Hematologic Malignancies
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Stefan H. Faderl
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Acute Leukemias

Second Edition

Hematologic Malignancies

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Acute Leukemias

Second Edition

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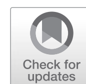
Part I

Acute Myeloid Leukemia



1 Acute Myeloid Leukemia: Epidemiology and Etiology

Kendra Sweet and Hannah Asghari

1.1 Introduction

Acute myeloid leukemia (AML) is a heterogeneous hematologic malignancy characterized by abnormal differentiation of cells of the myeloid lineage, leading to clonal proliferation of leukemic blast cells in the bone marrow, peripheral blood, and potentially extramedullary tissue. This in turn leads to decreased production of normal hematopoietic cells and associated complications related to ineffective hematopoiesis.

AML is the most common type of acute leukemia diagnosed in adults and is associated with the lowest survival [1]. Although survival remains poor overall, outcomes have improved over the past few decades with the advent of new therapeutic approaches. With growing understanding of the molecular pathogenesis, multiple new therapies have been recently approved for AML, and there is an ongoing investigation of novel agents [2, 3].

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1.2 Epidemiology

1.2.1 Prevalence

Acute myeloid leukemia comprises 1.1% of all new cancer diagnoses in the United States. It is estimated that 19,520 new cases of AML were diagnosed in the United States in 2018 [4, 5]. The overall incidence of acute myeloid leukemia in the United States is 4.3 cases per 100,000 and is higher in males, with an estimated 5.2 cases per 100,000 compared to 3.6 cases per 100,000 in females [4]. White individuals also have higher rates of AML compared to other ethnicities [4, 6]. The incidence of AML is generally higher in North America and Europe compared to other regions including countries in Asia and South America [1, 7].

The incidence of AML increases with age and is highest in adults aged 65 years and older (Fig. 1.1). The median age at diagnosis is 68 years. It is estimated that over half of new cases of AML are diagnosed in individuals 65 years and older, with approximately one-third of patients diagnosed at the age of 75 years or older [4].

Acute leukemia is the most common malignancy in children, accounting for approximately 30% of all pediatric cancers. AML is less common in children and adolescents compared to acute lymphoblastic leukemia (ALL), comprising approximately 18% of childhood leukemias [8, 9].

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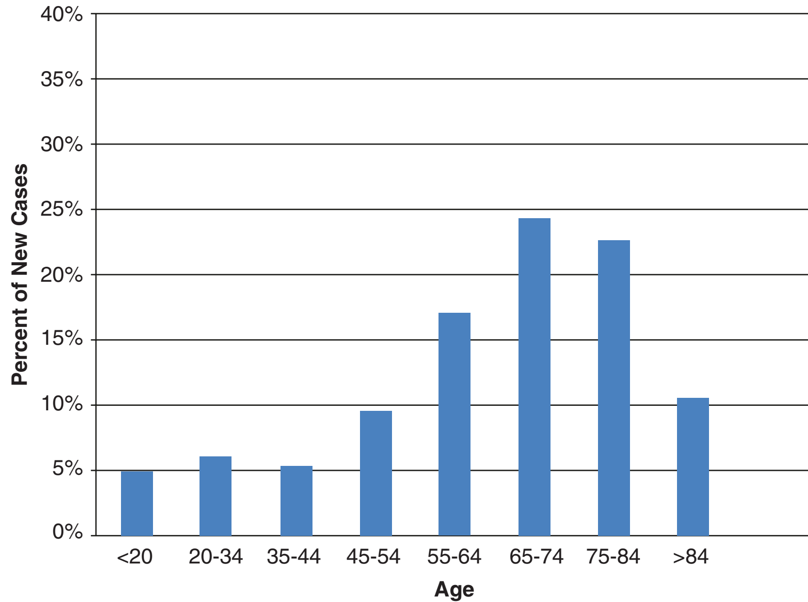

Fig. 1.1 Percent of new cases of AML by age group from 2011 to 2015. (Adapted from originally published data by SEER Cancer Stat Facts: Acute Myeloid Leukemia [4])

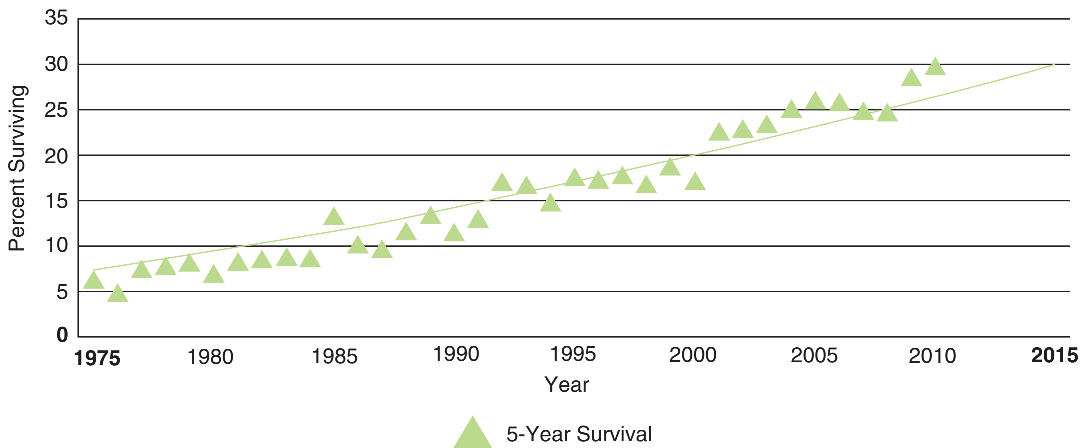

Fig. 1.2 Five-year relative survival from 1975 to 2010. (Reproduced from SEER Cancer Stat Facts: Acute Myeloid Leukemia [4])

1.2.2 Mortality

The estimated overall 5-year survival rate of individuals with AML is 27.4%, and in the United States, it is estimated that 10,670 patients died of AML in 2018 [4, 5]. Overall survival has steadily improved over the years (Fig. 1.2); however, survival and outcomes of older adults remain poor, and the estimated overall survival in AML decreases significantly with age [10]. A large population-based study in the United Kingdom from 2001 to 2006 estimated the 5-year relative survival rate for ages 15–24 years was 53% compared to 13% for ages 60–69 years, and 3% for ages 70–79 years [11].

1.3 Etiology

The process of leukemogenesis is not entirely understood; however, the pathogenesis of acute myeloid leukemia involves oncogenic transfor-

mation of a hematopoietic stem cell or progenitor cell to a leukemic clone that is capable of self-proliferation [12]. AML is a highly heterogeneous disease. Most cases develop de novo and are associated with acquired genetic abnormalities, including cytogenetic changes and somatic mutations. Secondary AML (s-AML) can arise in the setting of clonal evolution from an antecedent hematologic disorder or from prior exposure to cytotoxic therapy (therapy-related AML or t-AML) and is overall associated with worse prognosis compared to de novo AML [13].

Acquired chromosomal abnormalities are present in approximately 50–55% of cases of de novo AML and have higher incidence in secondary AML [14]. Cytogenetic abnormalities have been demonstrated to have prognostic significance in several studies and the European LeukemiaNet (ELN) classification incorporates cytogenetic and molecular abnormalities in risk stratification of AML [15–19].

Early somatic mutations are thought to confer selective advantage for clonal hematopoiesis and may later evolve to AML [17, 20]. One large study found that approximately 10% of individuals over age 65 years have somatic mutations with associated clonal hematopoiesis, most commonly with mutations in *DNMT3A*, *ASXL1*, and *TET2* [21]. In patients without a known hematologic malignancy, this is referred to as clonal hematopoiesis of indeterminate potential (CHIP) [22]. It has been demonstrated that the incidence of somatic mutations involved in clonal hematopoiesis increases with age and is associated with an increased risk of developing hematologic malignancies, cardiovascular disease, and all-cause mortality [23].

On average, patients with de novo disease have 13 genomic mutations, with an average of five genes that are recurrently mutated in AML [24]. The most common recurrent mutations that play a role in the pathogenesis of AML (Fig. 1.3) include genes involved in DNA methylation (*DNMT3A*, *TET2*, *IDH1*, *IDH2*), tumor suppression (*TP53*, *WT1*, *PHF6*), spliceosome complex, modification of chromatin, cohesin complex, sig-

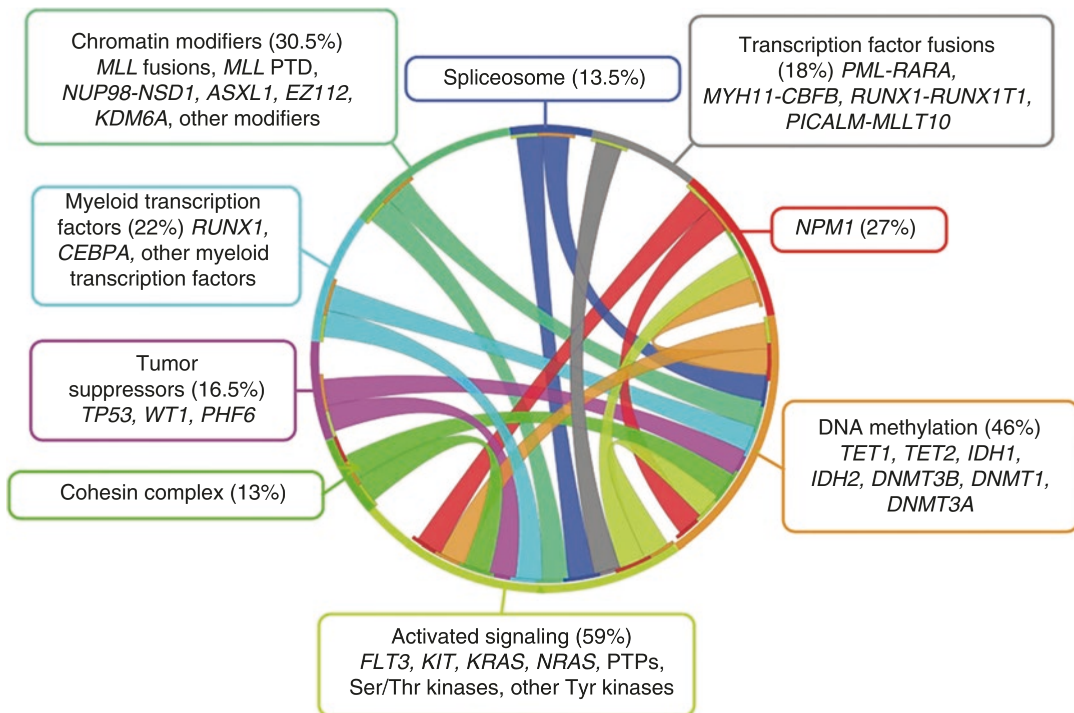

Fig. 1.3 Circos plot demonstrating functional categories of mutated genes involved in pathogenesis of AML. (Reproduced from Chen et al. [25])

nal transduction (*FLT3*, *KIT*, *KRAS/NRAS*), in addition to nucleophosmin (*NPM1*), myeloid transcription factors (*RUNX1*, *CEBPA*), and transcription factor fusion genes [24, 26, 27]. Targeted mutational analysis in one study noted that the presence of certain somatic mutations (*SRSF2*, *SF3B1*, *U2AF1*, *ZRSR2*, *ASXL1*, *EZH2*, *BCOR*, or *STAG2*) was highly specific for secondary AML [28].

1.3.1 Secondary AML

AML can develop from antecedent hematologic malignancies including myelodysplastic syndrome, myeloproliferative neoplasms (i.e., polycythemia vera, essential thrombocythemia, myelofibrosis), and myelodysplastic/myeloproliferative neoplasms (MDS/MPN like CMML, aCML) [29–33]. Paroxysmal nocturnal hemoglobinuria and aplastic anemia, which are nonmalignant hematologic conditions, have also been associated with increased risk of developing AML [34, 35]. In a large population-based Swedish study, AML from an antecedent hematologic disease and therapy-related AML were both associated with worse overall survival and were more likely to have high-risk cytogenetics and lower rates of complete remission (CR) compared to de novo AML [36]. Higher-risk AML is also more common in older adults (Fig. 1.4).

AML with myelodysplasia-related changes (AML-MRC) can arise from an antecedent myelodysplastic syndrome or MDS/MPN and can be associated with MDS-related cytogenetic abnormalities or multilineage dysplasia [13].

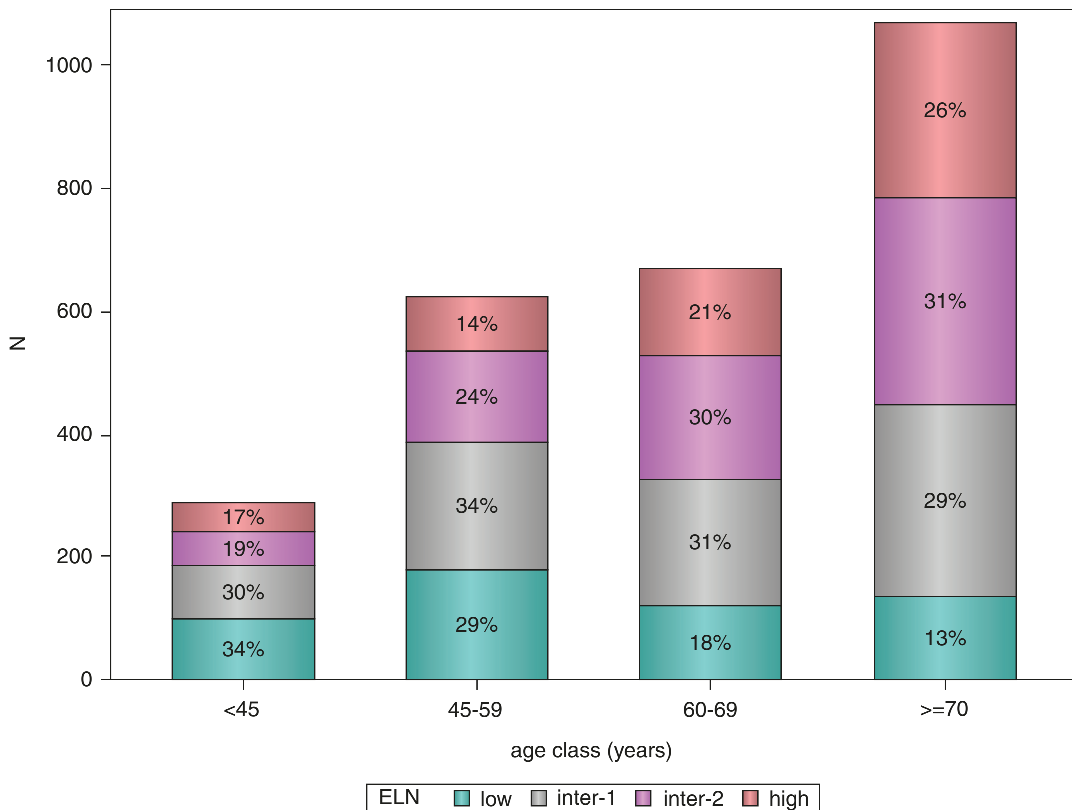

Fig. 1.4 Prevalence of AML in Germany and Austria as part of the AMLSG BiO registry. Prevalence stratified according to age and distribution of risk groups per 2010 European LeukemiaNet (ELN) classification. (Reproduced from Nagel et al. [37])

AML-MRC is associated with worse overall survival likely due to higher risk disease as affected individuals are more likely to be older and have unfavorable cytogenetics [38].

1.3.2 Therapy-Related AML

Treatment with cytotoxic therapy for a preceding primary malignancy is associated with predisposition to developing therapy-related acute myeloid leukemia (t-AML) [13]. Several agents have been implicated, including prior treatment with alkylating agents, topoisomerase inhibitors, and less commonly with exposure to other cytotoxic agents including taxanes or antimetabolites [32, 39]. Alkylating agents can have longer latency periods (5–7 years) prior to progression and can frequently be associated with myelodysplastic features as well as clonal cytogenetic abnormalities involving chromosome 5 and 7 (del(5q) or −7/del(7q)). Inhibitors of topoisomerase II can have a shorter latency period (2–3 years) prior to the development of therapy-related AML and have been more commonly associated with balanced cytogenetic translocations involving chromosome bands t(11q23.3) or t(21q22.1), as well as t(15;17) [29, 30, 40, 41].

1.3.3 Inherited Syndromes

There are also cases of inherited/familial syndromes associated with developing AML, including inherited bone marrow failure syndromes (i.e., Fanconi anemia, Shwachman–Diamond syndrome, dyskeratosis congenita) or telomere syndromes (associated with mutations in *TERT* and *TERC*) [42, 43]. Other inherited disorders associated with predisposition for AML or myelodysplastic syndrome include germline mutations in *CEBPA*, *DDX41*, *RUNX1*, *GATA2*, *SRP72*, *ANKRD26*, and *ETV6* [2, 43–45]. Certain cancer predisposition syndromes, i.e., Li-Fraumeni syndrome and germline BRCA1/2 mutations are also associated with increased risk of developing AML as well as other hematologic malignancies [17].

Individuals with Down syndrome (trisomy 21) have a significantly increased risk of AML (10- to 20-fold increased risk), particularly the subtype of acute megakaryocytic leukemia, and are associated with somatic mutations of the *GATA1* gene [46, 47].

1.3.4 Environmental Factors

Exposure to environmental agents, including radiation and certain chemicals, as well as lifestyle factors can cause DNA damage and associated genetic changes which have been associated with increased risk of developing acute myeloid leukemia. It is important to note that most patients with AML develop de novo disease without identifiable risk factors.

Historically, ionizing radiation was identified as a risk factor for AML in survivors of atomic bomb explosions in Japan [48]. Radiologists and technicians chronically exposed to high levels of radiation in the early twentieth century were also found to have increased risk of developing leukemia [49]. The use of ionizing radiation for the treatment of other primary malignancies have also been implicated in potential development of AML [50, 51]. Increased risks have also been identified with combined radiation and chemotherapy [52].

Occupational hazards may also play a contributing factor, including prolonged exposure to certain chemicals including organic solvents and pesticides [53]. Exposure to benzene also appears to be associated with increased risk of acute nonlymphocytic leukemia [54]. Other factors including cigarette smoking and obesity have also been associated with increased risk of developing AML [55–58].

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2 Clinical Presentation, Diagnosis, and Classification of Acute Myeloid Leukemia

Ridas Juskevicius, Mary Ann Thompson, Aaron Shaver, and David Head

2.1 Introduction

The acute myeloid leukemias (AML) are a diverse set of phenotypically similar diseases characterized by increased myeloblasts replacing the normal bone marrow, with variable involvement of peripheral blood and occasional involvement of extramedullary sites. In some cases, proliferating blasts replace normal hematopoiesis resulting in failure of the marrow to produce normal peripheral blood cells, with tumor burden itself becoming life-threatening. In other cases, while blasts are increased as a percentage of marrow cells, the predominant problem is primary marrow failure (resembling MDS) rather than blast tumor burden. Classification of AML has undergone fundamental changes over the last two decades, in part due to recognition of these varying scenarios [1]. Although not without areas of controversy, the introduction of the World Health Organization (WHO) classification framework in 2001, updated in 2008 and revised in 2016 [2], represents the official international consensus classification of AML, combining these two scenarios under the common heading of AML. The WHO classification of AML is based on clinical, phenotypic, and molecular genetic features with an attempt to define biologically and prognostically distinct entities which have uniform response to therapy. Although genetic heterogeneity of AML has been recognized for several decades, enormous molecular heterogeneity has become apparent only recently with the introduction of new molecular diagnostic methodologies including next-generation sequencing (NGS)-based assays. The massive amount of data generated utilizing these techniques is contributing to improved understanding of the biologic heterogeneity of AML. Incorporation of the data into the classification framework of AML is inevitable, but is still at its early stages, as we are only now beginning to understand the biologic and clinical implications of these newly discovered molecular alterations. In this chapter, we discuss the clinical presentation, diagnosis, and classification of AML, including appropriate diagnostic laboratory studies necessary for diagnosis and subclassification of biologically and clinically relevant types of disease. Understanding the basis for the current WHO classification of AML requires additional knowledge of the myelodysplastic syndromes (MDS) and their relationship to one subset of AML. Finally, we will address monitoring AML minimal residual disease during and after treatment.

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2.2 Clinical Presentation of AML

The classic onset of the symptoms of acute leukemia is rapid. The patient may have felt ill for only a few weeks prior to seeking medical attention. In other cases, the presentation may be more insidious, with prolonged symptoms related to cytopenias with or without prior diagnosis of underlying MDS. In either case, the most typical presentation is that of symptoms related to bone marrow failure. These include easy bruising and petechiae due to thrombocytopenia, frequent infections due to neutropenia, and/or symptoms related to anemia such as fatigue, pallor, or even cardiovascular effects of profound anemia. In this type of presentation, the primary care physician will typically obtain a complete blood count (CBC), which may show circulating blasts. The number of blasts in peripheral blood may be few or numerous. When blasts are present in the peripheral blood accompanied by anemia and thrombocytopenia in a newly presenting patient, the level of suspicion for acute leukemia is high and a bone marrow biopsy is typically obtained. When blasts are few in number, other morphologic clues on the peripheral smear that may increase the suspicion of marrow replacement by leukemia include leukoerythroblastosis (triad of immature myeloids, nucleated red blood cells, and teardrop red cells), dysplastic changes in neutrophils, and so-called leukemic hiatus where only blasts and few mature segmented neutrophils are present with the absence of other left-shifted myeloid cells that would typically be seen in reactive conditions. All these clues should serve as triggers to obtain a diagnostic bone marrow sample.

Since in the contemporary practice of medicine the initial examination of blood smear takes place in the clinical hematology laboratory, the ability of the hematology technologists to recognize blast morphology is crucial, as they serve as the frontline of diagnosis in patients where the diagnosis of AML may not be suspected. Laboratory quality control (QC) and continuing medical education (CME) activities to reinforce this ability are crucial. The morphologic characteristics of myeloid blasts on the Wright stained peripheral blood smear include immature chromatin ("ground-glass"), increased nuclear:cytoplasmic ratio, and variable granulation to the cytoplasm. The presence of Auer rods, needle-shaped cytoplasmic inclusions resulting from fusion of primary azurophilic granules, is pathognomonic for myeloblasts. In the more frequent absence of Auer rods, flow cytometry must be performed to determine unequivocally the lineage of blasts.

Several clinical manifestations of AML constitute medical emergencies, most notably (1) leukostasis due to hyperleukocytosis and (2) coagulopathy, typically associated with, but not restricted to, acute promyelocytic leukemia (APL). Hyperleukocytosis is usually defined as a white blood cell count greater than 100,000 per μL, but whether leukostasis occurs depends on many factors individual to the patient. Leukostasis is thought to be the result of increased blood viscosity due to the increased cellularity, reduced deformability of the blasts (versus mature cells), and direct and indirect blast–endothelium interaction, all causing occlusion of microvasculature [3]. Both the specific lineage of the increased cells and their rate of rise in the circulation are contributory factors, with monoblasts being the most problematic cell type. Leukostasis should be suspected if the patient has pulmonary, CNS, or cardiovascular symptoms that cannot be explained by other medical conditions: dyspnea, confusion, somnolence, headache, impaired vision, tinnitus, chest pain (myocardial ischemia/infarction), limb ischemia, thrombosis, and priapism [3]. Treatment options include hydration, leukemia-directed chemotherapy, and leukapheresis. The role of the latter is controversial [3, 4]. Hyperleukocytosis may also result in disseminated intravascular coagulation (DIC), which should be considered if the peripheral blood smear demonstrates schistocytes and decreased platelets, and confirmed by checking for decreased fibrinogen, elevated D-dimers, prolonged prothrombin time (PT), and activated partial thromboplastin time (aPTT). DIC occurs in 30–40% of patients with AML and hyperleukocytosis [4]. Finally, hyperleukocytosis may be associated with tumor lysis syndrome (TLS),

which occurs with treatment in approximately 10% of AML patients [4]. Chemistry laboratory values for potassium, phosphorus, calcium, and particularly uric acid should be monitored to detect TLS.

The clinical presentation of acute promyelocytic leukemia (APL) bears particular discussion as the associated coagulopathy may result in life-threatening hemorrhage or thrombosis. The risk of early death from hemorrhage in APL has been estimated at 17–29% in community studies [5], with most cases occurring before institution of treatment. At presentation, mucocutaneous bleeding is common, with immediate risk of hemorrhagic death due to intracranial or pulmonary bleeding. The characteristics of APL blasts on the peripheral blood smear will be described later in this chapter. The presence of low platelets is also obviously significant. Clinical signs are bleeding from gums, epistaxis, GI hemorrhage, and excessive ecchymoses and petechiae. When APL is suspected, coagulation studies including PT, aPTT, D-dimers, and fibrinogen should be obtained. The complex coagulopathy of APL is multifactorial but includes tissue factor (TF)-induced DIC and primary hyperfibrinolysis [5]. APL blasts have increased TF on their surface, which activates factor VII. The resultant factor VIIa activates FIX and FX, leading to thrombin generation, ultimately resulting in fibrin formation. In addition, the promyelocytic blast surface contains Annexin II, which binds plasminogen and tissue plasminogen activator (tPA), promoting plasmin formation and thus fibrinolysis [5]. Immediate treatment with all-trans retinoic acid (ATRA) is required when APL is suspected, before confirmation of the diagnosis with other studies. Treatment with ATRA causes blasts to mature and arrests the coagulopathy. This is essential prior to initiation of chemotherapy, when there will be massive lysis of the blasts. If diagnosis of APL is not subsequently confirmed, ATRA may be stopped with no compromise to other treatment options.

A rare presentation of AML is with myeloid sarcoma, which is defined as a tumor mass consisting of myeloid blasts in which tissue architecture is destroyed, to distinguish it from an area of simple leukemic infiltration [2]. The most common sites are skin, lymph nodes, gastrointestinal tract, bone, soft tissue, and testes. The presentation is usually as a solitary mass [2]. Myeloid sarcoma may be the first, and sometimes the only, early manifestation of AML. It may also be the first manifestation of blast crisis of an underlying myeloproliferative or myelodysplastic syndrome. Another common setting is at relapse, including post-hematopoietic stem cell transplant. Diagnosis depends on morphology (preferably including a Wright stained touch preparation) and immunophenotyping of the myeloid blasts using a combination of flow cytometry and immunohistochemistry. Cytogenetic analysis including FISH may be helpful, particularly if the lesion has monocytic differentiation which often lacks definitive immunologic markers of immaturity. Myeloid sarcoma is most often associated with monocytic differentiation. It has relatively high prevalence in children, which likely reflects a higher incidence of AML with core binding factor abnormalities (t(8;21) and inv16) in this age group, since myeloid sarcomas are prevalent in AML with core binding factor abnormalities [6, 7]. In several series of adults with myeloid sarcoma, there were many cases with a complex karyotype, monosomies, trisomy 8, and translocations involving 11q23 (*KMT2A*) [8, 9]. For diagnostic purposes, the antigens expressed most often in myeloid sarcoma are CD43, CD68, lysozyme, MPO, and CD117 [10]. Immunohistochemistry which includes antibodies to CD4, CD56, CD123, and TCL-1 may be helpful to rule out the possibility of a blastic plasmacytoid dendritic cell neoplasm (which typically is MPO negative, TCL-1 positive, and usually positive for both CD4 and CD56) [11, 12].

A very rare presentation of AML is CNS involvement with the first manifestation being blasts in the CSF, not the peripheral blood. CNS symptomatology suggesting a process involving cranial nerves, spinal cord, or meninges will trigger CSF cytologic examination of a Wright stained cytospin slide, showing blasts and requiring further testing such as flow cytometry to confirm diagnosis. In one study of 12,000 patients

diagnosed with acute leukemia (ALL and AML), only nine patients presented in this way with blasts present in the CSF prior to presence in the peripheral blood [13].

In patients with myeloproliferative or myelodysplastic disease, exacerbation (often insidious) of symptoms (fatigue, bruising, dyspnea), or deterioration of laboratory values (cytopenias, increased peripheral blood blast count, elevation in uric acid or LDH) may be a harbinger of blast crisis with evolution to acute leukemia. In this setting, the blasts are likely to be myeloid. Morphologic review of the peripheral blood smear and a low threshold for obtaining a bone marrow sample are recommended. A caveat about making the diagnosis of AML in this setting is that a leukoerythroblastic smear due to profound hypercellularity or myelofibrosis may have a few blasts on the peripheral blood smear. Therefore, review of the peripheral blood smear should be followed by a bone marrow biopsy. In patients with CML, approximately two-thirds of blast crises are acute myeloid leukemia, whereas one-third are acute lymphoblastic leukemia [2].

2.3 Laboratory Studies for the Diagnosis and Monitoring of AML

2.3.1 Morphology

A good bone marrow aspirate and biopsy sample are essential and require good technique at the bedside in acquisition and in the laboratory in processing the sample. Squash preps are discouraged except in the hands of experienced technologists. Preferable are push preps, performed identically to preparation of peripheral smears, or coverslip preparations. Touch preps should also be performed routinely. If a biopsy is to be obtained, it should be large enough to properly assess marrow characteristics and should be redirected to avoid the preceding aspirate site.

Morphologic evaluation of biopsy samples is the cornerstone of pathologic evaluation and still remains important even with the advent of other ancillary modalities. Review of morphology can focus on low-power, large-scale patterns, or high-power, fine-scale details. Low-power evaluation of the bone marrow sample can help detect patterns of infiltration and assess for disease burden. However, high power examination of individual cell features, often called cytomorphology, is of particular importance in hematopathology and especially in evaluation of AML, since the differential diagnosis often depends on morphologic features present in individual cells, such as Auer rods, cytoplasmic granules, and nuclear features.

The need for both low- and high-power examination of bone marrow specimens helps to explain some of the sample collection strategies employed in the evaluation of leukemias. Taking both aspirate and core biopsy samples of bone marrow, for example, allows evaluation of individual cytomorphology on smeared specimens of aspirate material, as well as evaluation of low power architectural distortion and geographic patterns using the core biopsy specimen. While examination of these two different tissue types historically was performed by different groups of physicians—pathologists were responsible for reviewing core biopsy specimens, and hematologists often reviewed aspirate specimens—modern practice, particularly in the United States, has moved toward combining the review of both specimen types under the auspices of the pathologist, which allows better integration of all sources of diagnostic data into one process and one report.

2.3.2 Immunophenotype

In addition to assessment of light microscopic morphologic features, modern diagnosis requires interpretation of the set of proteins and other markers expressed by the cell, which is referred to as the immunophenotype. In particular, the WHO classification of AML requires correlation with immunophenotype both for excluding other categories of acute leukemia and in aiding in subclassification. Myeloid-specific markers such as myeloperoxidase, or markers of immaturity such as CD34, are important diagnostic adjuncts built directly into the WHO classification system.

Most methods for immunophenotyping employ targeted antibodies (or other molecules with high specificity of binding, such as nucleic acid sequences), whose specific regions react with the phenotypic target of interest. Laboratory techniques for immunophenotyping differ in the method for assaying the binding of these targeted antibodies. While a range of techniques are available, two categories of the most prevalent techniques in the clinical diagnostic setting are tissue-based techniques such as immunohistochemistry (IHC) and in situ hybridization (ISH) and cell-based techniques such as flow cytometry. These categories have overlapping strengths and limitations and are often used in a complementary strategy in the diagnostic setting.

Immunohistochemistry and other tissue-based methods leverage the diagnostic information present in morphologic features of the tumor to help correlate with the immunophenotypic data, particularly in tumor populations that are heterogeneous or mixed with a significant non-neoplastic background population. This is brought about by performing the antibody reaction and subsequent development for visualization in the setting of an intact tissue block, with a counterstain added so that morphologic features can be appreciated at the same time. IHC uses specific antibodies conjugated to a reporting molecule, whose presence is detected by a secondary reaction after the initial antibody binding step. The result is a color change (typically brown or red, depending on the developer) in the cells/areas where the antibody has bound. The result is a pattern of color change on a tissue slide that correlates with the presence of the marker of interest. ISH is a similar technique that uses synthetic DNA/RNA sequences with attached reporter molecules to detect the distribution of complementary nucleic acid sequences, rather than proteins or other antibody targets.

Tissue-based methods like IHC have two primary areas of strength. IHC can be performed on formalin-fixed, paraffin-embedded material. Because of the longevity of this type of material and the lack of a need for viable, fresh specimen, this allows a range of studies to be performed both at the time of the initial acquisition of the material and at any point in the future when re-review of the specimen is needed. For small samples, such as bone marrow biopsies, the small amount of tissue received can be used for both morphologic and immunophenotypic interpretation, without having to triage the sample between two diagnostic techniques. The second area of strength is that, because the IHC stain is performed on the tissue in situ, morphologic correlates can be drawn with areas of abnormal IHC staining. In the case of AML, IHC can be particularly helpful in a heterogeneous, mixed sample where the morphologic features of blasts are striking. This is particularly important for morphologically unusual subclassifications of AML, such as acute promyelocytic leukemia or AML with erythroid or megakaryocytic differentiation. In these cases, IHC allows direct correlation of the phenotypic data with the morphologic diagnostic features.

Immunohistochemistry does have significant drawbacks, which limit its utility in certain situations. The most prominent of these limitations is the necessity to use only one (or at most two) labeled antibodies in a single reaction, due to the relatively limited number of different reporter tags available for routine use. For a neoplastic process such as AML in which it is necessary to assay a complicated immunophenotype with many markers, this requires laborious and error-prone comparison between individual markers tested on different slides. Focal areas of abnormality may not be present on every slide, and scant tissues may be entirely consumed in the process of testing before the entire immunophenotype can be measured. Another limitation is that, in the clinical setting at least, IHC and ISH stains are typically reviewed by eye under the microscope, and therefore evaluation of the results is necessarily qualitative (positive/negative, dim/bright) rather than quantitative. This can be a limitation for some markers of diagnostic or therapeutic importance, such as CD38, where expression is almost ubiquitous, and it is the degree of intensity of expression that is the important clinical consideration [14]. A separate issue with tissue-based techniques like IHC and ISH is the time required to perform the testing.

These techniques require several hours for binding and developing of the specific target molecules, which limits the rate at which diagnostic information can be incorporated. One or two rounds of IHC stains can add 1–2 days to the time required to render a final diagnosis for a case, which can have a clinical impact, especially in settings such as initial diagnosis or initiation of targeted therapy.

The prevalence of flow cytometry in clinical hematolymphoid diagnostics, and in particular in the evaluation of acute leukemia, is due to its ability to address many of the limitations described above for tissue-based immunophenotyping. In turn, flow cytometry itself has many limitations that can be backed up with the use of IHC or ISH. As a technique, flow cytometry shares some similarities with IHC: specific antibodies are linked to reporter molecules, which in the case of the most common form of flow cytometry are fluorophores that emit light at specific wavelengths upon excitation by a laser. These antibodies are allowed to hybridize with the cells of interest, and then exposed to a reporter reaction (in this case, excitation by a laser) which allows for detection of specifically bound antibodies. The major distinction from tissue-based techniques is that flow cytometry is performed on disaggregated, individual cells in suspension in a buffer fluid, rather than on intact sections of tissue. Additionally, multiple different antibodies conjugated to different fluorophores are used at once, allowing the measurement of multiple markers simultaneously on the same cells.

Flow cytometry's differences from tissue-based techniques like IHC lead directly to its advantages and disadvantages. Whereas interpretation of IHC for multiple markers on the same tissue can lead to frustration and ambiguity as multiple slides have to be compared, flow cytometry is a natural system for looking at multiple markers on the same specimen. This is especially important for subclassification within broader categories or for distinction between closely related diseases, where assessment of a complicated set of overlapping immunophenotypes needs to be made using a large battery of specific antibodies. Another advantage of flow cytometry is its ability to reproducibly measure relative quantitative intensity of staining, rather than the crude strong/weak/negative categorization with IHC. An example of the utility of this approach in myeloid neoplasia is in assessment of CD56 on bone marrow myeloid precursors: dim, variable CD56 expression may be seen in a variety of reactive conditions, while uniform brighter expression of CD56 is a much more specific marker of neoplastic abnormality. Properly calibrated flow cytometry can also often detect much lower intensity of staining than IHC, allowing the diagnostician to detect dim aberrant expression of markers not associated with normal populations that help definitively establish the presence of a neoplasm [15]. In the setting of a new presentation of acute leukemia, the rapid turnaround time of flow cytometry is an additional advantage. Total time in the laboratory from processing to data acquisition to analysis can take less than an hour, allowing rapid triage of an unstable patient.

The limitations of flow cytometry primarily stem from the need for individual cells in suspension. The process of disaggregating the cells results in a complete loss of the low-power, geographic context, in contrast to IHC, where the ability to map staining pattern onto morphologic pattern can often be vital to interpreting a complicated sample. The same processing requirements also remove the ability to correlate the immunophenotypic features detected by flow cytometry with specific high-power cytomorphologic findings. As discussed above in the section on immunohistochemistry, this can be relevant in cases with relatively rare leukemic cells with striking morphologic features. Finally, the requirement for disaggregation and suspension means that paraffin-embedded tissue is unsuitable for flow cytometry; fresh aspirate or disaggregated biopsy material, or carefully frozen archival material is required. This limits the utility of flow cytometry for returning to previous cases or as an adjunct test in cases where appropriate material was not reserved at the time of biopsy.

This set of opposing and complementary strengths and limitations has led to the adoption of both IHC/ISH and flow cytometry as routine

clinical tests in hematolymphoid disease, including myeloid neoplasms such as acute myeloid leukemia. Some diagnostic challenges are more suited to one modality over another. Fresh bone marrow aspirate material is an ideal specimen for flow cytometry, and in newly diagnosed disease, the abundant and often relatively homogeneous blast population makes correlation with specific morphologic patterns relatively unimportant. For these reasons, comprehensive flow cytometry panels are used as the first-line immunophenotypic assessment of new leukemia. On the other hand, tissues where disaggregation might be more difficult or not expected at the time of biopsy, such as cutaneous involvement by extramedullary deposits of acute leukemia, are less amenable to flow cytometry and the importance of IHC increases. Another area favoring overlapping use of the two modalities is in diseases such as myeloid leukemias with monocytic differentiation, where the flow cytometry immunophenotypic features are not always helpful for distinguishing between chronic and acute disease, and correlation of immunophenotypic abnormalities with morphologic features may be necessary to definitively establish the disease subtype.

Acute myeloid leukemia is well-studied and illustrative of how a careful analysis of immunophenotype can assist in the diagnostic process, while also serving as a reminder of the necessity of incorporating the immunophenotypic data into a broader context of morphologic and ancillary testing. Specific subtyping of AML can have a massive impact on prognosis and therapy for the patient, and specific subtypes often correlate with immunophenotypic differences. APL is a well-known example: it has profound prognostic implications due to its association with DIC, and it is amenable to a very specialized targeted therapy using retinoic acid derivatives. APL has a striking immunophenotype, often lacking many of the markers generally associated with immature myeloid cells, including CD34 and HLA-DR, while strongly expressing other myeloid phenotypic markers such as CD117 and myeloperoxidase. Detection of a population of leukemic blasts with this immunophenotype can help raise or confirm clinical and morphologic suspicion for APL, leading to proper targeted and supportive management of the patient. Unfortunately, detection of this special phenotype is neither entirely specific nor sensitive for APL. The prominent granules in APL tend to autofluorescence when exposed to laser light, leading to a well-known propensity for the leukemic blasts to show non-specific, non-antibody-mediated fluorescence for a wide range of markers [16], leading to false negatives in the sense that the immunophenotypic pattern of interest is not recognized. Relatively simple techniques exist to identify and account for this autofluorescence but neglecting to employ these techniques can lead to misdiagnosis on immunophenotypic grounds. On the other hand, even if the phenotype is correctly interpreted, it is not entirely specific for APL. Other leukemias may have a similar phenotypic pattern, with a prominent example being *NPM1*-mutated AML, a common category of AML with prognostic and therapeutic consequences much different than APL [17]. Thus, recognition of specific phenotypic patterns can be helpful in guiding the clinician onto the right track, but definitive diagnosis still generally relies on correlation with the entire suite of diagnostic testing, including morphology, cytogenetics, and molecular studies.

