although TFR is feasible in children and adolescents with CML, more data are needed to provide an accurate estimate of the chances of successful TKI discontinuation.

49.6.3 TKI Discontinuation after TKI Resistance or Prior Advanced Phase

The STOP-2G TKI study evaluated cessation of either first- or second-line nilotinib or dasatinib in 60 patients with >3 years of TKI exposure and > 2 years of MR4.5 [43]. Although the 12-month rate of TFR was 63%, this fell to 54% by 48 months [43]. Among 22% of this patient cohort who switched to 2G-TKI due to suboptimal response or resistance to imatinib, only 36% of patients remained in TFR at 48 months, compared with 77% who were treated with first-line 2G-TKIs or switched for intolerance [43]. Similarly, only 8% of patients who switched to dasatinib for imatinib resistance in the second-line DADI study remained in TFR at 12 months whereas the rate of TFR was 58% in the remaining patients [38]. Patients receiving second-line dasatinib also had an inferior likelihood of TFR in the DASFREE study, although this included a mix of resistant and intolerant patients [39]. This is in contrast to ENESTop where prior imatinib resistance had no observed impact on the rate of TFR [29]. The conflicting data observed in ENESTop may be secondary to differing definitions of resistance. ENESTop relied on clinicians to define the rationale for TKI switch to

nilotinib whereas in the second-line DADI and STOP-2G studies, resistance was defined according to the relevant ELN guidelines available at the time of the study. Patients with a history of TKI resistance are less likely to become eligible for TFR and the number of such patients with successful TFR outcomes is small. TFR is not routinely recommended for this particular population unless there are significant concerns about TKI toxicity.

49.6.4 Second TFR Attempts

Failing a TFR attempt is associated with substantial emotional upheaval for patients [61] and a common question is whether re-attempting a TFR will be possible in the future. Data concerning a second TFR experience are limited, but the French RE-STIM study addressed this question in 70 patients who had failed a first TFR attempt [62]. The enrolled cohort was composed of patients from STIM [30], A-STIM [32, 42] and EURO-SKI[12] who were eligible if an undetectable molecular response had been re-achieved following the first failed TFR attempt [62]. Molecular relapse in this second attempt was defined as loss of MMR and the 12-month TFR rate was 48%, falling to 35% by 36 months [62]. Once more, molecular relapses were most frequent within the first 6 months of the TFR attempt and the timing of molecular relapse during the first failed TFR attempt was predictive of the second TFR outcome [62]. Patients who remained in MR4.5 at 3 months following the first TFR attempt had a 72% probability of being in TFR at 24 months after the second TFR attempt compared with 36% for patients who lost MR4.5 within 3 months of the first TFR attempt [62].

49.7 Predictors of Successful TFR

As TFR continues to gain momentum as the ultimate goal of therapy in chronic phase CML, there is increasing impetus to identify clinical predictors or biomarkers that can be used to identify which patients will be able to achieve TFR.

49.7.1 Exploring the Optimal Duration of TKI Exposure and DMR Prior to TFR

Currently, the minimum requirement to qualify for a TFR attempt is 3 years of TKI exposure, following the inclusion criterion utilised in a number of clinical trials (Table 49.1). However, the median duration of TKI therapy in clinical trials is highly variable, ranging from 40 months in the first-line DADI study [35] to 7.5 years in EURO-SKI [11]. The STIM data suggested that patients treated with ≥50 months of imatinib had a lower probability of molecular relapse (53%) by 18 months compared with patients with <50 months of imatinib therapy (78%) [30]. An Australian retrospective analysis of 130 patients attempting TFR showed that longer TKI exposure was associated with a higher probability of remaining in TFR [63]. Patients who have a longer duration of TKI treatment will typically also have a longer duration of DMR, and an important question is whether it is the duration of DMR that is more strongly associated with TFR outcome. The EURO-SKI study demonstrated that the probability of remaining in MMR at 6 months was more closely related to the duration of MR4.0 than to the duration of imatinib treatment: for each additional year of MR4.0 during imatinib treatment the odds ratio for TFR (MMR at 6 months) was 1.14 [11]. The link between longer duration of DMR and TFR success is further corroborated by other studies such as ENESTop [29]. While the optimal duration of TKI exposure prior to a TFR attempt remains undefined, for most patients, it is almost certainly longer than the specified minimum of 3 years, and may be more closely related to the duration of DMR. Limited data suggest that the optimal TKI duration may be shorter if patients are treated with upfront 2G-TKIs, since the rates of TFR in ENESTfreedom and the DADI study with a relatively short duration of treatment were similar to those seen in imatinib-treated patients with a longer duration of TKI treatment [34, 35].

The optimal level of DMR prior to a TFR attempt is not established. The STIM [30] and TWISTER [31] studies required undetectable *BCR-ABL1*^{IS} levels, with a variable detection limit around MR4.5 to MR5.0. Subsequent studies have mostly used MR4.0 or MR4.5 (Table 49.1). While the optimal definition of DMR is still unanswered, the overall impression is that deeper molecular responses will have superior TFR outcomes [64, 65]. In the ISAV study, imatinib-treated patients with detectable *BCR-ABL1* transcripts by digital PCR, despite being deemed in compete molecular remission by conventional qPCR, had a higher likelihood of molecular relapse at 12 months (68%) compared with those having a more stringent complete molecular response (43% incidence of relapse) [66]. Similar results were observed from another Italian study assessing *BCR-ABL1* by digital PCR at the time of TKI discontinuation [67]. Patients with *BCR-ABL1* values below an empirically determined threshold at the beginning of the TFR attempt had an 85% likelihood of remaining in TFR at 1 year compared with 59% if the *BCR-ABL1* level was above the threshold [67].

49.7.2 Other Clinical Predictors

In a several studies, older age has been associated with TFR success [39, 66] although a systematic review investigating potential factors was not able to confirm this association [68]. History of interferon use before TKI therapy was

reported to be a possible predictor of TFR success from early data [31]. However, the EuroSKI study showed that this was possibly confounded by an association with Sokal risk score, with low-risk patients being more likely to respond to interferon. An additional confounding factor may be that patients who switched from interferon to imatinib subsequently had a very long duration of TKI treatment before attempting TFR. In the STIM study, only 13% of high Sokal risk patients had no evidence of molecular relapse 18 months into the TFR attempt compared with 54% of low-risk patients [30]. Similar associations have been observed in other studies, such as the ENESTfreedom [34] where 63% and 50% of low and intermediate risk patients, respectively ,remained in TFR at 96 weeks compared with 29% of high risk Sokal patients. However, as few high risk Sokal patients will actually reach TFR eligibility due to the reduced likelihood of reaching DMR [69], this means that even fewer patients will be able to achieve TFR.

The presence of the e14a2 *BCR-ABL1* transcript as opposed to e13a2 has also been associated with a higher likelihood of TFR success, and this has now been confirmed in independent studies [63, 70]. The cumulative incidence of molecular relapse at 48 months in e13a2 patients was 64% compared with 35% in 64 patients attempting TFR from the Hammersmith [70]. Similarly, 67% of e14a2 patients in an Australian cohort remained in TFR at 12 months compared with 40% of patients with e13a2 transcripts [63].

More recently, the initial rate of *BCR-ABL1* decline has been demonstrated to be predictive of the achievement of MR4.5 and TFR eligibility [63, 71]. Achievement of an early molecular response (EMR, *BCR-ABL1* $\leq$ 10% at 3 months following TKI commencement) in imatinib-treated patients was associated with a 41% rate of MR4.5 achievement at 4 years compared with 6% following EMR failure [71]. Even lower *BCR-ABL1* values at 3 months were associated with very high rates of stable MR4.5, a prerequisite for TFR eligibility. Achievement of MMR or better by 3 months was associated with a 78% rate of stable MR4.5 by 8 years compared with 9% if the 3-month *BCR-ABL1* value was >10% [72]. An alternative measure of *BCR-ABL1* kinetics is the halving time, the number of days taken for the *BCR-Abl1* value to halve [71]. This value, obtained at the time of initial TKI commencement, has shown a strong association with TFR eligibility and even eventual TFR success [63]. Patients with a very short *BCR-ABL1* halving time (<9.35 days), indicating a rapid fall in leukaemic burden, have a 71% probability of achieving TFR eligibility by 5 years compared with 9% in patients with substantially longer halving times (>21.85 days) [63]. Importantly, patients with a halving time of <9.35 days have 80% probability of remaining in TFR at 12 months whereas if the halving time was >21.85 days was associated with almost no chance of remaining in TFR [63]. In contrast, EMR failed to predict the 12-month TFR outcome [63]. Strategies to maximise the number of patients with rapid *BCR-ABL1* decline may be a potential future strategy to improve TFR success but this requires further validation before adoption into standard practice.

49.8 TFR in Current Guidelines

Recommendations regarding TFR have been incorporated into various national and international CML management guidelines (Table 49.2) [14, 15]. Patients with chronic phase CML with no history of TKI resistance or prior disease progression may be considered suitable for a TFR attempt. However, the guidelines differ in the minimum criteria for both duration of TKI therapy and DMR. The NCCN [15] recommends a minimum of 3 years of TKI exposure. In comparison, the ELN guidelines are more conservative, recommending at least 5 years of imatinib therapy or, if patients are treated with a 2G-TKI, shortening this threshold to at least 4 years of TKI exposure [14]. The NCCN specifies a minimum of 2 years of MR4 before a TFR attempt. The ELN recommendation on the other hand suggests a minimum of 3 years of MR4.0 or 2 years of MR4.5.

Table 49.2 Comparison of requirements for patients to be considered for a TFR attempt, adapted from the NCCN and the ELN guidelines

NCCN guidelines [15]	ELN guidelines, 2020 update [14]
Age ≥ 18 years	
Chronic phase CML, no history of accelerated or blast crisis CML	CML in first chronic phase, no prior treatment failure
On approved TKI therapy[a] for ≥3 years	Duration of TKI therapy >5 years (>4 years if 2G-TKIs are used)
Quantifiable *BCR-ABL1* transcript	e13a2 or e14a2 transcripts
Documented stable MR4 response ≥2 years	Duration of DMR (MR4 or better) >2 years[b]
Access to *BCR-ABL1* qPCR testing with sensitivity of ≥MR4.5 with result available within 2 weeks	Access to high quality *BCR-ABL1*^{IS} qPCR testing with rapid turn-around time
Monthly molecular monitoring for the first 6 months, followed by 2-monthly for the following 6 months and 3-monthly thereafter indefinitely	Monthly molecular monitoring for the first 6 months, followed by 2-monthly for the following 6 months and 3-monthly thereafter indefinitely
Prompt recommencement of TKI within 4 weeks of MMR loss with monthly monitoring until MMR re-established	Prompt recommencement of TKI with MMR loss

[a] Discontinuation trials have been reported in CML patients treated with imatinib, nilotinib and dasatinib. There is no substantial evidence for TFR after bosutinib or ponatinib treatment

[b] Optimal eligibility if either MR4.0 > 3 years or MR4.5 > 2 years

IS International Scale

49.9 Summary

Several thousand patients with CML have participated in TFR clinical trials over the past 15 years, and some of the patients in the earliest studies have remained in TFR for over a decade. Recognition of the safety and importance of this therapeutic option has led to its inclusion in treatment guidelines for CML. The challenge for the future is to find more accurate ways to predict TFR outcome for an individual patient.

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Treatment Options in CML Resistant or Intolerant to Second-Generation Tyrosine Kinase Inhibitors

50

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Abstract

Tyrosine kinase inhibitors (TKIs) are the mainstay of treatment in patients with chronic myeloid leukaemia (CML). Commonly available second-generation tyrosine kinase inhibitors (2G-TKIs) include nilotinib, dasatinib, and bosutinib. A majority of patients respond well to 2G-TKIs, yet some might develop resistance, intolerance, or both to the drugs. Based on the reason(s) for treatment failure, appropriate investigations should be arranged and therapeutic strategy should be formulated. In cases of intolerance, alternative 2G-TKI and imatinib are possible options if the issues cannot be resolved by temporary dose interruption and/or reduction and supportive care, with the co-morbidities and preferences of patients taken into account. On the other hand, alternative 2G-TKI and ponatinib are commonly considered in resistant cases. Recently, FDA-approved asciminib partially fills the treatment gap for patients who are resistant or intolerant to multiple TKIs, and is a much-welcomed addition to the artillery against CML. Allo-HSCT remains a standard salvage option for a small proportion of patients who fail all the above measures.

Keywords

Chronic myeloid leukaemia (CML) · Asciminib Ponatinib · Second-generation tyrosine kinase inhibitor (2G-TKI) · Haemopoietic stem cell transplantation (HSCT)

50.1 Introduction

A majority of patients with chronic myeloid leukaemia (CML) achieve optimal response with tyrosine kinase inhibitors (TKIs) and enjoy near-normal life expectancy [1]. However, resistance and/or intolerance of TKIs are observed in some patients which warrant switch of therapy. Second-generation tyrosine kinase inhibitors (2G-TKIs) include nilotinib, dasatinib, bosutinib, and radotinib, all of which are approved for treatment of patients with newly diagnosed CML in chronic phase and those with resistance or intolerance of prior therapy. Their superiority to imatinib, the first-generation TKI, in terms of the rate and depth of molecular responses, has been demonstrated in various clinical trials [2–5]. Nevertheless, no significant improvement in survival outcomes has been established so far. "Failure" of a TKI refers to patients failing for intolerance or resistance. When considering alternative treatment options in patients who are on 2G-TKI, the key is the reason(s) for switching therapy as it would affect the available options and our decision-making process.

50.2 CML Intolerant of Second-Generation Tyrosine Kinase Inhibitors

Despite being distinct from conventional cytotoxic chemotherapy, TKIs are not without side effects [6]. Their toxicities can be broadly divided into haematological and non-haematological. Some patients may develop intolerance of TKI as a result of drug-induced toxicities and eventually require switch of treatment.

50.2.1 Haematological Toxicities

Haematological adverse events, namely, neutropenia, thrombocytopenia and anaemia, are commonly seen during the early course of TKI treatment. Myelosuppression is believed to result from the combined effect of suppression of the leukaemic clone by the treatment and delayed recovery of the normal haemopoietic cells. Cytopenia may occur across various TKIs, and data suggest all 2G-TKIs but dasatinib seem to be at least non-inferior to imatinib [3, 5–7]. On the other

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hand, dasatinib (at 100 mg daily) is associated with higher rate of haematological adverse events than imatinib [2]. Should patients develop significant haematological toxicities with a 2G-TKI, they can be managed by temporary dose interruption and reduction, with or without growth factor and blood product support. One should be very cautious about prolonged (>1 month) TKI interruption and watch out for disease progression vigilantly.

Cytopenias mostly resolve by first few months after initiation of TKI treatment. Infrequently, some patients may have refractory/recurrent cytopenia despite dose adjustment. Any of the first-line TKI agents, including imatinib, can be used as alternative option with gradual up-titration. A trial of asciminib, the first-in-class Specifically Targeting the ABL Myristol Pocket (STAMP) inhibitor, may be considered though it is also associated with cytopenia [8, 9]. If a patient has failed two or more TKIs with severe myelosuppression, allogeneic haemopoietic stem cell transplantation (allo-HSCT) may be warranted.