2.3.3 Cytogenetics

A frequent and recurrent abnormality in many hematologic neoplasms, including AML, is the presence of large-scale chromosomal abnormalities, including gain or loss of large sections or even entire chromosomes, as well as translocations involving transfer of millions of base pairs of genetic material from one chromosomal section to another. The analysis of chromosomal structure for these classes of large-scale abnormalities is referred to as cytogenetics. Some of the best-established diagnostic categories in AML depend on the detection of cytogenetic abnormalities, most particularly in looking for the presence of balanced translocations, exchange of two portions of chromosomes in a way that

results in no net gain or loss of genetic material, or specific patterns of aneuploidy, gain or loss of chromosomal material in a non-balanced fashion that leads to a change in the total amount of genetic material. For this reason, cytogenetic diagnostic techniques are standard of care in AML. Three of the most common techniques, each with their own advantages and limitations, are conventional karyotyping, fluorescence in situ hybridization (FISH), and comparative genomic hybridization (CGH).

Conventional karyotyping is the oldest and perhaps the most straightforward of these techniques. In karyotyping, cells of interest are stimulated ex vivo with mitogens to induce chromosomal replication and then arrested at metaphase via treatment with cell cycle inhibitors such as colchicine. Cells treated in this fashion have their chromosomal material well organized into chromatids, and proper staining techniques lead to visualization of individual chromosomes, each with a recognizable and unique banding pattern due to alternating stretches of tightly and loosely packed DNA. With appropriate training, these banding patterns can be used to detect and enumerate chromosomes and even to detect whether chromosomal elements have been translocated, amplified, or deleted. This technique is very well suited for the detection of aneuploidy, since the presence or absence of major chromosomal segments is readily apparent. Translocations, duplications, or deletions involving large enough stretches of DNA can also be detected due to abnormalities in the banding pattern. Because the karyotype is analyzed in an untargeted, nonbiased fashion, conventional karyotyping is also an optimal technique for the detection of non-hotspot abnormalities, such as the wide range of aneuploidy that can be seen in AML with myelodysplasia-related changes.

The technique of conventional karyotyping leads to a set of trade-offs that limit its utility in certain areas. While manually enumerating every chromosome in the cell allows the technique to be broadly sensitive to a wide variety of changes, it makes the technique labor intensive and requires specialized training. Because of this, conventional karyotyping in clinical practice is usually limited to 20 or 30 cells from one sample; this is enough to reliably detect abnormalities in samples floridly involved by a neoplastic population but is extremely insensitive for trying to track low-level involvement by disease in the context of therapy or disease evolution. The reliance on banding patterns visible under the microscope also limits the resolution of the assay; changes involving chromosomal regions smaller than several megabases are generally invisible to this technique. Finally, the reliance on the experimental conditions necessary to induce and then arrest mitosis requires the collection of viable, unfixed cells that respond to ex vivo mitogen stimulation. This means that archival or other fixed specimens cannot be analyzed using this technique, limiting it to fresh sample only.

Some of these trade-offs are addressed by FISH, another cytogenetic technique. FISH is performed via base-pair hybridization between target DNA and long (several kilobase) fluorescently labeled nucleic acid probes. The probes and the target DNA are allowed to hybridize, and then fluorescence is assayed under the microscope. Depending on the way the probes are designed, different patterns of fluorescence can be observed which help detect gain or loss of chromosomal segments, as well as translocation or other chromosomal disruptions involving specifically targeted segments of DNA. For example, an increase in the number of signals observed in a single cell from two to three might indicate triploidy (acquisition of another chromosome) or simply focal amplification of the targeted DNA sequence. Translocations can be detected using probes aligned at either side of the common area of breakage; visualization of the probes distant from each other in the nucleus would indicate a translocation involving the targeted area. Due to the relative ease with which this kind of pattern can be interpreted compared to manual staining and karyotyping, a larger number of cells can be assayed with FISH; clinical assays typically assess between 200 and 500 cells per sample. This increases the clinical sensitivity of the test, allowing for better detection of relatively low-level disease involvement. The targeted probes

also allow for better resolution in FISH compared to conventional karyotyping; FISH can detect abnormalities at the level of tens to hundreds of kilobases, which is at least an order of magnitude more sensitive than the megabase-level resolution of conventional karyotyping. Finally, FISH can be performed on non-replicating cells and does not require ex vivo stimulation, meaning that it can be performed on formalin-fixed, paraffin-embedded archival material without the necessity for culturing viable cells.

The targeted nature of FISH's probe-based system leads to its disadvantages as well as its advantages. While conventional karyotyping can detect a wide range of abnormalities, including ones not suspected by the diagnostician before the test was performed, FISH can only detect abnormalities at areas covered by its target sequence, which represents a tiny fraction of the total cytogenetic material. Thus, FISH is poorly suited for detecting a non-specific karyotype and is liable to completely overlook unsuspected findings. FISH performs best when used in a small panel to test for abnormalities high on the pre-test differential diagnosis in new disease or to assess for the presence of a known abnormality in follow-up testing.

A newer technique that addresses some of the limitations of both conventional karyotyping and FISH, while introducing its own complications, is comparative genomic hybridization (CGH). This method can detect copy number alterations and map them to specific chromosomal locations with a relatively high resolution. The most widely available CGH technique in the clinical setting is array CGH, in which the target genome is fluorescently labeled (rather than the synthetic probes, as is the case in FISH) and hybridized to a standardized reference array. After hybridization, the degree of fluorescence is measured for each element of the standardized array, which gives a readout of relative abundance of chromosomal material mapped onto the array. The resolution of the technique is dependent on the number of elements used in the reference array and, with the current techniques, can be decreased to the order of tens or hundreds of kilobases. This allows for a mapping of chromosomal gains or losses with a resolution many orders of magnitude finer than conventional karyotype, with high confidence in the mapping of the abnormal areas to specific chromosomal regions without the need for specialized training in reading banding patterns. Because the procedure requires only genomic DNA from the target sample, archival fixed material can be used as well as fresh material, circumventing one of the other major limitations of conventional karyotyping. And because targets from across the genome are included in the standardized array, the CGH technique lacks the highly targeted "tunnel vision" issues that plague FISH.

Given the importance of recurrent cytogenetic abnormalities in the WHO classification of AML, cytogenetics continues to play a central role in disease diagnosis and classification. Clinically validated targeted FISH panels are readily available for all of the recurrent translocations and inversions. Additionally, FISH probes can be used to track recurrent patterns of aneuploidy, particularly those seen in AML with myelodysplasia-related changes. Conventional karyotyping still plays an important role, particularly at diagnosis, in order to assess for non-standard abnormalities that may not be picked up by targeted FISH probes. Given the greater clinical sensitivity of FISH relative to conventional cytogenetics, follow-up assessment of AML is best performed in conjunction with targeted FISH, while conventional karyotyping in this setting is reserved for the assessment of clonal evolution or, potentially, emergence of new therapy-associated dysplastic clones. Despite the cited advantages of CGH, its role in clinical practice is still evolving, and it is not yet in widespread clinical use for diagnosis or management of AML.

2.3.4 Molecular Genetics

Cytogenetic analysis is a powerful technique but is restricted in its scope to large-scale changes to chromosomal structure. An entirely different scale of genetic alterations occurs at the level of one or a few base pairs: point mutations, small

insertions or deletions, and other fine-scale genetic abnormalities. Testing for these abnormalities requires an entirely different set of tools, one that has exploded in scope and utility in the last decade or two. The results of this kind of molecular genetic analysis are being rapidly assessed and incorporated in classification and prognostic guidelines, with AML serving as a prime example of a disease process for which the entire diagnostic approach has changed as a result of these new techniques. As expected in an emerging field, many techniques are coming to the fore and being incorporated into clinical practice, but they fall into a few major categories: sequence-specific amplification techniques, Sanger sequencing, and next-generation sequencing.

Amplification-based assays are targeted via synthetic nucleic acid probes to particular areas of the genome. They use polymerase chain reaction (PCR) or related techniques to amplify segments of DNA or RNA including the targeted area of interest. Once the target area is amplified, follow-up studies are employed to investigate these amplified fragments, by their size, their hybridization characteristics, their abundance, or their specific sequence. With some systems, the targeted probes can be made to hybridize at hotspots of frequent mutation, so that the mere presence or absence of a product from a test reaction can indicate whether a particular genetic alteration is present. Using techniques such as real-time PCR, quantitative results can be obtained, allowing the levels of specific abnormalities to be followed over time, a technique which has been directly incorporated into the therapeutic strategies for some subtypes of AML [18].

Because of their targeted nature and their relative maturity as some of the first molecular assays developed, amplification-based assays are widely employed in clinical laboratories both in oncologic testing and elsewhere. Only DNA (or RNA, depending on the test type), not intact cells, is required to perform these tests, so testing is usually amenable to validation on fixed and archived material. In addition, the amplification of the sample over many cycles using targeted probes allows sensitive and reliable measurement of abnormalities present only at a very low level in the sample tissue, often at a level several orders of magnitude lower than the most clinically sensitive cytogenetic techniques, and at a level roughly comparable or even more sensitive than flow cytometry [19].

Amplification-based assays share the same Achilles' heel as other targeted techniques like FISH, in that only those abnormalities for which targeted probes have been designed and validated can be assayed. For certain common abnormalities with well-defined hotspots, these techniques are very well-suited and can readily be employed; excellent examples of this type of lesion in AML includes mutations in the *FLT3* and *NPM1* genes, as well as other genes with frequently occurring hotspot mutations such as *IDH1* or *IDH2*. However, the advent of other molecular techniques such as sequencing has revealed the breadth and diversity of non-hotspot molecular genetic changes in neoplastic populations, many of which have already been demonstrated to be clinically relevant. For these classes of mutations, there is no practical, efficient method to employ targeted amplification techniques. A separate issue with this class of assays is that, due to the necessity for binding of the targeted probes used to guide amplification, the tests rely on the presence of the complementary sequences to the probes in the diagnostic sample being assayed. If large-scale changes, such as deletion or extensive mutation, has removed the binding sites, the assay will fail with a false negative or equivocal result.

Sequencing methods directly read the genetic sequence in the area of interest. Sequencing may often be performed as a follow-on technique after some of the amplification-based systems described above, although in more modern, high-throughput incarnations, other systems for selecting areas for sequencing may also be used. Widely used sequencing assays in the previous generation relied on the well-known Sanger technique, named after its developer, which incorporates fluorescent or radio-labeled tagged terminating nucleotides to create an entire array of differently size fragments, with the size and

terminating label of each fragment spelling out the nucleotide present at that position. Sanger and other related previous-generation sequencing assays had the advantage of allowing direct review of the results to help with troubleshooting but were relatively laborious and expensive to scale up even to the level of coverage of a large gene such as *TP53*, much less an entire panel of genes relevant for one tumor type, and certainly were not feasible for projects such as whole exome sequencing.

Next-generation sequencing (NGS) is a blanket term for a family of related techniques which permit rapid upscaling of sequencing efforts to high-throughput environments, in which large, multigene panels and even whole exome or whole genome sequencing can be performed on a patient-by-patient basis in the clinical setting. In most NGS techniques, a large number of small sequencing reactions are typically carried out in parallel, allowing assessment of many targets (or of many samples for fewer targets) simultaneously. Selection of targets to sequence can be done via processing of whole exome or whole genome material, or by using targeted amplification-based or hybrid capture techniques. An important component of NGS techniques is an elaborate software "pipeline" to help filter the results for human review; the sequencing techniques employed are relatively error-prone, so testing errors need to be eliminated, along with the large number of benign variants identified that are unrelated to disease.

The advantages of sequencing in current practice center on the increased volume of data that the assays provide. With NGS techniques, the "tunnel vision" issue that smaller scale targeted techniques create can be minimized through sheer brute force, by sequencing more and more targets. At the current time, routine analysis of whole genome data is not economically or informatically practicable, but large panels (tens to hundreds) of clinically relevant genes can be tested at once, and not just at commonly mutated hotspots. This has led to increasing recognition of the clinical importance of large genes without significant targetable hotspots, such as *TP53*, both in AML and in other hematologic neoplasms. The high level of coverage provided by NGS leads to other benefits as well; in cases where fewer genetic regions need to be examined, the bandwidth of the assay can be used to provide deeper coverage of the smaller number of targets. This leads to better clinical sensitivity of the assay, where smaller abnormal populations can be detected due to the sequencing of many DNA segments from a mixed sample. Thus, NGS assays can be tweaked to provide some combination of broad coverage or high clinical sensitivity, based on the clinical needs.

This flexibility in assay design, as well as the sheer volume of data produced, leads to some of the most confounding challenges facing those who wish to use NGS in the clinical arena. Because the field is still in a state of active evolution, and because everything from the selection of the genes of interest to the method for filtering the resulting data is in a state of innovative flux without well-validated guidelines for standardization, comparison of data collected by different centers or using different techniques is less straightforward than it might appear by simply reviewing the final, synthesized reported information. Even with acceptably standardized and validated assays, the sheer volume of data produced by NGS studies can create its own problems. It can be difficult for clinicians and diagnosticians, either in the clinical trial setting or in the routine care of patients, to separate out genetic variants into clearly benign changes or polymorphisms, clearly disease-associated mutations, and potentially novel abnormalities that may be associated with the patient's disease.

Even for mutations that have been well-demonstrated to be definitively associated with disease, it can be surprisingly difficult to assign a particular diagnostic or therapy-guiding role. AML provides excellent examples of such challenges. Mutations in genes like *NPM1* and *FLT3* (particularly internal tandem duplications or ITDs) were thought to be well-understood on the basis of targeted amplification studies before the wide advent of NGS: *NPM1* mutations were associated with relatively good prognosis, and *FLT3* ITD mutations were associated with relatively poor prognosis, with the effects of *FLT3* to

some degree trumping those of *NPM1* in leukemias where the mutations co-occurred [20, 21]. However, with more data derived from NGS studies, it appears that the picture is muddier, with the prognostic effect of these mutations likely dependent to some degree on the presence or absence of mutations in other genes, including *IDH1*, *IDH2*, and *DNMT3A*, as well as the prevalence of the *FLT3* ITD mutation in the clone [22–24]. The importance of these genetic combinations is still in flux, owing to some degree to the problems of statistical power brought about by the need to measure so many different genetic combinations.

Current strategies for the diagnosis and follow-up of AML rely heavily on molecular genetic techniques. Given the increasing number of genetic targets that are relevant to the classification and prognostication of AML, new diagnosis is best done in conjunction with a multi-gene panel, typically performed via NGS, that assays for a wide range of genes. One potential issue with using a large NGS panel as an upfront diagnostic tool is the potentially long (5–10 days) turnaround time, particularly if the testing is performed at a reference laboratory. The mutational status of some AML-associated genes is playing an increasingly important role in initial therapy, both on clinical trials and in routine practice. For example, given the importance of FDA-approved inhibitors for AML with mutations in *IDH1* [25], *IDH2* [26], or *FLT3* [27], rapid assessment of mutational status for these genes, all of which have mutations primarily focused in hotspots, is necessary. For this reason, faster-turnaround amplification-based methods may be performed in conjunction with more comprehensive NGS panels in order to provide timely access to critically important clinical data.

2.4 Clinical Diagnostic Testing in Residual Disease

The laboratory testing modalities described above have been used in various forms for decades in the assessment and categorization of newly diagnosed oncologic disease, including AML. Diseases could be classified, stratified for prognostic purposes, and triaged for consideration for targeted therapeutic intervention. This remains an important and rapidly growing area for clinical diagnostics, but many of the same testing modalities also present opportunities for analysis in subsequent examination of the patient for residual disease.

Of particular interest to clinicians in hematologic malignancies, including AML, is determining whether the neoplastic clone remains in the patient at various stages of therapy. Older descriptors for disease status like “complete remission” (CR) are relatively insensitive; in AML, CR is defined as a decrease in blasts below 5% of total marrow cells (along with recovery of peripheral counts, return of marrow cellularity and normal hematopoiesis, and absence of extramedullary disease), but without necessarily distinguishing between normal marrow myeloblasts and malignant leukemic blasts. While this classification approach was a reasonable one based on the diagnostic modalities available at the time—primarily morphologic review and some basic immunophenotypic assays such as cytochemistry, and remains in clinical use today, modern methods of disease detection have made it abundantly clear that residual levels of abnormal cells can be readily detected after therapy in some groups of patients. Furthermore, the presence or absence of this low-level involvement can be shown to play a major role in determining prognosis and frank relapse risk. In many cases, the best predictor of relapse is the persistence of abnormal cells after therapy, often at levels of detection below the threshold for classic CR and other categories; this low-level persistent disease is termed “minimal residual disease” (MRD).

The utility of MRD detection was first and most extensively demonstrated in B lymphoblastic leukemia (B-ALL), particularly in the pediatric setting, and flow cytometric assessment of MRD is currently a well-established tool for the prognosis, monitoring, and treatment of B-ALL [28, 29]. The role of MRD testing in AML is an area of active and maturing development, with roles being defined for testing both by flow cytometry and by targeted genetic studies.

In general, detection of MRD by flow cytometry is based on a set of important technical principles. It is critical to be able to distinguish the abnormal leukemic population from normal precursors by its immunophenotypic characteristics. While B-ALL MRD detection has become at least somewhat standardized, approaches to AML MRD detection by flow cytometry are currently somewhat more variable between different centers. Some centers have emphasized more of a holistic different-from-normal approach to analysis, looking at whole populations of maturing myeloid cells and looking for abnormalities in those patterns, while other centers have focused more on looking at specific early populations of cells and evaluating their expression of various combinations of abnormal markers [30]. Both of these approaches are made challenging by the nature of the AML blast populations. AML shows more variation in the "leukemia-associated immunophenotype" (LAIP) at the time of diagnosis than B-ALL does, with less ability to rely on a reproducible gating scheme to routinely isolate the neoplastic population. Additionally, AML blasts show an even greater propensity than B-ALL blasts to alter their LAIP over time, requiring greater vigilance on the part of those monitoring for MRD. For these reasons, many of the largest AML trials have validated a cutoff of 0.1% for MRD detection, rather than the 0.01% used in B-ALL [31].

While flow cytometry is a reliable technique for MRD detection, able to be used in the vast majority of cases, the difficulty and relative lack of reproducibility due to the heavy analytic requirements of the assay have led to the use of molecular genetic techniques for MRD detection. As described in the previous sections, amplification-based targeted genetic assays can detect extremely small abnormal populations in a mixed sample. For those patients who have genetic lesions at diagnosis amenable to this type of testing, molecular methods for MRD detection are of great utility. *NPM1*-mutated AML is a prime example of this approach in myeloid disease; the detection of *NPM1* mutations by targeted amplification has been shown to be a powerful MRD detection tool [32]. Caveats apply to this approach, however; *NPM1*-mutated AML may relapse as a *NPM1*-negative clone [33], leading to potential false-negative results for MRD assays based on tracking of a single genetic lesion. The same problem arises for many other genetic abnormalities that could be tracked in patients, meaning genetic techniques for MRD detection must often be complemented with other tools, such as flow cytometry. The presence of clonal rearrangements of the *TCR* and *IGH* loci in T- and B-lymphoblastic leukemias that are almost never lost during clonal evolution provides a powerful tool for MRD monitoring in those diseases using specialized NGS panels with high clinical sensitivity [34, 35], but a similar common and invariant abnormality in AML has not yet been identified.

These methods for developing tools for MRD detection have been applied to AML with significant success [19]. Flow cytometry and molecular genetic MRD detection at the end of induction chemotherapy have been shown to be important for prognosis of AML patients to a much greater degree than standard CR status, both in the setting of conventional therapy [36, 37] and in allogeneic stem cell transplant (SCT) [38]. In the specific setting of SCT, MRD detection at the time of transplant has been shown to be strongly associated with the risk of relapse after transplant [39], potentially helping to determine which patients should be eligible for transplant and which should be triaged for additional pretransplant therapy.

2.5 AML Classification

2.5.1 The WHO Classification Framework

Acute myeloid leukemia (AML) is a heterogeneous disease clinically, morphologically, and genetically. In the latter half of the twentieth century, the FAB classification was in general use for the classification of AML. This classification, a standardization of the historical approach to classification, used morphologic, cytochemical, and later flow cytometric features to classify AML

based on how leukemic blasts recapitulate normal hematopoiesis (lineage of differentiation and level of maturation of blasts). While useful for laboratory description of leukemic blasts, by the end of the twentieth century, this approach had become obsolete for clinical practice and of limited utility for correlation with rapidly expanding cytogenetic, molecular genetics, and biologic knowledge.

An alternate approach was proposed in 1995 [1] which grouped most patients with AML into two broad biologically and clinically meaningful, although imprecisely defined, groups: de novo AML (DN-AML), meaning patients with no antecedent marrow abnormalities (not to be confused with the clinical usage of that term), and secondary AML (s-AML), meaning patients with antecedent hematopoietic disease, irrespective of whether it was recognized clinically (Fig. 2.1). DN-AML cases occur more frequently in younger patients, with a median age in the 30s and a relatively flat incidence curve for population at risk, implying a relatively simple pathogenesis. s-AML cases tend to occur in older patients, with a median age over 60 years and an exponential incidence curve implying a random multistep pathogenesis. This approach was adopted in the WHO classification of 2001, the third edition of the World Health Organization Classification of Tumours of Haematopoietic and Lymphoid Tissues, as "AML with recurrent genetic abnormalities" (AML-RGA) and "AML with myelodysplasia-related changes" (AML-MRC) respectively, with subsequent elaboration in the 4 th edition and in a revision (2017) (Table 2.1) [2, 40, 41]. This classification was designed to be

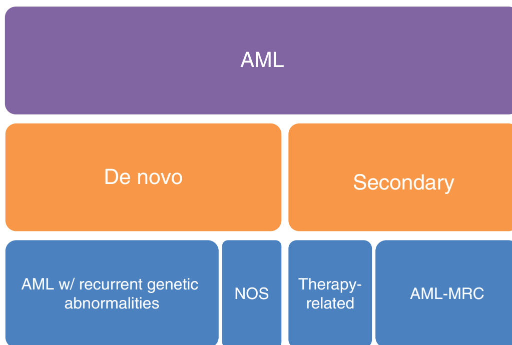

Fig. 2.1 Approximate representation of the broad biologically and clinically relevant categories of AML as they map on to the WHO 2016 classification framework. These categories are currently imprecisely defined by clinical features and currently available diagnostic methods. The proportional distribution of the de novo and secondary AML cases depends on demographics of the population at risk (i.e., de novo AML is more common in children and young to middle-aged adults with an incidence rate that is relatively flat throughout life. On the other hand, secondary AML with most cases corresponding to AML-MRC in the WHO classification framework is most common in the elderly patients comprising most AML cases beyond 60 years of age with a median age in the 70s)

Table 2.1 WHO classification of acute myeloid leukemia

AML with recurrent genetic abnormalities (mostly de novo AML)
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*
Acute promyelocytic leukemia (APL) with t(15;17)(q22;q12); *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*
Provisional entity: AML with BCR-ABL1
AML with mutated *NPM1*
AML with biallelic mutations of *CEBPA*
Provisional entity: AML with mutated RUNX1
AML with myelodysplasia-related changes (mostly secondary AML)
Therapy-related myeloid neoplasms (mostly secondary AML)
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

flexible to allow incorporation of new entities as they are recognized. It differs fundamentally from the historical (FAB) approach.

In the WHO classification, AML-RGA encompasses most cases of DN-AML, including cases with recurring balanced translocations. WHO AML-MRC encompasses most cases of s-AML, which are cases with often multiple genetic and mostly unbalanced chromosomal abnormalities, with background features frequently suggesting underlying myelodysplastic syndrome (MDS). Distinction between these pathogenetically different categories of AML is not always straightforward but is important clinically for therapeutic decisions. AML-RGA (DN-AML) usually has normal background hematopoiesis at presentation and in remission, with normalization of peripheral blood counts (complete remission). Patients with AML with recurrent genetic abnormalities tend to be younger and have relatively simple genomic aberrations. Most cytogenetically and molecularly defined subtypes under this category are recognized as distinct clinicopathologic entities and comprise approximately 65–70% of all AML cases [23, 42, 43] (Fig. 2.2). This is in contrast to AML-MRC (s-AML) which tends to occur in older patients, have MDS-like background hematopoiesis, with poor marrow reserve, the probability of reversion to clonal hematopoiesis (i.e., MDS), and persistent cytopenias during "remission" (sometimes called complete remission with incomplete recovery of counts [CRi]). AML-MRC also has a high frequency of resistance to conventional chemotherapy at presentation.

AML arising in the setting of prior cytotoxic therapy is classified under the WHO category as "Therapy-related myeloid neoplasms" [2]. The most common cause of therapy-related myeloid neoplasms is treatment that causes DNA interstrand crosslinks or DNA double-stranded breaks (alkylating agents, platinum derivatives, nitrosoureas, or ionizing radiation). AML cases in this group are typically associated with unbalanced chromosomal aberrations and major gains or losses of chromosomes and are an iatrogenic model of AML-MRC (Table 2.2). A second cause of therapy-related myeloid neoplasms is topoisomerase II inhibitor therapy, which is typically associated with balanced chromosomal translocations (especially involving the genes *KMT2A/MLL* and *RUNX1*); this subset appears to be an iatrogenic model of AML with recurrent genetic abnormalities. An uncommon third type of therapy-related AML, seen in patients receiving any mix of complex chemotherapy and/or radiation, is a several log increase in the incidence of the common balanced translocations of AML-RGA [44].

The precise biology of DN-AML and s-AML has been the subject of recent genomic investigation [45]. Most AML with recurrent genetic abnormalities is characterized by a single balanced translocation and a low number of other gene mutations, most frequently activating mutations in the signaling genes including *NRAS*, *FLT3*, *KIT*, other tyrosine kinases, and protein tyrosine phosphatases. Also included in this group are cases with a normal karyotype and mutations in *NPM1* or (biallelic) *CEBPA* genes.

Whole-genome sequencing studies have shown that progression from MDS to s-AML involves sequential acquisition of mutations at the stem cell level resulting in survival and proliferation advantages [46, 47]. At the MDS stage most differentiated cells contain identical mutations, indicating marrow involvement by a clonal process. At the AML stage, several clones defined by acquisition of new sets of mutations are present, as well the original set of stem cell mutations, indicating clonal evolution. The new mutations tend to be in genes involved in adhesion, cell death, cell cycle regulation, differentiation, metabolism, motility, signaling, transcription, and transporter proteins [47, 48].

An investigation of the genetic basis of AML ontogeny comparing the spectrum of genetic lesions in well-defined s-AML patients (including therapy-related disease) and DN-AML identified three distinct mutually exclusive patterns of mutations [45]. First, three abnormalities significantly under-represented in AML-MRC (s-AML) are *NPM1* mutations, *KMT2A*(*MLL*)/11q23 rearrangements, and core binding factor (CBF) rearrangements (so-called de novo-type alterations); it should be noted that by definition *NPM1*

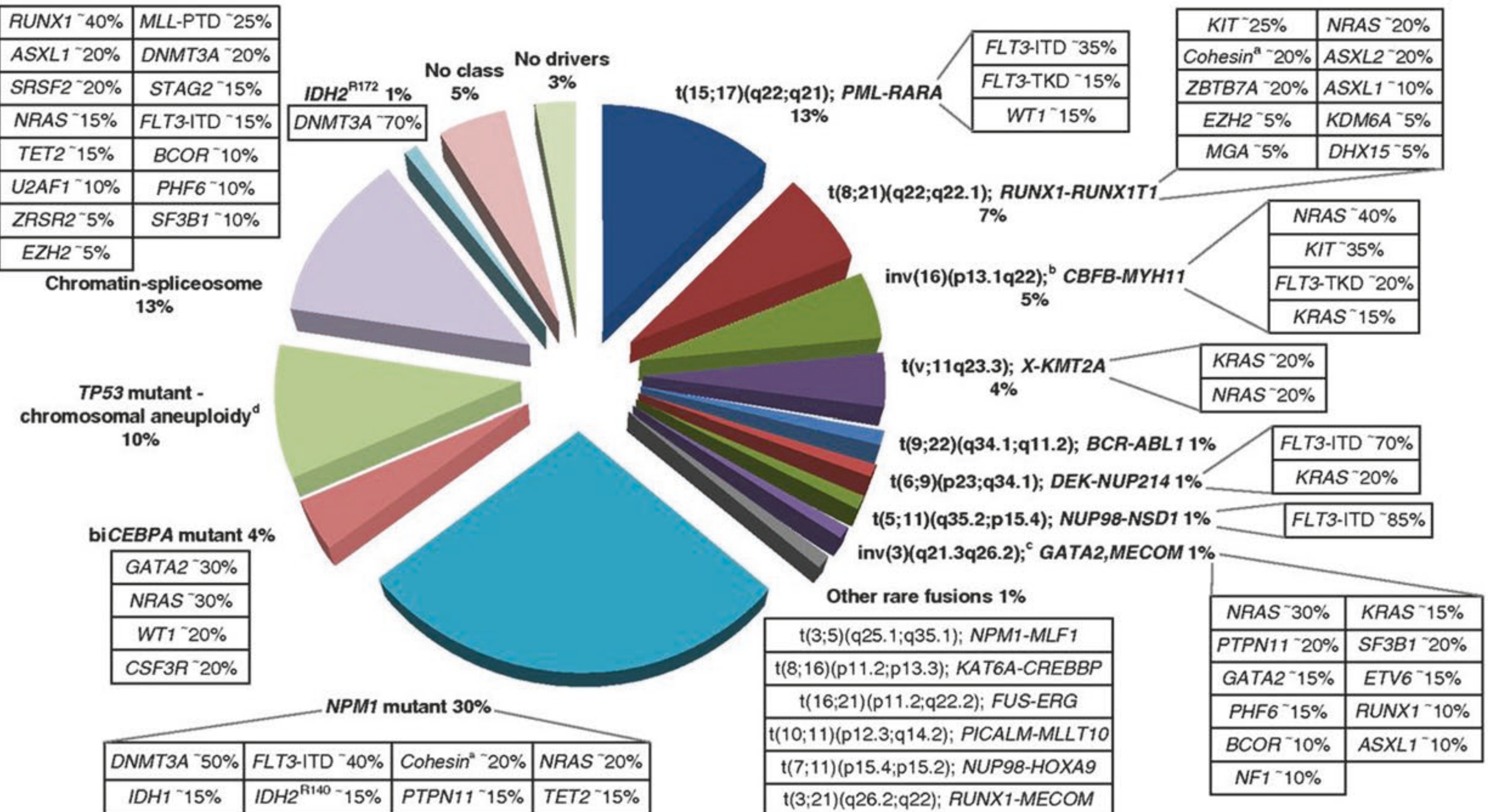

Fig. 2.2 Proportional distribution of AML cases associated with specific genetic lesions including most cases of de novo AML due to gene fusions and mutations with class definitions based on the study by Pappaemmanuil et al. [23]. For each AML class indicated in the pie chart frequent co-occurring mutations are shown in associated boxes. (Republished with permission of *Blood* from Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood.* 129:424–447, 2017; permission conveyed through Copyright Clearance Center, Inc.)

Table 2.2 Two major classes of therapy-related AML

	Alkylating agent class	Topo II inhibitor class
Cytogenetics	Del(5q), −7/del(7q), complex	Balanced translocations involving 11q23, 21q22, others
Frequency	~70%	~30%
Latency	Long (5–7 years)	Short (2–3 years)
Preceded by MDS phase	Typically, yes	No
Implicated medications	• Alkylating agents: bendamustine, busulfan, carmustine, chlorambucil, cyclophosphamide, dacarbazine, lomustine, melphalan, mitomycin C, nitrogen mustard, procarbazine, thiotepa • Platinum-based agents: cisplatin, carboplatin • Antimetabolite agents: azathioprine, fludarabine	• Anthracyclines: daunorubicin, epirubicin, doxorubicin • Other topoisomerase II inhibitors: etoposide, teniposide, amsacrine, mitoxantrone

mutations are placed in AML-RGA in the WHO classification and that AML with *PML/RARA* was not included in the study. Second, mutations (referred to as “secondary-type”) in eight genes mostly belonging to spliceosome and chromatin modifier functional classes (*SRSF2*, *SF3B1*, *U2AF1*, *ZRSR2*, *ASXL1*, *EZH2*, *BCOR*, and *STAG2*) appear to be highly specific (>95%) for AML-MRC (s-AML). The mutations in these genes are commonly seen in MDS, appear early in leukemogenesis, and persist in clonal remissions. Third, mutations in *TP53* are associated with a distinct clinical phenotype including complex karyotype, therapy resistance, and very poor survival [45]. No distinct genomic patterns were specific for WHO defined therapy-related AML, perhaps because these cases appear to be comprised of several different entities, as discussed above. These cases were distributed throughout the three mutational patterns mentioned above. This information suggests a genetic framework for future classification of AML into biological, pathogenetic, and clinically relevant groups.

2.5.2 AML with Recurrent Genetic Abnormalities

Biologically, the flat incidence curve of most AML-RGA suggests a single rate-limiting pathogenetic step (not a single step, but a single rate-limiting step) in development of disease (Fig. 2.3). To the extent that the molecular pathogenesis of AML-RGA has been clarified, most cases are characterized by one of a series of recurring genetic abnormalities that block differentiation of hematopoietic precursors, and a superimposed additional molecular abnormality(−ies) that drives proliferation.

2.5.2.1 Acute Promyelocytic Leukemia with *PML-RARA* and Variant Translocations

Acute promyelocytic leukemia (APL) with PML-RARA has a predominance of abnormal promyelocytes with characteristic nuclear morphology and cytoplasmic granulation. In AML it represents the best correlation of genetics with morphology. There is a spectrum from hypergranular to microgranular blast cell morphology in different patients. Recognition of the morphologic features of APL is extremely important for the early diagnosis and institution of targeted therapy with ATRA for this type of otherwise favorable prognosis AML, in order to prevent early and potentially serious complications of coagulopathy. The typical variant of APL has distinctive hypergranular blasts (abnormal promyelocytes) with prominent azurophilic granules which tend to obscure the boundary between the nucleus and the cytoplasm. They have distinctively shaped bilobed or grooved nuclei sometimes resembling an apple core. Some, but not all, cases have frequent Auer rods, and occasional cells with multiple Auer rods which sometimes are seen in bundles (so-

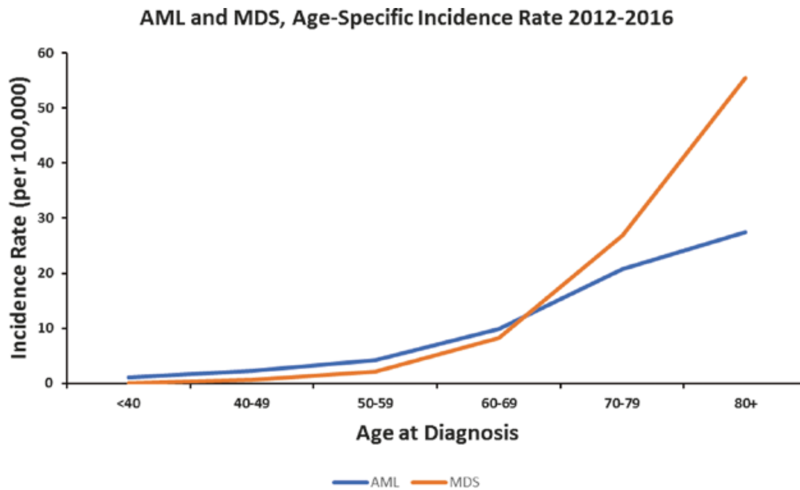

Fig. 2.3 Age-specific incidence rate of AML and MDS based on SEER data. MDS and secondary AML with most cases corresponding to AML-MRC in the WHO framework are most common in the elderly patients comprising most AML cases beyond 60 years of age with a median age in the 70s. While AML-MRC does occur in children and young adults, its incidence for population at risk comprises an exponential curve with progressive age, accounting for this feature of the incidence curve of AML as a whole

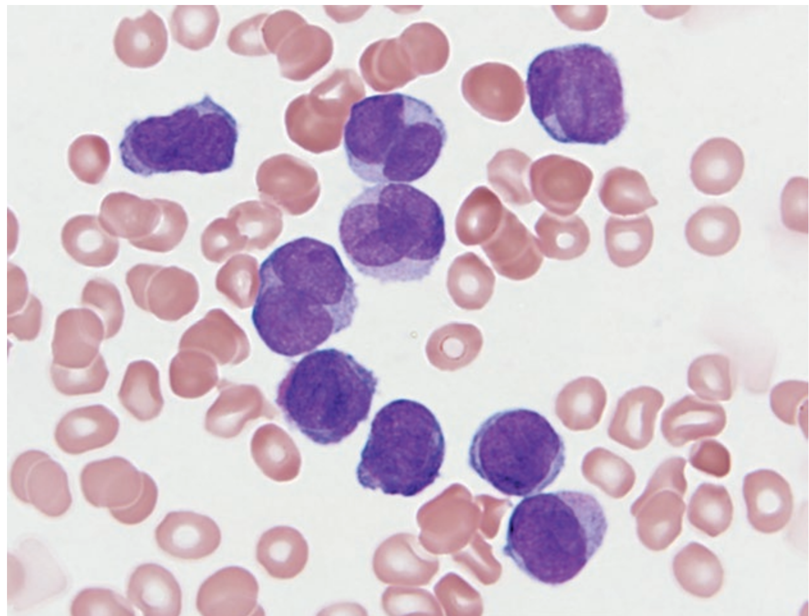

Fig. 2.4 Microgranular (hypogranular) APL (peripheral blood smear stained with Wright's stain at 1000× original magnification). Abnormal promyelocytes in this variant have few obvious cytoplasmic granules on Romanowsky stain but retain the characteristic distinctively shaped bilobed or grooved nuclei sometimes resembling an apple core

called faggot cells). The hypergranular variant often has low WBC counts and a low number of circulating blasts. The microgranular (hypogranular) variant (Fig. 2.4) may be more difficult to recognize, especially by an inexperienced observer, as the abnormal promyelocytes in this variant have few obvious granules with Romanowsky staining; however, even in the microgranular variant, the abnormal promyelocytes have similar nuclear features to the typical variant and if carefully searched for, at least a few typical cells with dense cytoplasmic granulation can be found, especially in the region of the perinuclear hof. These blasts still have the capacity to

release thrombogenic substances, as in the hypergranular variant. Patients with the microgranular variant tend to have a higher number of blasts in the peripheral blood.

By flow cytometry, there is a bright expression of myeloperoxidase and CD33 with variable expression of CD13 and CD117 and frequent expression of CD64 by the APL blast cells, which are typically negative for CD34 and HLA-DR. However, in the microgranular variant CD34 is frequently expressed, and there may be aberrant expression of CD2 [2]. Of note, as noted previously, APL blasts often display autofluorescence, complicating interpretation of data. An autofluorescence control should be run to correct for this problem [2, 49–51].

The successful treatment of APL with retinoic acid (RA) is a fascinating example of the potential power of targeted clinical application of molecular findings. As the association between t(15;17) (q22;q21) and APL was known, the near simultaneous reports [52] that oral all-trans retinoic acid (ATRA) induced complete remission in APL and that the retinoic acid receptor alpha gene (*RARa*) was located at 17q21 [53] led quickly to the demonstration that t(15;17) involves *RARa* and a previously unrecognized partner, *PML* [50, 51, 54].

The t(15;17) fuses the 5′ portion of the *PML* (ProMyelocytic Leukemia) gene at 15q24.1 and the 3′ portion of the *RARA* gene at 17q21.2. The breakpoint in *RARA* is invariant in intron 2, incorporating in the fusion protein the C-terminal portion of RARA including its DNA-binding, ligand-binding, dimerization, and repression domains. There are three possible breakpoint regions in PML. The most common bcr-1 in intron 6 includes the first six exons of *PML* and is designated PML(L)- RARA [55]. The second bcr-3 in intron 3 generates a shorter transcript, PML(S)-RARA. The third bcr-2 occurs within exon 6. RT-PCR using a single 3′ *RARA* primer and 2 *PML* primers to encompass the breakpoint sequences in introns 3 and 6 detects all three transcripts. FISH will also detect all *PML-RARA* fusion gene variants. These variant *RARA* breaks have no apparent clinical significance.

RARA is half (with retinoid X receptor or RXR) of a heterodimer ligand-dependent nuclear membrane receptor which mediates the cellular effects of RA. The heterodimer binds to RA response elements (RAREs) in the promoters of many genes important in myeloid differentiation. In the absence of RA, wild-type RARA/RXR on RAREs binds to the co-repressor proteins SMRT, N-CoR, mSin3, and histone deacetylases. Deacetylation of histone at the promoter, mediated by this complex, results in transcriptional repression, blocking cellular differentiation. Physiologic concentration of retinoic acid (10^{-8} M) causes a conformational change of the receptor, release of co-repressors, and recruitment of a co-activator complex (SRC-1) which associates with histone acetyltransferases [56]. This new complex mediates acetylation of histones at the promoter, relaxes chromatin conformation, and allows transcription to proceed (reviewed in [57]), resulting in cellular differentiation.

PML/RARA also heterodimerizes with RXR and binds to RAREs, competing with RARA/RXR in a dominant negative manner. In the absence of ligand, PML/RARA (via its 3′ RARA portion) binds co-repressor proteins similarly to RARA but requires pharmacologic concentration of retinoic acid, in the form of ATRA (10^{-6} M), to release them and bind to the co-activator complex. This is the mechanistic basis for the induction of differentiation of leukemic cells in APL with pharmacologic dosage of ATRA [56, 58].

Other translocations involving the RARA locus on 17q21.2 have been described. Studies of APL with variant RARA translocation t(11;17) (q23.2;q21.2); *ZBTB16-RARA* [58, 59] has furthered understanding of the mechanism of response of APL to ATRA. Patients with t(11;17) AML are resistant to treatment with pharmacologic dosage ATRA. The fusion partner gene *ZBTB16* on chromosome 11q23 encodes the promyelocytic leukemia zinc finger (PLZF) protein, a transcriptional repressor that contributes a second co-repressor binding site to the fusion protein. Although pharmacologic dosage ATRA induces release of co-repressors from the RARA portion of the fusion protein, those binding to PLZF are unaffected [56, 57, 60]. Addition of

Trichostatin A, which inhibits the deacetylase activity of PLZF-associated co-repressors [58, 61], allows induction of differentiation to proceed. Leukemia with *ZBTB16-RARA* has some morphological differences from typical APL, with blasts showing more regular nuclei. In some cases, the "blasts" approach the appearance of myelocytes [59]. Typically, Auer rods are absent. There may be an increased proportion of neutrophils with pseudo Pelger–Huet appearance [2]. Another rare fusion partner with RARA is *STAT5B* at 17q21.2. Similar to APL with *ZBTB16-RARA*, APL with *STAT5B-RARA* appears to be resistant to ATRA [2].

Finally, two additional partners involved in variant translocations in APL, both of which are ATRA responsive, are *NPM1* in t(5;17)(q35;q21) [62] and *NUMA1* in t(11;17)(q13.4;q21.2) [63]. All translocation partners of *RARA* encode proteins with multimerization domains, and all appear to contribute a block of differentiation to leukemia pathogenesis.

Wild-type PML protein is normally localized in subnuclear PML oncogenic domains (PODs), also called nuclear bodies (NBs), in which other nuclear factors colocalize [64]. PML may act as a tumor suppressor protein and is involved in growth suppression as well as in induction of apoptosis (reviewed in Ref. [57]). Although it does not bind DNA directly, it influences transcription by interacting with both the transcriptional activator CBP [65] and transcriptional repressor HDACs, possibly within the NBs. The protein encoded by the *PML-RARA* fusion transcript resulting from the t(15;17) translocation is delocalized from the NBs to a multigranular nuclear pattern with nucleolar exclusion [66].

Whole genome sequencing of de novo and relapsed APL patients has demonstrated approximately 8 non-silent somatic mutations per exome [67]. In de novo APL cases, mutations in *FLT3*, *WT1*, *NRAS*, and *KRAS* were predominant. In relapsed APL, there were frequent mutations in *RARA* and *PML*, with *RARA* mutations predominating in cases with a history of ATRA treatment and *PML* mutations associated with arsenic trioxide treatment. In addition, in relapsed APL, there were mutations in *ARID1A* and *ARID1B*, members of the SWI/SNF chromatin remodeling complex [67].

Although by convention an arbitrary level of 20% blasts is required for diagnosis of AML, the presence of the recurrent translocation t(15;17) is diagnostic of APL even when present in a small percentage of cells. Of note if the clinical setting is post chemo/radiotherapy, diagnosis should be therapy-related myeloid neoplasm as the main classification of the patient's disease [2].

2.5.2.2 AML with Core Binding Factor Translocations

Core binding factor (CBF) AML refers to AML-RGA characterized by the recurring structural abnormalities t(8;21)(q22;q22), involving *RUNX1(CBFA1)* and *RUNX1T1*, and inv(16)(p13.1q22), involving *CBFB* and *MYH11*. Together, these comprise 30% of pediatric AML cases after infancy and 15% of adult AML cases [68]. The *RUNX1* (Runt-related transcription factor 1, formerly called *AML1*) gene was cloned from the t(8;21)(q22;q22) breakpoint [69, 70]. In addition to involvement in this translocation, it is also mutated in another 3% of AML. The activity of the murine counterpart of RUNX1 was first described as part of the core binding factor complex (CBF), which binds to a core enhancer sequence of the Molony leukemia virus long terminal repeat (LTR) [71]. A second component of CBF, the non-DNA binding CBFβ, was subsequently found to be involved in AML-RGA with inversion 16 [72]. Finally, the fusion partner of *RUNX1* in t(8;21), named *RUNX1T1*, formerly *ETO* (eight-twenty-one), also encodes a transcriptional regulator [73]. The wild-type CBF complex recruits additional transcription factors, regulates hematopoietic differentiation, and is essential for hematopoietic development. Gene deletion of either *Runx1* [74] or *Cbfβ* [75] in mice results in fetal death at E11.5–12.5. These embryos lack all fetal hematopoiesis. Further transgenic experiments have demonstrated that RUNX1 is essential for the development of hematopoietic stem cells in the aorta/gonadal/mesodermal (AGM) region, the source of definitive hematopoiesis [76]. The essential role of RUNX1 in hematopoietic development appears

to be through its function as a transcriptional activator. *RUNX1*, located at chromosome 21q22.3, is encoded by 12 exons over 260 kb of DNA. The N-terminal portion of the protein is the DNA binding domain. This region is mutated in familial platelet disorder (FPD) and in AML associated with *RUNX1* mutations [77, 78]. CBFβ interacts via this domain and changes the conformation of RUNX1 to increase DNA binding affinity [79].

The blasts in AML with t(8;21) *RUNX1T1/RUNX1* translocation display a degree of granulocytic differentiation, with salmon pink coloration associated with the perinuclear hof (Fig. 2.5), but exceptions are frequent (about 20% of cases).

In the t(8;21) translocation, the *RUNX1* gene is fused to the *RUNX1T1* gene on chromosome 8. The RUNX1-RUNX1T1 protein specifically binds to the same DNA binding site as RUNX1, heterodimerizes with CBFβ [80] and acts as a dominant negative inhibitor of wild-type RUNX1. RUNX1-RUNX1T1 also functions as an active transcriptional repressor [81] by associating with class I histone deacetylases (HDACs) via RUNX1T1 [82]. Targets of RUNX1-RUNX1T1 repression are presumed to include genes important for granulocyte differentiation. In addition, RUNX1-RUNX1T1 represses the tumor suppressor genes *P14ARF* and *NF1* [83, 84]. P14ARF stabilizes TP53 by antagonizing MDM2, an inhibitor of TP53 [85]. Therefore, repression of *P14ARF* reduces the checkpoint control path of TP53 and may be a key event in t(8;21) leukemogenesis.

AML with inv(16) or t(16;16), the *CBFB/MYH9* translocation, present in about 8% of AML cases, correlates frequently (50–60% of cases) with myelomonocytic differentiation and with dysplastic eosinophils containing immature basophilic as well as eosinophilic granules (baso-eosinophils) (historic FAB category M4Eo) (Fig. 2.6) [86].

This cytogenetic abnormality fuses the first 165aa of *CBFβ* to the C-terminal of *SMMHC* encoding its coiled-coil region [87]. The CBFβ/SMMHC fusion protein associates with mSin3a and HDAC8 and interacts with RUNX1 to form a transcriptional repressor complex [88].

A number of experiments demonstrate that the CBF translocations are necessary but not sufficient for induction of leukemia. Support for the hypothesis that genetic mutations besides a mutant *RUNX1* locus are necessary for development of acute leukemia comes from the study of patients with familial platelet disorder with propensity to develop AML (FPD/AML). These patients have mutations in one allele of *RUNX1*

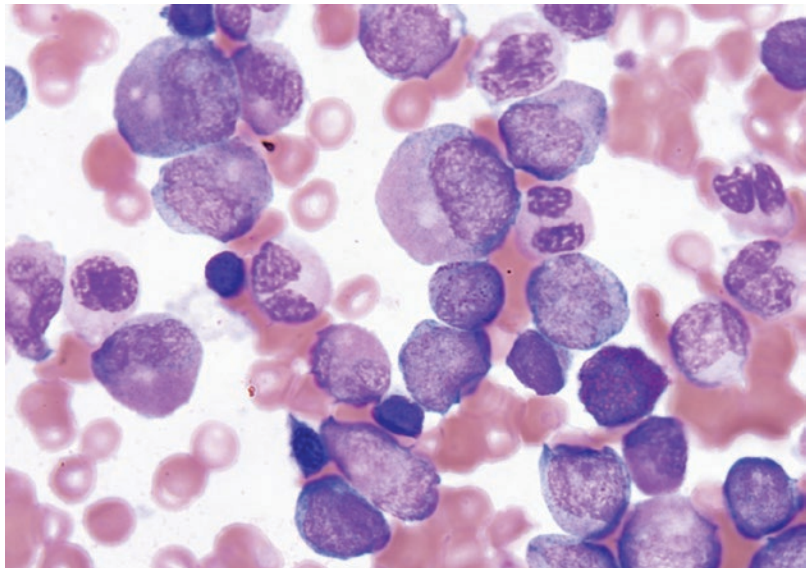

Fig. 2.5 AML with t(8;21);*RUNX1-RUNX1T1* (bone marrow aspirate smear stained with Wright's stain at 1000× original magnification). There is some degree of granulocytic maturation with characteristic salmon-coloration of the cytoplasm in perinuclear hof area

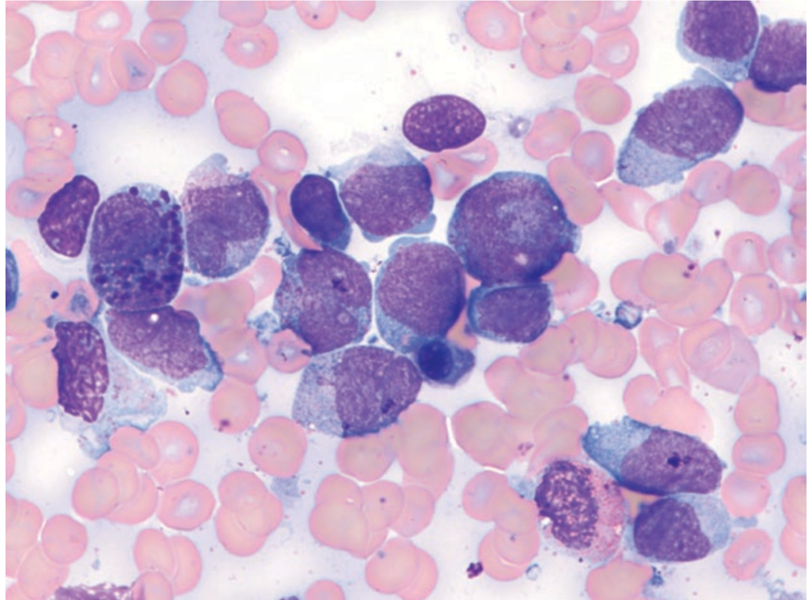

Fig. 2.6 AML with inv16 (bone marrow aspirate smear stained with Wright's stain at 1000× original magnification). There is myelomonocytic differentiation with dysplastic eosinophils containing immature basophilic as well as eosinophilic granules (baso-eosinophils)

[89]. They have defective platelets and progressive pancytopenia and develop myelodysplasia and a high incidence of AML with age. However, secondary mutations appear to be necessary before progression to AML occurs. Another set of studies also support this hypothesis. Guthrie spot studies (drop of blood obtained at birth and stored) have shown that many children up to age 10 years with AML with t(8;21) or inv(16) [and also t(15;17)] have identical translocations (including sequencing across intronic fusions) in their Guthrie spots and are asymptomatic for years before developing AML, again indicating that the translocations are necessary but not sufficient for induction of AML [90, 91].

The presence of additional mutations in CBF leukemia has been addressed directly by NGS experiments. In one experiment comparing patients with *RUNX1-RUNX1T1* and *CBFB-MYH11*, an average of 11.86 somatic mutations with functional consequences were present in *RUNX1-RUNX1T1* cases and 7.74 somatic mutations were present in *CBFB-MYH11* cases [68]. About 66% of mutations were in kinase pathway genes such as *NRAS, KIT, FLT3, KRAS, PTPN11, NF1*, and *CCND2*. *KIT* mutations were found in 45% of t(8;21) and 33% of inv(16) cases, with a mutant allelic ratio of 35% or greater required to confer a worse prognosis [68]. Similar findings were present in a subsequent study performing NGS on a series of 331 patients with t(8;21) [92]. Additional mutations in kinase pathway genes were detected in 63.4% of cases; additional mutations were also detected in genes encoding epigenetic regulators (45% of cases), as well as cohesion complex members, MYC-related regulators, and spliceosome complex proteins. Loss of a sex chromosome was the most common karyotypic abnormality, besides the defining t(8;21). A reduced complete remission (CR) rate was associated with del(7q), *FLT3*-ITD (high allele burden), and *JAK2* mutations. The factors most strongly associated with poor prognosis were a *cKIT* mutation in >25% of cells (KIT^{high}) and *JAK2* mutations. These results suggest that RTK inhibitors may be effective ancillary treatment alternatives for t(8;21) leukemia [92].

As with t(15;17) APL, although by convention an arbitrary level of 20% blasts is required for diagnosis of AML, the presence of the recurrent translocations t(8;21) and inv(16) or t(16;16) is diagnostic of acute myeloid leukemia even when present in a small percentage of cells. Of note, if the clinical setting is post chemo/radiotherapy, diagnosis should be therapy-related myeloid neo-

plasm as the main classification of the patient's disease [2].

2.5.2.3 AML with t(9;11)(p21.3;q23.3); *KMT2A-MLLT3*

KMT2A (previously named mixed lineage leukemia or *MLL*), which maps to chromosome 11q23.3, is a transcriptional regulator and chromatin remodeling gene frequently rearranged in AML-RGA (as well as in ALL) and characteristically rearranged in infant leukemia, epipodophyllotoxin therapy-related leukemia, and frequently in mixed phenotype leukemia [93]. *KMT2A* translocations are seen in leukemias of both myeloid and lymphoid origin, hence the older name (*MLL*). In AML, t(9;11) involving *MLLT3* (*AF9*) is the most common translocation partner and appears to define a distinct disease entity. Unusually however, compared to other recurring translocations in AML, there are multiple variant *KMT2A* translocation partners, with more than 70 partner genes characterized. This type of AML can occur at any age. It comprises the large majority of AML in infants up to 18 months of age. Although *KMT2A* translocations involving some of the partner genes such as *MLLT1* (*ENL*), *MLLT10* (*AF10*), *MLLT4* (*AF6*), or *ELL* predominantly occur in AML, they can also be seen in ALL. Cases have been described as switching from AML to ALL and vice versa with treatment.

The *KMT2A* gene is the mammalian homolog of *trithorax*, a *Drosophila* transcriptional regulator that encodes a methyltransferase and positively regulates homeobox (*HOX*) genes, a large family of genes involved in the regulation of development and essential for growth and differentiation [93, 94]. Wild-type KMT2A regulates *HOX* gene expression by methylation of histone H3 lysine (H3K4), resulting in transcriptional activation; this action requires the KMT2A SET domain, a domain shared by a number of transcriptional regulators with histone methyltransferase activity [95]. The multiplicity of fusion partners of *KMT2A* has been perplexing. Three of the most common fusion partners, *MLLT3*(*AF9*), *AF10*, and *MLLT1*(*ENL*), associate with DOT1L, a histone methyltransferase with different activity than wild-type KMT2A. DOT1L (in a complex, which consists of DOT1L, AF10, MLLT6(AF17), and MLLT1(ENL)) methylates histone H3 lysine 79(H3K79) [96, 97], which is also associated with transcriptional activation [93, 98]. Thus, many of the fusion partners of *KMT2A* normally associate in complexes that regulate transcription through histone methylation. *HOX* genes are expressed highly during early development, but then are downregulated during hematopoiesis. The end result of the altered methylation of these transcriptional regulatory complexes by the KMT2A-fusion proteins is thought to be abnormally sustained *HOX* gene expression [93]. Small molecule inhibitors of DOT1L are under development, with success in mouse models of *KMT2A* leukemia. One such molecule, pinometostat (EPZ-5676), has been evaluated in a phase I clinical trial in adult patients with advanced acute leukemia with *KMT2A* rearrangements and has shown clinically meaningful responses and modest efficacy as a single agent [99, 100].

KMT2A rearrangements are associated with several unique types of leukemia. In infant acute leukemia (birth to 18 months, both AML and ALL), there is a 60–80% incidence of 11q23.3 rearrangements [101]. In secondary acute leukemias (both AML and ALL) developing after treatment with DNA topoisomerase II inhibitors (epipodophyllotoxins), there is a 70–90% incidence of *KMT2A* rearrangements, particularly t(4;11)(q21;q23.3) and t(9;11)(p21–22;q23.3) [102, 103]. Topoisomerase II is involved in unwinding of DNA during replication and transcription by producing double-stranded nicks in DNA, after which the ends are rejoined by a ligase activity of topoisomerase II. Topoisomerase II inhibitors block this ligase function, and DNA-free ends accumulate, triggering apoptotic events. There are 11 possible topoisomerase II consensus binding sites in *KMT2A* breakpoint cluster areas [104]. Incorrect religation of DNA-free ends in these areas due to inhibition of topoisomerase II religase activity may explain the association of topoisomerase II inhibitors and translocations involving *KMT2A*. Interestingly, infant leukemia with *KMT2A* translocations has a similar distri-

bution of breakpoints to cases following epipodophyllotoxin treatment, whereas sporadic cases of *KMT2A* acute leukemia have more random breakpoints [105]. This observation has triggered speculation that in utero exposure to environmental topoisomerase II inhibitors such as flavonoids may have a role in the etiology of infant leukemia [106]. In addition, this hypothesis may be supported by the fact that in utero exposure to a common class of antibiotics with anti-bacterial topoisomerase II (gyrase) activity (fluoroquinolones), which have been shown to cross react with human topoisomerase II, results in an increased risk of leukemia development in infants and young children [107].

The latency of development of leukemia appears to be shorter for *KMT2A* rearrangements than for other leukemogenic rearrangements. Similarly, therapy-related leukemias based on *KMT2A* rearrangement occur sooner after therapy than those occurring after alkylating agents or radiation [103, 108]. This suggests that the oncogenic fusion protein produced by the *KMT2A* rearrangement can deregulate the cell without the accumulation of many secondary mutations.

Overexpression of *MECOM* (*EVI1*) is common in AMLs with *KMT2A* rearrangements, being seen in approximately 40% of cases with t(9;11). Secondary chromosomal abnormalities are commonly seen in AML with t(9;11)(p21.3;q23.3), with trisomy 8 most frequently observed in *MECOM* negative cases; the secondary translocations do not appear to influence prognosis.

AML with t(9;11) and other *KMT2A* fusions frequently has myelomonocytic and monoblastic morphology and immunophenotype including strong expression of CD33, CD65, CD4, and HLA-DR and low to variable expression of CD13, CD14, CD117, and CD34.

Controversial points in placing *KMT2A* rearranged AML cases in the WHO classification include occurrence of the same rearrangements in non-random settings, including MDS and therapy-related neoplasms discussed above. Diagnosis of AML-RGA with *KMT2A* translocations should be limited to de novo AML cases [2]. AML arising in the context of prior cytotoxic therapy should be classified as therapy-related disease with a *KMT2A* rearrangement. Likewise, AML with myelodysplasia-related changes and a *KMT2A* translocation should be diagnosed as AML with myelodysplasia-related changes. Furthermore, although t(9;11) cases with <20% blasts are not currently classified as AML, it is suggested that in the right clinical setting they should be treated as AML [2]. AML with t(9;11) has an intermediate survival which is superior to that of AML with other 11q23.3 translocations [109, 110]. Overexpression of *MECOM* associates with poor prognosis [111].

2.5.2.4 AML with Biallelic Mutation of CEBPA

CCAAT/enhancer binding protein-alpha (CEBPA) is a transcription factor that is required for granulocytic differentiation [112, 113] as demonstrated in *Cebpa* knockout mice lacking mature granulocytes [114]. CEBPA transactivates the genes for G-CSF and GM-CSF receptors and several granulocyte-specific proteins. Genetic aberrations in other myeloid leukemia-associated genes often lead to *CEBPA* down-regulation. Furthermore, the *CEBPA* promoter is methylated in half of AML cases [115]. Biallelic mutations in *CEBPA* have been identified in about 4–9% of children and young adult patients with AML [116, 117]. The biallelic mutation in CEBPA is associated with a specific gene expression pattern that is different from single mutations. It is now recognized that the favorable prognosis associated with CEBPA mutation in AML is related to biallelic mutations, a requirement to assign a case to this category [118, 119]. If biallelic CEBPA mutations are found in AML, especially in younger patients, an investigation should be undertaken of the possibility of a germline mutation, which would change the diagnosis to germline AML predisposition syndrome [120].

About 5–9% of AML cases with CEBPA mutations have *FLT3*-ITD mutations [121–123]. *GATA2* mutations are found in approximately 39% of cases [124]. Most cases (>70%) have a normal karyotype; of karyotypic abnormalities, del(9q) is commonly seen (similar to AML with

mutated NPM1); this does not appear to influence prognosis [122].

AML with biallelic mutation of *CEBPA* has no specific or distinguishing morphologic features and shows a myeloid phenotype with a possibly higher frequency of expression of HLA-DR, CD7 and CD15, and no expression of monocytic markers such as CD14 and CD64. Variable dysplasia is present in a significant minority of cases, similar to AML with mutated NPM1; it does not adversely influence prognosis [125].

2.5.2.5 AML with Mutated NPM1

AML with mutated *NPM1* occurs most commonly in adults and the elderly, typically without preceding abnormalities, and typically has a normal karyotype. *NPM1* mutation is one of the most common recurrent genetic aberrations in AML (35% of adult AML with a normal karyotype with lesser frequency in the pediatric population), and only rarely occurs in other myeloid neoplasms such as MDS or MDS/MPN [126] (Fig. 2.2). AML with mutated NPM1 requires other mutations prior to acquisition of the NPM1 mutation. While placed in AML-RGA in the WHO classification, these features share much in common with AML-MRC, rather than other types of AML-RGA.

NPM1 (nucleophosmin) is a molecular chaperone that shuttles between cytoplasm and nucleus [127]. It is essential for cell survival [128] with several major functions, including ribosome biogenesis, regulation of centrosome duplication during the cell cycle, chromatin remodeling, potentiating the p53 stress response, interaction with tumor suppressor proteins, and DNA repair functions (reviewed in [129–132]). *NPM1* mutations are stably expressed, being retained in leukemic blasts at relapse in the majority of cases [33, 133, 134]. By inspection of variant allele frequencies, it has been shown that *NPM1* mutations precede *FLT3* mutations that often co-occur in AML.

Wild-type NPM1 is composed of 294 amino acids and has two nuclear localization signals (NLS), two nuclear export signals (NES) that mediate the nuclear-cytoplasmic shuttling of wild-type NPM1, and a nucleolar localization signal (NoLS) at the C-terminal end containing two tryptophan residues at positions 288 and 290 that are critical for retaining NPM1 in the nucleolus. *NPM1* mutations occur in the portion of the gene previously known as exon 12, with over 50 mutations described and named alphabetically in the order of discovery (types A, B, C, D, etc.). All the subtypes share an identical biological consequence, leading to generation of a mutant NPM1 protein with four extra amino acids [132, 135, 136]. The mutant NPM1 appears to function in a dominant negative manner through heterodimerization with normal NPM1 to cause relocation of some normal NPM1, as well as the mutant NPM1, from its normal predominantly nucleolar location to the cytoplasm [137]. This can be detected in tissue sections by immunohistochemistry and is predictive of *NPM1* mutation [136]. The mutation is always heterozygous; homozygous mutation is embryonic lethal [128, 138].

NPM1 mutation was initially considered a founder event in leukemogenesis, because it usually is maintained at relapse. However, it is now thought that *NPM1* mutation occurs later in AML development due to its absence in preleukemic hematopoietic stem cells, and the fact that in 10% of patients the *NPM1* mutation is lost at relapse while further chromosomal and molecular changes are acquired [33, 134]. One of the mechanisms by which mutant NPM1 may promote leukemogenesis is by destabilization of proteins regulating the TP53 response. In addition, cytoplasmic NPM1c retains its ability to bind to cytoplasmic caspases 6 and 8, which may inhibit apoptosis, also enhancing leukemogenesis. Furthermore, NPM1 may interact with a protein that is part of the E3 ubiquitin ligase that degrades MYC protein; mutant cytoplasmic NPM1c disrupts this activity, thus indirectly increasing the levels of MYC protein [130]. It has also been reported recently that *HOX* overexpression is directly dependent on mutant *NPM1* and maintains the leukemic state in *NPM1* mutated AML. It has also been shown that relocalization or degradation of mutant NPM1 induces differentiation of AML cells, potentially providing the rationale for a novel therapeutic strategy [139].

NPM1 mutations occur most frequently in conjunction with *FLT3*-ITD and *DNMT3A* mutations, but mutations in *TET2*, *IDH1*, and *IDH2* also commonly co-occur [22, 130, 131, 140]. Patients with *NPM1* mutations in the absence of *FLT3*-ITD mutations with high variant allele frequency appear to have a favorable response to chemotherapy [136, 137]. This may be due to the role of wild-type NPM1 in DNA repair; if *NPM1* is mutated there is less efficient repair of DNA damage induced by chemotherapy [131]. Younger patients with a normal karyotype and no concurrent *FLT3*-ITD mutation have prognosis comparable to that of CBF AMLs and may not benefit from allogeneic stem cell transplantation in first remission [141]. Co-occurrence of *NPM1*, *FLT3*-ITD, and *DNMT3A* mutations has been associated with very poor outcome [142].

AML with mutated *NPM1* strongly associates with myelomonocytic or monocytic morphology, but other morphologic types occur including AML without maturation and pure erythroid leukemia. Some cases have characteristic morphology with nuclei showing cup-shaped or thumb imprint-like indentations (Fig. 2.7). This morphology may raise the differential diagnosis of APL, especially in the presence of the similar immunophenotypic features to APL (lack of CD34 and HLA-DR expression on blasts). Blasts otherwise show an immature myeloid or monocytic profile (CD36+, CD64+, CD14+). Multilineage dysplasia is seen in up to 25% of cases but appears to have no prognostic significance [143, 144].

2.5.3 Rare Subtypes of AML-RGA

2.5.3.1 AML with t(6;9)(p23;q34.1); *DEK-NUP214*

The t(6;9)(p23;q34.1) occurs in 0.7–1.8% of AML cases and occurs both in later childhood and adults. The t(6;9) results in a fusion of *DEK* on chromosome 6 and *NUP214* on chromosome 9. This fusion protein acts aberrantly, altering nuclear transport by binding to soluble transport factors [145]. *FLT3*-ITD mutations are common in this entity [146–148]. There are no specific distinguishing morphologic features of blast cells in the AML with t(6;9)(p23;q34.1) but blood and marrow basophilia, generally uncommon in AML, is seen in more than half of cases [146,

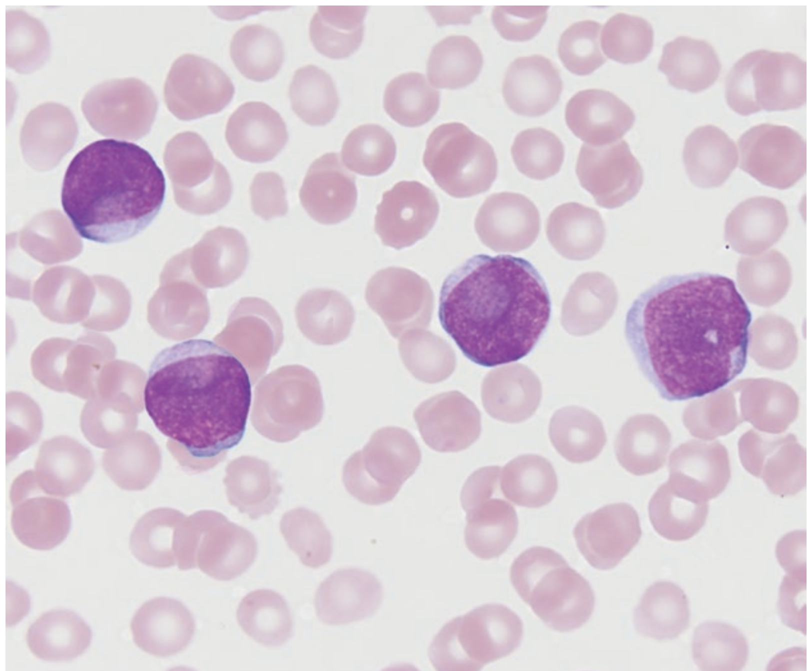

Fig. 2.7 *NPM1* mutated AML (peripheral blood smear stained with Wright's stain at 1000× original magnification). Some cases have characteristic morphology with nuclei showing cup-shaped or thumb imprint-like indentations. This morphology may raise the differential diagnosis of APL, especially in the presence of the similar immunophenotypic features to APL (lack of CD34 and HLA-DR expression on blasts)

149]. Some cases with t(6;9)(p23;q34.1) may have less than 20% blasts and are not currently classified as AML, but in an appropriate clinical setting, these patients may be treated as AML. The blasts cells have a non-specific myeloid phenotype, with co-expression of TdT seen in approximately 50% of cases. Basophils can be detected by flow cytometry as a separate population positive for CD123, CD33, and CD38 but negative for HLA-DR. The prognosis of AML with t(6;9)(p23;q34.1) is generally poor, with a high white cell count predictive of shorter overall survival [146, 148].

2.5.3.2 AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2, MECOM*

It is now recognized that inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2) in this type of AML does not represent a fusion gene but results in repositioning a distal *GATA2* enhancer to activate the oncogene *MECOM* at 3q26.2 (also known as *EVI1*), simultaneously resulting in GATA2 haploinsufficiency [150, 151]. These cases tend to occur in older patients with a median age approximating 60 years, suggesting differences with other AML-RGA subtypes. Secondary chromosomal abnormalities are common, with monosomy 7, del 5q, and complex karyotypes frequently seen. Mutations in genes activating RAS tyrosine kinase signaling pathways are present in most cases: *NRAS* (27% of cases), *PTPN11* (20%), *FLT3* (13%), *KRAS* (11%), *NF1* (9%), *CBL* (7%), and *KIT* (2%). Other commonly mutated genes are *GATA2*, *RUNX1*, and *SF3B1* [152]. This type of AML accounts for 1–2% of AMLs and is characterized by normal or elevated platelet counts and increased, dysplastic megakaryocytes typically with hypolobated or unilobed nuclei [153] (Fig. 2.8). Multilineage dysplasia in the non-blast marrow cells is also common, and marrow eosinophils and basophils may be increased [154, 155]. Bone marrow blasts have variable morphology with no specific characteristics. The blasts typically have a non-specific myeloid phenotype with aberrant expression of the T-associated marker CD7 and megakaryocytic markers CD41 and CD61 in a subset of cases. Cases with <20% blasts are not currently classified as AML; however, this is controversial since the outcome for patients with <20% or > 20% blasts are equally poor in this very aggressive disease with very short survival [156].

2.5.3.3 AML with t(1;22)(p13.3;q13.1); *RBM15-MKL1*

AML with t(1;22)(p13.3;q13.1) is characterized by blasts with megakaryocytic differentiation by morphology and immunophenotype. This type of AML-RGA is rare (<1% of all AML cases). It

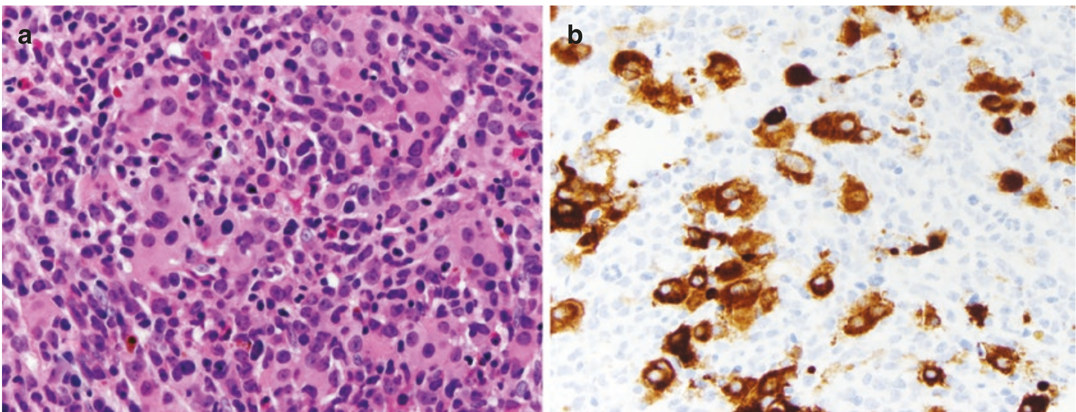

Fig. 2.8 AML with inv3/t(3;3) (bone marrow biopsy sections at 400× original magnification). H&E stained section showing numerous abnormal megakaryocytes with small hypolobated or unilobed nuclei (**a**), which are highlighted with CD61 immunohistochemical stain (**b**)

occurs almost exclusively in infants without Down syndrome in the first 6 months of life, presenting with marked organomegaly (commonly hepatosplenomegaly) due to leukemic infiltrates [157, 158]. Typically, the bone marrow is inaspirable due to dense marrow fibrosis. Careful search of the peripheral smear for blasts may be helpful in this diagnosis. In sections of biopsies of the marrow or liver, the blast infiltration mimics metastatic small round cell tumor, with cohesive clusters of small round cells in tissue or vascular spaces [157, 158]. There may be micromegakaryocytes associated with the megakaryoblast infiltrate; dysplasia in other cell lines is uncommon. Megakaryoblasts may be present in small numbers in the peripheral smear, facilitating diagnosis if recognized. Phenotypically the megakaryoblasts are often negative for CD34, CD45, HLA-DR, MPO, and lymphoid markers by flow cytometry and immunohistochemistry, contributing to mistaken diagnosis as a small round cell tumor, NOS. The blasts may show variable expression of myeloid markers CD13 and CD33, and if tested, are positive for megakaryocyte markers (CD41, CD61, and CD42b).

2.5.3.4 Provisional Categories of AML-RGA (WHO 2016)

Two provisional categories of AML-RGA are recognized in the 2016 WHO classification [2]. These categories exclude cases that meet criteria for other recognized types of AML.

De novo *AML with BCR-ABL1* cases may be difficult to distinguish from blast phase CML, especially without adequate clinical information, but the significance of this targetable fusion warrants the recognition of this entity [159, 160]. Preliminary data indicate that other molecular abnormalities may allow distinction of AML-RGA with *BCR-ABL1* from blast phase CML.

AML with mutated RUNX1 appears to represent a biologically distinct group of AMLs with some studies reporting worse prognosis versus other types of AML. Similar to biallelic CEBPA mutations, a subset of these patients may have germline mutations of *RUNX1*, and germline and family studies should be performed when these mutations are detected [161–164].

2.5.4 Myelodysplastic Syndromes (MDS) in Relationship to AML-MRC

Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal stem cell disorders characterized by ineffective hematopoiesis manifesting with low blood counts, typically normo- or hypercellular BM, and a variable degree of morphologic dysplasia in hematopoietic elements. The diagnosis of MDS relies on identifying and quantifying morphologic dysplasia, quantifying the proportion of blast cells, and/or demonstrating characteristic MDS-associated cytogenetic abnormalities [2]. This diagnosis can be challenging, especially in low-grade MDS (see below) with no increase in blasts, as the morphologic dysplasia may be subtle and subjective, and many patients (45–50%) lack characteristic cytogenetic abnormalities [2]. MDS is still a poorly understood set of diseases pathogenetically related to AML-MRC [2]. Distinction of MDS from AML is currently based, with exceptions, on an arbitrary marrow blast percentage, lowered from the historical ≥30% to ≥20% in the WHO classification; this is not based on an understanding of biological differences in the two sets of diseases [2]. For discussion of AML pathogenesis, the MDS subtypes in the WHO classification can be consolidated into two types: low-grade MDS (MDS with single lineage dysplasia, MDS with ring sideroblasts, MDS with multilineage dysplasia, MDS with isolated del(5q)), and high-grade MDS (MDS with excess blasts). High-grade MDS (see below) is usually fatal with or without progression to AML-MRC. Understanding the biology and pathogenesis of MDS remains elusive but would seem to be critical for improving the differential diagnosis of AML-MRC versus MDS and versus DN-AML and would possibly contribute to improved treatment strategies for AML-MRC and MDS, and possibly to prevention of progression of MDS to AML-MRC.

A variety of data (including progressive genetic damage, acquired structural and functional abnormalities in hematopoietic cells, and the high rate of transformation to AML) suggest

that high-grade MDS is a mutator phenotype with inherent genetic instability. AML-MRC appears to require both a block of differentiation and a drive to proliferate, but it is doubtful either of these events is the biologic basis of MDS. A solitary drive to proliferate (with associated inhibition of programed cell death) is the apparent cause of chronic myelogenous leukemia (reviewed in [165]) and the other myeloproliferative neoplasms (MPN) [166–168]; these diseases differ from MDS in being proliferative, lacking MDS-type morphologic dysplasia, and lacking MDS-type cytogenetic abnormalities. A solitary block of differentiation in hematopoietic progenitors has no clinical phenotype, except a possible propensity to be transformed by acquisition of a second genetic event that drives proliferation [90]. Both clinical and transgenic examples of each of these possibilities lack the phenotype of MDS, although acquisition of both appears to be required for MDS to transform to AML-MRC. A plausible hypothesis is that subsets of high-grade MDS represent acquisition of one of these events superimposed on the underlying biology of low-grade MDS. MDS with excess blasts (MDS-EB) is characterized by increased but relatively stable numbers of marrow blasts, with shortened survival of patients due to complications of MDS and with an increased propensity to progress to AML; hypothetically, MDS-EB may represent a block of myeloid differentiation superimposed on the underlying biology of low-grade MDS. The MDS/MPN diseases (CMML, JMML, and atypical CML) have mixed features of MDS (dysplasia, shared genetic abnormalities) and MPN (proliferation) [2]. A drive to proliferate through mutations leading to increased active RAS has been demonstrated in many MDS/MPN cases (inactivating *ATM* mutations; activating *RAS*, *PTPN1*, and *NF-1* mutations) [2], yet the cases also share morphology and cytogenetic abnormalities with MDS. In a possibly informative clinical scenario, septic patients with MDS may develop a reversible leukemoid reaction, often with monocytosis and mimicking CMML (apparently due to a physiologic proliferative drive superimposed on MDS); with successful treatment of sepsis patients revert to MDS. A second example of this possibility, patients with MDS with ring sideroblasts that progress to MDS/MPN with thrombocytosis often have coincidental acquisition of an activating *JAK2* mutation [2, 169]. Thus, while available data suggest that acquisition of both a drive to proliferate and a block of differentiation may contribute to MDS progression, and both are necessary for progression to AML-MRC, neither represents the underlying pathogenesis of MDS. The most tenable hypothesis is that the underlying pathogenic abnormality of high-grade MDS is its mutator phenotype, which causes random genetic damage, including in some cases acquisition of a drive to proliferate, with resultant progression to AML-MRC [46, 48].