50.2.2 Non-haematological Toxicities

Initial general approach to non-haematological toxicities is similar to that of haematological toxicities, i.e. dose reduction and/or interruption. Depending on the type of toxicity, specific management strategy may be offered and other TKI options should be actively discussed with patients. For example, if patients develop arterial occlusion events (AOE) while on nilotinib, they should be put on appropriate anti-thrombotic treatment; other cardiovascular risk factors should be optimally controlled. If patients have no specific reason to adhere to nilotinib, alternative TKI with no overlapping toxicity should be considered, e.g. imatinib, dasatinib, and bosutinib. On the other hand, if patients are in satisfactory molecular response and are contraindicated to other available TKIs, dose reduction with careful monitoring may be offered to strike a balance between drug efficacy and toxicity. Similarly, dose reduction and temporary interruption may be considered for patients who are intolerant of any of the 2G-TKIs. Medical treatment including diuretic and a short course of corticosteroid can be given to patients who develop dasatinib-associated pleural effusion. Again, if all the above measures fail, patients should be switched to alternative TKIs considering their co-morbidities and personal preferences.

50.3 CML Resistant to Second-Generation Tyrosine Kinase Inhibitors

Treatment resistance to 2G-TKI occurs in around 5–10% in first-line setting [2–4]. When patients have suboptimal response to treatment, their drug compliance should be assessed. BCR::ABL1 kinase domain (KD) mutational analysis should be arranged. A sensitive TKI should be chosen based on the mutational profile, if a mutation is detected. Otherwise, patients should be switched to an alternative 2G-TKI, or ponatinib. Dose escalation of the original TKI might be considered in selected patients without a mutation, e.g. those in the "warning" category under the European LeukemiaNet guideline [10]. Some clinical data and expert opinion support the early use of ponatinib as the second-line treatment after resistance to a first-line 2G-TKI [10–13], yet strictly speaking the label of ponatinib requires failure of at least two prior TKIs. In the meantime, HLA typing of the patients and donor search should be initiated, in the preparation of the potential need for allogeneic haemopoietic stem cell transplantation (HSCT). Failing at least two lines of TKI remains a standard indication for allo-HSCT [14, 15] [10]. Unless unavailable or contraindicated, a trial of ponatinib should be given to justify the decision for HSCT.

Asciminib was approved by the U. S. Food and Drug Administration (FDA) in October 2021 for patients with chronic phase CML who fail two or more TKIs, or those with T315I mutation. For patients who are resistant or intolerant to ponatinib, asciminib, if available, is a reasonable choice [16] as the last resort before proceeding with an allo-HSCT. Use of allo-HSCT in CML patients will be discussed in detail in Chap. 51.

50.3.1 Ponatinib

Currently, ponatinib is the only available third-generation TKI. It is approved for the treatment of patients with chronic phase CML who fail at least two prior TKIs, or those with T315I mutation. Ponatinib is a potent BCR::ABL1 inhibitor; it can overcome all clinically relevant single *BCR*::*ABL1* mutants which confer resistance to first- and second-generation TKIs, including the notorious gatekeeper T315I mutation [17, 18]. As shown in the pivotal phase 2 PACE trial [19] which studied the effect of ponatinib in patients who previously failed dasatinib or nilotinib, or had T315I mutation, 54% and 40% of CML-CP patients achieved complete cytogenetic response (CCyR) and major molecular response (MMR) with ponatinib, respectively. On the other hand, small studies showed that 2G-TKIs yielded CCyR of around 10–35% only when used in the third- or later-line setting [12].

However, ponatinib carries a black box warning of arterial occlusive events (AOE), which was observed in around 30% of patients in the PACE trial. Other side effects of ponatinib include hepatotoxicity, heart failure, hypertension and pancreatitis. The initial approved dosage of ponatinib was 45 mg once daily. Subsequently, the drug was found to carry significant toxicities which were dose dependent [20].

Indeed, the role of ponatinib in frontline setting was once explored in the EPIC trial [21]. But the study was prematurely terminated after excessive vascular adverse events were observed in the ponatinib group. As a result, the drug had been temporarily withdrawn from the market back in 2013 [22]. Ponatinib returned to the market less than 3 months after its suspension, with extra safety warnings and narrower indications. Its therapeutic role was refined to later-line treatment in CML patients.

The phase 2 OPTIC trial explored the effects of ponatinib dosage on efficacy and safety, in an effort to identify a dosing strategy to optimize the risk-benefit ratio of ponatinib [20]. It was concluded that optimal benefit-to-risk outcomes occurred with the 45-mg starting dose followed by reduction to 15 mg upon achievement of *BCR::ABL1* $\leq 1\%$, and hence the current dosing recommendation. Ponatinib may be taken with or without food. Three quarters of patients maintained a response after dose reduction. To minimize the risk of AOE, the importance of controlling cardiovascular risk factors cannot be stressed enough. Until the recent approval of asciminib, ponatinib has been the only agent that is active against T315I mutation. Despite its toxicities, ponatinib has been and remains a valued weapon against CML. It is still considered the treatment of choice in patients who have resistance to multiple TKIs without molecular response [9, 23].

50.3.2 Asciminib

Asciminib is the first-in-class STAMP inhibitor. Unlike the other approved TKIs which mainly target the ATP-binding site of BCR::ABL1, asciminib is an allosteric inhibitor that binds a myristoyl site of the protein and restores inhibition of kinase activity [16]. Asciminib is active against both native and mutated BCR::ABL1, and can overcome the resistance conferred by the gatekeeper T315I mutation when prescribed at high dose. In a cohort of heavily pretreated CML patients, around half of those without a complete cytogenetic response at baseline achieved CCyR with asciminib [16]. The superiority of asciminib to bosutinib, one of the 2G-TKIs, in patients with CML-CP after at least two prior TKIs had also been demonstrated in the phase 3 ASCEMBL trial [8]. The rate of MMR was 25.5% in the asciminib arm versus 13.2% in the bosutinib arm, albeit there was some imbalance in the baseline disease characteristics between treatment arms despite randomization. Regardless, the study outcomes resulted in the accelerated approval of asciminib by the U.S. FDA for chronic phase patients previously treated with two or more TKIs. Asciminib has also been approved for the treatment of patients with T315I mutation. The recommended dose of asciminib is 80 mg once daily or 40 mg twice daily in patients without T315I mutation, and 200 mg twice daily in patients with T315I mutation. Food intake should be avoided for at least 2 h before and 1 h after drug administration. Overall, asciminib is well tolerated. Common adverse events include fatigue, arthralgia, headache, hypertension, and thrombocytopenia. It is also associated with increased amylase and lipase, and uncommonly clinical pancreatitis [16].

50.4 Options Other than TKIs for Patients Ineligible for HSCT

50.4.1 Omacetaxine Mepesuccinate

Omacetaxine mepesuccinate, a protein synthesis inhibitor, is a semi-synthetic purified form of homoharringtonine (HHT) [24]. It is administered by subcutaneous injection twice daily and approved for the treatment of CML patients with resistance and/or intolerance to two or more TKIs. The CML-300 study [25] showed that omacetaxine had modest activity in this group of patients with the rate of complete cytogenetic response at around 10–20%. Significant side effects included haematological toxicities and infection. Omacetaxine has a different mechanism of action from that of BCR-ABL1 TKIs and thus is not affected by KD mutations. Nevertheless, its role in the management of CML is overshadowed by the potent TKIs. Its popularity is also undermined by its limited availability and inconvenience of administration.

50.4.2 TKI Plus Interferon-α Combination

Interferon-α- (IFNα-) based regimen used to be the standard-of-care in CML before emergence of TKI. In recent years, there has been a revival of interest in the role of IFNα in CML management, mostly with respect to deepening molecular response and treatment-free remission due to its immunomodulatory effects [26]. Combinations of TKI and IFNα or its pegylated formulations did result in improved efficacy. In the French SPIRIT study, the combination of imatinib and peginterferon produced higher rates of major molecular response and deep molecular response than imatinib monotherapy in newly diagnosed CML patients [27]. Small phase 2 studies of 2G-TKI and peginterferon combination also suggested promising molecular responses [28, 29]. In addition, there are case reports on overcoming TKI resistance by using TKI plus interferon/peginterferon combo [30, 31]. For patients who are running out of TKI options and ineligible for HSCT, TKI- IFNα combo may be considered, apart from participating in clinical trial of novel agents.

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Allogeneic Hematopoietic Cell Transplantation in CML: When and How?

51

Fiona Fernando and Andrew J. Innes

Abstract

Patients with chronic myeloid leukaemia (CML) presenting in chronic phase, who achieve and maintain optimal responses to tyrosine kinase inhibitors (TKIs), can potentially enjoy a normal life expectancy in many cases, with a subset maintaining their remission upon treatment withdrawal. However, this success is not universal and a sizable minority fail to achieve remission on any TKI. Additionally, for some, the treatment-associated toxicities of TKIs prohibit long-term use. In the setting of accelerated or blast phase disease, the TKIs have much less impact, and often the responses, if any, are short lived. The TKI-independent mechanism of allogeneic haematopoietic cell transplantation (HCT) means HCT can be exploited in these settings, and the exquisite sensitivity of CML to the graft-versus-leukaemia effect lends itself well to this modality. In this review, we explore the indications for HCT in CML in the TKI era, and consider the intricacies of transplant use in this setting.

Keywords

Transplant · CML · Chronic phase · Accelerated phase Blast phase

51.1 Introduction

The outcome of patients with chronic myeloid leukaemia (CML) has been transformed as a result of the remarkable efficacy of the tyrosine kinase inhibitors (TKI) such that many patients now enjoy a normal life expectancy on TKI treatment [1]. As the development of TKI therapy has evolved over the past two decades, transplantation, which used to be the mainstay of treatment for eligible patients, is now reserved for those patients with the most resistant or difficult to treat disease [2]. Over the same period of time, improvements in supportive care and the implementation of reduced intensity conditioning regimens, coupled with increasing donor availability, both from increasing registry sizes and the improved safety of haploidentical platforms, means allogeneic stem cell transplantation has become a feasible option for more patients [3]. This has led to a shift in the demographic of the transplant population in CML, with a rise in the median age for transplant, a bias towards transplantation in later phases of the disease, and a move towards more frequent use of reduced intensity regimens (Fig. 51.1). As with all haematological malignancies, the decision to embark upon transplantation in patients with CML is highly individualised, and relies on balancing the risk of disease progression and death from CML with the toxicities and long-term implications of transplantation. Transplantation remains an important curative modality for selected CML patients, with either TKI-resistant chronic phase disease, or advanced-phase CML, and can be considered in those with intolerable TKI toxicities. Early assessment for transplant is key to managing these patients, because stage of disease at time of transplant is one of the most important predictors of outcome [4].

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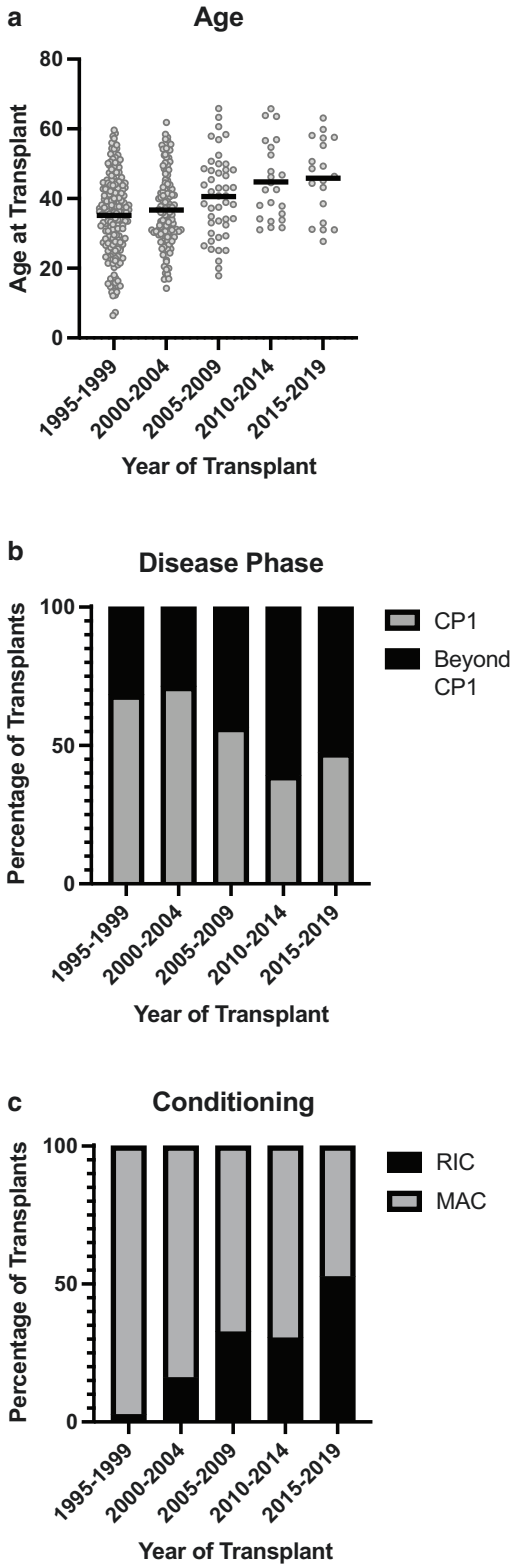

Fig. 51.1 The changing demographic of transplant in CML over the past 25 years, stratified in 5 years intervals. (**a**) Age of transplant, bars represent median age. (**b**) Disease phase at transplant. (**c**) Choice of conditioning regimen. CP1, first chronic phase; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning

51.2 Principles of Transplant and Their Application in CML

The role of haemopoietic cell transplant (HCT) in the management of patients with CML is ever evolving, and in parallel to developing experience in TKI use, transplant practice has evolved too. The use of reduced intensity conditioning regimens results in fewer toxicities, and there have been general improvements in supportive care such as better prophylaxis against infections [5, 6] and improved treatment options for graft-versus-host disease (GvHD) such as ruxolitinib [7]. These changes have been key to not only reducing transplant-related mortality (TRM), but also lowering long-term morbidity from HCT. Over the last 20 years, a number of strategies have seen a fall in the prevalence of chronic graft-versus-host disease (cGVHD) in a number of transplant settings [8], but this is particularly important in CML for a number of reasons. Firstly, while CML is particularly sensitive to the graft-versus leukaemia effect, molecular relapse necessitating donor lymphocyte infusions (DLI) are common [9], and the development of chronic GvHD can make their use challenging. Moreover, the late effects of chronic GvHD can be debilitating and in some cases fatal, and therefore exchanging one life limiting condition (CML) for another (cGvHD) achieves little.

With an increasingly aging population of HCT recipients in CML (Fig. 51.1a), it is important to consider the impact of recipient characterises and existing comorbidities on transplant outcome. The most widely used scoring system to quantify these risks is the HCT-comorbidity index [10] which can reproducibly predict co-morbidity-associated TRM.A number of scoring systems that focus more on the disease characteristics can also be used to help predict transplant outcomes. The European Society for Blood and Marrow Transplantation (EBMT) score identified five parameters predictive of overall survival: donor type (sibling vs unrelated donor), pretransplant disease stage, patient age, donor and recipient sex matching, and time from diagnosis to transplant [11]. The EBMT score is also strongly predictive of TRM, and was initially developed in a CML cohort before wider validation. Whilst this tool was developed in the pre-TKIs era, when RIC regimens were less common, it reinforces the importance of the principle of assessing disease specific factors, alongside both patient and donor characteristics, in order for clinicians to make an informed decision on transplant eligibility, and remains a robust predictor of outcome in current practice.