While high-grade MDS is a mutator phenotype, at least some low-grade MDS is not. Both MDS with isolated del(5q) and MDS with ring sideroblasts (MDS-RS), especially cases with *SF3B1* mutation, if defined stringently using WHO criteria, have very low rates of progression to AML with survival approaching age-matched peers [169–171]. While 5q− is one of the most common cytogenetic abnormalities in MDS, in most cases secondary to an underlying mutator phenotype, the specific subtype of MDS with isolated del(5q) may represent emergence of a 5q− clone with an associated clonal survival advantage, but a stable biologic state with no underlying mutator phenotype. MDS-RS defined as mostly unilineage erythroid abnormalities and the presence of mutations in the spliceosome gene *SF3B1*, may have a similar pathogenesis with additional superimposed consequences of mitochondrial damage due to iron loading [171, 172].

Progression of MDS to AML-MRC does not represent a continuum, but rather stepwise acquisition of specific genetic events is required for transformation and should equate with a rapid rate of accumulation of primitive precursors (blasts) in the marrow, differing qualitatively from MDS [46, 48] (Fig. 2.9). Thus, separation of MDS and AML-MRC often requires clinical–pathological judgment; it should be based on evidence of transformation with a distinct change in the rate of accumulation and the percentage of blasts, not

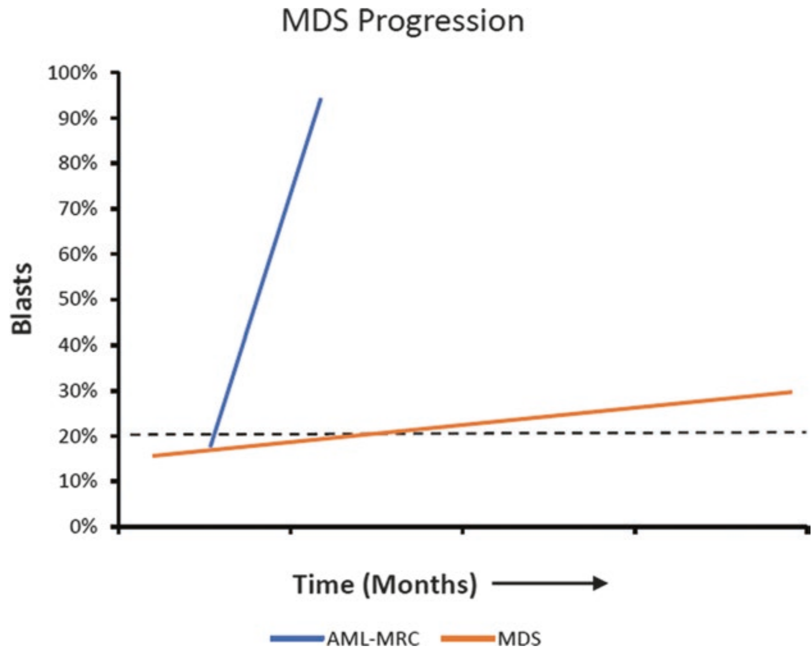

Fig. 2.9 Increasing proportion of blasts over time in MDS. Transformation to AML is denoted by rapid increase in bone marrow blasts (blue line). In contrast, if the blast proportion rises slowly over months exceeding or fluctuating around the arbitrary threshold of 20%, the situation should be considered to be a persistent MDS (orange line)

just on a marrow blast % rising slowly above an arbitrary threshold (whether 20% or 30%). While it is safe to assume that a high marrow blast % (>40–50%) represents transformation to AML-MRC, lower levels require clinical interpretation as to whether the patient's disease has shifted from primary marrow failure to proliferating blasts. If initial data are inconclusive, a repeat marrow examination after an interval may clarify whether the basic disease process has changed from one of marrow failure (MDS) to a proliferative state (AML) (Fig. 2.9). As our knowledge of these diseases increases, separation of MDS and AML-MRC may eventually include demonstration of specific genetic events leading to transformation [46, 48]. Finally, this perspective should not be interpreted to mean that MDS with a high blast % is a favorable disease; it is lethal with short median survival, but lacking transformation is resistant to cytotoxic chemotherapy.

2.5.5 AML with Myelodysplasia-Related Changes

AML with myelodysplasia-related changes (AML-MRC) is diagnosed when AML is preceded by MDS or MDS/MPN, there is an MDS-related cytogenetic abnormality, and/or there is otherwise unexplained multilineage morphologic dysplasia (Table 2.1). There should be no history of prior exposure to cytotoxic drugs or radiation therapy (which define therapy-related AML), and genetic abnormalities that define AML-RGA subtypes must be absent [2]. AML-MRC differs biologically and clinically from AML-RGA. Patients who fulfill criteria for AML-MRC can present clinically as “de novo” disease, in the sense of no prior diagnosed clinical abnormality, but with clinical, epidemiologic, genetic, treatment response, and prognosis data similar to other AML-MRC cases [45, 126]. It is reasonable to postulate that most clinically “de-novo” cases of AML-MRC have prior subclinical marrow disease only coming to medical attention at the time of progression to AML.

AML-MRC occurs most commonly in elderly patients, comprising the majority of AML cases beyond age of 60 years. It occurs at low frequency in children and young adults, with increasing incidence with age giving an exponential incidence curve for AML as a whole (Fig. 2.3). The exponential incidence curve of AML-MRC sug-

gests several random events are required for transformation in this set of AML.

AML-MRC is characterized by a series of cytogenetic changes shared with MDS. Despite extended efforts, the genes affected and the biologic impact of these cytogenetic changes (e.g., −7, 5q–, +8, 20q–, +21) remain incompletely understood. In most cases, these cytogenetic changes appear to be related to progression of MDS, rather than its initiation, as they are absent in up to 60% of MDS cases at presentation. AML-MRC also shares a subset of gene mutations with MDS [173, 174]. Recent NGS studies have found that mutations in a subset of spliceosome and chromatin modification genes are highly specific for AML-MRC. Similar genetic analyses may allow more precise definition of these diseases in the future [45, 175]. *TP53* mutations, which are also more frequent in secondary AML and MDS, usually associate with complex karyotypes and predict poor survival [176]. Some of these genetic abnormalities are now the target of specific treatment strategies (e.g. *IDH1/2* mutations, *FLT3*-ITD, *TP53* mutations) [43, 177].

Unlike AML-RGA, AML-MRC lacks clearly defined syndromes with specific morphologic, cytogenetic, or molecular genetic signatures. The genetic abnormalities cited above that have directed therapy lack specific morphologic or cytogenetic features to characterize them, short of molecular genetic identification. As discussed previously, some subtypes of AML currently placed debatably in WHO AML-RGA [AML with mutated NPM1, AML with inv(3) or t(3;3)] share features with AML-MRC.

2.5.6 AML, Not Otherwise Specified (the Historical Approach to AML Classification)

For much of the twentieth-century classification of AML was based on how leukemic blasts recapitulate normal hematopoiesis. Classification depended upon whether blasts in a given case had the appearance of myeloblasts, monoblasts, megakaryoblasts, promyelocytes, or erythroblasts and whether they appeared minimally, moderately, or well differentiated. This approach was formalized by the French-American-British (FAB) working group in a series of papers beginning in 1976, allowing analysis of its relevance. In the FAB classification, M0 designates AML with minimal morphologic or cytochemical differentiation, M1–2 AML with minimal or moderate granulocytic differentiation, M3 acute promyelocytic leukemia (APL), M4 AML with mixed myelomonocytic differentiation, M5a and M5b monoblastic leukemia with minimal or moderate differentiation, M6a myeloid leukemia with dysplastic background erythropoiesis, M6b acute erythroblastic leukemia, and M7 acute megakaryoblastic leukemia. Unfortunately, subsequent analyses showed a general lack of clinical and biological relevance to this approach. Analysis of 5848 patients with newly diagnosed AML demonstrated that when data on NPM1 and CEBPA mutations was available, the FAB classification added no further prognostic information [178]. The approach remains a useful shorthand descriptor of myeloblast morphology. The FAB categories are substantially retained in the WHO classification under the heading of AML, NOS (Table 2.1). AML, NOS was originally included for use in cases where cytogenetic and molecular data are not available for classification, as in developing countries where access to laboratories performing these tests is limited. This was controversial as these categories lack clear biologic and clinical relevance, and there is an ever-diminishing number of newly diagnosed AML cases that cannot be subclassified in other categories (Fig. 2.2). Despite these limitations, it is useful to revisit several categories that have unique morphologic or clinical presentations.

AML with Monocytic Differentiation: AML with monocytic differentiation may arise as AML-RGA, AML-MRC, or from a precedent chronic myelomonocytic leukemia (CMML). In the latter case, there may be progressive variation in the percentages of monocytes, promonocytes, and monoblasts over time, creating difficulty in determining when to call progression to AML. Monocytic precursors may not have distinctive immaturity markers by flow

cytometry, so morphologic review of the peripheral blood and bone marrow aspirate, and morphologic and immunohistochemical examination of the bone marrow biopsy/particle, are critical to the final designation of AML. In the peripheral blood and bone marrow aspirate, there may be a mixture of monocytes and monocytic precursors with varying maturity. Mature monocytes usually have irregular indented nuclei and abundant cytoplasm with pseudopods and cytoplasmic vacuoles. Promonocytes have delicate nuclear folds that one can see through, more immature chromatin, and blue cytoplasm which is more circumscribed than the mature monocyte (Fig. 2.10). These are considered "blast equivalents" in the calculation of the percentage of immature cells in the blood or bone marrow aspirate [2]. Finally, monoblasts have immature "ground glass" chromatin, nucleoli, folded nuclei, and variable amounts of cytoplasm, but usually with an increased nuclear:cytoplasmic (N:C) ratio (Fig. 2.10). Monoblasts are often negative for the immaturity markers CD34 and CD117. On aspirate and peripheral smears, cytochemical stains are occasionally performed to identify blasts as monocytic; alpha naphthyl esterase staining will stain cytoplasmic granules in monocytic cells; this stain is markedly reduced with fluoride treatment. Immunohistochemistry performed on the bone marrow biopsy/particle may include antibodies to MPO (negative in most monoblasts) and lysozyme (positive in monocytic cells, but not specific to monoblasts).

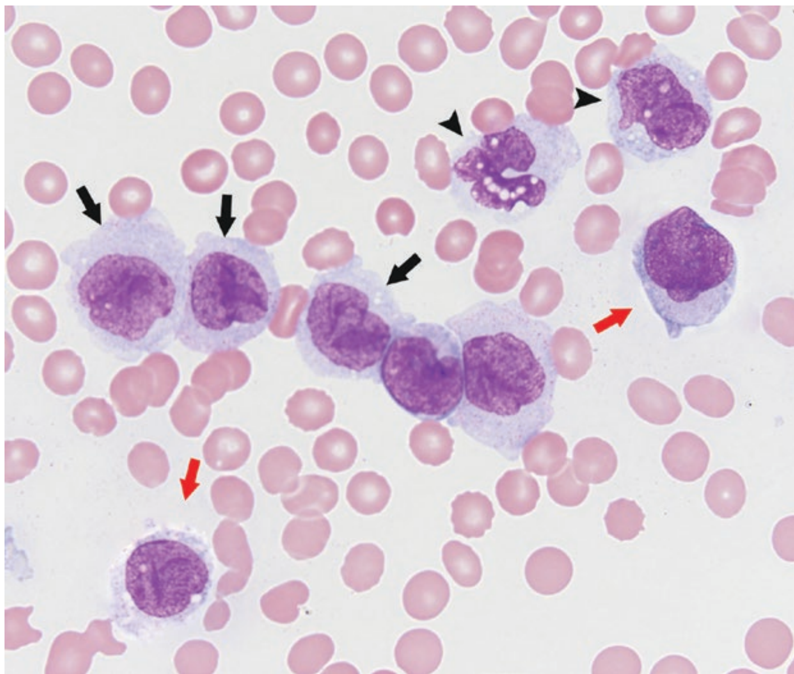

Fig. 2.10 AML with monocytic differentiation (peripheral blood smear stained with Wright's stain at 1000× original magnification). Mature monocytes usually have irregular indented nuclei and abundant cytoplasm with cytoplasmic vacuoles (cells in the top right indicated with black arrowheads). Promonocytes have delicate nuclear folds that one can see through, more immature chromatin, and variable amount of pale blue cytoplasm (a row of cells in the middle-left indicated with black arrows). These are considered "blast equivalents" in the calculation of the percentage of immature cells. Monoblasts (cells in the middle right and lower left indicated with red arrows) have immature "ground glass" chromatin, occasionally folded nuclei, variably prominent nucleoli, and an increased N:C ratio. Due to continuous morphologic spectrum of these monocytic cells, the recognition and enumeration of the promonocytes may be difficult and somewhat subjective especially in situations where smear and staining quality are less than optimal

Acute Erythroid Leukemia: Acute erythroid leukemia in the current WHO classification is limited to what was previously called "pure" erythroleukemia, FAB M6b, or Di Guglielmo's leukemia. It is rare and is defined as a neoplastic proliferation of immature precursors of the erythroid lineage where 80% of bone marrow cells are erythroid, with ≥30% proerythroblasts and no myeloblast population [2]. Blasts have morphologic features of proerythroblasts: large round nuclei with multiple nucleoli, deep basophilic cytoplasm, and cytoplasmic vacuoles. Immature chromatin and the cytoplasmic vacuoles are the main features that distinguish neoplastic from normal proerythroblasts. The vacuoles are often positive on a PAS cytochemical stain performed on an aspirate smear. On bone marrow biopsy/particle sections, immunohistochemistry using antibodies for E-cadherin and CD71 is recommended. Some morphologic features are shared with acute megakaryoblastic leukemia; however, the main differential to be aware of is similarity to benign conditions such as erythroid hyperplasia in response to erythroid growth factor or associated with megaloblastic anemia. A common clinical feature is profound anemia. Cytogenetic studies usually indicate a complex karyotype [2], which corresponds to the prevalence of *TP53* mutations in this leukemia [179].

Acute Megakaryoblastic Leukemia: Acute megakaryoblastic leukemia, also rare, is defined as an acute leukemia with greater than 20% blasts, greater than 50% of which are megakaryocytic in lineage. This category excludes cases of AML with myelodysplasia-related changes or therapy-related AML and also does not include cases with the recurrent cytogenetic abnormalities t(1;22) (p13.3;q13.1) and inv3(q21.3q26.2), or t(3;3)(q21.3;q26.2). Each of these leukemias with recurrent cytogenetic abnormalities are listed separately in the WHO classification [2]. Here we will mention general characteristics of megakaryocytic blasts. Megakaryoblasts are medium to large blasts with a high N:C ratio and basophilic cytoplasm which often has characteristic cytoplasmic blebs. On the aspirate smear and tissue sections, megakaryoblasts may form cohesive clusters and therefore may easily be mistaken for carcinoma, if marrow material is limited. Careful search of the peripheral smear for blasts may be helpful in this differential diagnosis. On the aspirate smear, alpha naphthyl acetate esterase stains megakaryoblasts and, in contrast to monocytic cells, is not quenched by fluoride. Immunohistochemistry with megakaryocyte-specific antibodies, including CD42b and CD61, is recommended, although these markers may be negative in poorly differentiated megakaryoblasts. Often the marrow is markedly fibrotic, resulting in hypocellular aspirate smears and compromising diagnosis.

A unique setting of acute megakaryoblastic leukemia is in the context of Down syndrome. Children with Down syndrome have a markedly increased incidence of acute myeloid leukemia, and over 50% of cases have megakaryoblastic differentiation (discussed in more detail below).

2.5.7 Therapy-Related AML

Therapy-related AML (t-AML) is classified within a distinct WHO category of therapy-related myeloid neoplasms (t-MN) which also includes therapy-related MDS (t-MDS) [2]. This is a distinct and well-recognized clinical syndrome which occurs as a late complication of cytotoxic chemotherapy for a primary neoplastic or non-neoplastic process [180, 181]. The incidence of t-AML is approximately 7% but is currently rising due to an increasing number of cancer survivors at risk [182–184]. There are two major biologic classes of t-AML (Table 2.2) (reviewed in [185]). The more common class is associated with prior exposure to alkylating agents and/or radiation therapy and occurs typically after 5–7 years. This type is usually preceded by an MDS phase and is associated with MDS type cytogenetic changes including abnormalities of chromosomes 5 and 7, complex karyotype and high frequency of *TP53* mutations. t-AML in general is associated with more adverse genetic lesions, and *TP53* may be the most commonly mutated gene in t-MDS and t-AML [186]. t-AML after therapy with topoisomerase II inhibitors has a shorter latency,

occurring 1–3 years after the exposure, typically with no antecedent MDS, and is associated with balanced translocations frequently involving *KMT2A (MLL)* at 11q23, *RUNX1* at 21q22 and other balanced translocations including *PML/RARA*. The precise distinction between these two classes may not always be possible or practical due to the use of multi-agent chemotherapy often in combination with radiation therapy. Genetically and phenotypically, these two classes of t-AML resemble AML-MRC and AML-RGA, respectively, with no distinctive genomic patterns that are specific for t-AML [45, 185]. Some recent studies have identified increased prevalence of CHIP, including mutations in the *TP53* pathway, in patients who eventually develop t-AML after treatment for other malignancy, suggesting that the hematopoietic progenitor cells with mutations in the *TP53* pathway may undergo selective unrepaired damage by chemotherapy, eventually leading to t-AML [185, 187]. In addition, some cases of t-AML have been shown to be associated with germline mutations in cancer susceptibility genes involving DNA damage response pathways such as *BRCA1, BRCA2, TP53,* and *CHEK2* [185, 188, 189]. The overall prognosis of patients with t-AML is poor, mainly due to consequences of prior therapy for the primary disease and to enrichment of this type of AML with adverse disease-related features.

2.5.8 Germline Predisposition to AML

The expanding availability of detailed molecular data in AML, and its integration with clinical and laboratory data, has led to recognition of predisposing germline mutations in a growing number of genes in patients with AML and other myeloid disorders [190]. Although these cases are currently considered to be rare, as more data accumulate, these neoplasms may be found to be more common than currently appreciated. As with myeloid disorders occurring in inherited syndromes associated with DNA damage (e.g., Fanconi anemia, dyskeratosis congenita), the recognition of these newly characterized autosomal dominant disorders arising from germline mutations will be essential for proper clinical management, long-term follow-up, and genetic counseling [191, 192]. Some of these patients may present with AML or MDS with no prior history of significant organ dysfunction or preexisting hematologic disorder, as seen in myeloid neoplasms associated with germline mutations in *CEBPA* or *DDX41*. Other patients may present with a pre-existing platelet disorder (e.g. mutations in *RUNX1*, *ANKRD26* or *ETV6*) [2]. Still others may present with additional non-hematological phenotypic abnormalities (e.g., germline *GATA2* mutation, inherited marrow failure disorders) [193]. Given the important clinical management considerations including donor selection for allogeneic stem cell transplantation, it is critical to distinguish diseases arising because of germline predisposition from those arising spontaneously or secondary to chemical or environmental exposures. Targeted myeloid gene sequencing panels are increasingly utilized as part of the routine diagnostic work up of AML and MDS cases. These panels include increasing numbers of known genes associated with germline predisposition syndromes. Types of mutations and mutant allele frequencies, in a context of a patient's clinical and family history, may raise suspicion of a germline predisposition, with a recommendation for confirmatory germline testing. An illustration of this is AML with germline *CEBPA* mutation. In these cases, the *CEBPA* mutation is biallelic with the germline mutation usually found in the 5′ end of one allele and a somatic mutation at the 3′ end of the other allele [120, 194]. Therefore, when a patient presents with a new diagnosis of AML with biallelic *CEBPA* mutations, testing should be undertaken to rule out a germline mutation, which if present would result in reclassification of the case, alter the clinical management of the patient, and lead to genetic counseling. A similar example is diagnosis of AML with mutation in *RUNX1* at high variant allele frequency (close to 50%, implying a constitutional abnormality), with similar consequences to the discussion of *CEBPA*.

2.5.9 Myeloid Leukemia Associated with Down Syndrome [195]

An unusual form of AML and MDS occurs with high frequency in children with Down syndrome (DS) under 4 years of age. The two are lumped together in the WHO classification as myeloid leukemia associated with Down syndrome (DS AML) because of similar excellent responses to chemotherapy, differing markedly from AML and MDS in non-DS children. The incidence of this set of disease is approximately 300–400 times that of MDS and AML variants in non-DS children. There is no increase in the incidence of standard non-DS AML and MDS in DS patients. DS AML may be preceded by transient abnormal myelopoiesis (TAM) in the neonatal period. TAM and DS AML both have a high frequency of mutations of *GATA1*, of interest since GATA1 protein mediates differentiation of erythroid and megakaryocytic precursors. About one third of TAM patients later develop DS AML; these patients usually have the same *GATA1* mutation as was present with TAM. Both MDS and AML in DS patients have unusual and characteristic features which appear to correspond to disruption of function of GATA1. Patients typically present with peripheral cytopenias and/or circulating or increased marrow blasts. Dysplasia is present and essentially restricted to erythroid and megakaryocytic precursors. Erythroids demonstrate megaloblastoid change and frequent hyperplasia. Megakaryocytes demonstrate hyperplasia, clustering, and hypolobate, often multiple nuclei, frequently including unusual morphology (peripherally displaced nuclei and a large central cytoplasmic inclusion giving the megakaryocyte the appearance of a Touton giant cell, or in smaller cells signet-like morphology) [195]. In AML cases, and if blasts are increased in MDS cases, blast lineage is most frequently megakaryoblastic, but erythroblastic, mixed erythroblastic/megakaryoblastic, and undifferentiated cases also occur [195]. Clinical evaluation is similar to that followed in non-DS cases, to include examination of peripheral blood and bone marrow samples, including blast characterization by IHC and flow cytometry. Blasts have a characteristic antigen expression pattern, with a near 100% frequency of positivity for CD33, CD117, CD38, and CD7, lower frequencies for CD13 and CD34, and variable expression of sublineage antigens depending on blast differentiation (CD41, CD61, and CD42b for megakaryocytic differentiation; CD36, CD71, and glycophorin A for erythroid differentiation). In 10% of DS AML cases, blasts are undifferentiated, lacking sublineage differentiation markers. Cases demonstrating significant myeloid dysplasia or myeloid blast differentiation may represent infrequent cases of standard non-DS AML. Given the expected ratio of DS to non-DS AML in DS patients, such cases should be reviewed by an experienced hematopathologist. With cytogenetic testing patients by definition must have constitutional +21 or mosaic +21. Additional abnormalities such as +8 are seen, but do not appear to affect prognosis. Presence of translocations typical of childhood AML in non-DS patients are not seen; such karyotypes may indicate that standard non-DS type disease should be confirmed by FISH testing and should be reviewed by an expert cytogeneticist. MRD testing in follow-up samples may be beneficial for predicting outcome. While *GATA1* mutations are usually present, such information is not required for clinical purposes, and comprehensive testing is difficult as mutations are not localized and require extensive sequencing not currently feasible in clinical laboratories. Other mutations have been demonstrated with advances in molecular testing, but no clinical significance has been shown. After 4 years of age, the incidence of MDS and AML in DS patients decreases markedly (to the approximate level of disease in non-DS children), patients lack *GATA1* mutations, usual subtypes of childhood disease become prevalent, and prognosis reverts to that of standard non-DS disease.

2.6 Summary

An array of testing to include morphology, flow cytometric immunophenotyping, cytogenetics, and increasingly molecular genetics is necessary

Table 2.3 Risk groups in adult AML based on cytogenetic and molecular analysis

Risk profile	Genetic abnormality	Other mutations
Favorable	t(8;21)(q22;q22.1); *RUNX1-RUNX1T1* inv(16)(p13.1;q22) *or* t(16;16)(p13.1;q22); *CBFB-MYH11* Mutated *NPM1* without *FLT3*-ITD or with *FLT3*-ITD[low b] Biallelic mutated *CEBPA*	Any mutation or combination thereof not classified as intermediate or adverse
Intermediate	Mutated *NPM1* and *FLT3*-ITD[high b] Wild-type *NPM1* without *FLT3*-ITD or with *FLT3*-ITD[low b] (without adverse-risk genetic lesions) t(9;11);(p21.3;q23.3); *MLLT3-KMT2A(MLL)* Cytogenetic abnormality not classified as favorable or adverse	Any mutation or combination thereof not classified as favorable or adverse
Adverse	t(6;9);(p23;q34.1); *DEK-NUP214* t(v;11q23.3); *KMT2A (MLL)* 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[a] Wild-type *NPM1* and *FLT3*-ITD[high b] Mutated *RUNX1* Mutated *ASXL1* Mutated *TP53*	Any mutation or combination thereof not classified as favorable or intermediate

Adapted from Dohner, H., Estey, E., Grimwade, D. et al. (2017). “Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel.” *Blood* 129(4): 424–447

[a]Complex karyotype defined as three or more unrelated chromosome abnormalities in the absence of one of the WHO-designated recurring translocations or inversions. Monosomal karyotype defined by the presence of one single monosomy (excluding loss of X or Y) in association with at least one additional monosomy or structural chromosome abnormality (excluding core-binding factor AML) [196]

[b]Low, low allelic ratio (<0.5); high, high allelic ratio (>0.5); semiquantitative assessment of *FLT3*-ITD allelic ratio (using DNA fragment analysis) is determined as ratio of the area under the curve “*FLT3*-ITD” divided by area under the curve “*FLT3*-wild-type”; recent studies indicate that AML with *NPM1* mutation and *FLT3*-ITD low allelic ratio may also have a more favorable prognosis [197–199]

to diagnose and subclassify AML as well as assign the patients into clinically meaningful risk categories (Table 2.3). Careful attention is required to assure an adequate sample is obtained at diagnosis to accomplish this array of testing. Expanding knowledge of the genetic basis of AML will continue to complicate diagnostic requirements and classification, as increased understanding of biology progresses to therapy directed at specific genetic targets in AML. The most important diagnostic and classification issues are:

- Distinction of AML-RGA from AML-MRC (because of the fundamental biologic differences in the two sets of disease).
- Recognition of specific molecular/genetic subsets of disease amenable to targeted therapy (e.g., currently AML with *PML-RARA*; AML with *IDH1/2*, *FLT3*-ITD, or *TP53* mutations).
- Recognition of specific molecular/genetic subsets of disease requiring specific prognostication-driven treatment strategies (CBF AML, AML with mutated *NPM1*, AML with biallelic *CEBPA* mutation, AML with *TP53* mutation).
- Recognition of specific molecular/genetic subsets of disease requiring possible genetic counseling (AML with germline predisposition).

Accumulating molecular genetic data generated by NGS-based and other technologies will continue to refine our understanding of the biology of AML, provide novel insights into its

pathogenesis, lead to new treatments for subsets of disease, and require an expanding array of laboratory testing. Incorporation of these developments into AML classification will be challenging and may eventually lead to fundamental revisions of AML classification [23, 45, 200–202]. The need for monitoring early response by MRD assessment with adjustment of treatment will require improvements in flow cytometry and/or application of molecular approaches such as NGS-based methods. A role for long-term monitoring of MRD remains to be established, except in APL. There remains a need for better understanding of the pathogenesis of MDS and AML-MRC, as we currently lack precise diagnostic methods and treatments for most patients with these entities outside of allogeneic stem cell transplantation.

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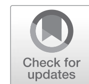

3 Insights into the Pathobiology of Secondary AML

Tania Jain and Raajit K. Rampal

Abbreviations

AHD	Antecedent hematological disorder
AML	Acute myeloid leukemia
CBF	Core binding factor
CIBMTR	Center for International Bone Marrow Transplant Research
EBMT	European Society for Blood and Bone Marrow Transplantation
HCT	Hematopoietic cell transplantation
JAK	Janus kinase
MDS	Myelodysplastic syndrome
MPN	Myeloproliferative neoplasm
SNP	Single-nucleotide polymorphism
TP	Tumor protein
WHO	World Health Organization

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3.1 Introduction: What Is Secondary AML?

Acute myeloid leukemia (AML) is the most common type of acute leukemia, the incidence of which increases with advancing age. The etiology remains elusive for the most part, but development following a prior cytotoxic agent or as a consequence of an antecedent myeloid disorder has been widely recognized. In the 1997 World Health Organization (WHO) classification of neoplastic disease of the hematopoietic and lymphoid tissues, AML with multilineage dysplasia, defined as dysplastic changes in two or more cell lines, and therapy-related AML were recognized as distinct entities due to morphological differences from de novo AML, characteristic cytogenetic abnormalities, and worse prognosis [1].

Secondary acute myeloid leukemia (s-AML) in the current era informally refers to the AML that evolves from an antecedent hematological disease (AML-AHD), usually a myeloid malignancy such as myelodysplastic syndrome (MDS), myeloproliferative neoplasms (MPN), or aplastic anemia; has myelodysplasia-related changes (AML-MRC); or develops after exposure to a cytotoxic chemotherapy or radiation treatment for a prior neoplasm (t-AML) (Table 3.1). Per the 2016 WHO classification, AML with MRC is defined as AML meeting at least one of the following criteria: (a) presence of 50% of more dysplastic cells in at least two cell lines and in the

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Table 3.1 What is included in secondary AML

	Details
AML-AHD	• Preceding MDS • Preceding MPN • Preceding MDS/MPN overlap syndromes • Bone marrow failure syndrome
AML-MRC	• Dysplasia in >50% cells in at least two cell lines and absence of favorable-risk mutations of *NPM1*, biallelic *CEBPA* or del(9q) • MDS-related cytogenetic abnormality • AML-AHD with preceding MDS or MDS/MPN
t-AML	• Type I – Alkylating agents—cyclophosphamide, bendamustine, melphalan, busulfan, carmustine, chlorambucil, thiotepa Platinum agents—cisplatin, carboplatin Antimetabolites—azathioprine, fludarabine – 5–7 years after exposure – Preceding MDS phase or MDS-related changes at diagnosis – Del(5q), del(7q), monosomy 7, complex karyotype, *TP53* mutations • Type II – Topoisomerase II inhibitors—anthracyclines (daunorubicin, doxorubicin, epirubicin), etoposide, mitoxantrone – 2–3 years after exposure – Balanced chromosomal translocations involving 11q23 (*KMT2A/MLL*) or 21q22 (*RUNX1/AML1*) • Radiation exposure

AML-AHD acute myeloid leukemia with an antecedent hematological disorder, *AML-MRC* acute myeloid leukemia with myelodysplasia related changes, *MDS* myelodysplastic syndrome, *MPN* myeloproliferative neoplasm, *t-AML* therapy-related acute myeloid leukemia

absence of favorable-risk mutations of NPM1 or biallelic CEBPA or del(9q) [2]; (b) an antecedent hematologic disorder (MDS or MDS/MPN); or (c) presence of an MDS-related cytogenetic abnormality.

t-AML has been associated with prior chemotherapy, typically alkylating agents and DNA topoisomerase II inhibitors, as well as prior radiation therapy. Alkylating agents such as melphalan, cyclophosphamide, and nitrogen mustard may lead to dysplastic changes similar to MDS, bi- or tri-lineage cytopenias, abnormalities of chromosomes 5 and 7 or both, and complex karyotype, typically with a latency of over 5 years from exposure [3–6]. These karyotypes have been reported to comprise 76% of all abnormal karyotype in one series of patients with t-AML or therapy-related MDS [7]. t-AML associated with topoisomerase II inhibitors, for example, doxorubicin, etoposide, and mitoxantrone, is characterized by shorter interval (around 2–3 years) between exposure and diagnosis, absence of preceding MDS, and a genetic abnormality involving translocation of *MLL* gene on chromosome 11, band q23, and *RUNX1/AML1* gene on chromosome 21, band q22 [7–10]. t-AML has also been described following the use of other chemotherapy agents such as azathioprine, 5-flourouracil, methotrexate, 6-mercaptopurine, and fludarabine [7, 11, 12]).

3.2 Why Does It Matter?

Two large population studies reported that s-AML constituted approximately one in every four cases of AML diagnosed between 1997–2006 and 2000–2013 in these respective studies [13, 14]). More specifically, AML-AHD has been reported in approximately 16–19% and t-AML in approximately 7% of all AML diagnoses in several studies [13–16]. This number may reasonably be expected to rise due to improved survival following chemotherapy for prior malignancies.

More importantly, numerous studies over the years have shown inferior survival in patients with s-AML compared to de novo AML, which makes it imperative to recognize this as a prognostic factor, and indeed an unmet need for treatment options [13–16]. Response to standard chemotherapy in patients with s-AML has also traditionally been reported to be lower than response rates seen with de novo AML. This is likely a result of a higher incidence of high-risk genetic and molecular features, genetic alterations, or selection of chemotherapy-resistant clones in cases of prior treatment of the AHD. The high-risk cytogenetic and mutational profile in

s-AML, such as mutations in tumor protein (*TP53*) and complex karyotype, may render leukemia cells less responsive to chemotherapy and a poorer overall survival [17]. Mechanism of resistance and resulting lower response to chemotherapy in s-AML have been attributed to an over-expression of multidrug resistance gene 1 (MDR1) which results in increase in efflux pumps such as p-glycoprotein leading to a decreased intracellular concentrations and overall exposure to anthracycline chemotherapy, hence rendering the resistance to drugs [18, 19]. Other plausible mechanisms of resistance include expression of proteins conferring multidrug resistance such as multidrug resistance-associated protein 1 (MRP1) and lung resistance protein (LRP) [20, 21]. Of these, MDR1 expression and the resultant drug efflux has been reported to increase with increasing age from 17% in age <35 years while being 39% in age >50 years, which at least partly contributes to decreased responses to chemotherapy in s-AML which is commonly seen in older patients [19]. Additionally, patients with s-AML are older and may have been treated previously with cytotoxic agents, both of which can possibly limit the ability to utilize high dose or cytotoxic chemotherapy to treat s-AML [16, 22].

To summarize, understanding the biology as well as management strategies for s-AML is important in treatment planning and risk-stratification as the incidence of this diagnosis is anticipated to rise, response as well as survival in these patients is inferior compared to de novo AML, and treatment options can be limited by age or other patient factors.

3.3 Biology and Genomics of Secondary AML: Are They Different and How?

Clonal hematopoiesis plays an important role in the development of s-AML from both an AHD and in t-AML [23, 24]. It is now being realized that clonal hematopoiesis exists early, likely even prior to exposure to cytotoxic therapy in t-AML. Subsequent exposure to chemotherapy results in selection of a drug resistant preleukemic clone. Mutant *TP53* clones have been found years prior to chemotherapy exposure or diagnosis of t-AML [25, 26]. Recent studies have also shown that both hematopoietic and stromal compartments of the bone marrow are involved in the malignant transformation of hematopoiesis and that mesenchymal stem cells undergo remodeling upon exposure to MDS cells [27].

3.3.1 AML-AHD from Preceding MDS

Transformation of MDS to AML (AML-MDS) is mediated by clonal evolution or increase in the number of mutations as well as expansion of existing mutant clones. A variety of cytogenetic abnormalities and mutations in genes affecting splicing machinery and chromatin modifiers have been reported more commonly in AML-MDS than in de novo AML, including *SRSF2*, *SF3B1*, *U2AF1*, *ZRSR2*, *ASXL1*, *EZH2*, *BCOR*, and *STAG2* [28]. Paired sampling of MDS and AML-MDS bone marrow samples suggested that at least one new driver mutation occurs in most (59% in the study) patients in the process of transformation, except in patients with *TP53* mutation [28]. Most of these mutations were in genes encoding myeloid transcription factors (*RUNX1*, *CEBPA*, *GATA2*) and signal transduction proteins (*FLT3* or *RAS*). Another study comparing samples of patients with s-AML versus high-risk MDS showed enrichment of *NRAS*, *FLT3*, *WT1*, *NPM1*, *IDH1/2*, *PTPN11* genes in s-AML [29]. Whole exome sequencing studies have demonstrated that mutations in signaling pathways (such as *NRAS* and *PTPN11*) occur or expand significantly during transformation of disease [30]. This was, however, not observed for mutations associated with DNA methylation or splicing machinery, which were noted to expand at the initial stages of MDS but not at the time of progression to s-AML. Another study using a comprehensive transcriptome sequencing in CD34+ bone marrow cells identified two subgroups of MDS by gene expression profiling: one with increased expression of

genes in the erythroid/megakaryocytic lineage and the second with upregulation of genes related to immature progenitor cells. The later demonstrated upregulation of various signaling pathways and downregulation of pathways related to metabolism and DNA repair and was exclusively associated with leukemic transformation and shorter survival [31].

Identification of the above-mentioned mutations in patients with MDS may herald emerging AML subclones and potentially identify patients at risk for transformation to s-AML.

3.3.2 AML-AHD from MPN

MPN are another set of myeloid disorders that can potentially transform into AML (AML-MPN) with a rather dismal prognosis [32]. In a single-center study of 91 patients, a clonal abnormality was identified in 91% patients including complex karyotype (54%), core binding factor (CBF) gene mutations (3%), and chromosome 5 or 7 abnormalities (32%) [32]. The three known driver mutations in myelofibrosis, *JAK2V617F*, *CALR* or *MPL*, also impact time to leukemia transformation. Mutations in *CALR* were associated with lesser risk of transformation when compared with "triple-negative" (absence of all three mutations) and *JAK2V617F*, but with no difference compared to mutations in *MPL* [33]. Subsequently, genomic profiling of samples from patients with AML-MPN has delineated differences from genetics patterns of de novo AML. Recurrent point mutations in *TET2*, *ASXL1*, *SRSF2*, *TP53*, and *IDH1/2* have been reported to be more common in AML-MPN than in de novo AML [34–37]. Single-nucleotide polymorphism (SNP) array analysis of 88 chronic phase MPN and 71 MPN-blast phase samples showed three-times higher genomic changes in the latter [38]). Aberrations in chromosomes 3p (*FOXP1*), 4q (*TET2*), 7p (*IKZF1*), 7q (*CUX1*), 8q (*MYC*), 12p (*ETV6*), 17p (*TP53*), 21q (*RUNX1*) were seen more commonly in AML-MPN samples (MPN-blast phase) than in chronic phase [38, 39]. Genomic profiling of AML-MPN samples demonstrated a higher frequency of somatic *TP53* mutations accompanying *JAK2V617F* mutations, in contrast to chronic phase MPN samples where *TP53* mutations were less frequent [40]. *TP53* loss in combination with *JAK2V617F* mutation led to expansion of blasts, in both the bone marrow and the peripheral blood, and fully penetrant AML in murine models, thus biologically validating these genomic observations.

Collectively, these data demonstrate that recurrent mutations in epigenetic regulators, transcription factors, spliceosome complex members as well as *TP53* have been associated with the leukemia transformation of MPNs. This mutational spectrum appears to differ from that observed in de novo AML, thus potentially explaining biological differences and clinical behavior of AML-MPN.

3.3.3 t-AML

t-AML has a high representation of high-risk or unfavorable cytogenetic abnormalities and somatic mutations. In a series of 306 patients with t-MDS/t-AML from a single center, over 70% patients had del(5q), del(7q), or monosomy 7 [7]. Of these, chromosome 7 abnormalities [del(7q) and monosomy 7] occur in almost half of these patients and is associated with mutations that activate the RAS pathway [41, 42]. *TP53* mutations occur in up to 37% of patients with t-AML and can be associated with del(5q) as well as complex karyotype [28, 43]. Around one-third patients with t-AML can harbor mutations in *SRSF2*, *SF3B1*, *U2AF1*, *ZRSR2*, *ASXL1*, *EZH2*, *BCOR*, or *STAG2* [28]. Additionally, association of *MLL* rearrangements and exposure to topoisomerase II has been described above.

Overall, the genetic composition of t-AML is notable for a higher frequency of unfavorable mutations that are associated with poor response to therapy and inferior outcomes.

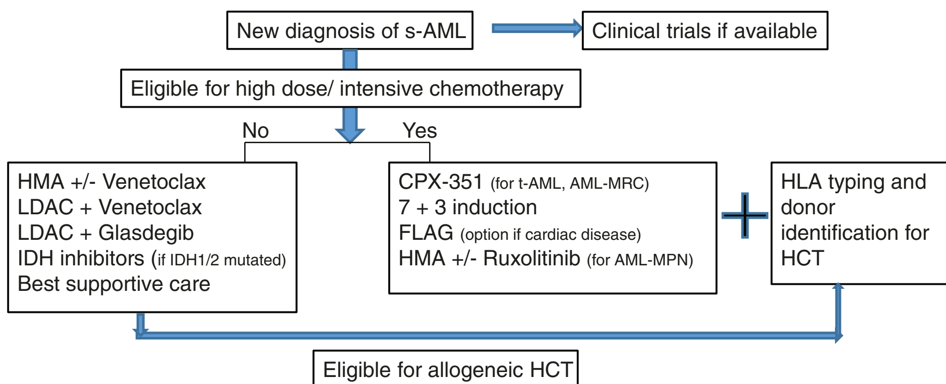
3.4 Management of s-AML: How to Think of Treatment Options?