Despite the exquisite sensitivity of CML to the GvL, disease recurrence remains a common problem post-HCT, something that is exacerbated by RIC regimens [12, 13]. Quantitative BCR-ABL1 monitoring is therefore mandatory post-transplant, in order to identify patients with relapse early. Whilst very low level BCR:ABL1 transcripts may be detectable post-HCT without evidence of relapse, criterial exist that are strongly associated with progressive disease [14]. With careful

monitoring, molecular relapse can be identified before overt disease recurs, and can be managed with early intervention with DLI or TKI. Close observation is recommended for the first 3 years post-transplant when most relapses are likely to occur, but it remains important to remain vigilant as late relapses can occur many years after transplant [15].

DLI remains the most effective salvage therapy in molecular relapse which results in restoration of molecular remission in between 60% and 90% of patients allografted in first chronic phase. The major complication of DLI is the development of GVHD, which typically occurs between 4 weeks and 8 weeks after the infusion. The development of escalating dose regimens of DLI have reduced the risk of concomitant acute GVHD [16], and this can still be used in the mis-matched donor setting, providing sufficient time has elapsed since transplant [17]. TKIs represent an alternative salvage strategy in patients who relapse after HCT, and can be particularly useful in those with evidence of active GVHD precluding the use of DLI, or those in whom DLI cannot otherwise be delivered because of donor availability. However, increasingly, most patients now reaching HCT have already demonstrated resistance to multiple TKIs, including the most potent, and therefore, TKIs may not be practical. This can often be compounded by sensitivity of the donor graft to TKIs, which often results in dose limiting haematological toxicities if used early post-transplant.

51.3 Current Indications for Allogenic SCT in CML

The decision to proceed to allogenic HSCT and consequently, the timing of HCT, in the TKI era, depends on the phase of CML: classically, chronic phase (CP), accelerated phase (AP) or blast phase (BP) (Table 51.1, [21, 22]). The poor outcomes seen with non-transplant strategies in advanced phase, and in particular CML-BP, continue to position HSCT as a standard of care for eligible patients. However, the decision to embark upon a transplant strategy for CML-CP can be more of a challenge. In 2022 the World Health Organization (WHO) proposed a revised classification from a triphasic (CP, AP and BP) to biphasic (CP and BP) disease [20], with the addition of high risk CP features.

Table 51.1 BC and AP definitions

	WHO criteria 2008 [18]	Provisional WHO 2023 [19]	ELN criteria [20]
Blast crisis			
Peripheral blood or bone marrow blasts	≥20%	≥20%	≥30%
Additional defining characteristics	Extramedullary blast proliferation (Except spleen), Large foci of blasts in spleen or bone marrow	Extramedullary blast proliferation, presence of lymphoblasts in the peripheral blood or bone marrow even if <10%	Extramedullary blast involvement (except spleen)
Accelerated phase			
Peripheral blood or bone marrow blasts	10–19%	No accelerated phase group	15–29% blasts; or blasts plus promyelocytes >30%, with blasts alone <30%
Peripheral blood basophils	≥20%	–	≥20%
Platelets	$<100 \times 10^9$/L unrelated to therapy, or platelets >1000 unresponsive to therapy	–	$<100 \times 10^9$/L not attributable to treatment
Evidence of clonal evolution	Additional clonal chromosomal abnormalities in Ph+ cells at diagnosis (Second Ph, trisomy 8, isochromosome 17q, trisomy 19), complex karyotype or abnormalities of 3q26.2 Any new clonal abnormality in Ph+ cells occurring on therapy	–	Appearance of additional genetic abnormalities on treatment
White cell count and spleen size	Persistent or increasing WBC ($>10 \times 10^9$/L) unresponsive to therapy. Persistent or increasing splenomegaly unresponsive to therapy	–	Not included
Provisional criteria—Response to TKI	– Haematological resistance (or failure to achieve a complete haematological response) to the first TKI – Any haematological, cytogenetic or molecular indications of resistance to two sequential TKIs – Occurrence of two or more mutations in the BCR-ABL1 fusion gene during TKI therapy	–	

This remains a controversial change, not least because the impact on interpreting the historical data in the context of the new classification. Here we discuss the data in the context of a triphasic disease, but acknowledge that those previously classified as AP may require more nuanced interpretation.

51.3.1 Transplant Indications and Outcomes in Chronic Phase

It is important to remember that whilst many patients with CML will achieve optimal responses to TKI therapy defined by the ELN criteria [20], this is not universal. Just as there are a group of patients who will enter a deep, durable remission and even develop an 'operational cure', there are a group of patients in whom an adequate response will never be achieved with TKIs alone, or in whom the toxicities are so severe that their long-term use is intolerable. For transplant-eligible patients in this group, HCT remains a worthy option. Moreover the independent mechanism by which GvL controls CML post-HSCT, means that transplant can be a very effective strategy even in those with ubiquitous resistance to TKIs.

Whilst the management of CML has been discussed extensively in Chaps. 48, 49 and 50 broadly speaking first-line imatinib will induce a major molecular remission (MMR) in ~60% of patients [23]. In those failing imatinib, second-generation TKIs (2GTKI, dasatinib, nilotinib, and bosutinib) can be expected to achieve durable remissions in approximately 50%, with the third-generation drug [24, 25], ponatinib capable of rescuing approximately 50% of those failing 2GTKI [26]. For the remaining cohort, which equates to approximately 10% of the starting group, there is either no, or suboptimal, responses to all generation drugs, and transplant or experimental therapies are the only remaining strategy with disease modifying potential. Whilst there is ongoing interest in novel BCR:ABL1 inhibitors, such as asciminib with molecular responses in up to 40% of patients with resistance to multiple TKIs [27], the long-term data are awaited.

Additionally, increasingly physicians feel uneasy about the long-term risks of TKIs, and in particular, the cardiovascular risks. For example, nilotinib has been reported to be associated with accelerated risk of atherosclerosis [28, 29] and ponatinib with heart failure in addition, in a dose-dependent manner [30]. Whilst lower-dose maintenance regimens are currently under investigation (OPTIC study [31]) open discussions with patients should be held about the role of HCT in this setting.

The up-front use of second (2GTKI) including dasatinib, nilotinib and bosutinib are more effective in achieving molecular responses, with ~75% of patients achieving an MMR at 4 years [32–34]; however, the toxicity profiles of 2GTKIs mean that discontinuation rates of upfront imatinib or upfront 2GTKI are broadly similar, but more patients will have discontinued imatinib for failure, while more will have discontinued 2GTKI because of toxicity [32].

A specific subset of patients who are destined to fare poorly are those with T315I mutation, which predicts resistance to imatinib, nilotinib, dasatinib, and bosutinib. It occurs in approximately 20% of patients who have not achieved a durable CCR post first-line TKI therapy [26]. Whilst the presence of this mutation was previously an indication in itself, to proceed to transplantation, ponatinib has been shown to induce a durable response in a cohort of patients harbouring these mutations [26, 35], and asciminib, at higher doses looks promising too [27], but this is not universal, and these patients require close supervision. A retrospective comparison of patients with CML harbouring T315I mutations treated with ponatinib alone or ponatinib followed by HCT favoured ponatinib alone in patients in CMP-CP, but favoured HCT in CMP-AP or CML-BP [36]. It is however important to note, that while a plateau in survival occurred at 4 years in the transplant cohort, there was a continued decline in survival for those treated with ponatinib alone, so longer-term follow-up is required to determine which strategy delivers the optimal long-term strategy.

Table 51.2 summarises the current indications for HCT in CML-CP, but with the development of novel therapies, this is a constantly evolving field. The strongest indications currently for HCT in CML-CP is treatment failure with a 3GTKI or severe intolerance to 3GTKI. Consideration should also be given to those patients who do achieve an optimal response to 3GTKI, particularly where a T315I mutation has been detected, especially where the risk of long-term toxicities

Table 51.2 Indications for transplant in CML

	Indication strength
Chronic phase disease	
T315I mutation with 3GTKI failure	Strong[a]
Failure of 3GTKI (No T315I mutation)	Strong[a]
Failure of 2 × 2GTKI	Moderate[a, b]
T315I mutation with optimal response to 3GTKI (Or equivalent)	Moderate[a, b]
Optimal response to 2GTKI or 3GTKI, but with concerns about long-term toxicity	Moderate[a, b, c]
Financial burden of long-term TKI	Moderate[d]
Advanced phase disease	
Progression to AP on treatment	Strong
De novo AP	Moderate[e]
Blast phase disease	
All patients with blast phase	Strong

2GTKI, second-generation tyrosine kinase inhibitor; *3GTKI,* third-generation tyrosine kinase inhibitor; *AP,* accelerated phase; *TKI,* tyrosine kinase inhibitor

[a] Give consideration to novel agents; e.g. asciminib

[b] Start transplant discussion early while perusing alternative treatments

[c] Consider long-term toxicity of current treatment vs long-term toxicity of transplant, consider dose-reductions of safe

[d] Consider prospect of TFR success vs need for long term use vs transplant cost

[e] Required close molecular monitoring if non-transplant strategies are pursued

cannot be mitigated with dose reductions. In practice, most patients undergoing HCT in CML-CP will have failed or been intolerant to ponatinib, but it remains important to give early consideration to HCT in those failing or intolerant to 2GTKS.

The outcomes of patients with first chronic phase (CP1) after a MAC allograft from a matched sibling or volunteer unrelated donor have continually improved over the last 3 decades, with retrospective registry data reporting 3-year survival rates ranging from 70% to 90% for those who received a myeloablative conditioned HSCT using a matched sibling donor, and similar outcomes for matched unrelated donors [2].

51.3.2 Transplant Outcomes in Accelerated Phase

CML patients that fall into the category of accelerated phase describe a group of CML patients with marked heterogeneity with clinical phenotypes found between the spectrum of chronic phase and near overt blast crisis. Therefore, there is great difficulty in implementing one single treatment strategy for this entire patient cohort. The outcomes of patients with CMP-AP treated with TKIs alone compare unfavourably who present in CP. Of all CML patients with accelerated phase, 20–40% will achieve a CCR after treatment with a TKI; however, this response may not be durable [37, 38]. There also appear to be distinct responses for those presenting in CML-AP compared to those progressing to CML-AP whilst on treatment, with the outcomes for the later significantly worse. While a risk-managed approach, with carefully molecular monitoring of response to TKI can be considered in de novo CML-AP, all eligible patients progressing to CML-AP on treatment should be referred for HCT [20].

Even within the cohort of de novo CML-AP, there is heterogeneity, with some early in the spectrum of CML-AP and others closer to the definition of CML-BP. For CML-AP patients who sit in the spectrum on the brink of transformation to CML-BP, HSCT undoubtedly offers the best chance of long-term survival. However, TKIs with careful monitoring might suffice for those who are in the early stages of the transition from CML-CP to CML-AP. As a result, attempts have been made to further risk stratify patients in accelerated phase. Jiang et al. [39] proposed a model that may predict TKI responses based on a number of disease characteristics: CML duration (≥12 months), haemoglobin (≤100 g/L), and peripheral blood blast percentage (≥5%) are used as predictive markers, with the high-risk group defined by those with two or more of these risk factors. The outcome for high-risk patients who did not receive a HCT was poor (5 year OS: 18% vs 100%). Whereas, those with no risk factors and deemed to be low-risk gained no survival advantage from HCT over treatment with imatinib alone. It is important to note however that this study used the 2008 WHO criteria for AP [18, 21], which is generally more conservative than the ELN criteria [20], and therefore, patients in this cohort would likely represent those earlier in the natural history of progression.

In practice, most physicians will initiate treatment in patients with CML-AP and institute close molecular monitoring. The majority of clinicians would elect to proceed to HSCT in those patients with high-risk accelerated phase disease, as it provides a potentially curative treatment modality, with 5-year progression free survival rates of 50–80% in subsets of patients who have undergone both sibling and unrelated donor HSCT.

51.3.3 Transplant Outcomes in Blast Phase

Despite marked improvement in outcomes of both chronic phase and accelerated phase CML with TKIs, overall survival in patients with blast phase CML remains poor. The impact of TKIs has been the least significant in this group of patients, with only a modest increase in median survival following their widespread use, with any benefit usually being short lived unless consolidated with HCT. HCT should be offered to all eligible patients with CML-BP as it provides the only treatment modality that can offer potential of long-term survival [40, 41].

Those presenting in CML-BP regimen immediate treatment, with the focus being to achieve a second chronic phase (CP2), and TKI alone is unlikely to achieve this. Intensive induction chemotherapy regimens are therefore necessary, and once a remission is achieved, consolidation with an HCT is the only strategy offering the prospect of long-term survival and cure.

The optimal induction regimen has not been defined, and there are no head-to-head studies comparing chemotherapy alone to chemotherapy plus TKI; however, given the aggressive nature of CML-BP, a pragmatic concurrent approach of chemotherapy and TKI is the most frequent choice [42, 43]. While in CML-CP, the BCR-ABL1 fusion drives an exclusively myeloid proliferation, CML remains a stem cell disorder, and at transformation, the blast population can be myeloid, lymphoid or biphenotypic/mixed lineage. Several induction chemotherapy regimens have therefore been used (FLAG-Ida, HyperCVAD, and ALL-type induction protocols), and some physicians may take into account the blast lineage when selecting the regimen. There are no randomised trials of TKI in CML-BP, and the choice will be guided either by mutations status (particularly in those transforming to CML-BP compared to those presenting in CML-BP), or by physician choice, but will typically be second or third generation drug.

Importantly, the non-relapse mortality of CML-BP, even if CP2 is achieved, is significantly higher than that of CP1, and even of acute leukaemias transplanted in remission. In those transplanted in active BP, the TRM can be as high as

40–50%, compared to around 20–30% for those who successfully achieve return to chronic phase (CP2) with chemotherapy [44, 45] which compares to around 10% for those in CP1 [46]. Long-term survival rates for patients who achieve a second CP range from 30% to 40%.

51.4 Transplant Optimisation

51.4.1 Pre-transplant Therapy

Patients undergoing transplantation for CML-CP will likely have cycled through several TKIs before the decision to transplant has been reached. The choice of treatment while awaiting HCT will be dictated by drug toxicities, intolerance and response. In the scenario of patients who are failing TKI, the priority is to move to transplant in a timely manner, and before accelerated or blast phase develops.

In the case of transplantation for patients with CML-BP, the best outcomes are seen in those achieving a remission, CP2, prior to HCT [45]. Whilst the optimal regiment to achieve a remission in CML-BP remains debatable, typically it will include an intensive 'salvage' type chemotherapy regime, in combination with a second or third generation TKI [42, 43].

There is no role for induction-type combination chemotherapy prior to HCT for those in chronic or accelerated phase.