The overall prognosis of patients with s-AML remains poor whether they arise from an antecedent MDS, MPN, or t-AML; and treatment options have not changed significantly in over four decades. A proposed treatment schema with contemporary options is depicted in Fig. 3.1.

3.4.1 Induction and Chemotherapy: Past, Present, and Future

Anthracycline-based therapy, in combination with cytarabine, in the historic "7+3" regimen has remained the traditional therapy for s-AML, although response rates have been lower than that with de novo AML [13, 14, 44]. While both AML-AHD and t-AML show inferior outcomes compared to de novo AML, s-AML arising from AHD other than MDS was associated with an even lower survival in patients over 60 years of age with adverse karyotype [13].

CPX-351 is a novel agent, recently approved by Food and Drug Administration (FDA) and European Medicine Agency (EMA), for treatment of newly diagnosed t-AML or AML-MRC. It is the dual-drug liposomal encapsulation of cytarabine and daunorubicin at a fixed 5:1 molar ratio at which maximal synergistic activity had been demonstrated in preclinical models [45–47]. The encapsulated liposomal formulation improves pharmacokinetic characteristics and allows for intracellular delivery which is enhanced in leukemia cells compared to normal cells. The particular benefit of CPX-351 in patients with s-AML was seen in a randomized phase 2 study conducted in patients with newly diagnosed AML of ages 60–75 years comparing CPX-351 with the standard 7+3 regimen [48]. While a statistical significant advantage in overall survival or event-free survival was not seen for the overall study population, a planned subgroup analysis in high-risk patients including those with s-AML showed a significantly superior overall survival in this subgroup (HR 0.46, p = 0.01). These findings were confirmed in a phase 3 trial of patients with ages 60–75 years with newly diagnosed t-AML or AML-MRC. The study showed significantly superior overall survival compared to 7+3 induction (9.56 vs 5.95 months, HR 0.69, p = 0.003) along with



FLAG, fludarabine, cytarabine, plus G-CSF; HCT, hematopoietic cell transplantation; HLA, human leukocyte antigen; HMA, hypomethylating agents; IDH, isocitrate dehydrogenase; LDAC, low dose cytosine arabinoside (cytarabine); s-AML, secondary acute myeloid leukemia; t-AML, therapy related AML; AML-MRC, AML with myelodysplasia related changes

Fig. 3.1 Treatment schema for secondary AML

higher remission rates (47.7% vs 33.3%, $p = 0.016$) and improved event-free survival (2.53 vs 1.31 months, HR 0.74, $p = 0.021$) [49]. An exploratory landmark analysis in patients who underwent allogeneic stem cell transplantation (HCT) after induction was notable for a significantly favorable overall survival with CPX-351 (median not reached vs 10.25 months, HR 0.46, $p = 0.009$). Although this analysis was influenced by confounding factors such as that the decision to undergo HCT was not randomized, these results do establish CPX-351 as a suitable treatment option as a bridge to HCT.

Azanucleosides are pyrimidine analogs which at lower doses have established themselves as potent inhibitors of DNA methylation and are commonly referred to as hypomethylating agents. Due to the noted significance of DNA methylation in transformation of MDS to AML, hypomethylating agents have an established role in treatment of patients with s-AML especially those who are not candidates for high dose chemotherapy [50, 51]. The two commonly used hypomethylating agents are 5-azacytadine and 5-aza-2′-deoxycytidine (or decitabine). Azacitidine was FDA approved for use in MDS based on data from the AZA-001 trial which included patients with up to 30% blasts, which would now be classified as AML for >20% blasts, and showed improved survival compared to best supportive care [52]. Subsequently, a phase 3 study (AZA-AML-001) was conducted in patients with AML, including AML-AHD and AML-MRC, who were ≥65 years old and continued to show improved overall survival with azacitidine versus best supportive therapy (median, 8.9 vs 4.9 months, HR 0.74) [53]. Similarly, decitabine was compared to conventional therapy in older patients with AML, resulting improved response rate (17.8% vs 7.8%, OR 2.5, $p = 0.001$) although no statistical improvement in survival was reported in the primary analysis (7.7 vs 5 months, $p = 0.108$) until an unplanned analysis a year later (HR 0.82, $p = 0.037$) [54]. Both azacitidine and decitabine have been reported to have responses in AML-MPN although most reports are relatively small series. Decitabine showed responses in 6/21 (29%) patients with AML-MPN, including one partial response, with the response lasting a median of 7 months [55]. Azacitidine therapy resulted in an overall response in 10 (38%) with complete response in 2 (8%) of the 26 patients with AML-MPN, with median duration of response of 9 months [56]. Ruxolitinib, a selective *JAK1* and *JAK2* inhibitor, as a single agent also has moderate activity with responses seen in 3/18 (17%) patients with AML-MPN, of which 2 were complete responses [57]. Given the individual activity with these agents and the synergistic activity demonstrated in murine models of *JAK2V617*-driven AML, a phase 1 trial was conducted using a combination of decitabine and ruxolitinib in patients with accelerated phase (10–19% blasts in peripheral blood or bone marrow) and blast phase (>20% blasts in peripheral blood and bone marrow) [58]. No dose-limiting toxicity was observed, and overall responses were seen in 5/13 (39%) patients with AML-MPN.

The more recent success story in the treatment paradigm of AML is venetoclax, an orally administered B-cell leukemia/lymphoma-2 (bcl-2) inhibitor, that has been studied in various combinations with hypomethylating agents as well as low-dose cytarabine in older patients with AML who are not candidates for intensive therapy. Approximately one quarter to half of the patients in these studies have s-AML and have shown responses ranging from 8% to 35% in these patients when utilized as upfront therapy [59–61]. It should be noted though that in these studies with venetoclax/hypomethylating agent combinations, no patients with prior hypomethylating agent exposure were included (Table 3.2).

3.4.2 Transplantation or No Transplantation? The Perpetual Enigma

Prospective or randomized studies to compare outcomes with versus without HCT do not exist and are unlikely to be conducted. Studies to date show variable outcomes regarding this, which is likely the result of patient selection in these sin-

Table 3.2 Broad therapeutic categories in secondary AML

	Study	Number of patients	CR/CRi	Median OS	Clinical pearls
CPX-351 (cytarabine:daunorubicin 5:1)	Phase 2 study (compared to 7+3) [48]	Total *N* in CPX-351 arm = 85 s-AML = 33 (39%)	58% in s-AML	12.1 months	Prolonged cytopenias, neutropenic fever but without infection-related deaths
	Phase 3 study (compared to 7+3) [49]	Total *N* in CPX-351 arm = 153 t-AML = 30 (20%) AML-MDS = 71 (46%) AML with preceding CMML = 11 (7%) AML with MDS related cytogenetic abnormalities = 41 (27%)	48% in s-AML	9.5 months	
Hypomethylating agents (±V)	Aza, randomized [53]	Total *N* in Aza arm = 129 AML-MDS = 44 (34%) AML-MRC = 72 (56%) (overall s-AML = 100%)	24.8% in s-AML	8.9 months in s-AML	Cytopenias/myelosuppression
	Aza, retrospective [56]	*N* with AML-MPN in Aza arm = 26	12% in AML-MPN	8 months in AML-MPN	
	Dec, open label phase 3 [54]	Total *N* in Dec arm = 242 s-AML = 87 (36%)	26% overall	7.7 months	Myelosuppression/febrile neutropenia
	Dec, retrospective [55]	Total *N* = 45: AML-MPN = 21	24% in AML-MPN	6.9 month in AML-MPN (10.5 months in responders, 4 months in non-responders)	
	V + Aza/ Dec, phase 1b [59]	Total *N* = 145 s-AML = 36 (25%)	67% in s-AML	Not reached (95% CI, 14.5 months—not reached)	Cytopenias; no tumor lysis observed; patients with prior HMA exposure excluded
	V + Dec10, phase 2 [60]	Total *N* = 48: s-AML = 7 (15%)	71% in s-AML	Not reached (95% CI, 1.8 months—not reached)	Neutropenia, febrile neutropenia, 4% patients had tumor lysis syndrome

(continued)

Table 3.2 (continued)

	Study	Number of patients	CR/CRi	Median OS	Clinical pearls
Rux ± HMA	Rux, phase 2 [57]	Total $N = 38$ AML-MPN = 18 (47%)	17% in AML-MPN	–	Thrombocytopenia
	Dec + Rux, phase 1 [58]	Total $N = 21$ AML-MPN = 13 (62%)	19% overall (in AML-MPN and MPN-AP)	7.2 months (95% CI, 2.2—not reached)	Myelosuppression, pneumonia
Low dose cytarabine + V	V + LDAC, phase 1b/2 [61]	Total $N = 82$ s-AML = 40 (49%)	35% in s-AML	10.1 months for overall cohort	Venetoclax dose interruptions between subsequent cycles in 55% patients, due to delayed neutrophil (10%) and platelet (12%) recovery

AML acute myeloid leukemia, *AML-MDS* acute myeloid leukemia transformed from underlying myelodysplastic syndrome, *AML-MPN* acute myeloid leukemia transformed from underlying myeloproliferative neoplasm, *Aza* azacitidine, *CI* confidence interval, *CMML* chronic myelomonocytic leukemia, *Dec* decitabine, *Dec10* decitabine 10 days, *MDS* myelodysplastic syndrome, *MPN* myeloproliferative neoplasm, *MPN-AP* MPN-accelerated phase, *N* number of patients, *Rux* ruxolitinib, *s-AML* secondary AML, *t-AML* therapy related AML, *V* venetoclax

gle institution or retrospective studies, but do favor improved outcomes in patients who undergo HCT after achieving a complete response [62–67]. While HCT remains the only potential curative option, s-AML has been identified as an independent risk factor for poorer outcomes after HCT [68]. Hence, careful consideration to the various clinical and genetic factors that impact HCT outcomes is warranted to identify who would benefit most from an HCT. Data from the Center for International Bone Marrow Transplant Research (CIBMTR) using HCT data between 1990 and 2004 for t-AML or t-MDS and from European Society for Blood and Bone Marrow Transplantation (EBMT) of HCTs performed between 2000 and 2016 for s-AML have demonstrated inferior survival with advancing age at HCT, poor risk cytogenetics, active disease at HCT, and alternative donors (other than HLA-identical sibling or partially or well-matched unrelated donor) [69, 70]. Among genetic markers, a study from Japan Marrow Donor Program, that included 24% patients with s-AML showed that patients with mutations in *NRAS* (HR 1.64, $p = 0.0075$), *TP53* (HR 1.49, $p = 0.0096$), *CBL* (HR 1.55, $p = 0.024$) as well as complex karyotype (HR 1.45, $p = 0.046$) had particularly inferior outcomes post-HCT [68].

Emergence of therapeutic options prior to HCT increases the optimism for ability to achieve deeper remissions and possibly improving the outcomes from HCT. The possibility of incorporation of the novel therapies as maintenance strategies after HCT and availability of these drugs for treatment of relapse post-HCT further pave the way to utilize the graft-versus-leukemia in combination with the targeted agents.

3.5 Future Directions

Conventional therapeutic options have demonstrated limited activity in s-AML. Identification of novel recurrent somatic mutations in MDS, MPN, as well as post-MPN AML have helped to improve our understanding of disease biology, as well as to elucidate novel therapeutic possibilities. An example is the presence of *IDH1/2* mutations that is seen in over 20% patients with AML-MPN (versus around 4% in chronic phase MPN) [71, 72]. Ivosidenib and enasidenib have shown promising activity in relapsed/ refractory AML with *IDH1* and *IDH2* mutations, respectively [73, 74]. Whether these agents can be used in combination with other active agents in patients with s-AML as front-line therapy is being actively studied with encouraging early results. As mentioned above, mutations in *TP53* are also enriched in patients with progression to AML following MDS or MPN, which presents a particularly challenging problem. The role of investigational drugs such as APR-246, with the potential ability to restore the function of point mutant *TP53* in tumor cells, is currently being explored in *TP53* mutated myeloid malignancies in combination with azacitidine (NCT 03072043). Additionally, novel venetoclax-based or CPX-351-based combination studies with targeted therapies are in progress. Collectively, these recent biological and clinical insights have the potential to alter the historically poor clinical course of patients with s-AML, and hopefully yield better options and outcomes.

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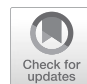

4 Selection of Patients for Individual Acute Myeloid Leukemia Therapies

Roland B. Walter

4.1 Introduction

In 2019, an estimated 21,450 people developed acute myeloid leukemia (AML) in the United States alone [1]. Until recently, treatment options were relatively limited, and decision-making followed an algorithm that has been invariant for several decades [2, 3]. If the person was felt to be medically fit, cure was considered possible: some form of intensive chemotherapy would be offered followed by further courses of chemotherapy and/or allogeneic hematopoietic cell transplantation (HCT) if a complete remission (CR) was obtained. On the other hand, if the person was felt to be medically unfit, cure was considered rare: in this situation, some form of nonintensive, "palliative" chemotherapy would be offered, most typically low-dose cytarabine or, more recently, single-agent treatment with an azanucleoside (e.g., azacytidine or decitabine) or AML-directed therapy is forgone altogether. Over the last few years, the U.S. Food and Drug Administration (FDA) has approved eight new drugs for AML, most of them being "targeted" therapeutics: midostaurin, gemtuzumab, ozogamicin, enasidenib, CPX-351, ivosidenib, gilteritinib, glasdegib, and venetoclax [4]. With these, the treatment options have substantially increased, and the line between intensive and non-intensive therapies has become blurrier. Still, although outcomes have gradually improved, AML remains difficult to cure. Many affected individuals will die from consequences of leukemia or treatment-associated complications, and only a minority will be long-term survivors [2–4]. There is thus ongoing need for new therapeutics in AML and need to identify patients most suitable for participation in a clinical trial. With an increasing number of available standard AML therapeutics and ongoing need for new drugs, treatment decision-making has become more complex: should my patient receive standard AML therapy, and if so, with what regimen? Or is my patient better served participating in the testing of an investigational drug? The ability to accurately predict the efficacy of individual treatments in individual patients would greatly improve clinical management as it could form the foundation for evidence-based decision-making regarding the most appropriate treatment. This review will summarize and appraise efforts taken so far to develop tools to predict the risks and benefits of AML therapies for individual patients, focusing on non-transplant treatments.

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4.2 Outcomes of Interest with AML Therapy

Arguably, the most desirable AML therapy is the one that effectively eliminates leukemia cells and restores normal hematopoietic cell function without treatment-associated morbidity and mortality but with maintenance of a high quality of life (QOL). Also arguably, we are far away from having such a therapy available. In fact, even though the value of successful AML therapy was quickly recognized once the first chemotherapeutics for acute leukemia became available [5], the presumption that potential risks are not commensurate with potential benefits often leads physicians and/or patients to shy away from AML-directed therapy, at least for older people. This is indicated by recent estimates that less than half of Americans aged >65 years with newly diagnosed AML, and as few as 10–20% of those aged >80 years, receive specific chemotherapy, and only a minority does so in specialized cancer centers [6, 7].

At least in the era of non-targeted AML therapy, multiagent chemotherapy was felt to be a pivotal component of a curative treatment strategy [2–4]. Although cautious interpretation of findings is warranted, data from the U.S. and European population-based registry data suggest value of intensive chemotherapy not just for younger patients but also for older individuals up to age 80 years or perhaps beyond [7, 8]. Considering that AML primarily affects older people, many of whom will have comorbidities that could limit drug tolerance, it is therefore not surprising that most efforts have focused on developing models to estimate the fitness to tolerate intensive multiagent chemotherapy. With continued improvements in supportive care, however, our abilities to support patients throughout the periods of disease/treatment-related cytopenias have progressively increased and early deaths with intensive chemotherapy have significantly decreased [9–11]. Likewise, non-relapse mortality with allogeneic HCT has substantially declined over time [12]. Thus, intensive therapies can now be given more safely to treat AML, even in older adults. With this, primary failure of AML therapy or disease recurrence after a period of remission—the two outcomes that constitute "therapeutic resistance"—has become the principal life-limiting problem in AML.

4.3 Brief Statistical Considerations

When considering approaches to estimating outcomes with AML therapy, it is important to distinguish between association and prediction (or classification) models. Association models, as the name implies, aim to identify associations between covariates and patient outcomes. Common measures of association in clinical studies are odds ratios and hazard ratios, which are interpreted as an average effect in the study population [13]. In contrast, prediction models aim to evaluate the ability of one or more covariates to predict outcomes for individual patients. Common measures of prediction models are sensitivity, specificity, positive predictive value, negative predictive value, and the area under the receiver operating characteristic curve (AUC) [14]. A strong association is usually necessary but not sufficient for a model to be able to predict well [15]. For a binary outcome (e.g., early death/no early death), the AUC measure can take values between 0.5 and 1.0, with an AUC of 0.5 being analogous to a coin flip and an AUC of 1.0 denoting perfect prediction. It is commonly accepted that AUCs of 0.6–0.7, 0.7–0.8, and 0.8–0.9 indicate poor, fair, and good predictive ability, respectively [16–18].

4.4 Predicting AML Therapy-Related Mortality

Most approaches to predict the toxicity of AML therapy have focused on deaths within 28–30 days (sometimes within 60 days) of beginning chemotherapy. A rationale behind this is the observation that weekly death rates sharply decline after 4 weeks, suggesting patients who die in this time frame are qualitatively different from those who do not [19]. However, considering early death to be equivalent to treatment-related mortality

(TRM) is flawed because deaths may be related to disease-associated myelosuppression or organ dysfunction or—as recently shown in an institutional trial using reduced-intensity CPX-351 [20]—early progression of AML rather than be a direct consequence of the therapy given. Thus, in many patients, early deaths may occur *despite* rather than *because of* the AML therapy. A cleaner way to assess treatment-related toxicities might be to model early deaths with post-remission therapy for patients who have attained full hematologic recovery with prior courses of treatment although the nature of the therapy given and the patients receiving it will limit the conclusions that could be drawn from such models.

Over the years, many factors have been associated with early death, including age and covariates such as albumin and creatinine that may serve as surrogates for biological (rather than chronological) age. Such factors allow building of multicomponent scores reflective of the probability of early death with AML therapy. Several scoring systems aimed at identifying patients at high early death risk after intensive chemotherapy have been developed [19, 21–27]. Some of these systems reach good predictive ability with AUC values above 0.8. While they differ in the details, they all indicate the accuracy of predicting early death is optimized when a combination of factors rather than just one factor (such as age or performance status) is considered. This observation underlies the recommendation by the European LeukemiaNet and the National Comprehensive Cancer Network to consider age in the context of other covariates when considering the appropriateness of intensive AML therapy [3, 28].

Although not perfect, existing models to predict early death offer an empiric approach of selecting patients who will not die early after receiving intensive chemotherapy. It is plausible that models could be improved by integrating additional covariates such as comorbidities not captured in current models, additional information on patient demographics (e.g., educational level), site of treatment, among others [4]. To what degree comprehensive geriatric assessments could improve early death predictions after AML therapy is currently unknown but important to determine. It is becoming more and more clear that geriatric assessments provide a framework for an individual patient's fitness for therapy and can help in personalized decision-making [29]. Several studies have demonstrated geriatric assessments provide information that, independently, is associated with survival in older patients with AML [30–32]. and it is possible such information could improve multicomponent early death prediction models. As a consequence of the progressively declining rates of early death with intensive AML therapy and increasing number of available treatments re-calibrating existing systems (to account for changes in the supportive care pattern) and developing new systems is becoming increasingly more challenging as larger and larger datasets of similarly-treated patients will be required to model early death mathematically.

4.5 Predicting Non-fatal Toxicities of AML Therapy

In contrast to the many efforts spent on trying to predict early death after intensive AML therapy, understanding the degree to which non-fatal toxicities can be predicted has not been of major interest, and it is not understood which patient characteristics are most strongly associated with occurrence of such toxicities. One recent study has examined these questions using data from 260 adults age 18–60 years with AML treated with 7+3 on a contemporary cooperative study group (SWOG) Phase 3 trial [33]. The following baseline covariates were assessed: age at study registration, gender, performance status, pre-study white blood cell (WBC) counts, pre-study platelets counts, pre-study hemoglobin (HGB), pre-study bone marrow blast percentage, secondary vs. de novo AML, cytogenetic risk, and *NPM1* as well as *FLT3*-ITD mutation status. In univariate models, no individual covariate was a strong predictor of toxicity. Only three pairs of toxicity/covariate had an AUC >0.65: older age predicting increased risk of endocrine

abnormalities (AUC = 0.67), higher baseline WBC predicting increased risk for bleeding (AUC = 0.67), and higher baseline HGB predicting increased risk of neurologic toxicity (AUC = 0.69). As incidence allowed, multivariable models were evaluated which showed increased AUCs compared to univariate models, but no multivariable model had an AUC larger than 0.70. Within the limitation that not all covariates important to predict toxicities may be captured in cooperative group datasets and that patients with significant organ dysfunction were excluded from trial participation, these findings indicate that there is a poor ability to predict commonly occurring grade 3 and higher toxicities that occur with multiagent AML chemotherapy.

4.6 Predicting the Efficacy of AML Therapy

At the cohort level, many disease characteristics, in particular cytogenetic and molecular abnormalities, have been associated with measures of therapeutic efficacy, e.g., achievement of complete remission (CR), relapse rates, event/disease-free survival, or overall survival [2–4]. Forecasting efficacy of therapy for individual people with AML, on the other hand, has proved relatively difficult. Using data from over 4500 adults treated with conventional intensive AML chemotherapy, it was found that there was only a fair ability to predict failure to achieve CR with the initial 1–2 courses of chemotherapy or to have a short relapse-free survival if CR was obtained. Various models that included basic patient characteristics (age, performance status) and commonly available disease characteristics (white blood cell count, secondary disease, cytogenetic risk, and *NPM1* as well as *FLT3*-ITD mutation status) had AUCs typically ranging from 0.71 to 0.78 [34]. This finding of only a fair ability to predict CR is consistent with a study by Krug et al. who observed AUCs of 0.72 and 0.68 with multivariable models in their study cohort [24]. These relatively low AUCs suggest caution to avoid overestimating our ability to predict resistance following standard therapy of AML, which is closer to a coin-flip than certainty in many instances when commonly utilized factors are considered.

To some degree, inclusion of additional disease characteristics can refine prediction of therapeutics. For example, data from a larger number of additional commonly occurring mutations improved the predictive accuracy of simpler models minimally (from AUCs of 0.70–0.76 to 0.72–0.80 in a cohort of 298 patients treated uniformly on a cooperative study trial) [35]. Moreover, a score derived from expression data from 17 genes associated with stemness of leukemia cells (17-gene LSC score, “LSC17”)—yielding an AUC of 0.78 for the prediction of failure to achieve CR after initial induction therapy—improved a multicomponent prediction model for this endpoint from an AUC of 0.73 to an AUC of 0.82 [36]. However, it is likely that even highly sophisticated genetic models will come short of high accuracy. Data from a comprehensive genetic analysis of over 1500 patients suggested that genomic features—while being the most powerful predictors—accounted for only about two thirds of the observed variation in survival. One third of this variation was attributed to demographics, clinical, and treatment variables [37].

4.7 Predicting the QOL Impact of AML Therapy

QOL is severely reduced in people diagnosed with AML and is affected over time as a patient goes through AML-directed therapy successfully or unsuccessfully [38]. Current evidence indicates that different treatments will affect the QOL in different ways. For example, QOL may further decrease early after receiving intensive chemotherapy but then improve, whereas it may be stable initially with non-intensive treatment but worsen over time. QOL considerations therefore need to play an important role in the daily care of AML patients. Undoubtedly, QOL is linked to treatment toxicities and efficacy, but there will be some elements that are independent. For example,

for two therapies that are equally toxic and effective, there might be strong preference to receive this treatment at home (e.g., as oral medication) or in the clinic rather than requiring administration in the hospital. To date, no efforts have been made to predict QOL (or, rather, changes in QOL) with different types of AML therapy. One barrier to modeling QOL endpoints in AML is the lack of a disease-specific QOL instrument that can efficiently capture the major QOL deficits in this population. Efforts to correct this deficiency are ongoing [39].

4.8 Outcome Prediction in the Era of Targeted AML Therapy and Rapidly Evolving Treatment Algorithms

With recent regulatory approval of several small molecule inhibitors and one antibody–drug conjugate, we have now entered the era of targeted therapy in AML. Unlike the treatments that target PML-RARA in acute promyelocytic leukemia (APL), however, so far none of the existing targeted AML drugs has near-perfect efficacy even in patients selected by the presence of documented abnormalities in the drug target. In a disease as heterogeneous as AML where genetic abnormalities typically appear to work in concert rather than single handedly to drive the leukemic process, they never may. Thus, having good tools available that can help select individual therapies will remain as important as it is today. To what degree relevant outcomes with targeted AML therapies can be predicted is not known. It will take a considerable amount of time to gather large-enough datasets that allow development and validation of such models.

Since outcome prediction models are reflective of the time when they were developed, capturing not only the anti-AML therapy given but also the supportive care provided, they are—by default—outdated at the time they are introduced into clinical use. That is true even if the general therapeutic strategy does not substantially change. Fortunately, after many years of no change, we have now seen rapid introduction of several new drugs for AML. With every additional drug approved for clinical use, there will be more treatments and treatment combinations available to choose from. As seen today by the shift away from low-dose cytarabine or azanucleoside monotherapy to lower-intensity doublet (or triplet) therapies, some treatments may become quickly obsolete and replaced by others. Constant shifts in treatment paradigms pose a real challenge for physicians and patients interested in empiric approaches to help choose the most appropriate treatment given the time it takes to establish a validated treatment selection tool. Rather than estimating outcomes with the treatment of interest, they may be left with estimating outcomes with "older" treatments and, indirectly, have to use that information to decide whether it is worth pursuing an alternative treatment. Similar to how we might think about deciding between "standard" and investigational therapy.

4.9 Conclusion

There is no shortage in the scoring systems aimed to identify patients at high risk of either early death or treatment resistance after conventional intensive AML therapy. They offer an empiric approach of selecting patients who will do well with standard AML chemotherapy. However, there are important caveats physicians and patients need to be aware of when utilizing these tools. First and foremost, the accuracy of these prediction models is imperfect even at the time of their development, highlighting our limitations in comprehensively capturing and mathematically describing the factors relevant for outcomes of AML therapies. As the rather small improvements in accuracy between relatively simple and complex resistance models indicate, it is very unlikely that we will reach perfect (or near-perfect) prediction accuracy, at least not when trying to forecast the results with conventional AML therapy. Second, scoring systems are likely not agnostic to the type of AML therapy given. Especially at times when newly approved drugs become available for routine use and the standard

of care approach changes, existing models are no longer capturing the clinical reality. It will take great, concerted effort and large patient datasets to refine prediction models based on data derived from patients receiving new standard therapies. And finally, scoring systems are a reflection of factors that mattered at the time the patient received treatment that contributed to the models. Already imperfect at the time of development, the models' accuracy will likely decrease over time with changes in AML care. For example, the rate of early death following intensive induction chemotherapy has declined considerably over the last 20 years because of improvement in supportive care [9–11]. Thus, early death prediction tools—but also resistance prediction tools that are affected by non-leukemia-related deaths—need to be re-assessed and re-calibrated periodically to account for our increasing ability to keep AML patients alive. The task of updating mortality prediction models will become more and more difficult as death rates decline.

Conflict of Interest The author declares no competing financial interests.

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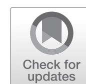

5 Therapy of Newly Diagnosed Acute Myeloid Leukemia (AML)

Anna B. Halpern and Elihu Estey

5.1 Pretreatment Disease Risk Stratification

Much progress has been made in identifying genetic features of AML that may predict resistance to classic cytotoxic therapy (and likely newer "targeted" therapies as well) and thus shape prognosis. These features encompass both classical cytogenetics and the mutational status of various genes. Further distinction is often made between de novo and secondary AML due to prior chemotherapy or following an antecedent hematologic disorder, as discussed in Chap. 3, although much of the prognostic relevance of secondary disease is accounted for by genetic risk [1]. The European LeukemiaNet (ELN) guidelines, most recently updated in 2017, are the most commonly used source for classifying risk of resistance, which even in patients in their 70s is the main cause of death in AML [2]. These guidelines group patients into "favorable," "intermediate," and "adverse" categories. The favorable category encompasses the core binding factor (CBF) leukemias, i.e., those with t(8;21)(q22;q22) resulting in the RUNX1-RUNX1T1 (AML1-ETO) gene and inv (16)(p13.1;q22) or t(16;16)(p13.1;q22) which creates the CBFB/MYH11 fusion gene. Patients with mutated NPM1 with wild-type FLT3-ITD or with FLT3 mutations with low allelic ratios (ratio of mutated to normal alleles <0.5) and those with biallelic mutations in CEBPA are also classified as favorable risk, regardless of other cytogenetic aberrations, although it now appears patients who have the NPM1+/FLT3-negative genotype but adverse cytogenetics have an adverse rather than a favorable prognosis. The intermediate risk category includes those with both a mutated NPM1 and FLT3-ITD with a high allelic ratio, those with wild-type NPM1 with a low-allelic ratio FLT-ITD, those with a t(9;11)(p21.3;q23.3) leading to the MLLT3-KMT2A fusion gene, and cytogenetic abnormalities not otherwise classified. Finally, the adverse-risk category encompasses those with TP53 mutations—perhaps the most dominant adverse-risk factor—the RUNX1 and ASXL1 mutations, along with complex and monosomal karyotypes and a few other gene specific cytogenetic abnormalities (e.g., monosomy 5 and 7). The National Comprehensive Cancer Network (NCCN) also published risk stratification guidelines whose recent 2017 update [3] are largely similar to the ELN 2017 guidelines, with a few differences in the classification of various FLT3-ITD mutated patients and CBF leukemia with the KIT mutation, who are placed in the intermediate-risk group.

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While the ELN 2017 guidelines are widely used, relatively easy to apply with currently available clinical genetic testing at most centers, and have been partially validated in subsequent studies (e.g., Japan Acute Leukemia Group Study patients [4]), there are some limitations. For example, age is not factored into these guidelines, although it does clearly play a role in prognosis, as Ostronoff et al. [5] found that patients aged >65 years with NPM1 mutations and wild-type FLT3-ITD (favorable risk) had poorer outcomes than younger adults in SWOG and MRC/NCRI trials. In addition to age, co-occurring mutations found on next-generation sequencing (NGS) likely interact with mutations in NPM1 and FLT3, for example, thus modifying prognosis. Eisfeld et al. [6] evaluated outcomes of 423 patients aged >60 years treated on Alliance protocols and found that while ELN favorable-risk patients did have longer survival than intermediate/adverse-risk patients, the intermediate and adverse risk had an indistinguishable prognosis. However, when they used an 80-gene NGS panel to more specifically attempt risk stratification, they were better able to risk stratify their study cohort. Similarly, Patel et al. [7] reported that both higher NPM1 mutation burden (measured as variant allele frequency) and the co-occurrence of DNMT3A mutations with NPM1 were associated with shorter EFS and survival.

These findings of the importance of gene–gene interactions demonstrate the significance of incorporating more comprehensive genetic data than what is included in the ELN 2017 guidelines when creating risk categories that then drive treatment decisions. The downside, however, of these large NGS panels is that while initial treatment decisions for AML therapy are usually made within the first few days after diagnosis, and often in community settings, these panels are not yet widely available beyond academic settings and results can take weeks to come back. Notably, however, multiple studies have demonstrated that delay in AML therapy often does not have a deleterious effect on outcome [8, 9]. Finally, although there has been a recent surge of drug approvals for AML, the majority of leukemia-associated mutations that have been identified are not yet targetable by available drugs. Even in cases where drugs are available for single mutations, the duration of response is relatively short, suggesting the future will likely see the use of such drugs in combinations with each other or with chemotherapy.

5.2 Pretreatment Patient Risk Stratification

In addition to the disease-associated factors noted above, patient-specific factors also play an important role in initial therapeutic decisions, especially whether to give an “intensive” or “less-intensive” regimen. With a recently expanding list of approved options with which to treat patients with newly diagnosed AML, in addition to many investigational agents, it is useful to have an “objective” or quantifiable approach to risk prediction. As described more fully in Chap. 4, there are a variety of scoring systems that have been developed whose goal is to identify patients at high-risk for “treatment-related mortality” (TRM) or early mortality following induction chemotherapy [10–14]. Some of these tools focus solely on patient-related factors, with recurring important predictive factors across tools including age, performance status, baseline leukocyte count, serum creatine, and antecedent hematologic disorder, while others combine both patient-specific factors and diseased-specific factors such as cytogenetics, which are more likely a predictor of efficacy rather than short-term toxicity [12].

While age is indeed a common variable in many of these models—and in real-world practice, it often is the primary factor used in treatment determination—the accuracy of each of these models seems to be improved when other covariates are added, underscoring the recommendation by both the ELN and NCCN that age should be considered in the context of other variables when deciding whether to proceed with intensive therapy [2, 3]. Further, with improvements in supportive care, intensive therapies have become safer and the overall TRM rate for AML has decreased over time [15]. In practice, however, even fitter older patients are

frequently offered less intensive therapy (commonly "hypomethylating agents" such as azacitidine or decitabine) rather than more intense therapy, based on the assumption that potential risks of intensive therapy are not commensurate with potential benefits. Indeed, records from large databases suggest that less than half of Americans aged >65 years with AML, and as few as 10–20% of those >80 years, receive intense chemotherapy [16, 17]. Data from population-based AML registries and one retrospective analysis from patients treated at five U.S. cancer centers support the use of intensive rather than low-intensity chemotherapy—in terms of disease-related and survival outcomes—in most AML patients up to age 80 years even with comorbidities [17, 18]. However, retrospective data should be interpreted cautiously since information on exact regimens is often not available and differences in supportive care and selection bias may confound the apparent benefit of intensive therapy. Further, although it is commonly assumed that quality of life (QOL) is better in recipients of non-intensive therapy rather than intensive therapy, recent data from a prospective observational study [19] in older AML patients comparing QOL measures between those treated with intensive vs. non-intensive therapy found QOL scores to be *higher* in the intensive group, supporting the notion that the benefits of better disease control can outweigh the detriment of treatment-related toxicities to QOL. There are data indicating that incorporation of formal geriatric assessment tools (such as the "Get-up and Go Test" and the timed 4-min walk test) into the evaluation of an older patient's health may provide a more nuanced picture of their medical fitness and tolerance of intensive chemotherapy [20, 21].

In sum the choice of therapy should take into account not only disease-specific cytogenetic and mutational data but also patient-specific features including (but not limited to) age, functional status, and organ function. These should ideally be quantified within a risk-prediction model. Taking these steps may help move us away from using age as our primary determinant of initial AML therapy.

5.3 Goal of Induction Chemotherapy

For younger and fitter patients (particularly those with intermediate and adverse risk disease), getting to allogeneic stem cell transplant (HCT), with as little disease and decline in functional status as possible, should be the goal; the intent being cure. For medically less fit, and potentially older patients, a more realistic goal is prolongation of life with the preservation of some measure of quality. But how to translate these goals into objective measures of drug efficacy for empiric evaluation in clinical trials is a challenge.

As in other malignancies, overall survival (OS) is likely the most relevant drug efficacy endpoint in AML. However, death is often delayed even when therapy is unsuccessful. This suggests the use of event-free survival (EFS) rather than OS as a measure of drug efficacy. Earlier-observed endpoints have also been suggested as a means to allow more efficient early-phase drug testing [22]. For many years, complete remission (CR) was regarded as such an endpoint. However, while achieving a CR appears necessary for long-term survival in AML, CR in itself is not sufficient to prolong survival [23, 24]. One potential explanation is that morphologically defined CRs vary widely in quality, with only "high quality" CRs translating into a survival advantage. In particular, the presence of measurable (formerly "minimal") residual disease (MRD) at the time of CR, or CR with incomplete hematologic recovery (CRi)—the latter likely more prone to relapse than the former—is associated with higher relapse and shorter EFS/OS—likely because MRD indicates a poor-quality CR [25, 26]. Indeed, once account is made for response (CR vs CRi) and presence/absence of MRD at CR, pretreatment covariates conventionally predictive of relapse (adverse cytogenetics, secondary AML, newly diagnosed vs. relapsed disease) lose much of their significance. Consequently, the goal of induction therapy in medically fit patients with AML should be attainment of a CR without MRD (with MRD defined by multiparameter flow cytometry and potentially per-

sistent cytogenetic abnormalities, with the contribution of mutational data to MRD evaluation remaining under investigation [27, 28]). The ability to demonstrate improved rates of MRDneg CR may be a more specific measure of efficacy when evaluating new induction regimens. Finally, speaking to the goal of induction therapy for medically less fit patients, there is some limited evidence that QOL is better for those who achieve a CR than those who do not (and thus are more likely to be transfusion dependent and at increased risk for infection and its sequelae); however, this is an area that requires further study.

5.4 Intensity of Induction Chemotherapy

Although 7+3 still remains the most commonly given induction regimen to newly diagnosed AML patients, many attempts have been made to “intensify” this backbone, both via increasing the dose of the anthracycline and the cytarabine, and via incorporation of a third agent—most commonly a nucleoside analog—into the regimen.

5.4.1 Anthracycline Dose and Intensity

There is no definitive evidence that higher doses of anthracycline (e.g., daunorubicin 90 mg/m^2 vs. 60 mg/m^2) is more efficacious, nor more toxic, than lower doses of anthracycline, although 90 mg/m^2 does appear superior to 45 mg/m^2. On the one hand, two well-controlled studies have indicated that escalated doses of anthracyclines given during initial induction chemotherapy can improve response rates and survival [29, 30]. Fernandez et al. randomized 657 adults aged <60 years to daunorubicin 45 mg/m^2 vs. 90 mg/m^2 as part of standard 7+3 and found higher rates of CR and improved OS in the higher-dose group, with similar rates of serious adverse events [29]. Similar results were found by Löwenberg et al. [30] in an older age group (age 60–83 years), although in this case higher remission rates in the higher-dose arm did not translate into a survival benefit. On the other hand, the UK NCRI AML17 trial randomizing 1206 patients treated with 7+3 to daunorubicin 90 mg/m^2 vs. 60 mg/m^2 found no difference in survival (except in the FLT3 ITD-mutated subgroup which appeared to benefit from the higher-dose arm), although there was higher 60-day mortality in the 90 mg/m^2 arm, which may have attenuated long-term benefit [31]. Ultimately, the lack of conclusive data on the benefit of anthracycline dose intensification largely stems from differences in designs of studies (e.g., choice of anthracycline and dose and differences in controls arms and consolidation strategies) that limit clear comparisons between studies.

5.4.2 Cytarabine Dose and Intensity and the Addition of a Nucleoside Analog

There are a few randomized studies asking whether increasing the cytarabine dose in combination with an anthracycline can improve outcomes. A large German study randomized 3375 adults to 7+3 vs. high-dose cytarabine containing therapy and found no difference in 5-year event-free or relapse-free survival (EFS, RFS) [32]. In the SWOG12033 trial (full results not yet published), 739 adults aged <60 years were randomized to standard dose 7+3 (with daunorubicin 90 mg/m^2) vs. idarubicin + high-dose cytarabine (IA; cytarabine dose 1.5 g/m^2 daily × 4 days) and IA + vorinostat (IAV). Although rates of CR were higher in the IA arm after the first course, there were no differences seen in EFS, RFS, or OS. As a limitation, patients receiving IA and IAV received less cytarabine with post-remission therapy than those given 7+3. Further, patients with favorable-risk AML actually did better on the control arm, although this outcome may also have been confounded by their post-remission therapy containing high-dose cytarabine rather than attenuated dosing of induction.