51.4.2 Optimal Conditioning Regimen

There are no prospective trials comparing myeloablative conditioning (MAC) directly with reduced intensity conditioning (RIC). For younger, fit patients, myeloablative regimens are often the preferred conditioning modality, principally because of the lower risk of GvHD and lower relapse risk, nevertheless, RIC protocols remain a feasible strategy for patients ineligible for myeloablative protocols.

MAC conditioning frequently consists of chemoradiotherapy given to condition the marrow for stem cell transplantation and typically consists of total body irradiation (TBI) with additional chemotherapy, which is commonly cyclophosphamide. Cyclophosphamide—busulfan is comparable to cyclophosphamide-TBI, without the toxicity of irradiation [47, 48] and has become a frequently employed regimen. TBI will often be favoured in the setting of extramedullary disease.

As previously discussed, given the high TRM associated with MAC regimens for patients with comorbidities, increased age or poorer performance status, RIC regimens have allowed many patients who may be otherwise considered ineligible for transplant to be considered for HCT. However, the optimal RIC regimen has not been defined, many centres use a combination of fludarabine and busulfan at non-myeloablative doses.

MAC regimens remain extremely well tolerated in patients with CML-CP compared to those with other malignancies, with recent studies reporting TRMs of less than 10% [46]. Additionally, RIC regimens, while less toxic, are associated with higher rates of chronic GVHD, complicating the use of donor lymphocyte infusion, which is commonly required for disease eradication in this context.

Recent retrospective studies have shown that RIC and MAC have broadly similar outcomes in CML-CP, but that the lower TRM of RIC regimens is offset by the higher risk of relapse [49], and so the decision will typically rest on the balance of patient fitness and disease pressure. Traditionally most physicians would have favoured MAC regimens for those beyond chronic phase, but the clearly increased in TRM associated with transplant in BP does seem to be better offset by the lower TRM risk of RIC, which translated to better overall outcome in recent reports from EBMT analysis [45]. Extreme cation must be exerted when employing RIC regimens in BP however, as post-HCT relapses in this setting are exceptionally resistant to treatment, and almost universally fatal.

51.4.3 The Effect of Graft vs Leukaemia

CML remains one of the most sensitive haematological malignancies to the graft-versus leukaemia (GvL) effect. However, whilst T-cell mediated GvL is the cornerstone of long-term disease control following HCT, there remains a careful balance between optimal GvL, and the occurrence of GvHD, which can be catastrophic. T-cell depletion can either be achieved by negatively selecting donor T-cells in the graft *in vitro* or by treating recipient with monoclonal antibodies (alemtuzumab) or antithymocyte globulin *in vivo*. T-cell depletion was introduced partly to circumvent high rates of TRM associated with the use of volunteer unrelated donors, and whilst this appeared effective in reducing the incidence and severity of acute GVHD, it rapidly became apparent that this benefit came at the expense of a higher risk of relapse, providing direct evidence for the role of the GVL effect.

The exquisite sensitivity of CML to the GvL is evident in the responses to DLI. Administration of DLI is used to manipulate the GvL effect and to restore full donor chimerism and bolster T-cell mediated responses to induce durable remissions. The responses to DLI in CML exceeds 80%, and once molecular negativity is achieved, subsequent relapses are rare [9].

There are no consensus guidelines on whether to T-cell deplete prior to stem cell infusion for CML, or on which

strategy is optimal if T-cell depletion is employed. This decision is ultimately made based on physician and institution preference, and will likely take into account a number of variables. e.g. while T-cell depletion might be advantageous for an HLA—mismatched recipient undergoing HCT in chronic phase, there may be less benefit for a patient in second chronic phase undergoing an HLA-identical sibling HSCT where the risks of acute GVHD are less, and the risk of relapse significantly higher.

51.4.4 Source of Stem Cells

Peripheral blood stem cells (PBSCs) have now largely replaced BM derived stem cells as the stem cell source of choice in most situations, predominantly because of donor preference, but also because of the earlier engraftment they produce. However, the use of PBSCs for patients in CML-CP1 is associated with increased non-relapse morality, driven by higher rates of chronic GvHD compared to bone marrow grafts [50, 51]. Given that the majority of patients undergoing transplant for chronic phase CML will subsequently develop molecular relapse necessitating DLI, a strong case can be made for maximising strategies to avoid chronic GvHD in this setting. Active chronic GVHD precludes the use of DLI, thereby hindering the management of relapse, on top of which, there is a significant burden of morbidity associated with chronic GVHD which should not be overlooked. Nevertheless, while this data clearly favours the use of bone marrow derived stem cells in patients with first chronic phase CML, the final choice of the harvest method lies with the donor, and PBMC continue to be the most frequent source.

Haploidentical transplantation can extend the opportunity for transplantation for patients who lack an HLA-matched sibling or unrelated donors. Significant advances in haploidentical platforms have led to a marked decrease in TRM historically associated with them. Due to widespread availability of haploidentical donors, improved TRM and development of haploidentical transplanting expertise, haploidentical donors are becoming a readily available source of stem cell donors, and CML is no exception [3].

51.5 Conclusion

The success of TKI therapy in CML has resulted in a marked decline in the number of patients requiring allogeneic HCT. However, HCT remains a highly effective therapeutic modality in high-risk patients with resistant and/or advanced disease.

Advances in transplantation and donor availability have simultaneously increased access to transplant and improved patient outcomes for a number of patients who may have previously been considered ineligible.

The use of TKIs has undoubtedly transformed the therapeutic landscape of CML-CP, but not all patients will respond to, or can tolerate, these drugs. The dismal outcome associated with progression to BP means that this should be avoided at all costs, and it is therefore important to identify non- or suboptimal-responders early, in order to move to transplant before progression beyond CP occurs. HST remains the cornerstone of treatment for CML-BP, and in CML-AP patients unless rapid deep responses are achieved (Fig. 51.2).

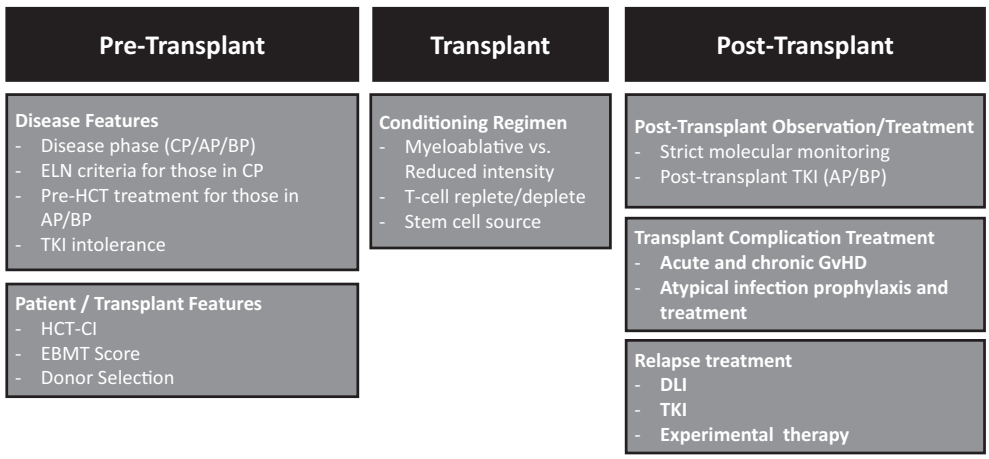

Fig. 51.2 Considerations for transplant in CML. AP, accelerated phase; BP, blast phase; CP, chronic phase; DLI, donor lymphocyte infusions; EBMT, European group for blood and marrow transplantation; GvHD, graft versus host disease; HCT-CI, haematopoetic cell transplantation-comorbidity index; TKI, tyrosine kinase inhibitors

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In the Pipeline: Emerging Therapy for CML

52

Harinder Gill, Emily Lee, and Pinky Mo

Abstract

The development of BCR-ABL1 tyrosine kinase inhibitors (TKI) has revolutionized the treatment of CML. A major limitation of TKI therapy is the relative inability to eradicate quiescence CML leukemic stem cells (LSC) that persist in the bone marrow microenvironment (BMM), which limits the achievement of treatment-free remission (TFR) in patients who have discontinued TKIs. This chapter will discuss novel and combination therapeutic strategies in targeting abnormal HSCs and TKI resistance in CML patients, with an aim of inducing TFR through the eradication of the LSC population. These strategies include the following: (a) targeting of signaling pathways in CML stem cells, (b) targeting the interaction between LSCs and the bone marrow niche, (c) targeting cell cycle and apoptosis via p53 modulation, (d) targeting differences in epigenetic regulation between normal HSCs and LSCs, (e) targeting autophagy, and (f) immunotherapy.

Keywords

Chronic myeloid leukemia · Leukemia stem cell
Targeted therapy

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52.1 Introduction

Chronic myeloid leukemia (CML) is a hematological malignancy arising from mutation of a single progenitor cell. It is characterized by the presence of the Philadelphia (Ph) chromosome resulted from the specific chromosomal translocation t(9, 22) (q34.1;q11.2), forming an oncogenic fusion gene BCR-ABL1 on chromosome 22 [1, 2]. BCR-ABL1 is a constitutively active tyrosine kinase that causes aberrant activation of downstream signaling pathways, such as phosphoinositide 3-kinase (PI3K)/murine thymoma viral oncogene homolog (AKT)/mammalian target of rapamycin (mTOR), Rat Sarcoma proto-oncogene (RAS)/extra—cellular signal-regulated kinase (ERK) and Janus Kinases (JAK)/Signal Transducer and Activators of Transcription (STAT) [3]. This results in CML initiation, maintenance, and progression.

The development of BCR-ABL1 tyrosine kinase inhibitors (TKI) has revolutionized the treatment of CML, yielding promising outcomes in the contexts of hematological, cytogenetic and molecular response [3]. In a clinical trial evaluating the long-term outcome of imatinib-based therapy, the 10-year overall survival (OS) rate was estimated to be 83.3% [4]. However, 15% of patients failed to obtain a satisfactory response due to imatinib resistance [3–5]. TKI resistance can be BCR-ABL-dependent and BCR-ABL-independent, the study of resistance mechanisms has led to the discovery of novel therapeutic targets.

Another major limitation of TKI therapy is the inability to eradicate quiescence CML leukemic stem cells (LSC) that persist in the bone marrow microenvironment (BMM), which obscures the achievement of treatment-free remission (TFR) in patients who have discontinued TKIs. In CML, TFR refers to having a stable deep molecular response (DMR) without the need for ongoing TKI treatment [6]. Although CD34+/CD38—is widely accepted as the principal immunophenotype of CML LSCs, the CML stem cell subpopulations exhibit a considerable degree of heterogeneity [7]. Variations in immunophenotype lead to difference in

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leukemogenicity among CML LSCs and their response to TKI therapy [8–11]. TKIs are able to eliminate LSCs with myeloid and proliferative molecular signatures to a greater extent compared to the subfractions exhibiting more primitive and quiescence signatures, leading to their persistence throughout the course of the therapy and expansion upon cessation of TKI therapy, thus is rendered inadequate in the eradication of abnormal HSCs found in CML patients [11–13].

52.2 Current Therapy for CML

52.2.1 TKIs

TKIs are ATP-analogues which competitively bind the ATP-binding site of BCR-ABL1, blocking the constitutively active tyrosine kinase via reducing aberrant phosphorylation to inhibit downstream signaling pathways and subsequent leukemogenesis [14–16]. Despite possessing strong anti-proliferative on LSCs, it only induces modest level of apoptosis [17]. Quiescence LSCs are especially resistant to TKI-mediated apoptosis and eradication as a result of BCR-ABL-1-independent mechanisms, contributing to disease relapse and progression. Although 50% of CML patients are able to achieve deep molecular remission (DMR) post TKI treatment, most harbor residual CML LSCs [18–20]. The first-generation TKI imatinib (IM) is able to target BCR-ABL+ cells via antagonizing inositol-triphosphate (IP3)-mediated calcium mobilization and oxidative stress through IP3 receptor inhibition on the endoplasmic reticulum. This results in reversal of dysregulated intracellular calcium signaling and uncontrolled expression of pro-inflammatory cytokines like IL-6, IL-8 and NF-kB in CML stem cells [21–23]. Second-generation TKIs like nilotinib and dasatinib improved 3-month major molecular responses ($p < 0.05$) as they are more potent in terms of inhibiting IP3R [21, 24]. The third-generation TKI ponatinib is indicated for patients with BCR-ABL1 T315I mutation or those refractory to two or more TKIs [25, 26]. Recently, FDA has approved a fourth-generation TKI, asciminib (ABL1001), which acts as an allosteric inhibitor that binds the BCR-ABL1 myristoyl-pocket (STAMP) and has been proven to be effective against BCR-ABL1-dependent and -independent mutations as monotherapy or in combination with other TKIs [16, 20, 27, 28]. It restores TKI sensitivity and synergizes with other TKIs by reducing CRK-like protein (CRKL) phosphorylation in CML stem cells [17, 27].

52.2.2 Interferon-α

IFNα was formerly the frontline treatment for CML prior to the introduction of TKIs. It has multiple mechanisms in targeting CML LSCs including induction of apoptosis, altering their interaction with the BMM and immune activation [29]. It induces LSCs apoptosis through the upregulation of Fas-receptors and release of cytochrome-c and activation of FADD/caspase-8 pathway, resulting in mitochondrial outer membrane permeabilization (MOMP) and apoptosis [30–32]. IFNα also disrupts the protective interaction offered by the BMM to CML LSCs by restoring its normal function via β1-integrin [30, 33, 34]. In terms of immune activation, IFNα induces major histocompatibility complex (MHC) class I expression, which presents tumour-specific antigens leading to cytotoxic T lymphocyte (CTL)-mediated cytotoxicity against LSCs [30, 31]. In a meta-analysis including seven randomized trials with 1554 patients, the 5-year OS was 57% [35]. In another study investigating IFNα monotherapy, the 10-year OS was 72% and complete cytogenetic response (CCyR) was 46% [36]. Promising results demonstrated the potential for re-introduction of IFNα for treatment of CML, yet the toxicity profile for long-term use and its role in achieving TFR remain to be elucidated.

52.3 Limitations of Current Therapeutic Options: TKI Resistance and LSCs Persistence

TKI resistance can be attributed to BCR ABL-dependent and BCR-ABL-independent pathways. BCR-ABL-dependent resistance is conferred by ABL1 kinase domain mutations sterically modifying the ABL1 protein structure, resulting in failure of TKI attachment or stabilization of the active conformation of the kinase. They can be further categorized into mutations in the activation loop that regulate entrance to the catalytic site, mutations affecting the ATP-binding loop and ATP-binding sites [37]. These mechanisms of resistance are known to account for 50–90% of the patients that experience hematological relapse after receiving IM [38, 39]. The "gatekeeper" mutation T315I is resulted from the replacement of threonine with isoleucine, rendering IM, nilotinib, dasatinib and bosutinib ineffective, leading to the development of ponatinib, a pan-BCR-ABL inhibitor exhibiting broad-spectrum inhibition against all BCR-ABL mutants [37]. Other mechanisms of resistance include amplification of the BCR-ABL1 oncogene and increased expression of BCR-ABL1 mRNA.

BCR-ABL independent mutations are conferred via aberrant expression of the regulatory pumps which controls influx and efflux of the TKI, hence drug bioavailability. These mutations are commonly overcome by administration of higher dose TKIs as demonstrated in in vitro studies [37]. Other BCR-ABL-independent mutations involve activation of signaling pathways (e.g., P13K/AKT/mTOR, JAK/STAT3/STAT5, and RAF/MEK/ERK) which promotes LSCs survival, proliferation, quiescence and stemness, rendering TKIs insufficient as a single agent in terms of disease eradication [37]. Mutations may also arise from epigenetic regulators (e.g., DNMT3A, EZH2, IDH1/2, 14, and 40) or tumour suppressor genes (e.g., TP53, PTEN, and TET1/2), as well as genes that code for anti-oxidant systems (e.g., FoxO and EPAS1) [14, 19, 40, 41]. Persistence of residual LSCs may lead to progression into the accelerated and/or blast phases. Novel therapies targeting these mechanisms of resistance will be discussed further below.

52.4 Novel Therapies

52.4.1 Novel Therapies that Target the Signaling Pathways in CML LSCs (Fig. 52.1)

52.4.1.1 Novel TKIs

Although ponatinib may inhibit the T315I mutation, its broad-spectrum kinase inhibitory activities result in off-target effects and may cause adverse cardiovascular events, giving rise to the development of newer tyrosine kinases [42]. PF-114 is a highly potent and selective fourth-generation TKI that inhibits native and mutated BCR-ABL. It is able to target T315 and other BCR-ABL-dependent mutations to lower the incidence of undesirable off-target effects and overcome non-mutational resistance in CML cells [42]. It antagonizes the BCR-ABL1 kinase domain and possesses STAMP inhibitory activity, thereby suppressing the hyperactive JAK/STAT3/5 and PI3K/AKT/ERK1/2 signaling pathways, as well as increasing p27 levels, resulting in G1 cell cycle arrest in LSCs [42–45]. Preclinical studies have shown ability to induce apoptosis in K562 and KCL-22 cell lines [42, 45]. In a K562 nude mouse xenograft, it completely eradicates leukemic bulk ($p < 0.001$) with no recurrence observed [42]. By sparing receptors of VEGF, FGF, EGF, PDGF, c-KIT, FLT3, and RET kinases, the potential of causing adverse effects such as myelosuppression, pancreatitis, cardiac and pulmonary complications has been dramatically reduced [42]. In phase 1/2 trials, PF-114 was given to patients who demonstrated refractoriness to previous TKI treatments. Major cytogenetic response (MCyR) was achieved in 55% of the subjects and 36% of them achieved major molecular remission (MMR) [43, 46]. Owing to its excellent safety and efficacy, it is considered a promising agent for patients resistant and/or refractory to first-line therapies.

52.4.1.2 miRNA as Direct Inhibitors of BCR-ABL

MicroRNAs (miRNAs) are short single-stranded non-coding RNAs that regulate gene expression on the post-transcriptional level by binding to their messenger RNAs

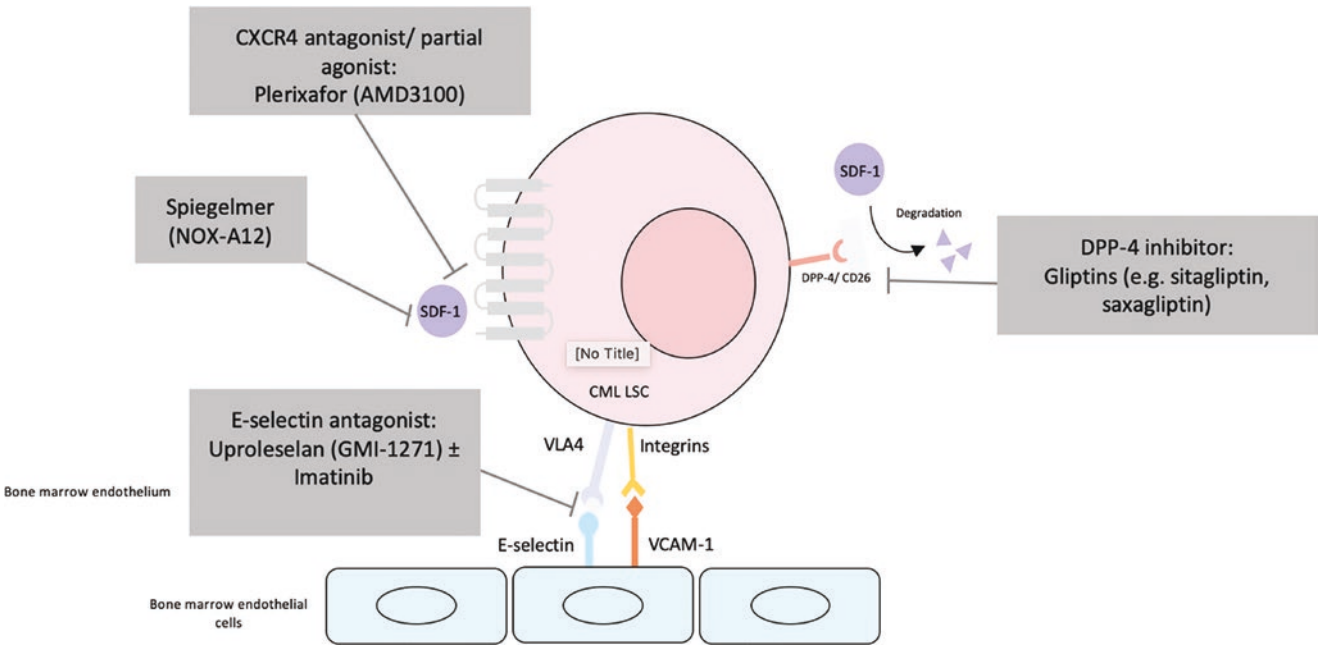

Fig. 52.1 Targeting interactions between CML LSCs and the bone marrow endothelium

(mRNAs). In general, such binding leads to mRNA degradation and represses expression of that particular protein [47, 48]. Aberrant miRNA expression drives leukemogenesis, maintenance and self-renewal of CML LSCs and TKI-resistance [47, 49, 50]. Low miR29 expression is believed to be associated with the high expression of BCL-2 and MCL-1 in the peripheral blood mononuclear cells (PBMC) of CML patients [47]. Preclinical studies also correlated low miR-29a expression with TET2 downregulation, which protects CML cells from IM-induced apoptosis, rendering IM-therapy ineffective [51]. miR29b functions as a tumour suppressor, shown to downregulate the expression of BCR-ABL1 and ABL1 in K562 cells. It also reduces cell proliferation and survival [50]. Long-term TKI exposure to K562 cells result in the development of drug resistance, which can be attributed to high DNA methyltransferase (DNMT) and low miR-217 levels [47, 52, 53]. The reduced miR-424 expression in CML LSCs aids maintenance and self-renewal, restoration of its expression results in inhibitory effects towards BCR-ABL1 and increase LSC sensitivity towards TKIs [47, 54, 55]. In vitro studies demonstrated that overexpression of miR-217 may restore tumour suppressive effects [47, 54, 55]. miR-142 expression is also shown to be downregulated in CML LSCs and TKI-resistant cells, which is associated with the increased expression of oncoproteins like MCL-1, c-KIT and SRI, leading to overactivation of downstream PI3K/AKT, JAK/STAT, and RAS/RAF/MEK/ERK signaling. Ultimately, low miR-142 levels yield anti-apoptotic and therapy-resistant effects, conferring LSCs with survival advantages [47, 54, 56–59]. Not only is the targeting of mi-RNA dysregulation a novel approach to reducing leukemic load in CML, it also possesses diagnostic and prognostic value to aid prediction of treatment response [60].

52.4.1.3 Inhibition of Grb2 Phosphorylation

The ABL portion of BCR-ABL phosphorylates tyrosine kinase residue 177 (Tyr177). This leads to binding of growth factor receptor-bound protein 2 (Grb2) to the SRC Homology 2 (SH2) domain of BCR-ABL. Subsequent Grb2-SOS complex formation activates the downstream RAS/MAPK pathway to promote leukemogenesis [61–63]. Grb2 binding to phosphorylated Tyr177 leads to association with Grb2-associated binder 2 (Gab2), and Gab2 phosphorylation activates downstream PI3K and SHP2 pathways, further contributing to the CML pathogenesis [61, 64]. L-Grb-2 Antisense Oligonucleotide (BP1001) is an RNA-targeted therapy which inhibits Grb2 expression to halt the RAS/MEK/ERK pathway. In a phase 1 study, BP1001 enhanced the effects of dasatinib by twofolds to sixfolds via the reduction of ERK1/2 phosphorylation and Grb2 levels. In patients with T315I mutation, reduction in circulating blasts was observed to be as high as 87% [65]. Preclinical studies have also demonstrated the effectiveness of Trametinib, an MEK inhibitor, in the restoration of TKI-sensitivity and inhibition of MEK/ERK and NF-κB-mediated LSC survival when used in combination with TKIs [66, 67].

52.4.1.4 Inhibition of mTOR

mTOR is a serine/threonine kinase that catalyzes the formation of mTORC1 and mTORC2 [68–72]. mTORC1 activation leads to the phosphorylation of its substrates p70-S6K, rpS6 and 4EBP1, resulting in the initiation of mRNA translation, cellular proliferation and autophagy inhibition [70, 72, 73]. mTORC2 activation results in Akt phosphorylation, leading to biosynthesis and cytoskeletal rearrangements which influence the migratory and apoptotic phenotype of tumour cells [74–76].

In CML, BCR-ABL is associated with increased activation of mTORC1 and mTORC2 via the induction of the P13K/Akt pathway [3, 68, 72]. The PI3K/Akt/mTORC pathway plays an important role in regulating quiescence and deregulation of HSCs; hence, dysregulation of such pathway from BCL-ABL expression leads to leukemia [70, 77, 78].

mTOR inhibition reduces viability and proliferation of CML cells, and increases IM efficacy and sensitivity in resistant cells [3, 69, 79–81]. In a preclinical study, treatment of K562 cell line with rapamycin resulted in a significantly reduced cell viability, which can be attributed to decreased BCL-2 and cyclin D1 expression, increased p21 expression and caspase-3 activation [79]. A dual mTORC2/mTORC1 inhibitor, OSI-027, is found to suppress primitive leukemic progenitors and induce apoptosis in TKI-resistant cells expressing the T315I-BCR-ABL mutation in human Ph + cell lines [69, 80]. Metformin, an anti-diabetic drug that activates AMPK, demonstrates the ability to inhibit constitutive P13K/AKT/mTOR signaling, which reduces oxidative catabolism of glucose and fatty acids, halting LSCs proliferation and ultimately induces apoptosis [81].

52.4.1.5 Inhibition of MNK1/2

The MAPK/MNK1/2 signaling is upregulated in CML cells but not in normal HSCs. This leads to constitutive phosphorylation of eukaryotic initiation factor 4E (eIF4E), which is an oncoprotein that regulates proliferation and self-renewal of LSCs, promoting the translocation of β-catenin into the nucleus. Such signaling plays a role in leukemogenesis and mediates TKI-resistance in LSCs [82]. The MNK1/2 inhibitor ETC-1907206 is shown to suppress phosphorylation of eIF4E and subsequent β-catenin signaling in preclinical studies [82].

52.4.1.6 Inhibition of B-Cell Lymphoma 2 (Bcl-2)

Bcl-2 is an important antiapoptotic protein regulating mitochondrial-mediated apoptosis, and promoting maintenance and survival of LSCs. [83–86] In CML cells, Bcl-2

levels are higher than in normal HSCs [87, 88]. One of the mechanisms by which BCR-ABL signaling supports the survival of CML cells is the upregulation of Bcl-2 proteins, including Bcl-XL and MCL-1 [89, 90]. A selective BCL-2 inhibitor, venetoclax (ABT-199), mimics the binding of BCL-2 Homology 3 (BH3) onto Bcl-2 [83]. Subsequently, this inhibits the BCL-2-associated X protein (BAX), leading to transcription-independent activation of BAX by p53 [83, 91]. In vitro studies demonstrated eradication of CD34+ cells via mitochondrial oxidative phosphorylation with the combined use of venetoclax and TKI [83]. In a retrospective study, the overall response rate was 75% among nine BP-CML patients who received venetoclax plus TKIs. The median OS and median relapse-free survival was 10.9 and 3.9 months, respectively [92].

52.4.1.7 Inhibition of JAK2

JAK2 is the upstream mediator of JAK/STAT3/5 signaling in CML cells as constitutive BCR-ABL activity leads to aberrant hyperactivation of this pathway. STAT3/5 binds the SH2 domain of BCR-ABL1, leading to phosphorylation, and such conformation is stabilized by the Abelson helper integration site 1 (AHI-1). AHI-1 is an oncogenic protein upregulated in CML LSCs, which synergizes with BCR-ABL to influence leukemogenicity in vivo, and is also shown to be associated with quiescence and persistence of primitive cells in vitro [93–97]. STAT5 is activated by a number of cytokines and growth hormones, controlling transcription of genes that regulate cellular proliferation, apoptosis, differentiation and inflammation [98]. Persistent STAT5 phosphorylation by BCR-ABL activates the downstream hypoxia inducible factor-2α (HIF-2α)/CITED pathway, which maintains quiescence and stemness of LSCs by aiding cells adaptation in the hypoxic BMM [99–102]. It is also associated with the production of reactive oxygen species (ROS) and aberrant p53 apoptotic signaling [102, 103].

STAT3 regulates important physiological processes such as cell proliferation, survival and angiogenesis [104, 105]. In the haematological context, it influences signal transduction in growth factor-mediated control of haematopoiesis and cellular differentiation in the myeloid lineages. Increased STAT3 phosphorylation has been shown to be associated with malignant transformation of several human cancers and drug resistance [104–107]. JAK2/STAT3 is an adaptive survival pathway that is significantly reinforced in the BMM, as the phosphorylation of STAT3 is observed to be increased in IM-treated CML cell lines, including CD34+ cells. It has been postulated that BCR-ABL inhibition leads to a shift to an alternative pathway to maintain CML cell viability [108], This results in IM resistance as LSCs persist independently of the BCR-ABL pathway, which may be attributed to the increased levels of STAT3 targets like Bcl-XL and MCL-1 [109]. In vitro studies using K562 cells shows that JAK2 inhibitors CYT387 and TG101209 reduce the adaptive survival pathway, restoring TKI sensitivity [108].

Ruxolitinib, a JAK2 inhibitor, shows eradication of primitive CD34+/CD38—CML cells when combined with TKIs in preclinical studies. In murine models, it also reduces CD34+ cellular engraftment to the bone marrow [110]. In a phase 1 trial, ruxolitinib and nilotinib leads to increased reduction of BCR-ABL1 transcripts with 44% of the patients achieving deep molecular response (MR4.5) [111]. In a similar trial, 40% of the patients achieved complete molecular remission (CMR) within six months [112]. In a phase 2 trial evaluating ruxolitinib monotherapy, overall response rate (ORR) was 60% with clinical benefits in 33% patients including improvement in platelet and hemoglobin counts, spleen size reduction by <50% and symptom reduction by <50% [113]. A phase 1/2 trial investigating TKI plus ruxolitinib, CCyR was 87.5% and MMR was 37.5% [114]. Some CML LSCs express high levels of MPL, further enhancing JAK/STAT signaling and increasing leukemogenicity in vivo, these cells show lower sensitivity to TKIs but are more sensitive to JAK2 inhibition, which demonstrate the potential of ruxolitinib-based therapy [7, 29, 110].