The MRC/NCRI AML 15 trial, on the other hand, involving approximately 3200 newly diagnosed patients with AML suggested that FLAG-Ida (fludarabine, high-dose cytarabine, G-CSF, and idarubicin) is a more effective anti-AML regimen than daunorubicin with standard-dose cytarabine (DA) or DA with addition of etoposide ([ADE]; 1268 patients participated in the direct comparison of FLAG-Ida vs. ADE [33]). This study found that patients randomized to FLAG-Ida had a lower cumulative incidence of relapse than the other regimens (at 3 years: 38% vs. 55%, $p < 0.01$), potentially reflective of more patients achieving CR after 1 course of therapy. Death in CR was more common with FLAG-Ida (17% vs. 11%), thus narrowing the difference in survival [15]. Despite a lack of unequivocal data, high-dose cytarabine containing regimens have, at some institutions, replaced 7+3 as the standard induction regimen for newly diagnosed AML, especially in light of improvements in supportive care and declining rates of TRM.

At the authors' institution, we use GCLAM (G-CSF, cladribine, high-dose cytarabine, and mitoxantrone) rather than FLAG-Ida based on data from the Polish Acute Leukemia Group and our own phase 1/2 trial [34, 35]. GCLAM differs from FLAG-Ida in the use of mitoxantrone rather than idarubicin and, primarily, in the substitution of cladribine for fludarabine. Cladribine is a more active single agent in AML than fludarabine; a Polish randomized trial in 652 adults aged <60 years found that while addition of fludarabine to 7+3 did not improve survival, however addition of cladribine to 7+3 did, with results principally due to superior outcomes in patients with adverse cytogenetics [35]. Single-arm studies from the Moffitt Cancer Center suggest that cladribine plus high-dose cytarabine is more effective than mitoxantrone, etoposide, and cytarabine (MEC) for relapsed/refractory disease [36], and the combination of cladribine, cytarabine, and mitoxantrone has also produced encouraging results in similar patients at Moffitt Cancer Center [37] and in Poland [38]. Nonetheless there are no randomized comparisons of GCLAM with FLAG-Ida or 7+3.

5.5 Newly Approved ("Targeted") Agents

In the past 2 years, the Food and Drug Administration (FDA) approved five new drugs for newly diagnosed AML (midostaurin, gemtuzumab ozogamicin, CPX-351, venetoclax, and glasdegib) along with another three in the relapsed/refractory setting (the IHD1 and 2 inhibitors ivosidenib and enasidenib and the FLT3 inhibitor gilteritinib) that may eventually move into the frontline setting. As the drug labels do not always reflect the populations in which these drugs were initially studied, there remains much to be learned about how to best integrate these drugs into our current treatment pathways, in which areas they might make the most impact, and how best to monitor response.

5.5.1 Midostaurin

Midostaurin—an oral tyrosine kinase inhibitor that is active against the FLT3 internal tandem duplication (ITD) and tyrosine kinase domain (TKD) mutations—was approved by the FDA in 2017 for the treatment of adults with newly diagnosed AML who are positive for the FLT3 mutation, in combination with standard cytarabine and daunorubicin induction and cytarabine consolidation. FLT3 ITD mutations occur in about 25% of patients aged <60 years and in about 15% of older patients, with mutations in the TKD occurring in another 5–10% [39]. Approval was based on a large randomized study by Stone et al. [40] comparing 7 + 3 + Midostaurin to 7 + 3 + placebo in 717 patients aged <60 years with a FLT3 mutation. Midostaurin was dosed at 50 mg orally twice daily (BID) on days 8–21 of induction and consolidation (cytarabine consolidation given at the commonly used dose of 3 g/m^2 BID on days 1, 3, 5) and then as maintenance at the same dose for 1 year. Although CR rates were similar, OS and EFS were longer in the midostaurin group compared to placebo (hazard ratio for death 0.78 for both; $p = 0.009$ for OS and $p = 0.002$ for EFS). Results were not affected by censoring at HCT. Midostaurin was superior regardless of

ITD allelic ratio (high vs. low) or type of FLT3 mutation. The benefit of the maintenance portion of this regimen remains unclear (maintenance therapy has been approved in Europe but not the United States) [41]. Regardless, the addition of midostaurin to 7+3 is now standard therapy for adults aged <60 years with newly diagnosed AML and a FLT3 mutation, although the FDA approval is not age-limited, despite a lack of data in older patients. Notably, while potentially more specific for the FLT3 domain than other multikinase inhibitors [42], midostaurin does inhibit kinases other than FLT3, and thus its multikinase properties could be inhibiting other growth pathways in cancer cells, plausibly providing benefit in patients without this mutation. Rollig et al. [43] demonstrated that the addition of the multikinase inhibitor sorafenib to 7+3 improved EFS compared to placebo in patients aged <60 years, regardless of FLT3 ITD mutation status. It remains to be worked out whether more "potent" and specific inhibitors of FLT3 (e.g., quizartinib and gilteritinib) provide more benefit or whether the less specific multikinase inhibitors are ultimately more effective due to ability to limit the development of resistance to a single mechanism.

5.5.2 Gemtuzumab Ozogamicin

Gemtuzumab ozogamicin (GO, Mylotarg), a CD33 targeting antibody–drug conjugate which delivers a DNA-damaging calicheamicin derivative into the cell, was initially approved in 2000 for CD33+ AML patients aged older than 60 years in first relapse, but then removed from the market in 2010 after a large phase 3 study from SWOG comparing induction with 7+3 vs. 7+3+GO (at a dose of 6 mg/m^2 on day 4) demonstrated a higher induction mortality in the combination arm without improvement of CR rate, disease-free survival, or overall survival [44]. Following the withdrawal, four more randomized trials were done: MRC AML15, ALFA-0701, NCRI AML1642, and GOELAMS AML2006IR [45–47], which demonstrated survival benefit with the addition of GO to induction therapy. In particular, the ALFA-0701 trial, which randomized patients aged 50–70 years to 7+3 with or without GO in fractionated doses of 3 mg/m^2 on days 1, 4, and 7, and demonstrated improvement in EFS from 9.5 to 17.3 months [47], and the NCRI AML17 trial compared GO doses of 3 and 6 mg/m^2 and found lower rates of veno-occlusive disease (VOD) in the lower dose arm without decrement in survival or higher relapse rates [48]. Further, meta-analyses have suggested particular benefit in favorable-risk disease [49]. Correlative studies attempting to identify genetic predictors of response—such as having the rs12459419 CC splice variant of CD33 as demonstrated in the pediatric phase 3 trial AAML0531 [50]—are ongoing.

This large body of data subsequently led GO to be re-approved by the FDA in 2017 for the treatment of both newly diagnosed and relapsed/refractory CD33-positive AML in adults and pediatric patients (Mylotarg prescribing information, 9/2017). It was approved in induction in combination with daunorubicin and cytarabine dosed at 3 mg/m^2 on days 1, 4, and 7 and in consolidation at a dose of 3 mm/m^2 given on day 1. It was also approved as a single agent given as 6 mg/m^2 on day1 and 3 mg/m^2 on day 8 of induction or in "continuation" at 2 mg/m^2 every 4 weeks for up to eight courses.

However, GO is not necessarily the "magic bullet" antibody therapy we have been looking for to revolutionize AML treatment for a variety of reasons. Mechanisms of resistance to GO include low and variable CD33 expression levels on AML, making CD33 a difficult target, part of a broader problem in AML contributing to the lag in development of antibody-based approaches compared to other leukemias such as acute lymphoblastic leukemia (ALL) and some lymphomas [51]. Further, GO is a first-generation antibody–drug conjugate, with technology that was not perfected, and thus about half of the antibody molecules are not labeled with the toxin leading to binding site competition with unconjugated CD33 antibody. Additionally, CD33 is only slowly internalized, leading to limitations in bringing the toxin into the cell, and AML blasts frequently express drug transporters

able to successfully expel the toxin. These shortcomings have led to active development of newer generations of antibody–drug conjugates targeting CD33, such as SGN-CD33A [52], and bispecific antibodies targeting CD33/CD3 such as AMG330 [53, 54], which are being designed to overcome these limitations. Novel and investigational therapies in AML will be further addressed in Chap. 8.

5.5.3 CPX-351

In 2017, the FDA-approved CPX-351 (Vyxeos) for the treatment of newly diagnosed therapy-related AML (t-AML) or AML with myelodysplasia-related changes (AML-MRC). It is a liposomal formulation of cytarabine and daunorubicin at a fixed 5:1 molar ratio that leads to prolonged exposure of AML blasts to the drugs. The approval was based on a randomized trial of 309 patients aged 60–75 years with therapy-related, secondary, or de novo AML with MRC to CPX-351 or standard 7+3 [55]. CR/CRi rates were higher with CPX-351 (48% vs. 33%), there was longer EFS and OS in the CPX-351 arm (HR 0.74, $p = 0.021$ and HR = 0.69, $p = 0.005$, respectively) and similar toxicity and 60-day mortality were observed between arms (13% with CPX-351 and 21% with 7+3). More CPX-351 patients went to HCT (34% vs. 25%) and were more likely to be in CR prior to HCT. Further, transplanted CPX-351 patients had better post-HCT survival than the 7+3 group [56], although this may only reflect that a higher proportion of these patients entered transplant in a CR or that more of them were actually MRD-negative, although MRD rates were not reported in the study. Notably the benefit for CPX-351 vs. 7+3 was limited to patients who had not received prior hypomethylating agents (HMA), although that is a likely frequent past therapy for patients with secondary AML or AM-MRC. Further, although the trial was limited to patients aged 60–75 years, the FDA approved the drug regardless of age. Thus, further evaluation of this drug in expanded clinical scenarios is warranted, including its use in patients who are medically unfit or as a backbone in combination with some of the newer targeted agents (e.g., midostaurin or venetoclax). However, despite the limitations of this initial trial, CPX-351 is currently a reasonable option for fit older patients with newly diagnosed secondary AML.

5.5.4 Venetoclax

Venetoclax is a selective oral inhibitor of B-cell lymphoma 2 (BCL-2), an anti-apoptotic protein that is thought to play an important role in survival of AML blasts and promote resistance to typical AML therapy. In 2018, the FDA approved Venetoclax in combination with azacitidine, decitabine, or low-dose cytarabine (LDAC) for the treatment of newly-diagnosed AML in adults aged >75 years or who have "comorbidities that preclude use of intensive induction chemotherapy." This approval, however, was based on only two non-randomized, single arm, open-label studies. The first, by Wei et al. treated 82 adults aged 60 years and older with untreated AML (prior HMA allowed) who were ineligible for intensive chemotherapy with venetoclax 600 mg daily in combination with LDAC 20 mg/m^2 daily for days 1–10 [57]. They demonstrated a CR rate of 26% and CRi rate of 28% with a median duration of remission of 8.1 months and median OS for all patients of 10.1 months. The 30-day mortality rate was 6%. Similarly, DiNardo et al. [58] evaluated venetoclax at doses of 400–1200 mg daily in combination with either azacitidine or decitabine in 145 patients aged >65 years and ineligible for standard induction chemotherapy for reasons such as: age >75 years, cardiac disease, prior anthracycline use, secondary AML, and "high probability" of treatment-related mortality. They demonstrated a CR rate of 37% and CRi rate of 30%, with 29% of patients achieving MRD negativity at at least one time point. Median time to first response was 1.2 months (range 0.9–13.5) and to best response 2.1 months (range 0.9–13.5), with median duration of response of 11.3 months. With a median follow-up 15.1 months, the median OS was 17.5 months. Similar response rates were seen with either

azacitidine or decitabine and at either venetoclax 400 or 800 mg daily.

Despite the FDA approval, several questions remain about in which settings the drug should be employed. Although patients in both the Wei and DiNardo studies were required to be "ineligible" for intensive therapy, they were required to have a performance status of 0–2 and adequate renal and hepatic function in the DiNardo study, and 71% and 84% of patients enrolled respectively in each study had a PS of 0 or 1. Therefore, it is worth assessing this regimen in an objectively unfit population, as it is likely to be used in real-world situations. Further, it might be worthwhile to evaluate venetoclax in combination with higher intensity therapies such as 7+3 or FLAG-Ida in patients at low risk of TRM or in the consolidation or MRD-positive settings.

5.5.5 Glasdegib

Glasdegib, an oral inhibitor of smoothened (SMO), a key regulator of the Hedgehog pathway, was also approved by the FDA in 2018 in combination with LDAC for newly diagnosed AML patients aged over 75 years or who have comorbidities that preclude intensive induction chemotherapy. This was based on a study by Cortes et al. [59] randomizing 132 patients to Glasdegib 100 mg daily in combination with LDAC (n = 88) vs. LDAC alone (n = 44) and demonstrated CR + CRi rates of 24% in the glasdegib/LDAC arm compared to 5% in the LDAC arm, with a median duration or CR in the combination arm of 9.9 months. Overall survival (the primary endpoint) was found to be longer in the glasdegib arm at 8.8 months compared to 4.9 months in the LDAC-alone arm (hazard ratio 0.51, $p \leq 0.01$). Criticisms of this study include an open-label design without a blinded, placebo-controlled arm, short duration of exposure to LDAC on the LDAC-alone arm, and lower than expected response rates observed in the LDAC-alone arm compared to prior studies. Since randomized trials have shown that LDAC is associated with shorter survival than azacitidine or decitabine, it is possible that the proper control arm for glasdegib + LDAC should be azacitidine or decitabine rather than LDAC. Further, like the venetoclax studies noted above, about 50% of enrolled "unfit" patients actually had performance status of 0–1. Therefore, more studies are needed before the role and true benefits of glasdegib in AML can be determined.

5.6 Overall Recommendation for Initial Induction Therapy

Favorable-risk disease: A 37-year-old, previously healthy woman presents with a leukocyte count of 38 × 10^3/μL with 54% blasts, platelets of 40,000/μL and hemoglobin of 7.8 g/dl. Cytogenetics revealed a t(8;21)(q22;q22) in 20 cells, and NGS testing is unremarkable, including negative for c-KIT.

> We would favor induction with 7+3+GO in this patient, without plan to transplant in CR1 as long as she achieves >3-log reduction in RUNX1-RUNX1T1 transcripts after induction [60].

Intermediate-risk disease: A 55-year-old man with diabetes mellitus and hypertension presents with a leukocyte count of 81 × 10^3/*μL* including 70% blasts, *platelets of 12,000/μL and hemoglobin 8.4 g/dl. Bone marrow confirms AML. Cytogenetics show a normal male karyotype, and NGS testing reveals a FLT3-ITD, NPM1, and DNMT3A. The FLT3 ITD allelic ratio is 1.2.*

> We would give this man 7+3 (with daunorubicin dosed at 90 mg/m^2) for induction in conjunction with midostaurin, along with midostaurin and high-dose cytarabine for post-remission cycles. We favor allogeneic HCT in CR1 with midostaurin maintenance following HCT [61].

Adverse-risk disease: A 78-year-old female with a history of invasive ductal carcinoma of the left breast, who received doxorubicin, paclitaxel and cyclophosphamide, presents with progressively worsening cytopenias down to a leukocyte count of 1.5 × 10^3/*μL, platelets of 20,000/μL and hemoglobin 6.7 g/dl. Bone marrow confirms AML with MRC, cytogenetics reveal a complex karyotype in 16 cells and normal karyotype in four cells, and NGS testing demonstrates a TP53 and*

STAG2 mutation. Medical history includes hypothyroidism, hyperlipidemia, and osteoporosis. She lives in a nursing facility and walks with a walker.

This patient has poor-risk disease and is likely to do poorly with standard chemotherapy, and thus we recommend participation in a clinical trial if possible. One of the more interesting investigational agents in myeloid neoplasms is APR-246, which has been shown to reactivate mutant and inactivated p53 protein. By restoring wild-type p53 conformation and function, the drug is able to induce apoptosis, and a small, early phase clinical trials in humans showed the drug to be very efficacious in combination with azactidine [62]. If the patient did not have access to clinical trials, a hypomethylating agent (azacitidine/decitabine) or LDAC in combination with venetoclax or glasdegib would be a reasonable option.

5.7 Conclusion

Where is the future of therapy for newly diagnosed AML headed? Despite advances in NGS techniques leading to a deeper understanding of AML pathogenesis and prognosis, cytotoxic chemotherapy remains the standard of care for inducing remission in most patients with newly diagnosed AML. However, refinements of this cytotoxic backbone are finally coming to fruition with the expansion of drug options targeting specific mutations and drug resistance pathways. Now that we have many new therapeutic options for this disease, the next challenge is to—through rigorously designed, prospective controlled clinical trials—evaluate in which situations and patient populations they will provide most benefit. Further attention needs be paid to a more precise evaluation of "ineligibility" for intensive chemotherapy and evaluating the benefits of less intense vs more intense therapy in medically less fit patients. And finally, we need better understanding of how genes interact in AML pathogenesis to allow us to more precisely combine our growing arsenal of therapeutic options to translate short-duration remissions into genuine long-term gains in survival.

Conflict of Interest The authors declare no competing financial interests.

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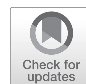

6 Management of Relapsed/Refractory Acute Myeloid Leukemia

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6.1 Introduction

Acute myeloid leukemia (AML) is an uncontrolled clonal proliferation of undifferentiated myeloid stem cells leading to an accumulation of immature myeloblasts [1]. In the United States, the incidence of AML ranges from three to five individuals per every 100,000 [2]. For over the past three decades, the gold standard induction therapy in AML consists of a cytarabine- and anthracycline-based chemotherapy (e.g., “7+3” regimen), resulting in cure rates as high as 45% [3]. However, approximately 25% of patients will fail to respond to induction therapy (refractory AML), and approximately 50% of patients will relapse after transient remission to initial treatment (relapsed AML) [4]. Patients with relapsed and refractory (R/R) AML have poor survival outcomes, with a median survival of less than 6 months [4]. Various factors have been associated with worse outcomes including age greater than 65 years, unfavorable cytogenetics, and a duration of first complete remission (CR1) of less than 12 months [3, 5, 6]. It has been reported that 60% of patients are likely to achieve a second CR (CR2) if the duration of CR1 is greater than 12 months, in contrast to less than 20% in patients with CR1 duration less than or equal to 6 months [7, 8]. Treatment of R/R AML is associated with low CR rates ranging from 0% to 33% after first salvage therapy and further declines with subsequent lines of therapy [9].

The only curative salvage option for R/R patients who achieved CR is hematopoietic stem cell transplant (HSCT). However, due to high risk of transplant-related mortality, many patients are not eligible for this approach. Over the last few decades, additional cytotoxic agents were found to have some benefits, but outcomes were still poor [5, 6]. In recent years, with advancements in understanding the biology of the disease and the prognostic impact of specific gene mutations and chromosomal abnormalities in AML, new treatment modalities have been developed. Novel treatment options including targeted therapies (e.g., Fms-like tyrosine kinase 3 (FLT3) inhibitors, isocitrate dehydrogenase 1 and 2 (IDH1, IDH2), BCL2 inhibitors), monoclonal antibodies (e.g., anti-CD33, anti-CD47, anti-CD123), and immunotherapies have demonstrated promising results with tolerable toxicity profile in R/R AML patients. In this chapter, we will highlight the current management strategies for R/R AML, and we will discuss the novel and investigational agents under development.

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6.2 Intensive Chemotherapy

Intensive induction chemotherapy is often reserved for younger or fit older patients. There are no standard guidelines regarding the optimal regimen to achieve CR prior to allogeneic HSCT. Cytarabine (Ara-C), a pyrimidine analog, plays a crucial role in treating R/R AML and serves as the backbone for most chemotherapeutic salvage regimens. Cytarabine converts to Ara-cytosine triphosphate (Ara-CTP), an active metabolite and interferes with DNA synthesis in the S-phase of the cell cycle. Ara-C has shown to be efficacious as monotherapy when given in high doses (≥1 g/m^2/dose) and in combination with other chemotherapeutic agents (e.g., purine analogs and/or anthracyclines) in the R/R setting [10].

6.2.1 Purine Nucleoside-Based Regimens

Purine analogs, such as fludarabine, cladribine, or clofarabine, are antimetabolites that inhibit ribonucleotide reductase and DNA polymerases, thereby inhibiting DNA synthesis. Although purine analogs are effective as monotherapy, clinical outcomes are improved when combined with Ara-C, with or without an anthracycline for the treatment of R/R AML. Preclinical and clinical studies have demonstrated that fludarabine or cladribine enhances the intracellular concentration of Ara-CTP, resulting in synergistic effects with Ara-C [11, 12]. In addition, in vitro studies have shown that granulocyte colony-stimulating factor (G-CSF) may improve the effect of fludarabine or cladribine-based regimens by stimulating resting-phase leukemic cells into the cell cycle, thereby enhancing cytotoxic activity. GCSF also potentiates Ara-C-induced apoptosis of leukemic cells [13]. Promising salvage options with purine nucleoside-containing regimens are shown in Table 6.1. With these regimens, profound myelosuppression was seen in most patients, with median time to neutrophil (≥0.5 × 10^9/L) and platelet recovery (≥20 × 10^9/L) of approximately 21 and 26 days, respectively [13–17]. Although CR rates greater than 45% are

Table 6.1 Purine nucleoside-containing salvage options for R/R AML

Trials	Regimens	Agents	*N*	Median age, year (range)	CR, %	HSCT, %
Parker et al. (1997) [13]	FLAG-IDA	Fludarabine 30 mg/m^2 days 1–5 Cytarabine 2 g/m^2 days 1–5 Idarubicin 10 mg/m^2 days 1–3 G-CSF 5mcg/kg day 0 until ANC recovery	19	44 (18–72)	63	50
Montillo et al. (1998) [15]	FLAG	Fludarabine 30 mg/m^2 days 1–5 Cytarabine 2 g/m^2 days 1–5 G-CSF 5 mcg/kg day 0 until ANC recovery	38	41 (11–70)	55	41
Wierzbowska et al. (2008) [16]	CLAG-M	Cladribine 5 mg/m^2 days 1–5 Cytarabine 2 g/m^2 days 1–5 G-CSF 5 mcg/kg day 0–5 Mitoxantrone 10 mg/m^2 days 1–3	114	45 (20–66)	53	48
Price et al. (2011) [14]	CLAG	Cladribine 5 mg/m^2 days 1–5 Cytarabine 2 g/m^2 days 1–5 G-CSF 5 mcg/kg day 0–5	162	55 (23–83)	38	36
Becker et al. (2011) [17]	GCLAC	Clofarabine 25 mg/m^2 days 1–5 Cytarabine 2 g/m^2 days 1–5 G-CSF 5mcg/kg day 0 until ANC recovery	50	53 (19–69)	46	50
Jabbour et al. (2012) [128]	BIDFA ± GO	Fludarabine 15 mg/m^2 every 12 h days 1–5 Cytarabine 0.5 g/m^2 every 12 h days 1–5 ± Gemtuzumab 3 mg/m^2 on day 1 G-CSF 5 mcg/kg day 0 until ANC recovery	107	62 (19–85)	23	–

Abbreviations: *CR* complete response, *G-CSF* granulocyte colony stimulating factor, *HSCT* hematopoietic stem cell transplant, *N* number of patients

seen with the combination of purine analogues and Ara-C, a high relapse rate greater than 40% and short remission duration (4–11 months) suggest that HSCT should be pursued as soon as CR is achieved [9].

6.2.2 Other Regimens

Combinations of mitoxantrone, etoposide, and cytarabine (MEC) have been extensively studied in the R/R AML setting. In a single-center, retrospective study, 162 patients were treated with MEC or CLAG (cladribine, Ara-C, GCSF). The overall CR rate was higher in patients receiving CLAG compared to MEC (38% vs. 23.8%; $P = 0.048$), with median overall survival (OS) of 7.3 and 4.5 months, respectively ($p = 0.05$) [14]. Although limited by the small sample size and retrospective nature of the study, results suggest possible superiority with the CLAG regimen. Notably when MEC was compared with CLAG plus mitoxantrone (CLAG-M), 61% (19/31 patients) in the CLAG-M arm compared with 56% (60/108 patients) in the MEC arm achieved CR/CR with incomplete count recovery (CRi) [18]. The median OS was similar in both groups (9.5 vs. 10 months; $p = 0.59$). The median OS was significantly higher post-HSCT of 13 months with CLAG-M and 31 months with MEC. A trend toward improved survival in those treated with MEC followed by allogeneic HSCT was noted.

CPX-351 is a liposomal formulation containing a 5:1 molar ratio of cytarabine and daunorubicin. A phase II, multicenter trial evaluated 125 AML patients in first relapse (median age 52 years in the CPX-351 group and 56 years in the comparator group) who were randomized to receive CPX-351 or investigators' choice of first salvage chemotherapy [19]. Most patients (68%) were classified as poor-risk based on the European Prognostic Index. CR rates were higher in the CPX-351 compared to the control group (all patients: 37% vs. 32%; poor-risk: 29% vs. 21%). Rates of post-induction allogeneic HSCT were similar in both groups, with no difference in survival after transplant. Interestingly, patients with a prior history of HSCT appeared to have poorer outcomes, with 27.3% of patients deceased within 60 days. In patients without a prior history of allogeneic HSCT, similar rates of 30-day mortality were seen between study arms (3.4 vs. 5.4%) However, lower rates of 60-day mortality (10.2% vs. 16.2%) were reported in the CPX-351 arm [19]. Although promising responses were seen with CPX-351 in patients with no prior history of HSCT, higher infection-related events due to delayed hematologic recovery was noted. CPX 351-based combination (e.g., with BCL-2, FLT-3 or IDH2 inhibitors, gemtuzumab) studies are currently ongoing and will be further touched on in subsequent sections (ClinicalTrials.gov Identifier: NCT03826992, NCT04209725, NCT03825796, NCT03904251, NCT03672539).

6.3 Low-Intensity Chemotherapy

Low-intensity chemotherapy including hypomethylating agents (HMA) and low-dose Ara-C (LDAC), doses under 1 g/m^2/dose, have been studied in elderly patients above the age of 60 years, who do not qualify for intensive chemotherapy. Elderly patients cannot tolerate intensive therapies due to the presence of comorbidities, poor performance status, and compromised organ function. In addition, elderly patients typically presents with unfavorable cytogenetics (e.g., abnormalities of chromosome 5 and 7 or complex chromosomal aberrations), presence of dysplastic changes, and secondary AML (includes therapy-related AML and AML evolving from antecedent hematological disorder). All of these factors are associated with increased resistance to treatment and adverse prognosis [20].

6.3.1 First-Generation Hypomethylating Agents

HMAs, such as decitabine and azacitidine, are nucleoside analogs that inhibit DNA methylation. An international multicenter retrospective study evaluated the efficacy of HMA in the R/R AML setting [21]. Of the 655 elderly patients

(median age 65 years) included, 290 (44%) had refractory and 365 (56%) had relapsed disease. Forty-percent of patients had poor-risk karyotypes, and 30% had secondary AML. Although the median OS was 6.7 months, a better OS was observed in responding patients with median survival of 25 and 15 months among those who achieved CR 11% and CRi 5.3%, respectively. In a multivariate analysis, patients with NPM1 and FLT3 mutational status did not significantly impact response or OS [21]. Another study evaluated the increased 10-day schedule of decitabine (20 mg/m^2) in 102 R/R AML patients (median age 66 years) [22]. Of the 102 patients, 16 (15.7%) achieved CR, with a median OS of 6 months. The responses with 10-day cycles of decitabine appear to be slightly better than other low-intensity therapies in the last decade; however, no direct comparison can be made. In addition, there has been inconsistent data suggesting that a 10-day schedule in comparison to a 5-day schedule of decitabine may improve responses in AML patients with unfavorable risk and/or TP53 mutation [23]. Further prospective studies with a larger sample size are warranted to validate these findings in the R/R AML setting. HMAs are a reasonable option for older R/R AML patients who are ineligible for clinical trials. HMA-based combination therapies with BCL-2, FLT-3, or IDH2 inhibitors are ongoing and will be further discussed in subsequent sections.

6.3.2 Second-Generation Hypomethylating Agents

Guadecitabine (SGI-110) is a dinucleotide of decitabine and deoxyguanosine, with longer half-life exposure of its active metabolite (decitabine) due to its resistance to degradation by cytidine deaminase. In a phase II study, 103 R/R AML patients, with a median age of 60 years, were treated with SGI-110. Of the 103 patients, 41% had poor-risk karyotypes and 18% had prior HSCT. The median OS was 6.6 months, with 1- and 2-year OS rates of 28% and 19%, respectively. The median OS was significantly longer in those who obtained CR (23%) compared to those who did not (not reached vs. 5.6 months; $p < 0.01$) [24]. Overall response and survival outcomes were significantly worse in patients with poor-risk cytogenetics compared to those with favorable or intermediate cytogenetics (CR: 19% vs. 26%; median OS: 5.4 vs. 8.3 months; $p < 0.001$). SGI-110 was well tolerated; the most common grade 3–4 adverse events were febrile neutropenia (60%), pneumonia (36%), thrombocytopenia (36%), and anemia (31%). SGI-110 may be a reasonable trial option in R/R AML patients who cannot tolerate intensive chemotherapy [24]. A phase III randomized trial comparing SGI-110 and investigators choice of salvage chemotherapy in R/R AML patient is currently underway (ClinicalTrials.gov Identifier: NCT02920008).

6.3.3 Other Regimens

The combination regimens of either clofarabine or cladribine plus LDAC were developed to improve the outcomes in elderly patients who cannot tolerate intensive chemotherapy. A retrospective study evaluated 16 patients (8 patients with AML, 7 patients with myelodysplastic syndrome (MDS), and 1 patient with chronic myelomonocytic leukemia) who had failed azacitidine therapy. In this study, patients received cladribine (5 mg/m^2/day × 5 days) plus LDAC (40 mg/m^2/day × 10 days) [25]. The patients had previously received a median of 15 cycles of azacitidine (range 4–33). A total of 9 patients (56%) achieved remission, including 6 in CR and 3 patients in CRi. The median OS was 10.6 months, with an estimated 6 and 12 months OS rate of 79.3% and 44.6%, respectively [25]. Another small study evaluated 16 patients, with a median age 58 years, diagnosed with R/R AML who were treated with clofarabine (20 mg/m^2/day × 5 days) plus LDAC (20 mg twice daily × 10 days) [26]. Of the 9 patients with poor-risk karyotypes, 2 patients with mutated TP53 achieved CR. The median OS was 13.25 months [26]. Future studies with larger sample sizes are warranted in order to optimize therapy for high-risk and heavily pretreated R/R AML patients who are ineligible for intensive treatment.

6.4 Hemopoetic Stem Cell Transplant

AML patients with R/R disease typically respond poorly to subsequent chemotherapies with low CR rates and poor survival outcomes [7–9, 27]. Allogeneic HSCT is the only potential curative salvage option for these patients based on its graft-versus-leukemic effect but carries the risk of life-threatening graft-versus-host disease. In a retrospective study, 177 evaluable primary refractory AML patients who have failed induction therapy containing high-dose cytarabine (>1 g/ m^2/dose), received salvage chemotherapy alone ($n = 149$) or allogeneic HSCT ($n = 28$). Patients who underwent allogeneic HSCT had superior median OS compared to chemotherapy alone (15.7 vs. 2.9 months; $p < 0.001$). The 3-year OS was 39% in the allogeneic HSCT arm and 2% in the salvage chemotherapy arm [27]. Due to the superior survival benefit of allogeneic HSCT, it is imperative to offer effective intensive salvage chemotherapy to fit R/R AML patients with a goal to achieve CR followed by allogeneic HSCT. Unfortunately, allogeneic HSCT may not be suitable for many patients due to age and/or concomitant comorbidities. Options for elderly patients include less-intensive chemotherapy (e.g., HMAs, LDAC), palliative strategies, and clinical trials. Encouraging novel therapies including checkpoint inhibitors, targeted therapies, and monoclonal antibodies may increase the duration of the response and decrease the likelihood of relapse.

6.5 Targeted Therapy

6.5.1 FLT3 Inhibitors

FLT3 mutations are the most common genomic alterations in AML, occurring in nearly one-third of cases. FLT3 is a tyrosine kinase receptor expressed on the surface of AML cells that play a role in cell signaling and proliferation. Within the FLT3 domain are two types of activating mutations, the internal tandem duplication (ITD) of the intracellular juxtamembrane and point mutations in the tyrosine kinase domain (TKD) [28]. The FLT3-ITD is present in about 25–30% of cases and has been associated with shorter durations of remission and increased relapse. In comparison, FLT3-TKD mutations are present in up to 10% of cases, with its prognostic relevance remaining unknown. Additional findings have shown that the co-occurrence of nucleophosmin 1 (NPM1) and FLT3-ITD is associated with an improved prognosis [29]. According to the National Comprehensive Cancer Network (NCCN) guidelines for AML, FLT3-ITD mutated patients are stratified into all three different risk categories based on allelic ratio and presence of NPM1 [30].

The discovery of FLT3 inhibitors for the treatment of patients with FLT3 mutation has improved survival outcomes. The first-generation FLT3 inhibitors (midostaurin, sorafenib, sunitinib, lestaurtinib) are multikinase inhibitors with lower affinity for FLT3 and more off-target toxicities. In contrast, second-generation FLT3 inhibitors (crenolanib, quizartinib, gilteritinib) are more specific and potent at inhibiting FLT3 and have fewer toxicities associated with off-target effects. FLT3 inhibitors are also categorized as type I or type II, which determines their activity against FLT3. Type I inhibitors bind to FLT3 receptors in the active conformation, near the activation loop or the adenosine triphosphate (ATP) binding site, and are active against FLT3-ITD and FLT3-TKD mutations. Type II inhibitors bind to FLT3 receptors in the inactive conformation, adjacent to the ATP binding domain, and are only active against FLT3-ITD mutations [31]. Although a number of FLT3 inhibitors have been studied and utilized for the treatment of FLT3-mutated AML, only midostaurin and gilteritinib have received the United States Food and Drug Administration (US FDA) approval for induction therapy in combination with "7+3" in FLT3-positive AML and FLT3-positive R/R AML, respectively. A summary of promising studies in FLT3-positive AML can be seen in Table 6.2 and are discussed below.

Gilteritinib is a potent type I FLT3 and AXL inhibitor that was rationally designed to overcome resistance seen with other FLT3 inhibitors.

Table 6.2 FLT3 containing salvage options for R/R AML

Trials	Regimens	N	Median age, year (range)	CR + CRi, %	OS, months
Ravandi et al. (2013) [37]	Sorafenib 400 mg twice daily Azacitidine 75 mg/m^2 days 1–7	43	64 (24–87)	43	6.2
Muppidi et al. (2015) [39]	Sorafenib 200/400 mg twice daily Decitabine 20 mg/m^2 days 1–10	6	56 (33–70)	83	5.2
Strati et al. (2015) [43]	Azacitidine 75 mg/m^2 days 1–7 Midostaurin 50 mg twice daily days 8–21	54	62 (21–85)	26	5.1
Cortes et al. (2016) [44]	Crenolanib 100 mg three times daily	57	–	31–39	3.1–7.8
Cortes et al. (2019) [41]	Quizartinib 40 mg daily vs. MEC, FLAG-IDA, LDAC, HMA	367	55 (46–65)	48 vs. 27	6.2 vs. 4.7
Perl et al. (2019) [33]	Gilteritinib 120 mg daily vs. MEC, FLAG-IDA, LDAC, HMA	371	62 (19–85)	34 vs. 15.3	9.3 vs. 5.6

Abbreviations: *CR* complete response, *CRi* complete response with incomplete hematologic recovery, *FLAG-IDA* fludarabine + cytarabine + idarubicin + granulocyte colony stimulating factor, *HMA* hypomethylating agent, *LDAC* low-dose cytarabine, *MEC* mitoxantrone + etoposide + cytarabine, *N* number of patients, *OS* overall survival

Overexpression of AXL is commonly seen after treatment with FLT3 inhibitors and is a known mechanism of resistance [32]. Gilteritinib was FDA approved based on the randomized phase III (ADMIRAL) study in R/R AML [33]. A total of 371 R/R FLT3-mutated AML patients were randomly assigned to receive gilteritinib 120 mg/day or salvage chemotherapy (MEC, FLAG-IDA, LDAC, or azacitidine) in a 2:1 ratio. Gilteritinib was associated with an improved OS of 9.3 months compared to 5.6 months with salvage chemotherapy ($p < 0.001$). Additionally, a slight trend toward improved OS was seen with gilteritinib across all subgroup analyses, especially in patients with both DNMT3A and NPM1 mutations (median OS of 10.8 months). More patients in the gilteritinib group achieved CR at 21.1% compared with 10.5% in the chemotherapy group. Although, more patients in the gilteritinib group underwent allogeneic HSCT (25.5% vs. 15.3%), the OS benefit was maintained when survival data was censored at the time of HSCT. Gilteritinib was well tolerated and with a lower incidence of adverse events in comparison to the salvage chemotherapy arm. The most common gilteritinib-related adverse events were, pyrexia (42.7%), increased alanine aminotransferase (41.9%), and increased aspartate aminotransferase (40.2%). [33] Ongoing trials are being conducted in the R/R setting combining gilteritinib with venetoclax, and in newly diagnosed AML in combination with azacitidine as well as in comparison to midostaurin when given with "7+3" induction therapy (ClinicalTrials.gov Identifier: NCT03625505, NCT02752035, NCT03836209).

Sorafenib is a type II FLT3 inhibitor that has limited activity as monotherapy, with bone marrow remission achieved in <10% of patients [32]. A number of studies have shown more promising results when sorafenib is used in combination with HMAs in the R/R setting [34–36]. Sorafenib in combination with azacitidine demonstrated an overall response rate (ORR) of 46%, with 27% achieving CRi and 16% achieving CR. Although the median duration of CR/CRi was only 2.3 months, 85% of patients were able to achieve adequate FLT3 inhibition within one cycle of therapy [37, 38]. In a small case series of 6 patients with R/R AML treated with sorafenib plus decitabine, 5 patients (83%) had a response and 4 of the 5 patients (80%) achieved CRi [39]. Sorafenib is currently not FDA approved for the treatment of FLT3-mutated R/R AML. Given the promising results, it is listed as an option in the NCCN guidelines to be used in combination with

HMA in individuals who are not candidates for intensive chemotherapy [30, 40].

Quizartinib is a more potent and selective type II FLT3 inhibitor that has strong activity as monotherapy in the R/R setting, in which composite CR rates greater than 40% have been reported [32]. In a randomized phase III (QuANTUM-R) study, 367 FLT3-mutated R/R AML patients received quizartinib or salvage chemotherapy (MEC, FLAG-IDA LDAC) in a 2:1 ratio. With a 23.5-month follow-up, the OS was 6.2 months with quizartinib compared with 4.7 months with salvage chemotherapy ($p = 0.02$). The most prevalent adverse event seen in the quizartinib group was QTc prolongation, with a 3% (central reading) incidence [41]. Despite this evidence, quizartinib was not granted an FDA approval in the USA, but received approval for use in Japan. A phase I/II study evaluating the use of quizartinib in combination with low-intensity chemotherapy (azacitidine or LDAC) in 52 R/R AML patients has shown an ORR of 67%, with 38% ORR in patients with prior FLT3 use. A median OS of 14.8 months was reported when combined with azacitidine, and 7.4 months with LDAC, suggesting this combination has benefit in FLT3-ITD mutated AML patients [42]. Currently, a number of studies are underway in the R/R setting including quizartinib in combination with FLAG-Ida, venetoclax, decitabine with venetoclax or cladribine, idarubicin, and cytarabine (ClinicalTrials.gov identifier: NCT04112589, NCT03735875, NCT03661307, NCT04047641). In the frontline setting, quizartinib is being studied in combination with either standard care chemotherapy or in combination with CPX-351 (liposomal cytarabine and daunorubicin) (NCT04047641, NCT04128748).

Midostaurin and crenolanib are both type I FLT3 inhibitors known for their efficacy when used in the frontline setting of FLT3-mutated AML patients. Both have data to show efficacy in the R/R setting as well. Midostaurin was studied in a phase I/II trial with 54 R/R AML patients in combination with azacitidine and found to have an ORR of 26% (CR + CRi + morphologic leukemia-free state (MLFS) = 24%; partial response (PR) = 2%) [43]. Crenolanib was studied in the R/R setting as monotherapy in 36 patients who had been treated previously with a FLT3 inhibitor and found to have an ORR of 31% [44]. Crenolanib is being studied in a phase III trial with and without chemotherapy in the R/R setting (ClinicalTrials.gov identifier: NCT02298166), as well as in the frontline setting in combination with standard chemotherapy (ClinicalTrials.gov identifier: NCT02283177) [45, 46].