52.4.1.8 PPAR-γ Agonists

PPAR-γ is a repressor of STAT5 transcription and the expression of its downstream targets, contributing to the exit of quiescence in CML LSCs and an increased sensitivity to IM, thus increased PPAR-γ activity with the use of PPAR-γ agonists is shown to have promising therapeutic effects [101, 115]. Thiazolidinediones are synthetic PPAR-γ ligands shown to inhibit LSC adhesion to the BMM by upregulating MMP-9 and MMP-2, as well as activating pro-apoptotic caspase-3 [7, 115–117].

In a preliminary clinical study, three CML patients with residual disease were given pioglitazone and IM combination therapy, in which all of them resulted in CMR for up to 4.7 years even after pioglitazone withdrawal [115]. In the phase 2 "ACTIM" trial (ClinicalTrials.gov identifier: NCT02889003), patients receiving IM and pioglitazone resulted in a cumulative incidence of MR4.5 of 56% by 12 months, compared to 23% in the control arm receiving IM, and no major drug-induced toxicities was reported [17, 29, 100, 115]. Aside from their synergistic effects, thiazolidinediones like clofibrate and WY-14,643 are able to increase IM uptake via upregulating the expression of human organic cation transporter 1 (hOCT1) in KCL22 cells, further increasing the efficacy of imatinib [116].

52.4.1.9 Prostaglandin E (PGE) 1 Analogue

BCR-ABL1 upregulates the proinflammatory PGE2, which induces β-catenin accumulation, stabilization and translocation into the nucleus to activate downstream signaling, which contributes to LSC maintenance and progression into accel-

erated phase and/or blast crisis [118, 119]. Despite being structurally similar, PGE1 is mechanically distinct from PGE2 and possesses anti-leukemic effects [118]. PGE1 acts on E-prostanoid receptor 4 (EP4) to suppress activator protein 1 (AP-1) factors in LSCs, mediating repression of Fos/FosB and downregulation of TCF1/LEF1, thus resulting in suppressed β-catenin activation [118]. Targeting LSCs with the combined use of IM and PGE1 shows synergism without affecting normal haematopoietic stem cells (HSC), demonstrating PGE1 as a promising therapeutic target in the elimination of CML cells [118].

52.4.1.10 Activation of Promyelocytic Leukemia: Nuclear Bodies (PML-NB)

The promyelocytic leukemia protein (PML) is a tumour suppressor protein which multimerizes to form the PML-Nuclear body (PML-NB). It regulates numerous metabolic processes including DNA-damage response, cellular apoptosis and senescence, as well as asymmetric division of HSCs via the PML/PPAR-δ/FAO pathway [120]. Preclinical studies have demonstrated that PML upregulation in mesenchymal stromal cells leads to increased production of inflammatory cytokines such as IL-6 and CXCL1, which maintains the protective interaction between the BMM and CML LSCs, conferring TKI-resistance [121]. Ito et al. correlated high PML expression with poor prognosis as they observed that CML patients with low PML expression display higher complete molecular and cytogenetic response, as well as improved OS compared to those with high PML expression [122].

Prior to the emergence of TKIs, arsenic trioxide (ATO) was employed as frontline treatment for CML but rendered insufficient in targeting LSCs as monotherapy [123]. Yet, combined use with TKIs demonstrated effective targeting of LSCs through intrinsic (BAX) and extrinsic (caspase-8/-10) apoptotic pathways, downregulation of VEGF receptors and angiogenesis, as well as upregulation of NKG2D ligands, which induces NK-cell-mediated cytotoxicity, cell cycle arrest and inhibition of P13K/AKT and RAS/MAPK pathways [123–125]. Researches have also proven the efficacy of ATO/IFNα combination therapy in mediating autophagy-induced cell death of CML cells via cycling of dormant LSCs and the inhibition of the Hh pathway [125]. In vitro studies have shown that such combination is able to eliminate TKI-resistant CML stem cells [126, 127]. In a phase 1 trial, 100% of patients achieved a decrease in BCR-ABL1 fusion transcript and 87.5% of patients achieved MR4.5 or above post-trial [128]. Twelve months later, 87.5% of the patients maintained a decreased BCR-ABl1 transcript while MR4.5 or above was maintained in 55.6% of the patients [128].

52.4.2 Targeting the Bone Marrow Microenvironment

The complexity of the BMM can be attributed to the vast interactions between the mixed cell population that reside, including endothelial cells, neural cells, osteoclasts, mesenchymal stromal cells and osteoblasts [129–132]. The homeostasis in the complex BMM is tightly regulated by a multitude of chemokines and cytokines; for instance, homing and engraftment of HSCs in the BMM is coordinated by the activation of various adhesion molecules called selectin and integrins [130, 133, 134]. Integrins are expressed on the surface of HSCs, which enable their binding to the vascular cell adhesion molecule 1 (VCAM-1) on the BM endothelium and to fibronectin in the extracellular matrix [130, 131, 135]. The rolling and homing process of HSCs depend on interactions between VLA4 and E- and P- selectins, which are expressed constitutively on BM endothelium. Another regulation of this process is via the SDF1-CXCR4 axis, which communicates with β1 and β2 integrins via signal transduction [130, 136, 137].

In CML, there are multiple dysregulations in the processes controlling LSCs homing and engraftment [130]. In vitro and in vivo studies have demonstrated there are marked alterations in the expression of chemokines and cytokines which can be attributed to leukemic development, leading to growth impairment of normal HSCs and conferring survival advantages to CML LSCs [29]. Although there are normal expressions of VLA4 and VLA5, CML LSCs possess defective β1 integrin function [130]. CML progenitor cells are shown to have reduced expression of CXCR4, thus the SDF1-CXCR4-axis is downregulated [130, 138, 139]. Despite multiple adhesion abnormalities, LSC homing and engraftment is made possible by the adaptation of BMM during leukemic transformation, providing alternative pathways for LSC residence in the niche and shielding them from destruction by conventional pharmacological agents [130, 140–142]. Here, we discuss possible therapeutic agents that target the abnormal interaction between BMM and CML LSCs.

52.4.2.1 Inhibition of Dipeptidyl-Peptidase (DPP-4)

Although CD34+/CD38—is a common immunophenotype in which CML LSCs reside in, the CML stem cell subpopulations exhibit a considerable degree of heterogeneity by expressing different additional cell surface antigens [7, 143]. DPPIV/CD26 is found to be expressed on CML LSCs but not normal HSCs, it possesses enzymatic functions to inactivate stroma-derived factor-1(SDF-1) and disrupt the SDF1-CXCR4-axis [143]. SDF-1 is a chemotaxin that recruits

CXCR4+ CML LSCs through binding to CXCR4, disruption of this axis results in dysregulation of LSCs homing, facilitating their escape from the BM niche, leading to niche-independent spread of leukemic cells [143, 144]. In a preclinical study, sitagliptin and vildagliptin reverted the DPPIV effect that blocked SDF-1-induced homing of CXCR4+ CD34+/CD38-/CD26+ cells [136, 143, 145]. Another study investigated the expression of DPPIV/CD26 on CD34+/CD38—cells in the peripheral blood (PB), revealing a substantial inverse correlation between the number of circulating CD26+ LSCs and the duration of TFR [146]. In a retrospective study, two patients receiving nilotinib but did not achieve MMR resulted in decrease in BCR-ABL1 transcripts after receiving saxagliptin or sitagliptin for their uncontrolled diabetes mellitus [147]. In the same cohort, BCR-ABL1 transcripts either increased or remained unchanged in other CML patients [147].

52.4.2.2 E-Selectin Antagonist

E-selectin inhibition by Uproleselan (GMI-1271) prevents binding of LSCs to the BM endothelium and directs their entry to the PB for cellular differentiation [130, 148, 149]. In a phase 3 trial, addition of Uproleselan to a chemotherapy regimen demonstrated promising remission rates and survival outcomes in relapsed AML patients [149]. This led to preclinical studies evaluating its effectiveness in targeting CML LSCs [148].

Improved survival is observed in a transgenic murine model treated with GMI-1271 with IM, which can be attributed to a reduced number of CML-initiating clones, impaired homing to the BMM, reduced leucocyte counts, reduced myeloid blast counts and smaller spleen size [130, 148]. In vitro studies have shown that GMI-1271 is able to increase expression of CDK6 which is a cell cycle promotor, and decreased p16 expression, which is a cell cycle inhibitor, causing decrease in G0 phase such that the proportion of cells in the G2-S-M phase increases [130, 148]. Further in vitro studies demonstrated E-selectin inhibition increases SCL/TAL1 expression in BCR-ABL positive cells, which downregulates CD44 expression on CML cell surface [148]. Binding of CD44 expressed on the surface of CML LSCs to E-selectin on the BM endothelium leads to their engraftment in the BM niche for protection against IM elimination [130, 148].

52.4.2.3 Inhibition of SDF1-CXCR4

SDF1-CXCR4 activity in the BM stroma prevents terminal differentiation of HSCs and supporting their proliferation, to offer stromal protection to CML cells and lowers their sensitivity towards TKI-mediated apoptosis [150, 151]. It is proposed that, by antagonizing CXCR4, the abnormal communication between stromal cells and CML cells can be disrupted, thereby mobilizing the leukemic cells to the peripheral circulation for TKI-mediated apoptosis [150, 152–154]. The NOX-A12 Spiegelmer, an L-enantiomeric RNA oligonucleotide, inhibits SDF1 and antagonizes the SDF1-CXCR4 interactions [155, 156]. In vitro studies showed marked reduction of SDF1-induced migration in BCR-ABL1+ cells and enhanced IM-mediated apoptosis when NOX-A12 was given in combination with IM [155]. Plerixafor, a CXCR4 receptor antagonist/partial agonist that is currently used as an immunostimulant to mobilize stem cells post hematologic stem cell transplantation, is shown in multiple studies to be effective in enhancing TKI-mediated apoptosis of stroma-protected AML [157–160], multiple myeloma [161] and CML [150, 152, 162]. In vitro and in vivo studies yielded results to support the idea of the combined use of CXCR4 inhibitor and TKI to override drug resistance in CML and suppress or eradicate residual disease. In K562 and KU812 cell lines, plerixafor reduced cellular migration and adhesion to BMM, sensitizing BCR-ABL+ cells to TKI by disrupting the stromal protection effect from the SDF1-CXCR4 interactions. In vivo murine models also demonstrated increased LSCs mobilization to PB and potentiation of TKI-induced tumour bulk elimination [150, 152, 162]. However, contradicting observations were presented by Agarwal et al., showing in stem cell infiltration into the central nervous system and the development of neurological deficits in a murine model [163].

52.4.2.4 Inhibition of Hypoxia-Inducible Factor (HIF)

BCR-ABL upregulates the expression of HIFs and HIF-responsive genes, in which interactions depend on the oxygen tension of the BMM [164]. HIFs aid cell adaptation to low oxygen by regulating gene transcription relevant to cell energy metabolism, survival and angiogenesis [164–167]. The hypoxic BMM is critical in maintaining survival, stemness and self-renewing capacity of both normal HSCs and LSCs [164, 168–170]. Under such environment, HIF-1α and HIF-2α are upregulated due to high levels of ROS, conferring LSCs the ability to survive, remain quiescent and escape TKI-mediated apoptosis [164, 166, 171]. HIF activities also include increased glycolysis, p21 upregulation, p53 downregulation, transcription of antioxidant enzymes (FOXO and NRF2), and evasion of cellular immunity via nitric oxide and B7H1/programmed death ligand 1 (PD-L1) expression [165]. Acriflavine (ACF), an HIF-1 inhibitor, decreases HIF transcriptional activity by inhibiting dimerization of the HIF complex and leads to reduced LSC proliferation, survival maintenance and stem cell potential [164]. The anti-leukemic effect of ACF can be explained by its upregulation of tumour suppressor proteins (e.g., p57, p19Arf and p16Ink4a) and inhibition of genes that maintain the stem cell potential of LSCs (e.g., NANOG, Oct4, and Sox9) [164]. ACF also exerts antiproliferative effects on CML LSCs as it represses the

expression of c-Myc, which is a proto-oncogene that regulates the cell cycle and is responsible for the BCR-ABL-driven transformation [164, 172, 173],

52.4.2.5 Inhibition of Hedgehog (Hh) Pathway

The hedgehog (Hh) signaling pathway is one of the major pathways that influence self-renewal of HSCs [174, 175]. Hh binding to the Patched (Ptch) receptor results in the activation of smoothened (Smo) and transcription factor Gli1, contributing to tissue homeostasis, regeneration and healing [7, 29, 175–177]. The upregulation of this pathway is found to be associated with the pathogenesis and disease progression of CML. Overactivation of Hh is seen in 50% of chronic phase (CP)-CML, 70% of accelerated phase (AP)-CML and >80% of blast-phase (BP)-CML patients [178–181]. Hyperactivation of Shh and Smo in CD34+ and c-kit+ LSCs contributed to cyclin-D1-mediated LSC quiescence and maintenance, as well as clonal expansion via the Wnt/β--integrin pathway [181].

Sonidegib (LDE225) is a synthetic small molecule Smo inhibitor of high potency and selectivity [175]. Both in vitro and in vivo studies demonstrated its effectiveness as monotherapy or in combination with TKIs in the elimination of Hh-mediated self-renewal capacity of CD34+ and BCR-ABL1+ CML cells [29, 175]. In a phase 1 trial, another SMO inhibitor BMS-833923 was given with dasatinib to CML patients with suboptimal TKI response [176]. Results showed no evidence of efficacy as there was only minimal reduction of BCR-ABL1+ progenitor cells. Despite expected toxicities and no drug interaction observed, further testing of this combination in CP CML was not supported [29, 176]. Due to, however, promising preclinical studies, the Hh pathway remains a therapeutic target of great potential in the treatment of CML.

52.4.2.6 Inhibition of Wnt/β-Catenin Signaling

Wnt signaling from the BMM supports the LSC self-renewal, quiescence, maintenance, and TKI resistance [182]. The secretion and activity of Wnt-ligands requires palmitoylation by Porcupine (PORCN), which is an endoplasmic reticulum membrane-bound O-acyl transferase [182–184], BCR-ABL is responsible for the constitutive secretion of Wnt-ligands and the substantial upregulation of frizzled-4 (FZD4) receptors, subsequently stabilizing B-catenin and mediating TKI-resistance [182, 185, 186]. Riether et al. hypothesized that long-term exposure to TKI leads to depletion of miR29 and upregulation of CD70, resulting in increased CD27-mediated Wnt activation [187]. WNT974, a potent PORCN inhibitor, effectively reduced neutrophils, white blood cells and myeloid cells in PB when used with nilotinib in a transgenic murine model with CD34+ cells [188]. CML progenitors also reduced in number in the BMM alongside splenic size reduction [182, 188]. C82, a novel inhibitor of Wnt/β-Catenin signaling, demonstrated synergism with nilotinib in eliminating LSCs. In vitro and in vivo studies showed its effectiveness in downregulating CD44, c-Myc, survivin, STAT5 and CRKL in E255V and T315I mutant cell lines, suggesting its potential to restore TKI-sensitivity [189].

52.4.2.7 Re-establishment of PP2A Activity

PP2A is a serine-threonine phosphatase that possesses tumour suppressor activity, working with PP1, contributing to >90% of intracellular phosphatase due to having a wide range of substrates, and is involved in the regulation of numerous oncoprotein signaling pathways [190, 191]. Aberrant BCR-ABL kinase activity leads to overexpression of oncogenes like MYC, cancerous inhibitor of PP2A (CIP2A) and Inhibitor 2 of PP2A (SET). This results in persistence of CML LSCs and disease progression [190, 192, 193].

BCR-ABL induces the transcription and translation of MYC [190, 194]. Increased expression of MYC forms a positive feedback loop that further upregulates BCR-ABL kinase activity [172, 190]. When MYC binds to BCR promoter together with MAX, the transcriptional and translational activities of BCR-ABL are enhanced [190]. This facilitates CML progression into blast crisis [190, 193]. PP2A activity results in MYC degradation via dephosphorylation [190]. CIP2A and SET are endogenous PP2A inhibitors [190]. CIP2A interacts specifically with PP2A-B56a holoenzyme, which disables the negative regulation of MYC expression via PP2A-B56a-mediated control [190]. In CML, CIP2A activity is also linked to the upregulation of BCL-XL [195]. SET interacts with SETBP1 upon binding to the catalytic subunit C of PP2A [196]. In CML, SETBP1 is known to be overexpressed due to SETBP mutations. SETBP1 stabilizes SET binding to PP2A and promotes self-regeneration of LSCs in vivo [197].

Re-establishment of PP2A activity can be a potential therapeutic target in the context of CML. MYC inhibitor 10058-F4 prevents its interaction with MAX [198]. It has led to the suppression of CIP2A in 80% of CD34+ cells ($p = 0.04$) and in 85% if K562 cells ($p = 0.01$), restoring PP2A function and halting LSC maintenance [190, 192, 198]. FTY720, a SET antagonist that induces apoptosis in CML K562, KBM5 and MYL cells by activating both intrinsic and extrinsic apoptotic pathways [198]. When used in combination with IM, FTY720 can also overcome TKI resistance induced by BIM deletion and GAL-3 [198, 199]. Another SET antagonist, OP449. results in inhibition of JAK/STAT5, P13K/AKT and B—catenin signaling in in vitro K652 and CD34+ cell lines [200]. Both FTY720 and OP449 are shown to enhance the efficacy of TKIs and overcome TKI resistance [29, 190, 198–200],

52.4.3 Modulation of P53, a Key Regulatory Network

The p53 protein is a key transcription factor that regulates cell cycle and apoptosis, it also regulates the self-renewal of normal HSCs and possesses tumour suppressor activities [29, 201–203]. BCR-ABL activity is shown to be associated with the alteration of the p53 pathway via promoting the formation of a ternary complex with IκBα and p53 in the cytoplasm, resulting in loss of p53 tumour suppressive nuclear pool [201, 204]. Additional mechanisms of p53 inactivation can be attributed to p53 mutations and deregulation of its regulators such as HDM2 and SIRT1 [202, 205, 206]. The loss of p53 leads to progression of CML into the blast crisis [201, 203, 204] and renders CML cells to be resistant to apoptosis [204, 207]. These findings suggest that p53 activation may be efficacious in targeting CML LSCs.

Abraham et al. demonstrated that alongside BCR-ABL, p53 and c-MYC influences the LSC phenotype and hence their effects on CML disease presentation and progression [173]. Preclinical studies have revealed that depletion of E3 ligase FBXW7 leads to upregulation of both c-MYC and p53, thus promoting p53-dependent modulation of cell cycle and apoptosis in CD34+ and HeLa cell lines [173, 208, 209]. Prevention of p53 degradation by RITA (NSC652287) in in vitro studies using the CD34+ and K562 cell lines shows degradation of IκBα and downregulation of m-MYC and anti-apoptotic (BCL-XL, MCL-1, cIAP1, and XIAP) genes [210]. RITA also abrogates PI3K/AKT and JAK/STAT5 signaling pathways, leading to eradication of CML cells [173, 210, 211]. In vivo murine CML models treated with RITA plus CPI-203 (a BET inhibitor) resulted in significant decrease levels of progenitor marker-positive cells (CD11b, CD19, CD33, CD34 and CD45, and CD133), suggesting that such combination is effective against the elimination of CML progenitor cells and reducing LSC engraftment [173, 210]. However, studies presented contradictory results as to whether RITA is effective in targeting p53-mutant cells [210, 212]. Ma et al. have proven RITA ineffective in targeting p53 mutant cells due to lacking phosphorylation at Serine-46 (Ser46), which is necessary for p53-dependent apoptosis [212]. On the other hand, Mobaraki et al. demonstrated that RITA can induce CML cell apoptosis in a p53-independent manner, via targeting the PI3K/STAT5 pathway, as well as mediating caspase-dependent apoptosis [210].

52.4.3.1 Inhibition of Sirtuin 1 (SIRT1)

There is shown to be an overexpression of Sirtuin 1 (SIRT1), which is a NAD+ dependent deacetylase in CML LSCs in comparison to normal HSCs, contributing to LSCs maintenance and TKI resistance [213–215]. Through the activation of PGC-1α, SIRT1 promotes mitochondrial oxidative phosphorylation to aid the maintenance of bioenergetic demands in CML LSCs [216]. Both in vitro and in vivo studies have showcased the suppression of mitochondrial genes expression and increased p53 acetylation in CML LSCs with SIRT1 deletion [213, 214, 216]. Inhibition by SIRT1 can be done by using Tenovin-6, a small molecule inhibitor, as well as RNAi [217]. Combination of SIRT inhibitors with TKIs also demonstrated the restoration of TKI-sensitivity in CML cells via increased TKI-induced apoptosis [214, 216, 217].

52.4.3.2 Inhibition of Human Double Minute 2 Protein (HDM2)

HDM2 binds the transactivation domain of TP53 and inhibits its transcriptional activities, leading to suppressed p53 expression [202, 218]. In a promotor-driven CML mouse model, administering HDM2 antagonist DS-5272 with IM restored TKI sensitivity through reactivating p53 and upregulating downstream pro-apoptotic proteins NOXA and BAX in CML cells [219]. NOXA induces the degradation of anti-apoptotic proteins such as MCL-1 while BAX promotes apoptosis by Mitochondrial outer membrane permeabilization (MOMP), leading to the eradication of LSCs [220, 221]. A small molecule inhibitor, MI-219, disrupts the interaction between p53 and HDM2 [29, 202]. MI-219 stabilized and reactivated p53, reducing homing and engraftment of LSCs. It is also associated with the downregulation of genes that are crucial for the self-renewal of LSCs (JARID2, PRDM16), without imposing significant effects on normal HSCs as demonstrated in both in vitro and in vivo studies [29, 202].

52.4.4 Epigenetic Regulators

Histone modifying processes are often involved in leukemogenesis and leukemic progression as they significantly impact on the expression of genes regulating cell cycle and apoptosis. Histones can be modified in numerous ways, including but not limited to methylation, acetylation, ubiquitination, phosphorylation and glycosylation. The targeting of epigenetic regulators is of great therapeutic potential when it comes to novel approaches to CML management, with an aim of restoring the normal balance between tumour suppressor and oncogenic proteins.

52.4.4.1 Inhibition of Histone Deacetylase (HDAC)

HATs and HDACs are epigenetic enzymes that control patterns of gene expression and cell signaling pathways by acetylation and deacetylation of their protein targets [222]. In CML, overexpression of HDACs leads to aberrant acetylation status of histone and non-histone proteins, resulting in uncontrolled cell proliferation via p21 repression and overexpression of cyclin D1 [223, 224]. Dysregulated HDAC activity also contributes to the development of BCR-ABL-

independent IM resistance via hypoacetylation of Hsp90, p53 and Ku70 [225–227]. HDAC inhibition induces histone H3 acetylation, enhances apoptosis and restores TKI-sensitivity in CML LSCs contributing synergism when used in combination with TKIs [222, 227–230]. Panobinostat (LBH589), an HDACi used in combination with Ponatinib in preclinical studies, showed increased apoptotic killing of CML cells in in vivo CD34+ mice and in vitro cell lines K562, K562/IM-R1, Ba/F3 and Fa/F3/T315I [230]. When used with Ponatinib, Panobinostat contributes extended cytotoxicity through distinct cell-killing effects on CML LSCs through the intrinsic and extrinsic apoptotic pathways, inhibition of the AKT-mTOR axis, and enhanced inhibition of BCR-ABL, STAT5, AKT and ERK1/2 phosphorylation [227, 230]. Such combination also shown effectiveness in tackling the primitive quiescence CML LSCs in in vitro CD34+/CD38—cell line [231]. This subfraction of CML cells is especially resistant to IM-mediated cytotoxicity [232, 233].

Panobinostat has been investigated in clinical trials in recent years [17, 29]. In a phase 1 study, 44% achieved >1log reduction in BCR/ABL transcripts with no dose-limiting toxicities observed, the study was however discontinued due to slow accrual [29]. Subsequent phase 2 studies demonstrated neither MCyR nor molecular response [17]. As preclinical results are promising, a phase Ib trial is being conducted to evaluate its safety and efficacy (ClinicalTrials.gov identifier: NCT03878524).

Other HDACs include Pracinostat (SB939) and Chidamide. In in vitro genome-edited K562 cells with BIM deletion polymorphism, Pracinostat with IM is found to lower viability of CML cells with BIM deletion and restores TKI-sensitivity, demonstrating its effectiveness in overcoming BIM deletion polymorphism-induced TKI resistance [228]. Chidamide induces apoptosis of CD34+ cells in vitro through increased H3 acetylation, activated caspase-3/-9 and decreased levels of β-catenin, survivin and Myc [229]. HDACs are orally bioavailable and do not induce apoptosis in normal HSCs [222, 227–230]. While HDACs show promising effect when used in combination with TKI, its efficacy as a single agent remains questionable [3, 17].

52.4.4.2 Inhibition of Enhancer of Zeste Homolog 2 (EZH2)

EZH2 is the enzyme subunit of the Polycomb Repressive Complex 2 (PRC2), which regulates gene silencing through its H3K27 methyltransferase activity and recruitment of Polycomb Repressive Complex 1 (PRC1) to target gene promotor region. In CML, overexpression of EZH2 constitutes a BCR-ABL1-independent differentiation arrest in myeloid blast cells, resulting in LSC expansion, persistence and TKI-resistance [234–237]. Inhibition of EZH2 leads to depletion of LSCs in in vitro cell lines K562, HEL, Kasumi-1, ME-1, Mv4–11 and MOLM13 through the upregulation of p16, a tumour suppressor regulated by PRC1 and PRC2 [235, 238]. Inactivation of EZH2 in CRISPR/Cas9 genome-edited murine models has showed disease regression and improved survival as lower WBC count and absence of features of advanced disease like splenomegaly and lung haemorrhage were observed in these mice compared with the control group [237]. Combination of nilotinib with an EZH2i, EPZ-6438 shows efficacy in eliminating primitive CML progenitors, achieving a near complete elimination of CD45 + CD34+ progenitor cells and a 70% reduction of CD45 + CD34 + CD39—cells [239]. Effectiveness of such combination therapy in restoring TKI sensitivity is demonstrated in the same study [239]. When potentiated by TKIs, EZH2 can increase p53 levels in CML CD34+ cells by rescuing previously repressed p53-mediated apoptosis via BCL-6 and EZH2-mediated mechanisms, as well as reactivation of pro-apoptotic targets (NOXA, PUMA, BIM) downstream of p53. This effectively induces apoptosis in TKI-resistant cells, which were previously refractory to apoptosis [240, 241].

52.4.4.3 Inhibition of PRMT5

PRMT5 is a type II arginine methyltransferase which catalyses the symmetrical transfer of two methyl groups to the arginine residues of both histone and non-histone proteins [242]. It is a class of epigenetic enzymes that regulates transcription, RNA metabolism, ribosome biogenesis and the cell-cycle [243, 244]. High PRMT5 expression is associated with numerous oncogenic processes in human cancers to promoting tumour growth [245, 246]. In CML, PRMT5-mediated modifications of histone tail repress the miRNA targeting tumour promoting genes (BCR-ABL, STAT3/5, CRKL), and supporting CML LSCs survival and self-renewal [243, 245, 246]. Furthermore, PRMT5 is associated with additional oncogenic drivers seen in AP-CML patients, including the activation of Wnt/B-catenin pathway [29, 247]. PRMT5 inhibition can be achieved with the use of a small-molecule inhibitor, PJ-68 or PRMT5 silencing with lentiviral shRNA [247]. In xenograft murine models, PRMT inhibition results in reduced CML LSC viability and self-renewal, eradication of quiescence LSC subpopulation, prolonged survival and inhibition of long-term multilineage engraftment of human CD34+ cells [29, 247].

52.4.4.4 Inhibition of Bromodomain and Extra-terminal Motif (BET)

BET proteins function as epigenetic regulators of transcription, cell-cycle and numerous inflammatory processes [248–250]. Bromodomain-containing protein 4 (BRD4) maintains the stability of chromatin and influences the cell cycle by regulating cellular transition of the G2/M phase via binding to the positive transcription elongation factor b (P-TEFb) [249, 250]. BCR-ABL1+ LSCs are able to acquire the secretory-associated senescent phenotype (SASP) to increase

BRD4 activity and MYC expression, resulting in the release of pro-inflammatory cytokines which supports senescence of LSCs (IL-1, IL-6, IL-8, IL-17, IL-23, BMP2, TNFα, CCL9, IFN-γ, and NF-κB). Abnormal hyperactivation of BRD4 activities induce PD-L1 transcription, leading to an increased expression on the surface of CML stem cells for immune-evasion [248–252]. BRD4 inhibition directly restricts PD-L1 transcription and indirectly suppresses MYC activity in MYC-driven malignancies [252]. JQ-1, is a BRD4 inhibitor that targets LSCs via upregulating IL-12β, reducing VEGF receptor-mediated angiogenesis, mediating CTL-mediated cytotoxicity via targeting the PD-1/PD-L1 axis and promoting IL-6-mediated Jagged1/Notch1 cellular invasion and migration [248–252]. Degraders of BET, dBET6 and dBET1, are shown in in vitro studies using the K562 and KU812 cell lines to be more potent over JQ-1 in suppressing BRD4 and MYC levels [249]. They can also eradicate BCR-ABL1+ cells and progenitor CD34+ LSCs, in which JQ-1 has failed [249]. In vivo studies revealed the ability of dBET6 in overriding niche-induced TKI resistance in CML LSCs while JQ-1 could only partially restore TKI-sensitivity [249]. However, all three BET inhibitors demonstrated the ability to inhibit PD-L1 expression induced by IFN-γ [249, 252]. A phase I trial evaluating a novel BRD4 inhibitor CPI-0610 is currently underway (ClinicalTrials.gov identifier: NCT02158858).