6.5.2 IDH Inhibitors

Isocitrate dehydrogenase (IDH) mutations occur in the arginine residue catalytic pathway, resulting in abnormal production of oncometabolite R-2-hydroxyglutarate (2-HG) levels, leading to abnormal methylation of histones in DNA, triggering improper cell differentiation [47, 48]. IDH1 (IDH1-R132) and IDH2 (IDH2-R172 and IDH2-R140) mutations are seen in 20% of AML and are known to be mutually exclusive [49, 50]. IDH mutations are commonly seen in elderly patients and those with normal or intermediate-risk cytogenetics and often co-occur with NPM1 and FLT3-ITD mutations [50, 51]. The prognostic relevance of IDH mutations in AML remains controversial. However, when looking closely at the types of IDH alterations, IDH1 leads to a more negative prognosis, while prognosis conferred by IDH2 varies based on the subtype. IDH2-R127K infers high sensitivity to chemotherapy, similar to a double-mutant CEBPA, whereas an IDH2-R140 mutation has a more neutral impact that can vary based on co-mutations [52]. Currently two mutant IDH inhibitors, ivosidenib (IDH1 inhibitor) and enasidenib (IDH2 inhibitor), are FDA approved for the treatment of IDH-mutated R/R AML. Table 6.3 summarizes published and up-and-coming studies in the realm of IDH-mutated R/R AML therapy.

Enasidenib is an oral selectively allosteric inhibitor of IDH2, which is located in the mitochondria of cells [53]. Enasidenib received FDA approval for use as monotherapy in R/R AML with IDH2 mutations after a successful phase I/II clinical trial demonstrating an ORR of 40.3% and

Table 6.3 IDH inhibitor containing salvage options for R/R AML

Trials	Regimens	*N*	Median age, year (range)	CR + CRi, %	OS, months
Stein et al. (2017) [54]	Enasidenib 100 mg daily	239	70 (19–100)	40.3	5.8
Dinardo et al. (2018) [57]	Ivosidenib 500 mg daily	179	68 (18–89)	42.2	9.3 *(all arms)*
Tallman et al. [56] Phase III, NCT02577406	Enasidenib 100 mg daily vs. HMA, LDAC, Int-dose Ara-C, BSC	–	–	–	8.0 vs. 5.0
Watts et al. [58] Phase I/II, NCT021719574	FT-2101 daily ± Azacitidine days 1–7	35	–	38 *(alone)* 27 (+*Azacitidine*)	–
Phase II, NCT04044209	Ivosidenib 500 mg daily Nivolumab 480 mg day 1 (cycle 2+)	–	–	–	–
Phase I/II, NCT03471260	Venetoclax days 1–14 Ivosidenib days 15–28, then daily ± Azacitidine days 1–7	–	–	–	–

Abbreviations: *BSC* best supportive care, *NCT* ClinicalTrials.gov Identifier, *CR* complete remission, *CRi* complete remission with incomplete hematologic response, *HMA* hypomethylating agent, *Int-dose Ara-C* intermediate dose cytarabine, *LDAC* low-dose cytarabine, *N* number of patients, *OS* overall survival

a median OS of 9.3 months [54]. Approximately 87.3% of patients reached their first response within 5.8 months; for this reason, enasidenib is recommended to be used for at least 6 months. The most common treatment-related adverse events were elevated bilirubin (81%), nausea (50%), diarrhea (43%), and vomiting (34%). Differentiation syndrome, characterized by fever, hypotension, and leukocytosis, occurred in 14% of patients [55]. A phase III randomized study evaluating the safety and efficacy of enasidenib compared to conventional regimens in elderly patients with IDH2-mutated R/R AML is currently ongoing (ClinicalTrials.gov identifier: NCT02577406). In an interim report, longer median OS was reported with enasidenib compared to conventional chemotherapy (8 months vs. 5 months) [56]. Enasidenib is also being studied in combination with azacitidine in patients with R/R AML and IDH2 mutation (ClinicalTrials.gov identifier: NCT03683433) and in the frontline setting in combination with induction therapy (ClinicalTrials.gov identifier: NCT02632705).

Ivosidenib is an oral inhibitor of mutated IDH1, located in the cytoplasm of cells [53]. Ivosidenib received FDA approval for use as monotherapy in R/R AML with IDH1 mutations after a phase I/II clinical study showing an ORR of 41.6% including a CR rate of 21.6%. The median duration of CR was 9.3 months, and median duration of response was 6.5 months [57]. The most common treatment-related adverse events were diarrhea (30.7%), leukocytosis (29.6%), prolonged QT interval (24.3%), and peripheral edema (21.8%). Differentiation syndrome occurred in 11% of patients. Ongoing studies are evaluating the use of ivosidenib in combination with a checkpoint inhibitor, nivolumab, and a BCL2 inhibitor, venetoclax, with or without azacitidine for the treatment of R/R AML (ClinicalTrials.gov identifier: NCT04044209, NCT03471260). In the frontline setting, ivosidenib is being studied in combination with azacitidine with and without venetoclax (ClinicalTrials.gov identifier: NCT03471260, NCT02677922).

Currently a number of additional IDH1, IDH2, and pan-IDH inhibitors are being studied in phase I/II clinical trials showing promising responses and side effect profiles. FT-2101, an IDH1 inhibitor, is being studied as monotherapy

and in combination with azacitidine in R/R AML, showing ORR of 38% and 27%, respectively [58]. Vorasidenib, also known as AG-881, is a pan-IDH1/2 inhibitor currently being studied for safety and efficacy in a phase I clinical trial (ClinicalTrials.gov identifier: NCT0242737) [59].

6.5.3 BCL-2 Inhibitors

BCL-2 is an anti-apoptotic protein that regulates the mitochondrial pathway by maintaining a proper balance between signal proteins that trigger apoptosis. By inhibiting the BCL-2 protein on the BH4 domain, pro-apoptotic proteins are released, thereby triggering apoptosis of the tumor cell [60]. BCL-2 overexpression in AML has been associated with poor survival outcomes and an increased resistance to chemotherapy [61]. One of the first BCL2 inhibitors discovered was navitoclax. It has yet to be FDA approved, but preclinical data shows promising outcomes in leukemia. Navitoclax is a BCL-2 and BCL-XL inhibitor that is known to cause thrombocytopenia, likely due to the BCL-XL inhibition, which limited the maximum dose tolerated by patients. The need to overcome this toxicity has led to the development of a highly selective BCL-2 inhibitor, venetoclax, with hope to decrease toxicity and increase clinical efficacy [60, 61]. Venetoclax is well-established for its efficacy and safety in chronic lymphocytic leukemia, mantle cell lymphoma, and more recently, in newly diagnosed older AML patients [62–64]. Published data of venetoclax in the R/R setting is limited [62]. In a phase II study, 32 R/R AML patients treated with venetoclax monotherapy with a dose of 800 mg a day demonstrated an ORR of 19%, with 6% achieving CR and 13% achieving CRi [65]. The most common adverse events included nausea (59%), diarrhea (56%), vomiting (41%), and febrile neutropenia. A number of retrospective analysis of R/R AML patients receiving venetoclax in combination with an HMA showed an ORR of 40–60%, which is higher than responses seen with HMA or venetoclax alone [66, 67]. Currently, a randomized clinical trial is being conducted to evaluate the efficacy of venetoclax in combination with decitabine in the R/R setting (ClinicalTrials.gov identifier: NCT03404193).

6.6 Immunotherapies

Harnessing the power of the human immune system is a relatively new concept that has exploded in the world of cancer care. One of the unique resistance pathways of cancer, leukemia included, is the ability to evade the immune system [68]. Upon understanding the potential benefits of the immune-mediated graft-versus-leukemia response after an allogeneic HSCT, researchers have found mechanisms to upregulate the immune system to more easily target cancerous cells to achieve a similar response. In recent years, various immune-mediated drug mechanisms have been studied, including the ability to upregulate effector T-cell activity and the creation of monoclonal antibodies. Monoclonal antibodies display an array of mechanisms of action within its class including the ability to block activation signals for cell growth, trigger complement-mediated or antibody-dependent toxicity, and the ability to enhance the anti-tumor response of the cell. [69] A summary of pertinent and promising studies utilizing monoclonal therapy can be seen in Table 6.4. In this section, we will discuss the various therapies currently available that illustrate these treatment modalities and the promising therapies that continue to be actively studied in AML.

6.6.1 Checkpoint Inhibitors

Numerous checks and balances exist in the immune system that allow for appropriate physiological immune responses to unwanted stimuli. Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1) are checkpoint proteins commonly seen on active T cells. When these proteins adhere to ligands either on antigen-presenting cells (CTLA-4 binding to CD80/CD86) or cancer cells (PD-1 binding to PD-ligand 1), antitu-

Table 6.4 Monoclonal Antibody containing salvage options for R/R AML

Trials	Target	Regimens	*N*	Median age, year (range)	CR + CRi, %	OS, months
Sievers et al. (2001) [82]	CD33	Gemtuzumab 9 mg/m² every 2 weeks	142	61 (21–84)	26	12.6
Taksin et al. (2007) [84]	CD33	Gemtuzumab 3 mg/m² on days 1, 4, 7	57	64 (22–80)	33	8.4
Ravandi et al. [91] Phase I, NCT02520427	CD3/CD33	AMG-300240 μ/day on days 1–14	35	58 (18–80)	6	–
Subklewe et al. [93] Phase I, NCT03224819	CD3/CD33	AMG-673 for 2 days	30	67 (25–84)	–	–
Zeidan et al. (2019) [94]	CD47	CC-90002 once weekly for 4 weeks	24	70 (28–85)	–	–
Phase I, NCT03248479	CD47	Hu5F9-G4 twice weekly Azacitidine days 1–7	–	–	–	–
Phase I, NCT03113643	CD123	SL-401 every 4 weeks Azacitidine days 1–7 ± Venetoclax days 1–21	–	–	–	–
Daver et al. (2019) [104]	CD123	IMGN632 0.015–0.45 mg/kg on days 1, 4, 8	74	69 (33–83)	20	–
Aftimos et al. (2017) [107]	CD70	ARGX-110 2 mg/kg on day 1	26	60 (22–78)	SD	–

Abbreviations: *BSC* best supportive care, *NCT* ClinicalTrials.gov Identifier, *CR* complete remission, *CRi* complete remission with incomplete hematologic response, *N* number of patients, *OS* overall survival, *SD* stable disease

mor response from the immune system is suppressed. Therefore, inhibition of CTLA-4 and PD-1 results in active T-cell proliferation and immune-mediated tumor response [70]. Preclinical data available showed an upregulation of CTLA-4 and PD-1 in AML patients inferring sensitivity to CTLA-4 inhibitors such as ipilimumab and PD-1 inhibitors such as pembrolizumab and nivolumab [71].

In the realm of solid tumors, immune checkpoint inhibitors have well-established data on its safety and efficacy [72–74]. More recently, these concepts have been explored in leukemia. In a phase I/IB multicenter, investigator-initiated study, 28 patients with R/R AML post HSCT received ipilimumab at a dose of either 3 or 10 mg/kg. Of the 28 patients included, 22 patients were treated with high-dose ipilimumab (10 mg/kg) resulting in CRs in 5 patients (23%) [75]. Similar outcomes were seen in a phase IB/II study of nivolumab in combination with azacitidine in 51 R/R AML patients. Six patients (18%) achieved CR/Cri, and 9 patients (26%) had a reduction in BM blasts by 50% [76]. In a phase II non-randomized study, 70 patients with R/R AML received a combination of azacitidine and

nivolumab. The ORR was 33%, including 22% in CRi [77]. What was most profound was the OS seen in patients receiving this therapy as their first salvage, which made up 50% of the study population. The OS seen was 11 months, nearly double the most OS seen with azacitidine alone. Common immune-related adverse events seen with checkpoint inhibitors were pneumonitis, nephritis, and rash. These symptoms were resolved in nearly all incidences with a course of high-dose steroids. A number of studies evaluating the safety and efficacy of checkpoint inhibitors including atezolizumab, an anti-PD ligand antibody (ClinicalTrials.gov identifier: NCT03154827, NCT03730012), nivolumab in combination with ipilimumab (ClinicalTrials.gov identifier: NCT03600155), and nivolumab in combination with azacitidine with or without ipilimumab are presently ongoing (ClinicalTrials.gov identifier: NCT02397720).

6.6.2 CD33 Monoclonal Antibodies

6.6.2.1 Gemtuzumab Ozogamicin

CD33 is a transmembrane cell surface glycoprotein receptor on a majority of myeloblast cells [78]. An anti-CD33 monoclonal antibody conjugated to a cytotoxic derivative of calicheamicin known as gemtuzumab ozogamicin (GO) was approved in 2000 and then removed from the market in 2010 due to lack of efficacy seen in confirmatory studies versus risk of harm, with increased incidences of veno-occlusive disease (VOD) [79–81]. However, over the years, strong data has been further established for its safe and efficacious use in AML, both in the frontline and relapsed setting. The first study that showed the efficacy of GO was a phase II study conducted in 2000. Patients were treated with GO utilizing a higher dose, 9 mg/m^2/dose, than the currently approved 3 mg/m^2/dose [82]. The study showed an ORR of 26% and a median OS of 12.6 months. Additional studies done utilized the fractionated dose of GO (3 mg/m^2/dose) and found similar outcomes. The rationale behind this is twofold, with the ultimate goal of decreasing maximum concentrations (Cmax) associated with toxicity, while maintaining a proper area under the curve (AUC) for efficacy. First, it was found that the fractionated dose resulted in 90% saturation of CD33. Additionally, regardless of the dose, CD33 re-expression occurred on the cell surface 72 hours after exposure to GO. Therefore, re-dosing the medication would result in greater efficacy, produce a larger AUC and reduce the Cmax, thereby avoiding toxicity [83].

A study conducted in 2007 that utilized this lower dose was MyloFrance-1, an investigator-initiated phase II study utilizing the fractionated dose of 3 mg/m^2/dose in CD33 positive AML patients in first relapse. Among the 57 patients evaluated, 33% had a response with a median OS of 8.4 months [84]. No incidences of VOD were reported with the lower doses, including those who underwent HSCT 90 days after therapy. Overall, adverse effects seen with GO included hyperbilirubinemia, transaminitis, and cytopenias [82, 84]. Additionally, a pilot study evaluated the safety and efficacy of CPX-351 in combination with GO in CD33-positive R/R AML and post-HMA failure high-risk MDS patients. Of the 10 evaluable patients, 5 patients (50%) achieved CR/CRi (4 CR, 1 CRi), including 2 patients with negative minimal residual disease (MRD) at CR. With a median follow-up of 6.1 months, the 6 month OS rate was 79% and the median OS had not been reached. A meta-analysis showed that patients with favorable cytogenetics had the highest response to GO [85, 86]. Continued studies are underway to find the subset of patients most suitable for GO in the R/R setting.

6.6.2.2 CD33-CD3 Bispecific T-Cell Engager (BiTE)

The concept of bispecific T-cell engager (BiTE) technology incorporates the activity of the host T cells in combination with a targeted monoclonal antibody [87]. AMG-330 is a BiTE therapy that binds to CD33 on leukemic cells and CD3 on T cells [88]. In preclinical studies, AMG 330 was shown to have higher activity against newly diagnosed AML specimens and those with favorable risk disease. Additionally, when given after chemotherapy agents that increase CD33 expression,

such as azacitidine and panobinostat, AMG 330 was found to have higher activity on those leukemic cells [89, 90]. Currently, a phase I study is underway, with promising tolerability and activity in R/R AML patients. Of the 35 patients included, two patients achieved CR at the target dose of 240 μg/day which was administered as a continuous infusion for 14 days, one patient in CRi and one patient in MLFS. The side effect profile was also tolerable, with cytokine release syndrome (CRS) being the highest incidence of toxicity, mitigated with steroid use, tocilizumab, and/or drug interruption. Other side effects seen include leukopenia, thrombocytopenia, and subdural hematomas [91]. The idea that PD-1/PD-L1 expression is upregulated with AMG 330 prompted the concept that combining checkpoint inhibitors with AMG 330 may result in an effective mechanism of therapy [92]. Currently no studies exist with the combination of a checkpoint inhibitor and AMG 330. For now, the phase I study of AMG 330 is ongoing with hopes of finalizing outcomes in the years to come (ClinicalTrials.gov identifier: NCT02520427). Another phase I study has looked at AMG 673, an extended half-life CD33-CD3 BiTE, which is dosed as two short and intermittent infusions during a 14-day cycle. This study looked at 30 patients with R/R AML, with a median age of 67.5 years, and showed a decrease in bone marrow blasts in 44% of patients, with one patient achieving CRi. Similar to AMG 330, CRS was also seen with its use, along with abnormal hepatic enzymes, leukopenia, and febrile neutropenia (ClinicalTrials.gov identifier: NCT03224819) [93].

6.6.3 CD47 Monoclonal Antibody

CD47 is a transmembrane protein that is overexpressed in various malignancies including AML and MDS. CD47 interacts with signal-regulatory protein alpha (SIRPα) expressed on macrophages to inhibit phagocytosis. Inhibition of this interaction with anti-CD47 monoclonal antibody enables macrophage-mediated killing of leukemic cells [94, 95]. In preclinical studies, anti-CD47 has demonstrated antibody-mediated phagocytosis of leukemic cells and rapid reduction of tumor burden in AML xenograft models. In a phase I multicenter study, 24 patients with R/R AML and 4 patients with high-risk R/R MDS (median age 70 years) received anti-CD47 monoclonal antibody (CC-90002) [94]. The best overall response observed was stable disease in 2 patients with MDS. The most common adverse events were diarrhea (46%), thrombocytopenia (39%), febrile neutropenia (36%), elevated liver transaminases (34%), and anemia (32%), in which 82% were dependent on red blood cell transfusions. Given that CC-90002 showed a lack of response in R/R AML and MDS patients as monotherapy, the CC-90002-AML-001 study was discontinued. A phase Ib study evaluated the use of Hu5F9-G4, an anti-CD47 monoclonal antibody in both untreated and relapsed AML and MDS patients. In 25 evaluable untreated patients, CR/CRi was achieved in 50% (5/10) of AML and 60% (3/5) of MDS patients. Of the 10 R/R patients, one patient had a response (MLFS) to Hu5F9-G4 alone. In addition, 50% of those who had a response achieved MRD negativity. The therapy was well tolerated even with the addition of azacitidine. The most common adverse events were anemia (25%), thrombocytopenia (20%), and infusion reactions (15%) [96]. Ongoing trials are evaluating the efficacy of Hu5F9-G4 (anti-CD47) as monotherapy and in combination with atezolizumab in R/R AML and MDS (ClinicalTrials.gov identifier: NCT03248479, NCT03922477).

6.6.4 CD123 Monoclonal Antibody

CD123 is an interleukin-3 receptor expressed on leukemic progenitor cells and is absent on normal hematopoietic stem cells. A number of humanized antibodies currently being studied function as bispecific T-cell engager utilizing antibodies, with affinities to CD3 on T-cells and CD123 on leukemic cells, to redirect T-cells against the leukemic cells [97–99]. SL-401 (tagraxofusp) is a fusion protein that consists of a truncated diphtheria toxin conjugated to the interleukin-3 recep-

tor, which serves as another method of targeting CD123+ cells [100]. Currently, SL-401 has received FDA approval for its use in blastic plasmacytoid dendritic cell neoplasm (BPDCN), where the hallmark of disease is overexpression of CD123 on plasmacytoid dendritic cells [100, 101]. Continued studies are underway with SL-401 for the treatment of R/R AML patients, as well as consolidation therapy in patients after their first CR (ClinicalTrials.gov identifier: NCT03113643, NCT02270463) [100]. One lead-in stage report showed results of the use in 8 AML patients who were able to maintain stable disease for 6–12 cycles of therapy, with one patient having resolution in transfusion dependency [102]. IMGN632 is another CD123-targeting antibody drug conjugate that has shown promising results in a phase I trial, demonstrating ORR of 20% and a reduction in bone marrow blasts in 55% of patients [103, 104]. Further studies are evaluating the efficacy of IMGN632 as monotherapy in patients with CD123+ disease and in combination with azacitidine and/or venetoclax in the R/R and frontline AML setting (ClinicalTrials.gov identifier: NCT03386513, NCT04086264).

6.6.5 CD70 Monoclonal Antibody

CD27 is a member of the tumor necrosis factor receptor family that binds to its ligand CD70. It was found that the signaling of CD70 and CD27 promotes myeloblast proliferation, and inhibiting this interaction induces cell differentiation of AML blasts [105, 106]. Cusatuzumab (ARGX-110) is a glycol-engineered anti-CD70 monoclonal antibody. A phase I dose-escalation trial studied the safety and pharmacokinetics of ARGX-110 in CD70+ patients with advanced malignancies. The best overall response was stable disease in 14 patients (54%). ARGX-110 was generally well tolerated in a dose escalation study with doses proportionality ranging from 1 to 10 mg/kg, with one R/R AML patient who was able to tolerate a dose of 2 mg/kg [107]. These findings led to the development of a phase II study where ARGX-110 was studied in 12 newly diagnosed AML patients. All patients had a response to therapy, with 67% in CR, 17% in CRi, and the remaining 17% with a PR. An average reduction in bone marrow blasts of 30% was observed. The median time to response was 3.3 months. ARGX-110 was well tolerated, with the most common adverse events being anemia, febrile neutropenia, and infection [108]. In vitro studies have shown efficacy for the combination of ARGX-110 with venetoclax and with azacitadine [109]. Multiple studies have shown promising results of ARGX-110 in the frontline setting; however, further studies are warranted to evaluate the safety and efficacy of ARGX-110 in the R/R setting.

6.7 P53 Targeted Agents

6.7.1 APR-246

TP53 is a cellular tumor antigen that is responsible for cell cycle arrest and apoptosis triggered upon DNA damage [110]. It is also one of the main driving forces behind numerous cancers, playing a strong role in AML. Patients with mutated TP53 have a high incidence of relapse as well as decreased sensitivity to cytotoxic therapy [110]. The need for targeted therapies in these patients is vital. PRIMA-1, also known as APR-246, is a small molecule with a mechanism of action that remains unclear [111]. It is thought that APR-246 covalently binds to and modulates an open pocket in the p53 molecule, converting it back to its wild-type form and allowing the normalization of its function including cell cycle arrest and apoptosis [112–114]. Other modalities for its mechanism of action include the increase of pro-oxidant activity and endoplasmic reticulum stress, which degrades misfolded proteins [113]. An open-label phase I dose-escalation study of APR-246 was able to isolate a tolerable dose in a small number of patients, with one patient maintaining a CRi of 3 months [115]. Currently a phase Ib/II safety and efficacy study of APR-246 with azacitidine is underway for R/R AML patients with TP53 mutations

(ClinicalTrials.gov identifier: NCT03072043). Preliminary results have shown that 9 patients (3 AML, 6 MDS) were included, all of which had responses. Four patients achieved CR, one with marrow CR and two with minimal residual disease negativity. The most common side effects seen included peripheral neuropathy, infection, neutropenia, and nausea [116].

Upcoming data is surfacing with the use of APR-246 in the frontline setting. In a study of HMA-naïve TP53 mutated MDS and AML patients, 55 patients were treated with APR-246 in combination with azacitidine in 28-day cycles. The ORR was 87%, with a median time to response of 2.1 months. CR rate for AML patients alone was 50%. OS was found to be 11.6 months, with an OS of 12.8 months in responders, and those undergoing all-HSCT resulting in an OS of 16.1 months [117]. Another study, conducted in France, with 53 HMT-naïve MDS and AML patients treated with APR-246 in combination with azacitidine, resulted in a response in 63% of patients. A CR was seen in 47% of patients. Of those patients, 79% achieved a complete cytogenetic CR, and 100% were negative for the TP53 mutation (variant allele frequency less than 2%) [118]. Among these two studies, adverse events included neutropenia and neurological symptoms (e.g., confusion, dizziness). Neurological symptoms resolved days within discontinuation of the drug.

6.7.2 MDM2 Inhibitors

Murine double minute-2 (MDM2) is a physiologic antagonist of p53, thereby preventing apoptosis and arrest in cell cycles of leukemic cells [119]. The formation of MDM2 inhibiters, like idasanutlin, is theorized to be useful in wild-type p53-expressing AML patients. In a phase IB trial, 75 patients with R/R AML were treated with idasanutlin in combination with cytarabine. The ORR was 33%, with 25% achieving CR [120]. An ongoing study is combining idasanutlin with venetoclax in R/R AM patients, with preliminary data showing a 46% ORR and 33% CR rate [121]. In another phase I study DS-3032b, an MDM2 inhibitor known as milademetan was studied as monotherapy in 38 R/R AML/high-risk MDS patients. Fifteen patients had reduction in bone marrow blasts and three patients achieved CR/CRi [122]. It was noted that the three patients who had CR/CRi durations of greater than 4 months had all developed TP53 mutations [122]. Currently, DS-3032b is being studied in combination with azacitidine, in hopes of more promising outcomes (ClinicalTrials.gov identifier: NCT02319369).

6.8 Chimeric Antigen Receptor T-Cell (CAR-T) Therapy

CAR-T-cell immunotherapy involves T cells that have been genetically engineered to effectively target cell surface tumor antigens and eradicate malignant cells. CAR-T-cell therapy has been successful in treating lymphoid diseases, but the favorable results have not yet been seen in AML due to the lack of a suitable targetable surface antigen [123]. Two antigens found to be highly expressed on the majority of AML blast cells are CD33 and CD123. With this, studies of CAR-T cell designed to target CD33 (CART33) and CD123 (CART123) are currently being explored. A case report describes a 41-year-old patient with R/R AML who received autologous CART33 cells [124]. A significant reduction of blasts (>50% to <6%) in the bone marrow was observed after 2 weeks of CAR-T-cell therapy. However, shortly after a gradual increase of blasts was detected with disease progression at 9 weeks after cell infusion. The patient then developed cytokine release syndrome requiring an inter-

leukin-6 receptor inhibitor. On the other hand, preclinical studies have demonstrated significant reduction of circulating myeloblasts and improved survival in CART123-treated AML xenograft models. Notably, the favorable results were seen regardless of the initial level of CD123 expression in the AML sample. [125] Current evidence suggests that CART123 may have a more favorable toxicity profile compared to CART33, with less activity against normal hematopoietic cells, while maintaining similar anti-leukemic activity [126]. Studies also evaluated the efficacy of alemtuzumab-mediated depletion of CART123. Sustained leukemia remission was observed in CART123-treated animals at week 1, followed by alemtuzumab at week 5, indicating that alemtuzumab can successfully eradicate T cells [125]. Future studies are needed to evaluate the benefit of incorporating CART33 and CART123 to conditioning regimens prior to HSCT. Ongoing clinical trials are currently underway evaluating the safety and efficacy of allogeneic CART33, combined CD33-CLL1 CART (CLL1 = C-type lectin molecule-1), and CART123 for R/R AML (NCT02799680, NCT*03795779,* NCT03556982).

6.9 Mechanism of Resistance

Treatment of AML remains challenging due to acquired drug resistance and mutations after treatment initiation. One mechanism of drug resistance is the overexpression of P-glycoprotein (P-gp) in resistant leukemic cells or mutations to topoisomerase II, thereby blocking fundamental effects of cytotoxic agents. A second mechanism pertains to gene alterations of common proto-oncogenes, which include FLT3, Wilms tumor, and those in the RAS family (e.g., KRAS, HRAS, NRAS), resulting in abnormal cell proliferation and differentiation. The PI3K/AKT signal pathway is an additional mechanism of resistance through its role in promoting cell growth and apoptosis inhibition. Abnormalities to this pathway lead to downstream effects of P-gp expression or dysregulation of secondary pathways leading to the emergence of additional drug resistance [127].

6.10 Conclusion

From treatment with HSCT to manipulations of cytotoxic therapy regimens and pairings with targeted agents, it can be seen that numerous drug classes have surfaced over the past 10 years, and many more are on the rise. Though the backbone of therapy at the moment is cytotoxic chemotherapy, as we better understand the genomics and molecular abnormalities associated with AML, we can continue to explore therapy options and isolate unique therapies suited to fit each form of this disease. Figure 6.1 summarizes treatment strategies discussed in this chapter and illustrates their place in therapy for R/R AML patients. To best understand which therapy can be studied and become successful in the front line setting, it must be well vetted through the R/R AML population first. It is in these studies, that if a clinical benefit is seen, the odds of an even stronger benefit will be observed in a frontline patient, without the burden of heavy pretreatments. The goal in better understanding how this disease functions, what targets work most efficiently and what side effects are tolerable is all in hopes that the disease can be eradicated entirely with the first treatment, before it ever has to reach a relapsed and refractory setting.

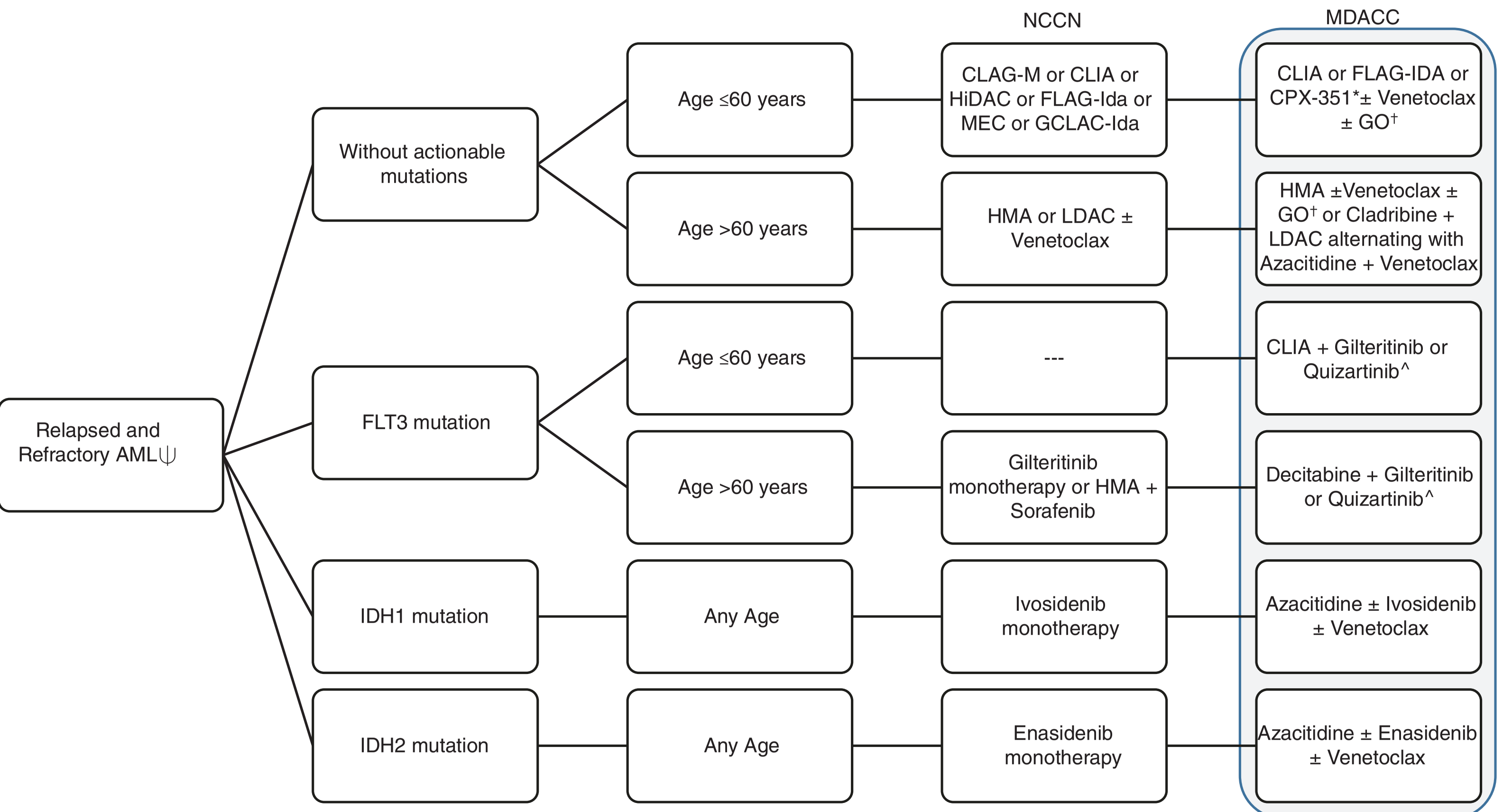

Fig. 6.1 Treatment strategies for relapsed and refractory AML. ΨMay omit anthracyclines if unable to tolerate. *Preferred in patients with therapy-related AML, antecedent myelodysplastic syndrome, and AML with myelodysplasia-related changes. †In patients with CD33-positive and core binding factor AML. ^Only for AML with FLT3-ITD mutation. Abbreviations: *AML* acute myeloid leukemia, *Anthracyclines* idarubcin, daunorubicin, mitoxantrone, *CLAG-M* cladribine + cytarabine + G-CSF ± mitoxantrone, *CLIA* cladribine ± idarubicin + cytarabine, *CPX-351* Vyxeos: liposomal daunorubicin and cytarabine, *FLAG-Ida* fludarabine + cytarabine + GCSF ± idarubicin, *GCLAC-Ida* clofarabine ± cytarabine + GCSF ± idarubicin, *GO* gemtuzumab ozogamicin, *HiDAC* high-dose cytarabine, *HMA* hypomethylating agents: azacitidine or decitabine, *LDAC* low-dose cytarabine, *MDACC* MD Anderson Cancer Center, *MEC* etoposide + cytarabine ± mitoxantrone, *NCCN* National Comprehensive Cancer Network

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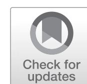

7 The Role of Stem Cell Transplant in the Therapy of Acute Myeloid Leukemia (AML)

David Sanford, Mahmoud Elsawy, and Florian Kuchenbauer

7.1 Introduction

Allogeneic hematopoietic stem cell transplantation (alloHSCT) is generally considered the most effective post-remission therapy in acute myeloid leukemia (AML) to prevent disease relapse. The efficacy of alloHSCT to treat AML is dependent on two factors: (1) high-dose conditioning chemotherapy and (2) recognition and killing of leukemia cells by the donor immune system, or graft-vs-leukemia (GvL) effect. AML is the most frequent indication for alloHSCT with over 3000 transplants performed annually in the USA and Canada which appears to be increasing [1]. The use of alloHSCT is still limited by several factors including donor availability and high rates of non-relapse mortality (NRM) compared to non-transplant strategies. Nevertheless, even with the availability of several new agents in AML, alloHSCT remains a key part of therapy for many AML patients and is more frequently being used to treat older patients. We review indications for alloHSCT in AML, approach to assessing patient fitness, autologous HSCT, conditioning regimens, donor sources, and post-transplant monitoring and interventions to prevent relapse.

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7.2 Allogeneic Stem Cell Transplant in First Remission

7.2.1 Impact of Genetics

Patients with newly diagnosed AML are assigned to a risk group to estimate the probability of remission, relapse, and long-term overall survival (OS). Risk status is primarily determined through genetic testing, incorporating results from cytogenetic and somatic mutational testing. Estimation of risk of relapse with chemotherapy treatment in first complete remission (CR1) is essential when considering up-front HSCT. As a rule of thumb, alloHSCT reduces the risk of relapse by approximately half compared to consolidation chemotherapy or autoHSCT [2]. Nevertheless, alloHSCT does not abrogate genetic and other disease prognostic factors, and the probability of cure is still related to these characteristics.

Early studies often showed a benefit of HSCT in AML patients in CR1 relative to chemotherapy consolidation [3]. These studies were limited by

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a lack of randomization to account for numerous confounding factors influencing outcomes in transplant and non-transplant groups. Several prospective trials have attempted to address this using "Genetic Randomization." In these trials, AML patients were assigned to alloHSCT or no alloHSCT (chemotherapy consolidation, auto-transplant, or observation) based on the presence of a matched sibling donor. This allows for a less biased comparison of the "donor vs. no-donor" groups using intention-to-treat (ITT) analysis.

A meta-analysis including 24 prospective trials using genetic randomization to assess the impact of alloHSCT in AML patients in CR1 found improved RFS (HR 0.80, 95% CI 0.74–0.86) and OS (HR 0.90, 95% CI 0.82–0.97) in patients with an available matched related donor [4]. In subset analysis, this benefit was only observed in patients with intermediate and adverse but not favorable risk cytogenetics. Based on the calculated HRs, the estimated 5-year OS in the intermediate and adverse risk group was 45% vs. 52% and 20% vs. 31% in no-donor vs. donor group, respectively. These results also have limitations and included trials published over a 20-year time span, and supportive care for HSCT and AML care has improved over time. In addition, not all patients in the donor group received alloHSCT which could underestimate the treatment effect of this intervention. Despite these limitations, these results support the use of alloHSCT in eligible patients with intermediate and adverse risk cytogenetics in CR1. This meta-analysis did not define prognostic risk using somatic mutations in genes (e.g., *NPM1*, *FLT3*, and *CEPBA*) that also inform risk assessment. A retrospective analysis by the German–Austrian AML Study Group did not find a benefit of having an HLA-matched donor in patients with normal karyotype and $NPM1^{mut}$ without $FLT3^{ITD}$ suggesting that other genetically defined favorable risk AML patients also do not have an OS benefit from alloHSCT in CR1 [5].

A decision to offer alloHSCT in CR1 depends on a balance between the anticipated reduction in the risk of relapse and risk of non-relapse mortality (NRM). In general, most patients within the ELN intermediate risk group and all patients in the adverse risk group should be considered for alloHSCT if eligible and a suitable donor exists. Nevertheless, estimated NRM with alloHSCT in AML patients is usually at least 20% which must be offset by a reduction in the risk of relapse [6]. Several groups, including the ELN, have published guidelines outlining the balance between relapse risk and NRM to consider alloHSCT, and adaptation of this is shown in Table 7.1 [2]. This type of decision-making framework is useful, but an individualized approach is still needed and additional considerations include: (1) Patient preferences and expectations, (2) additional prognostic information from next-generation sequencing (NGS) panels, and (3) minimal residual disease (MRD) testing and other disease characteristics.

Given the complexity of estimating prognosis in an individual patient, computer-assisted modeling may be an important approach to incorporate clinical, cytogenetic, and somatic mutational data into transplant decision-making. Gerstung et al. [7] have reported on a precision-medicine-based tool developed using large datasets of AML patients to support clinical decision making (https://cancer.sanger.ac.uk/aml-multistage/). This tool estimates relapse, NRM, and OS outcomes at 3 years using alloHSCT and non-HSCT therapy, although its use has not yet been validated in a clinical setting.

7.2.2 Impact of MRD

Minimal or measurable residual disease (MRD) testing can also be used to risk stratify patients for transplant decision-making. The HOVON-SAKK group has reported a study investigating the prognostic impact of MRD using NGS and multiparameter flow cytometry (MFC) [8]. This study measured MRD in AML patients in first CR/CRi after two cycles of intensive induction chemotherapy. The 4-year relapse incidence was 73.3% for patients in whom both NGS and MFC were positive, 52.3% among those who were positive by NGS only, 49.8% among those who were positive by MFC only, and 26.7% for those not positive by either technique. The combined use

Table 7.1 Factors influencing relapse risk and NRM and transplant decision-making in CR1

ELN risk	Genetics	MRD considerations	Relapse risk		HCT-CI	Risk of NRM to justify AlloHSCT
			Chemotherapy	AlloHSCT		

Good	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-ITDlow
	Biallelic mutated CEBPA
		<3 log reduction of CBF transcripts or detectable NPM1^{mut} after first cycle consolidation	35–40%	15–20%	<1	<10–15%

Intermediate	Mutated NPM1 and FLT3-ITDhigh Wild-type NPM1 without FLT3-ITD or with FLT3-ITDlow (without adverse-risk genetic lesions) t(9;11)(p21.3;q23.3); MLLT3-KMT2A Cytogenetic abnormalities not classified as favorable or adverse	AutoHSCT is possible option if MRD-ve by MFC	50–55%	20–25%	≤3–4	<20–30%

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)
	25 or del(5q); 27; 217/
	abn(17p)
	Complex karyotype,
	monosomal karyotype
	Wild-type NPM1 and
	FLT3-ITDhigh
	Mutated RUNX1
	Mutated ASXL1
	Mutated TP53
		Unknown	70–>90%	40–50%	≤5	<40%

of the two methods was predictive of relapse, RFS and OS in multivariate analysis. The study found that receipt of alloHSCT was associated with lower relapse incidence and better RFS, suggesting that patients with positive MRD after two cycles of chemotherapy should be considered for alloHSCT in CR1.