52.4.5 Targeting Autophagy

Autophagy is a stress-induced pro-survival pathway that helps CML cells to resist TKI-mediated metabolic stress and apoptosis, it functions by degrading and recycling aged and/or defective cellular components to meet cellular oxygen and nutritional requirements, hence maintain homeostasis [73, 253–255]. There are observed alterations in the cellular metabolism of LSCs which can be associated with their leukemogenesis and maintenance, including increased glycolysis and induction of the Warburg effect characterized by increased ROS [41, 255–257]. There is also an upregulation in Beclin-1 in CML cells, which is a protein associated with the initiation of autophagy, thus protecting CML LSCs from oxidative stresses and apoptosis [41, 253–256].

52.4.5.1 Tigecycline

Tigecycline is a third-generation tetracycline which possesses anti-leukemic effect [255]. In CML cells, it reduces cell viability, inhibits mitochondrial biogenesis, suppresses glycolysis and mediates apoptosis via activation of the cytochrome-C/caspase-9/caspase-3 pathway as demonstrated by Lu et al. employing the BCR-ABL positive KBM5 and K562 cells [255]. It also contributes to the downregulation of signaling pathways that are related to the formation of autophagosomes, including Wnt/β-catenin, PI3K/AKT/mTORC1, $p21^{CIP1}$/Warf1, hypoxia-inducible factors (HIF) and c-MYC [253, 258, 259]. When used with IM or as a single agent, it is shown to be efficacious in reducing leukemic cells in both in vitro studies employing the CD+/CD38—cell lines and in vivo studies using mouse models [258].

52.4.5.2 Chloroquine and Hydroxychloroquine

Chloroquine (CQ) is classically used as an antimalarial agent which inhibits autophagy by altering the acidic environment in lysosomes, thereby preventing fusion with autophagosomes and accumulation of degraded debris in the intracellular environment, resulting in persistent endoplasmic reticulum stress and apoptosis [260–262]. In vitro studies have demonstrated the effectiveness of CQ in eradicating BCR-ABL1+ cells by blocking lysosomal degradation and sensitizing the progenitor CD34+/CD38—cells to synergize TKI-induced apoptosis [73, 263]. Strong preclinical studies have led to Chloroquine and Imatinib Combination to Eliminate Stem cells (CHOICES), a randomized phase II clinical trial that demonstrated the ability of CQ in enhancing the therapeutic effects of IM [73, 264]. In a group of patient treated with hydroxychloroquine plus IM, MMR was 92% with 75% of them achieving qPCR level with ≥0.5 log reduction, which was 80% and 67%, respectively, in the control arm where patients were given IM alone.

52.4.6 Immunotherapy

CML is known to be responsive to immunotherapy due to the immunobiology underlying the disease, including the expression of leukemic-specific antigens and susceptibility to CTL-mediated cytotoxicity [265–268]. Here, we discuss possible immunotherapeutic targets exhibited by CML LSCs which allows elimination of residual disease in patients who received TKI treatment.

52.4.6.1 Vaccinations

CML is characterized by the formation of the highly specific BCR-ABL1 gene rearrangement, giving rise to the p210 BCR-ABL protein [265–267]. This chimeric fusion protein is tumour-specific as the amino acid sequence at the junction of p210 is not expressed in normal cells, rendering it the potential as a target antigen for immune therapy [265–267]. Although the intact p210 protein is located intracellularly, products derived from the cellular processing of the fusion proteins can be expressed on cell surface and recognized by T-cells [268, 269].

Identification of appropriate peptides to be used for vaccination is done by selecting fusion peptides from the CML breakpoint with high affinity to HLA molecules, and are able to mount HLA-restricted cytotoxicity and specific helper responses in vitro [270]. Immunogenic peptides that are more commonly employed in current treatment are usually

resulted from the amino acid sequence of the e13a2 or e14a2 breakpoint region [17, 270, 271].

There has been clinical trials evaluating the safety and efficacy of peptide immunization in the context of CML. In the Evaluation of Peptide Immunization in CML (EPIC) study, CCyR was achieved post-IM treatment and patients received vaccination with e14a2 peptides, while 68% showed late T-cell immune response to the BCR-ABL peptides with 1-log reduction in BCR-ABL transcripts [272]. In a phase 2 trial (ClinicalTrials.gov identifier: NCT00267085), vaccinations using the e13a2 or e14a2 peptides were given to 10 patients previously treated with IM, achieving 30% CCyR with 1-log decrease in BCR-ABL1 mRNA levels but no MMR [273]. Although results vary between clinical studies, it has been demonstrated that vaccines targeting the BCR-ABL1 breakpoints via MHC-restricted cytotoxicity can reduce residual disease in patients treated with TKI that have achieved CCyR [272, 273].

52.4.6.2 Immune Checkpoint Inhibitors (Fig. 52.2)

CML LSCs are able to escape cytotoxic T lymphocyte (CTL)-mediated immune attack due to dysregulated T-cell inhibitory pathways known as immune checkpoints.

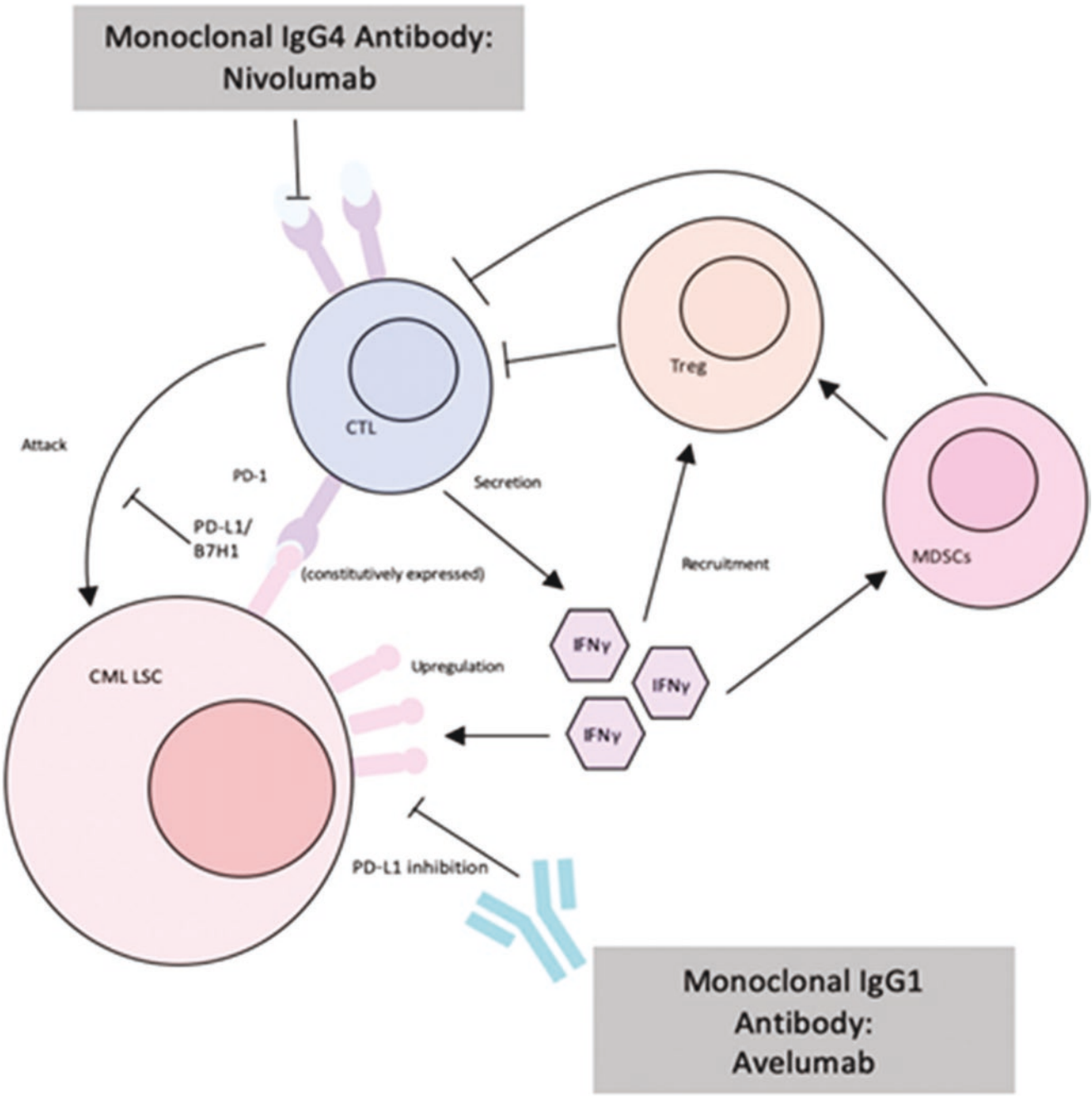

Fig. 52.2 Immune checkpoint inhibitors

Programmed death 1 (PD-1) is a negative immune-regulator checkpoint responsible for self-tolerance. It was found that while PD-1 is up-regulated on CTLs from CML patients, where CML cells express its corresponding ligand programmed cell death ligand 1 (PD-L1) under the influence of IFN-γ [274, 275]. Preclinical studies showed reduced PD-1 expression on CTLs led to increase in OS in murine model and in vitro studies have demonstrated the effectiveness of PD-1 inhibition in eradicating CML LSCs [274–276]. An 82-year-old man refractory and intolerant to multiple TKIs received nivolumab, which is a monoclonal antibody to PD-1, achieved MMR maintenance and undetectable BCR-ABL1 signals [275, 277]. Nivolumab is currently under a phase I trial evaluated for use in combination with dasatinib (ClinicalTrials.gov identifier: NCT02011945). Another trial evaluating combined use of TKIs with Avelumab, a monoclonal antibody to PD-L1, is also currently underway (ClinicalTrials.gov identifier: NCT02767063) [278].

52.5 Conclusion

There is a need of developing novel therapeutic approaches in the treatment of CML due to the insufficiency of TKIs in eradicating LSCs that are responsible for relapse, disease development and progression (Fig. 52.3 and Table 52.1). The understanding of LSCs persistence and TKI-resistant mechanisms has led to selective targeting of cellular physiological processes manifested in abnormal HSCs, which play a substantial role in leukemogenesis.

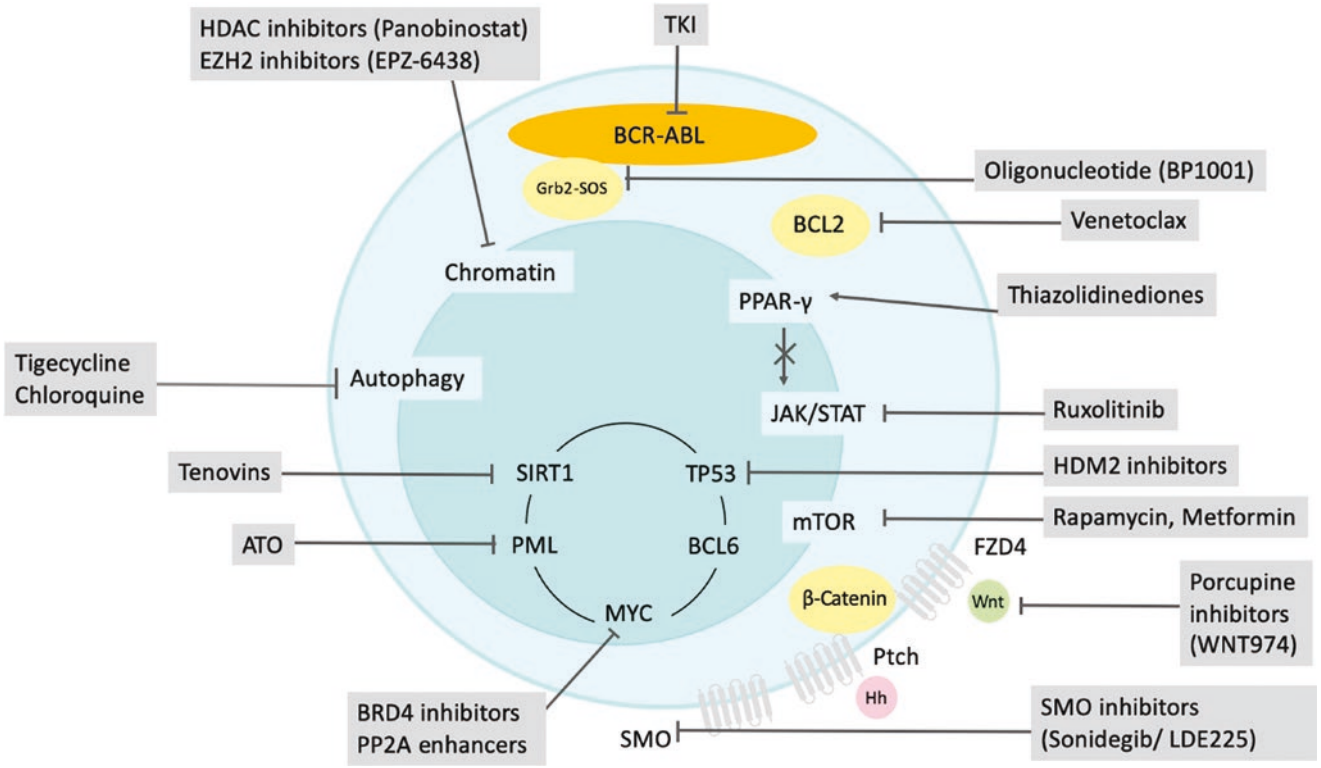

Fig. 52.3 Summary of novel therapeutic agents and targets

Table 52.1 Summary of novel therapeutic agents that have entered clinical trials

Agent	Target	Mechanism of action	Indication	Drug combination	ClinicalTrials.gov identifier (phase)
Asciminib	BCR-ABL1	Targets BCR-ABL1 kinase	Resistance or failure to TKI therapy	Monotherapy or with bosutinib or ponatinib	NCT02081378 (I) NCT03595917 (I) NCT03106779 (III)
Rapamycin	mTOR	Inhibits mTOR	Resistance or failure to TKI therapy	With cytarabine or etoposide	NCT00776373 (I)
Sonidegib	SMO	Inhibits SMO	Resistance or failure to TKI therapy	Monotherapy	NCT01456676 (I)
Ruxolitinib	JAK2	Inhibits JAK2/STAT5 downstream signaling	Residual disease due to persistence of CML LSCs	With nilotinib	NCT01702064 (I) NCT02253277 (I)
Panobinostat	HDAC	Inhibits HDAC which influences gene expression epigenetically	Resistance or failure to TKI therapy	Monotherapy or with other chemotherapies	NCT00451035 (II/III) NCT00449761 (II/III)
Pioglitazone	PPAR-γ	Agonizes PPAR-γ to downregulate STAT5 expression	Residual disease due to persistence of CML LSCs	Monotherapy or with Imatinib	NCT02889003 (II)
Vaccination (e13a2)	Immune activation	Induce delayed T cell response	Residual disease due to persistence of CML LSCs	With TKIs	NCT00267085 (II)
Immune checkpoint inhibitors	Immune activation	Monoclonal antibodies against negative immune-regulator checkpoints, increase susceptibility of CML LSCs to CTL	Resistance or failure to TKI therapy	Monotherapy or with dasatinib	NCT01822509 (I) NCT00732186 (I)

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Owing to an oversight on the part of the Springer, this book was inadvertently published with errors in chapter 3. The textual corrections and the latest updates were missed in chapter 3 and this has now been amended in the book.

The updated version of this chapter can be found at https://doi.org/10.1007/978-981-99-3810-0_3

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