The relative impact of MRD in AML patients in CR1 treated with alloHSCT, autologous HSCT (autoHSCT) and chemotherapy have also been reported in a separate retrospective study of HOVON-SAKK trials [9]. MRD, as measured by MFC, was "positive" (>0.1%) in ~25% of patients and was associated with higher relapse incidence (4 years: 54% vs. 32%, $p < 0.001$) and OS (4 years: 50 vs. 65%, $p = 0.002$) [9]. Interestingly, patients with MRD ≤0.1% and those >0.1% had a similar reduction in relapse incidence with alloHSCT relative to chemotherapy or autoHSCT consolidation, suggesting that GvL is present

regardless of MRD status. In patients with MRD <0.1% OS at 4 years was greater in those that received chemotherapy or autoHSCT vs. alloHSCT (71 vs. 60%), suggesting no benefit and possibly harm with alloHSCT in the low-risk group. In comparison, in patients with MRD >0.1% OS at 4 years was similar (47 vs. 53%).

Mutations in *NPM1* are present in approximately 30% of AML patients, and the prognostic value of MRD monitoring of *NPM1*^{mut} has been reported in several studies [10, 11]. A subset analysis of the ALFA-0702 trial reported on outcomes of *NPM1*^{mut} patients treated with alloHSCT or chemotherapy [10]. In this study, *NPM1*^{mut} was measured by RT-qPCR following induction in blood and bone marrow samples taken in CR1. A <4 log reduction in *NPM1*^{mut} was associated with a higher relapse incidence and poorer OS outcomes, when compared to those that achieved >4 log reduction. In patients achieving >4 log reduction, OS and DFS were similar regardless of receipt of alloHSCT, whereas DFS and OS were significantly improved in patients receiving alloSCT as post-remission therapy with <4 log reduction. This is a small retrospective analysis but suggests that MRD results can be used to select patients for alloHSCT.

Presence of MRD detected immediately prior to undergoing alloHSCT also has prognostic significance. The Fred Hutchinson Cancer Research Center (FHCRC) has reported on MRD testing in AML patients (n = 359) undergoing myeloablative alloHSCT in CR1 or CR2 [12]. MRD was assessed on pre-transplant bone marrow samples by MFC and was detected in 24% of patients. Within this group, twice as many patients had adverse cytogenetics and secondary AML. The incidence of relapse at 3 years was 67% and 22% (p < 0.001) in the MRD-positive and -negative groups, respectively. In fact, outcomes of the MRD-positive group were identical to patients with active disease, suggesting that detecting MRD by flow cytometry before alloHSCT is associated with a poor prognosis. The authors of this study did not find a threshold at which MRD appeared to confer a worse prognosis.

Not all detectable MRD pre-transplant appears to confer an equally poor prognosis. A similar study by the FHCRC group measured MRD in patients with *NPM1*^{mut} AML in remission by MFC and NGS [13]. The authors reported that the NGS methodology was approximately tenfold more sensitive than MFC. Patients in this study with detectable MRD by MFC prior to alloHSCT had significantly higher relapse incidence and lower RFS. In contrast, patients with detectable MRD by NGS alone did not have a higher relapse incidence or worse RFS than MRD-negative patients, suggesting that prognostic relevance of MRD pre-transplant depends on the level of disease and molecular subtype. MRD is a useful tool to further refine prognosis, particularly in favorable and intermediate risk AML patients. Nevertheless, the optimal use of MRD testing in decision-making around transplant requires well-designed prospective studies and ideally standardization of testing between centers.

7.3 Allogeneic Stem Cell Transplant Beyond First Remission

7.3.1 Outcomes Beyond First CR

The prognosis for patients with relapsed or refractory (R/R) AML is poor with a chance of long-term survival less than 20% [14–16]. This may be improved with the availability of new treatments; however, alloHSCT remains the main curative option for these patients. Factors associated with improved outcome include younger age, favorable risk cytogenetics, late relapse (~>1 year), and no prior receipt of alloHSCT. The Dutch-Belgian group has reported a simple prognostic score incorporating these four factors which divides patients into favorable, intermediate, and poor risk categories [14]. The 5-year OS was reported as 46%, 18%, and 4%, respectively. This type of tool is useful in counseling patients; however, the majority of this cohort fit into an intermediate (24%) or poor risk category (67%).

The majority of R/R AML patients do not have durable remissions following reinduction chemotherapy or targeted agents and alloHSCT

should be considered in eligible patients [17]. Nevertheless, outcomes of alloHSCT beyond CR1 appear to be poorer, which may relate to increased TRM and incidence of relapse. Longer-term DFS in AML patients undergoing alloHSCT in CR2 has been reported to range between 40% and 50% [15, 17]. It is important to emphasize that the majority of patients with R/R AML do not go on to receive alloHSCT, in part due to failure to achieve a second durable remission. An analysis of younger patients treated on MRC trials found that only 37% in first relapse received alloHSCT [17]. Survival at 5 years was 44% for those treated with alloHSCT and 21% for those who did not undergo alloHSCT. Interestingly, in subset analyses, patients with favorable risk cytogenetics did not have improved 5-year survival with allograft (35% (alloHSCT) vs. 44% (no-alloHSCT)), whereas patients with intermediate (47% (alloHSCT) vs. 15% (no-alloHSCT)) and poor risk (34% (alloHSCT) vs. 0% (no-alloHSCT)) cytogenetics did benefit. Despite this, we suggest alloHSCT should be offered to eligible patients in CR2 or greater regardless of cytogenetics [2, 18].

7.3.2 Outcomes with Active Disease

Previous studies report the probability of achieving a CR with intensive chemotherapy in R/R AML patients is approximately 50% in younger patients and 20–30% in older patients [17, 19]. In patients who do not achieve CR, prognosis is very poor and alloHSCT with active disease is an option for some patients. There have been several studies of AML patients with chemo-refractory active disease treated with alloHSCT, and the longer-term DFS is reported as ~10–30% [20]. An EBMT registry-based report of AML patients (n = 852) undergoing myeloablative (MA) alloHSCT with active disease between 2000 and 2012 found a 2-year OS and DFS of 30% and 25%, respectively [21]. A similar study of CIBMTR patients reported outcomes for AML patients with active disease undergoing myeloablative alloHSCT from 1995 to 2004 [22]. OS at 3 years was 19% with the incidence of death at 100 days post-transplant 39%, primarily related to leukemia. This study also included patients (19%) who were in first relapse and did not undergo reinduction chemotherapy before conditioning, although results were similar in this group. Multivariate analysis of baseline factors showed that survival was worse with duration of remission <6 months, circulating blasts, a mismatched unrelated donor, a related donor other than an HLA-matched sibling, poor PS (Karnofsky or Lansky score less than 90), and poor-risk cytogenetics. The authors developed a prognostic score based on these factors with four categories: 0, 1, 2, ≥3. These categories accounted for 13%, 29%, 30%, and 28% of patients, respectively, and 3-year OS in these risk groups was 42%, 28%, 15%, and 6%, respectively.

These results suggest that some patients with active disease can be cured with alloHSCT; however, the reported outcomes are based on a selected group and are not applicable to all patients with active disease. AlloHSCT has potential for significant harm in this setting and risk for significant morbidity related to transplant coupled with a low chance of cure, makes this type of approach impractical for many centers with limited resources. Other treatment options including clinical trials, lower intensity therapy or best supportive care, and palliative care may be a preferred approach in many patients with AML and active disease.

7.4 Autologous Stem Cell Transplant

AutoHSCT is also a potential post remission therapy in AML, however is less commonly used than alloHSCT. AutoHSCT relies on high-dose conditioning chemotherapy to eradicate residual leukemia cells, without the potential for GvL effect. There is no GVHD with autoHSCT and NRM and longer-term morbidity is lower than alloHSCT, making its use appealing in some patients including those without a suitable donor. Three prospective RCTs comparing autoHSCT to chemotherapy consolidation have reported a lower relapse incidence with autoHSCT which is

shown in Table 7.2 [23–25]. Of these studies, one showed a late OS benefit with autoHSCT [24] although this was not found in the other studies [23, 25]. Retrospective registry analyses show a higher relapse incidence with autoHSCT relative to alloHSCT using matched sibling, T-cell replete haplo-identical sibling and unrelated donors [26–28]. However, all of these studies showed similar long-term OS for both autoHSCT and alloHSCT, related to higher NRM in the latter group [26–28]. One study reported an OS advantage with autoHSCT compared to alloHSCT using mismatched unrelated donors [28].

Despite evidence of efficacy, treatment of AML with autoHSCT is less frequently used in the modern era, and in Europe accounts for

Table 7.2 Selected studies of autologous transplant in AML

Reference	Design	Patients	Intervention/ comparison	Main findings
Vellenga et al. [23]	RCT	AML patients in CR1, age 16–60 years following induction and two cycles consolidation, not eligible for alloHSCT	Randomization to third cycle consolidation or autoHSCT using BuCy conditioning	Lower RI with autoHSCT compared to chemotherapy consolidation 5-Year RI: 57% (auto) vs. 70% (chemo), $p = 0.002$ 5-Year RFS: 38% (auto) vs. 29% (chemo), $p = 0.065$ 5-Year OS: 44% (auto) vs. 41% (chemo), $p = 0.86$
Burnett et al. [24]	RCT	AML patients in CR1, age <55 years, following two cycle induction and one cycle consolidation	Randomization to fourth cycle consolidation alone or fourth cycle then autoHSCT using CyTBI conditioning	Lower RI with autoHSCT compared to no further treatment (NFT) 5-Year RI: 37% (auto) vs. 58%% (NFT), $p = 0.0007$ 5-Year DFS: 54% (auto) vs. 40% (NFT), $p = 0.04$ 5-Year OS: 57% (auto) vs. 45% (NFT), $p = 0.2$ NRM 12% (auto) vs. 4% (NFT)
Zittoun et al. [25]	RCT	AML patients in CR1, age <60 years following one cycle induction and one cycle consolidation	Randomization to second cycle consolidation or autoHSCT using CyTBI conditioning	Lower RI with autoHSCT vs. chemo. consolidation 4-Year RI: 57% (chemo) vs. 40.6% (auto) 4-Year DFS: 30% (chemo) vs. 48% (auto), $p = 0.05$ 4-Year OS: 46% (chemo) vs. 56% (auto), $p = 0.43$
Gorin et al. [26]	Retrospective	AML EBMT registry patients with HSCT 2007–2012 in CR1 or CR2	Comparison between autoHSCT vs. haploidentical HSCT	Higher RI with autoHSCT vs. haplo HSCT but no OS difference Improved OS with autoHSCT in intermediate-risk karyotype in CR1 3-Year RI: 50 (auto) vs. 27% (allo), $p < 0.01$ 3-year NRM: 4% (auto) vs. 25% (allo), $p < 0.01$ 3-year LFS: 47% (auto) vs. 48% (allo), $p = 0.7$ 3-year OS: 64% (auto) vs. 57% (allo), $p = 0.12$
Keating et al. [27]	Retrospective	AML patients from CIBMTR with transplant 1995–2004 in CR1	Comparison between autoHSCT vs. matched sibling donor (MSD) HSCT	Lower RI and better LFS with MSD HSCT vs. autoHSCT but no OS difference 5-Year RI: 46% (auto) vs. 26% (allo) 5-Year LFS: 46% (auto) vs. 57% (allo) 5-Year OS: 54% (auto) vs. 59% (allo-PB) vs. 64% (allo-BM)

<10% of transplants in CR1 [1, 29]. This may be related to increased donor availability for alloHSCT with the use of haploidentical sibling, cord, and unrelated donors and perhaps a perceived decrease in NRM over time with alloHSCT. AutoHSCT is also limited by concerns around graft contamination with leukemic cells, and historically, there have been many attempts to perform graft-purging although the impact of this is unclear [30].

It appears that autoHSCT is most effective in AML patients with intermediate-risk genetic changes [28, 31–33] and recent ELN guidelines include autoHSCT as a post-remission therapy option for this group [34]. There is evidence that MRD can also be used to select patients who will benefit from autoHSCT. The GIMEMA AML1310 trial addresses this and performed MRD testing by MFC in intermediate-risk patients following the first consolidation cycle [35]. Patients with negative MRD received consolidation with autoHSCT, whereas MRD-positive patients were preferentially treated with alloHSCT. The results of this study have been presented in abstract form and 2-year OS and DFS of 78.6% and 61.4% vs. 69.8% and 66.6% in the MRD-negative vs. -positive groups, respectively. Outcomes in the intermediate-risk group receiving autoHSCT were similar to good-risk patients (also treated with autoHSCT), providing some evidence for autoHSCT in a selected MRD-negative group. Further work needs to be done to define the role of this therapy, and autoHSCT should be included as a post remission option in frontline clinical trials in fit patients with intermediate risk and favorable risk disease and negative MRD.

7.5 Assessing Fitness for HSCT

Several models are available to estimate both risk of NRM and survival benefit to optimize decisions about a patient suitability for HSCT. Historically, age has been used as a selection criterion for alloHSCT referrals. Nevertheless, age alone is a poor prognostic factor. Reduced intensity conditioning (RIC) has extended the use of alloHSCT to older adults and patients ineligible for MAC alloHSCT. This necessitates the requirement for objective tools to further refine the balance of risk–benefit associated with HSCT.

7.5.1 The HCT-Specific Comorbidity Index (HCT-CI)

The HCT-CI was initially developed from the Charlson Comorbidity Index (CCI) and is the most widely accepted model for the assessment of fitness prior to alloHSCT [36]. It was developed in a cohort of 1055 patients with different hematological diseases who received alloHSCT after nonmyeloablative (n = 249) or myeloablative (n = 761) conditioning. The HCT-CI includes 17 comorbidities with each scored from 1 to 3 (Table 7.3). In the validation set, HCT-CI scores showed higher sensitivity than the CCI scores in capturing comorbidities. HCT-CI scores of 1–2 and ≥3 were found in 34% and 28% of patients, while only 10% and 3% of patients had CCI scores of 1 and ≥2, respectively. The HCT-CI scores of 0, 1–2, and ≥3 showed good discrimination of NRM (14%, 21%, and 41%) and survival (71%, 60%, and 34%), respectively.

The prognostic capacity of the HCT-CI has been augmented by the addition of age to build a composite comorbidity/age index using a dataset of 3033 allogeneic HCT recipients [37]. In multivariate models, age >40 years has been shown to impact NRM as equivalent to a single comorbidity with a score of 1. The composite comorbidity/age index provides a more accurate estimate of biological age, and patients should be evaluated with the composite comorbidity/age score, incorporating the impact of comorbidities and age, as well as disease characteristics for selection of the most beneficial transplant strategy. Regardless of age, patients with low scores should be considered for randomized clinical trials or offered higher-intensity regimens. An exception is patients older than 65 years as there are limited data on usage of high-dose regimens beyond this age. Likewise, regardless of age, patients with

Table 7.3 Definitions of comorbidities included in the augmented comorbidity/age index and their corresponding scores

Comorbidity	Definition	Score
HCT-CI		
Arrhythmia	Any type of arrhythmia that has necessitated the delivery of a specific anti-arrhythmia treatment at any time point in the patient's past medical history	1
Cardiac	Coronary artery disease,[a] congestive heart failure, myocardial infarction, or EF ≤50%	1
Inflammatory bowel disease	Crohn's disease or ulcerative colitis requiring treatment at any time point in patient's past medical history	1
Diabetes	Requiring treatment with insulin or oral hypoglycemic agents continuously for 4 weeks before start of conditioning	1
Cerebrovascular disease	Transient ischemic attack or cerebrovascular accident	1
Psychiatric disturbance	Any disorder requiring continuous treatments for 4 weeks before start of conditioning	1
Hepatic, mild	Chronic hepatitis, bilirubin > ULN to 1.5 × ULN, or AST/ALT > ULN to 2.5 × ULN; at least two values of each within 2 or 4 weeks before start of conditioning	1
Obesity	Patients with a body mass index >35 kg/m^2 for patients older than 18 years or a BMI-for-age of ≥95th percentile for patients of ≤18 years of age	1
Infection	Requiring antimicrobial treatment starting from before conditioning and continued beyond day 0	1
Rheumatologic	Requiring specific treatment at any time point in the patient's past medical history	2
Peptic ulcer	Based on prior endoscopic or radiologic diagnosis	2
Moderate/severe renal	Serum creatinine >2 mg/dl (at least two values of each within 2 or 4 weeks before start of conditioning), on dialysis, or prior renal transplantation	2
Moderate pulmonary	Corrected DLco (via Dinakara equation) and/or FEV1 of 66–80% or dyspnea on slight activity	2
Prior malignancy	Treated at any time point in the patient's past history, excluding non-melanoma skin cancer	3
Heart valve disease	Of at least moderate severity, prosthetic valve, or symptomatic mitral valve prolapse as detected by echocardiogram	3
Severe pulmonary	Corrected DLco (via Dinakara equation) and/or FEV1 ≤65% or dyspnea at rest or requiring oxygen	3
Moderate/severe hepatic	Liver cirrhosis, bilirubin > 1.5 × ULN, or AST/ALT > 2.5 × ULN; at least two values of each within 2 or 4 weeks before start of conditioning	3
Augmented comorbidity/age index: all of the above plus		
High ferritin	Values ≥2500 as measured the closest prior to start of conditioning	1
Mild hypoalbuminemia	Values <3.5–3.0 as measured the closest prior to start of conditioning	1
Moderate hypoalbuminemia	Values <3.0 as measured the closest prior to start of conditioning	2
Thrombocytopenia	Values <100,000 as measured the closest prior to start of conditioning	1
Age	≥40 years	1

Abbreviations: *EF* ejection fraction, *ULN* upper limit of normal, *DLco* diffusion capacity of carbon monoxide, *FEV1* forced expiratory volume in 1 s

[a]One or more vessel-coronary artery stenosis requiring medical treatment, stent, or bypass graft

higher scores are more suitable candidates for less intense regimens.

The HCT-CI can be further augmented by the addition of three markers: ferritin, albumin, and platelet count [38]. The prognostic capacity of an augmented comorbidity/age index was investigated among 724 recipients of allogeneic HCT from HLA-mismatched (n = 345), haploidentical (n = 117), and umbilical cord blood (UCB, n = 262) grafts between 2000 and 2013. Patients with scores of <4 had better survival compared to those with scores of ≥4 and received HLA-

mismatched (55% versus 39%, $p < 0.0008$), HLA-haploidentical (58% versus 38%, $p = 0.01$), or UCB grafts (67% versus 48%, $p = 0.004$), respectively. These results support the use of comorbidity assessment as a valid prognostic tool among the recipients of allo-HCT from alternative graft sources. Table 7.3 describes the definitions of the augmented comorbidity/age index [39].

The prognostic role of comorbidities has been specifically assessed among patients with AML ($n = 391$) or MDS ($n = 186$) who received either nonmyeloablative ($n = 125$) or high-dose conditioning ($n = 452$) [40]. In multivariate analyses of risk factors, high HCT-CI scores and high disease risk were the most significant factors predicting NRM ($p < 0.0001$ and $p = 0.004$), OS ($p < 0.0001$ and $p < 0.0001$), and RFS ($p < 0.0001$ and $p < 0.0001$), respectively. Therefore, all patients were stratified into four risk groups incorporating both comorbidities and disease-risk (Table 7.4). Rates of 2-year OS were 70% and 78% among AML/MDS patients with HCT-CI scores of 0–2 and low-risk disease following nonmyeloablative and high-dose HCT, respectively, and they were 57% and 51%, respectively, if patients had high-risk AML/MDS. Results suggested that AML/MDS patients with low comorbidity burden are candidates for prospective randomized studies to determine the role of conditioning intensity. Unsurprisingly, patients with higher HCT-CI scores (≥3) overall had inferior survivals, in particular, those with high-risk AML/MDS (OS of 29% and 24%, respectively). The poor survival rates were due to more relapses (49%) among nonmyeloablative recipients and more frequent NRM (46%) among high-dose recipients (Fig. 7.1) [40]. A systematic methodology for data acquisition and consistent guidelines for comorbidity coding are summarized in a Web-based calculator (www.hctci.org) [41].

7.5.2 The HCT-CI and Disease Risk Index

While patient-specific variables have a significant impact on NRM, disease-specific variables should be also taken into consideration as a predictor of post-transplant relapse when counseling patients regarding the feasibility of alloHSCT. In a recent analysis including 942 alloHSCT recipients with AML/MDS, a novel prognostic model, hematopoietic cell transplant-composite risk (HCT-CR), was developed and validated by combining the refined disease risk index (DRI-R) and comorbidity/age index to prognosticate for outcomes [42]. The HCT-CR index stratified patients into four risk groups: low-risk patients with low/intermediate DRI-R and comorbidity/age ≤3 ($N = 272$); intermediate-risk patients with low/intermediate DRI-R and comorbidity/age >3 ($N = 168$); high-risk patients with high/very high DRI-R and comorbidity/age ≤3 ($N = 284$); and very high-risk patients with high/very high DRI-R and comorbidity/age >3 ($N = 184$). Patients with higher HCT-CR scores had significantly worse outcomes compared to those with lower scores. The low-risk group, intermediate, high, and very high-risk groups had adjusted HR

Table 7.4 Two-year NRM, relapse, OS, and RFS incidences among nonmyeloablative compared to myeloablative patients as stratified into four risk groups based on HCT-CI scores and disease status

Risk groups	Patients	NRM (%)	Relapse (%)	OS (%)	RFS (%)
Group I (HCT-CI scores 0–2 and low-risk diseases)	Myeloablative ($n = 138$)	11	14	78	75
	Nonmyeloablative ($n = 28$)	4	33	70	63
Group II (HCT-CI scores 0–2 and intermediate and high-risk diseases)	Myeloablative ($n = 176$)	24	34	51	43
	Nonmyeloablative ($n = 34$)	3	42	57	56
Group III (HCT-CI scores ≥3 and low-risk diseases)	Myeloablative ($n = 52$)	32	27	45	41
	Nonmyeloablative ($n = 19$)	27	37	41	36
Group IV (HCT-CI scores ≥3 and intermediate and high-risk diseases)	Myeloablative ($n = 86$)	46	34	24	20
	Nonmyeloablative ($n = 44$)	29	49	29	23

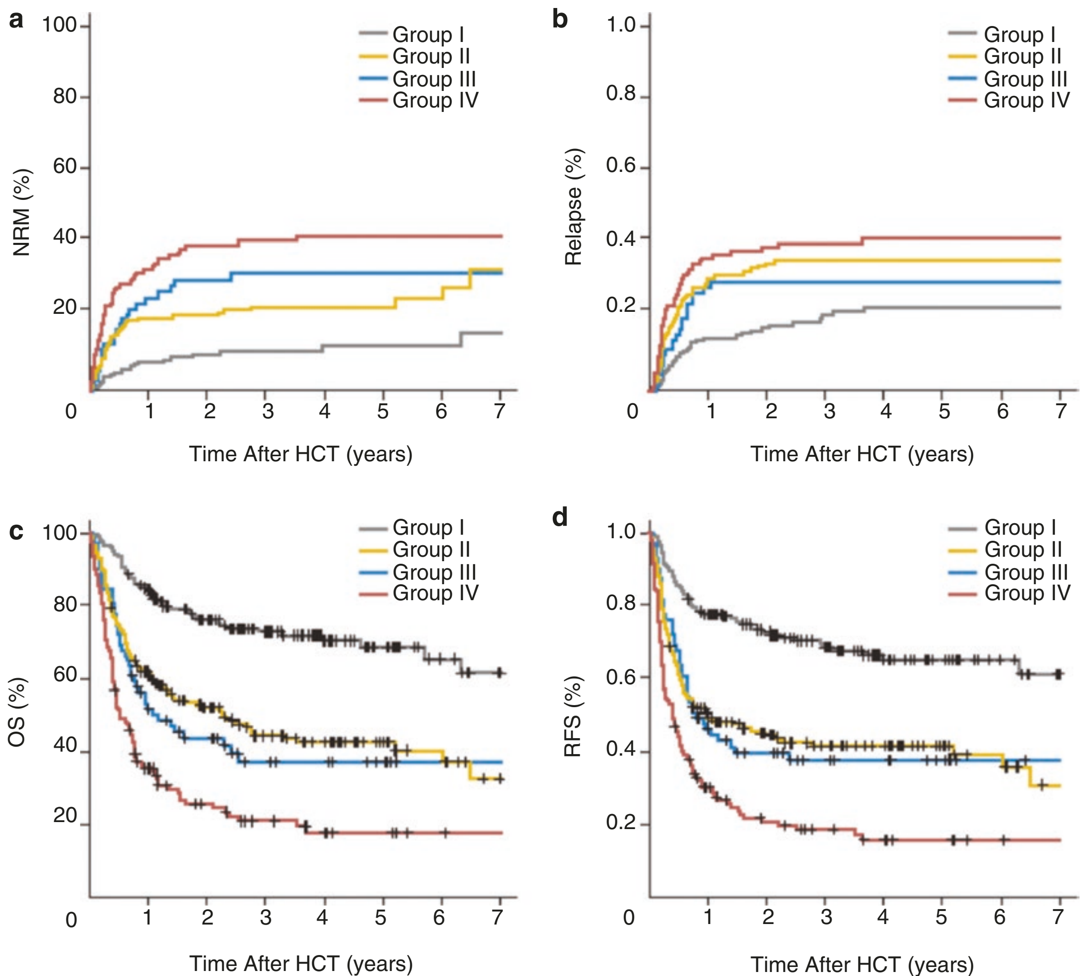

Fig. 7.1 Risk stratification of patients with acute myeloid leukemia/myelodysplasia and receiving allogeneic hematopoietic cell transplantation (HCT). Group I (gray) included HCT-specific comorbidity index (CI) scores 0–2 plus low disease risk; group II (yellow) included HCT-CI scores 0–2 plus intermediate and high disease risks; group III (blue) included HCT-CI scores ≥3 plus low disease risks; and group IV (red) included HCT-CI scores ≥3 plus intermediate and high disease risks. NRM, nonrelapse mortality; OS, overall survival; RFS, relapse-free survival. (From Sorror et al.: J Clin Oncol Vol. 25, 2007: 4246–4254. Reprinted with permission. © 2007 American Society of Clinical Oncology. All rights reserved)

of 1.37 ($p < 0.04$), 2.08 ($p < 0.001$), and 2.92 ($p < 0.001$), respectively. The HCT-CR model provided better discriminative capacity for OS prediction compared with all prior models independently, including cytogenetic risk group, DRI-R, and comorbidity/age model (C-statistics: 0.62, 0.55, 0.60, and 0.54, respectively) ($p < 0.001$).

Similar results were demonstrated in another recent analysis of 959 alloHSCT recipients between 2000 and 2013 at the University of Minnesota for hematological malignancies (66% with leukemia) [43]. The HCT-CI was combined with DRI to produce a composite disease risk and HCT comorbidity index (DRCI). The new model stratified patients into six risk groups with discrete outcomes. Patients with very-high risk DRCI had worse 2-year OS compared with patients with very low-risk DRCI, 34% versus 74%, respectively ($p < 0.01$). In multivariable model adjusted for patient age and donor type, the DRCI was an

independent predictor of overall mortality, relapse risk, DFS, and GVHD-free/RFS. These results suggest that patient-related factors should simultaneously be considered with disease-specific variables to better risk stratify outcomes of patients with AML/MDS undergoing alloHSCT.

7.5.3 Comprehensive Geriatric Assessment (CGA)

Older patients experience additional age-specific health vulnerabilities relative to younger patients. Unfortunately, performance status assessment tools such as KPS and European Cooperative Oncology Group performance status (ECOG) do not provide a comprehensive evaluation of health status. CGA could potentially reveal these vulnerabilities; however, it has several domains, and identification of the most relevant predictors on outcomes in alloHSCT requires further studies. In a recent analysis of outcomes of 527 alloHSCT recipients who were 60 years or older, 40% with AML, the incidence of post-transplant delirium was strongly associated with pre-transplant fall in the preceding year, use of potentially inappropriate medications, low platelet count, and impaired renal function in multivariate models; while age older than 70 years and impaired activities of daily living were significantly associated with post-transplant fall, both assessed at day 100 [44]. Both delirium (HR 1.66; 95% CI, 1.09–2.52; $p < 0.023$) and fall (HR 2.14; 95% CI 1.16–3.95; $p < 0.026$) were significantly associated with increased NRM at 100 days; further fall (HR 1.93; 95% CI 1.18–3.14; $p = 0.016$), but not delirium, was significantly associated with reduced OS. Improved understanding and identification of the most significant predictors of outcomes among CGA tools is of prime importance. The majority of these variables are potentially targetable with preemptive interventions to improve the outcomes in older patients.

7.5.4 Recommended Models

The augmented comorbidity/age index is a relatively simple tool that could be used in the daily clinical practice to council patients on potential risks of post-transplant NRM. An online calculator is available to provide the HCT-CI score at www.hctci.org. All patients 60 years or older should additionally be assessed using domains of CGA. This approach aims at revealing potential targets for peri-transplant interventions to improve outcomes and post-transplant quality of life.

7.6 Conditioning Regimens and Donor Type

7.6.1 Conditioning Regimens

Conditioning regimens given prior to alloHSCT create immunologic and physical space to allow for engraftment and for eradication of residual leukemia cells. Therefore, conditioning consists of a lymphodepleting component, which targets the host lymphoid system and a myeloablative component which theoretically should target the host stem cells as well as the remaining malignant cells. The conditioning regimen influences risk of GVHD, TRM, relapse incidence, and OS. Nevertheless, there is no “one-size fits all” approach to the selection of a conditioning regimen, which needs to be tailored to disease characteristics, patient fitness, and comorbidities.

Traditionally, conditioning regimens can be classified based on the reversibility of cytopenia into myeloablative conditioning (MAC), non-myeloablative (NMA) and reduced intensity conditioning regimes (RIC). MAC leads to irreversible cytopenia and requires stem cell support. In contrast, NMA protocols cause minimal cytopenias and can be given without stem cell support but are currently not favored especially in AML patients with advanced disease due to high relapse rates [45]. RIC is positioned between MA

and NMA, causes profound, but not irreversible cytopenia which requires stem cell support [46].

Early studies comparing myeloablative CyTBI and BuCy regimens suggested that TBI resulted in improved survival outcomes with lower relapse incidence, TRM, and improved OS [47, 48]. These findings have not been confirmed in recent registry-based studies, which report equivalent or improved outcomes with BuCy compared to CyTBI [49, 50]. This may relate to widespread use of IV busulfan dosing in modern eras, which has more predictable absorption and pharmacokinetics with lower toxicity and risk of veno-occlusive disease (VOD) than oral busulfan used in older studies [51, 52]. High-dose TBI regimens are infrequently used in AML partly due to the long-term toxicities with TBI, such as endocrine dysfunction, cataracts, and risk of second cancers.

Several strategies may reduce toxicity associated with MAC regimens including the use of pharmaco-kinetic (PK)-guided busulfan dosing, avoidance of dual alkylator regimens, and treosulfan-based regimens [53–58]. Reduced intensity conditioning (RIC) regimens have improved the tolerability and safety of alloHSCT in older patients and those with comorbidities.

Retrospective studies have found that RIC regimens result in lower rates of TRM with a higher risk of relapse [59]. Two recent prospective trials have compared RIC vs. MAC alloHSCT in relatively young, fit patients with AML with conflicting results. One study by the BMT-CTN found a significantly higher relapse incidence with RIC regimens with worse OS, and the trial was stopped early due to this [60]. In contrast, two European trials comparing RIC vs. MAC regimens in AML in CR1 found similar OS with each regimen [61, 62]. However, differences in trial design, donor source, and age adjustment make comparisons between these studies difficult.

There is also evidence that MAC is less beneficial in AML patients with high-risk disease and complex karyotypes [63–67]. With this in mind, FLAMSA-RIC, a sequential approach combining intensive chemotherapy, RIC, and prophylactic donor lymphocyte infusions (DLI) was developed demonstrating promising results in adverse risk AML patients [68–72]. However, to further improve and personalize conditioning regimens, there is a need to better understand the interaction between the microenvironment and disease biology. In addition, the potential incorporation of targeted agents into conditioning regimes should be explored to reduce disease burden and thus relapse after alloHSCT. In general, we suggest that fit, younger patients (<65 years) should receive MAC regimens although this remains an individualized decision based on patient comorbidities and estimated risk of NRM.

7.6.2 Donor Type

Historically, a graft from an HLA-matched sibling donor (MSD) has been the ideal and in general is still the preferred donor choice. There are three graft sources for alloHSCT: bone marrow (BM), peripheral blood stem cells (PBSC), and cord blood (CB). Anasetti et al. recently compared in a large randomized Phase 3 trial PBSC and BM from unrelated donors in patients undergoing alloHSCT [73]. The authors did not detect significant survival differences between both arms, although the PBSC group had a higher risk of cGvHD and lower risk of graft failure. Although the trial did not exclusively focus on AML patients (~50% of patients), it confirmed previous retrospective analyses [74, 75]. In the setting of RIC, the positive effect of PBSC on OS and LFS was more pronounced as recently shown by Savani et al. in a retrospective analysis [76].

Despite PBSC being the main stem cell source for HSCT in adults, the use of alternative graft sources such as haploidentical donors, mainly through the introduction of post-transplant cyclophosphamide (PT-Cy) is increasing [1, 29]. In a large retrospective analysis, Ringden et al. [77] compared relapse and survival in AML/ALL patients, receiving MSD HSCT to patients that received T-cell-replete or T-cell-depleted haploidentical HSCT. There was no difference in RI between recipients of haploidentical and matched sibling grafts, but a significantly higher NRM in the haploidentical group. Similar to unrelated

donors, using PB as graft source for haploidentical HSCT has been associated with increased grade II–IV GvHD, but without a significant effect on survival outcomes [78].

Comparing the outcomes of haploidentical HSCT with MSD in AML in CR1, Salvatore et al. found that in intermediate-risk AML patients receiving a haploidentical graft exhibited a less favorable outcome with decreased LFS and OS and higher NRM, whereas in adverse-risk AML patients, the outcomes were similar [79]. Although the use of CB as graft source has been decreasing, data from Milano et al. suggest that OS is superior in acute leukemia and MDS patients receiving CB compared to HLA-mismatched unrelated donors in the presence of MRD [80]. Several reports have shown that CB as graft can provide a therapeutic benefit in AML patients regardless of age and risk stratification [81–84]. There is no clear evidence for superiority of haploidentical grafts vs. HLA-mismatched unrelated donors [85]. Therefore, based on the available data, MSD remain the first-choice donor for AML patients in first remission.

7.6.3 Role of T-Cell Depletion

GvHD is one of the leading causes of NRM and morbidity after allogeneic HSCT. In addition to standard GVHD prophylaxis strategy, in vivo T-cell depletion with antithymocyte globulin (ATG) has been developed to reduce the incidence of especially cGvHD. Multiple randomized studies evaluated the use of ATLG (former ATG-Fresenius) or thymoglobulin, both produced from immunized rabbits, for GvHD prophylaxis in patients who underwent alloHSCT from unrelated as well as HLA-identical matched donors [86–89]. Overall, the use of ATG was associated with a lower incidence of cGvHD without affecting OS and relapse incidence, suggesting a broader use of ATG for related and unrelated donor transplants. Due to its potent effect on proliferating T cells as GvHD prophylaxis for haploidentical HSCT, PT-Cy has been investigated for allogeneic HSCT in the setting of matched related and unrelated donors alone or in combination with immunosuppressive therapy. Initial promising results for PT-Cy for alloHSCT with matched related and unrelated donors receiving BM as graft as single agent in hematological malignancies with cGVHD rates <15% prompted the further investigation of PT-Cy in alloHSCT [90, 91]. PT-Cy as single immunosuppressive agent for allo-HSCT with PBSC as graft source was associated with severe aGvHD and an increased NRM, prompting investigations of PT-Cy in combination with other immunosuppressive drugs. In fact, the combination of PT-Cy with a calcineurin inhibitor enhanced its effect on GvHD prophylaxis in particular severe cGvHD for PBSC and BM from MSD and MUD, thus reducing mortality and improving survival [92–94]. Although randomized trials are still lacking, PT-Cy appears to be a reasonable alternative for in vivo T-cell depletion and may have a cost advantage over ATG. A promising approach for ex vivo T-cell depletion is αβ T-cell depletion to overcome the HLA disparity for patients undergoing haploidentical HSCT as well as HLA-matched HSCT [95]. However, clinical trials comparing αβ T-cell depletion with other immunosuppressive approaches are still absent.

7.7 Maintenance Therapy and Monitoring for Relapse

7.7.1 Maintenance Therapy

Disease relapse remains the most frequent cause of death following HSCT, underscoring the need for novel approaches with maintenance and preemptive therapies [96]. Hypomethylating agents (HMAs) are attractive options for maintenance therapy as they can be delivered in the outpatient setting and are well tolerated. Maintenance using decitabine has been compared in an RCT following chemotherapy and does not reduce relapse in this setting [97]. Following alloHSCT, HMAs may augment GvL through increased expression of tumor antigens such as WT1 on leukemic cells [98]. In addition, pre-clinical models show that HMAs expand regulatory T-cell populations, suggesting they may not increase risk of GVHD [98,

99]. Several non-randomized studies have evaluated HMAs post-HSCT [100–103]. These agents have acceptable safety and tolerability, although the optimal dose appears to be lower than for upfront treatment [100, 103]. The EFS and OS reported in these trials is promising; however a prospective, randomized trial is needed to evaluate the efficacy of HMAs in the post-HSCT setting.

Targeted therapy, such as tyrosine kinase inhibitors, can prevent relapse in Philadelphia positive ALL and CML post-HSCT, and targeted agents could play a similar role in AML (PMID 20005967). FLT3-mutated AML is a suitable candidate to investigate post-HSCT maintenance as relapse remains high despite the availability of several FLT3-inhibitors. Promising results using sorafenib maintenance in FLT3-positive AML have been reported in retrospective studies relative to historical controls [104, 105]. Recently, the results of an RCT by the German/Austrian group evaluating sorafenib maintenance was reported in abstract form [106]. In this trial, patients with FLT3-ITD mutated AML in CR1 or beyond were randomized to either placebo or sorafenib 400 mg PO BID started day +60 to +100 post-HSCT and continued for 24 months. The primary endpoint of RFS was 85% vs. 53% at 2 years in the sorafenib and placebo group, respectively (HR = 0.39; 95% CI 0.18–0.85; $p = 0.013$). There was a higher rate of relapse in the placebo-treated group but no difference in NRM between groups. OS was also higher in the sorafenib-treated patients (HR = 0.45; 95% CI 0.20–0.97, $p = 0.03$). The rates of acute and chronic GVHD were similar between groups; however, it appeared that skin toxicity and electrolyte abnormalities were more frequent with sorafenib. This suggests sorafenib maintenance can prevent relapse in this group of AML patients, and several studies evaluating maintenance therapy using other FLT3-inhibitors are ongoing.

7.7.2 Pre-emptive Treatment for MRD and Mixed Chimerism

There is growing evidence that pre-emptive treatment for early or low burden disease with immunomodulation and other therapies may prevent relapse in AML following HSCT [107–109]. Monitoring of chimerism following alloHSCT is used to assess risk of disease relapse and graft failure. "Full chimerism" refers to a state of complete engraftment of donor cells in the recipient, and this is often determined in a specific lineage (e.g., lymphoid, CD34+). Chimerism is frequently assessed by monitoring polymorphic short-tandem repeats (STR) of <10 nucleotides or microsatellite regions. Several other methods are possible including FISH for X and Y chromosomes in opposite sex donors, qPCR analysis of single-nucleotide polymorphisms (SNP), and insertion/deletions (Indels) [110]. Multiple studies have shown that mixed or increasing chimerism in lymphoid or CD34+ compartments is associated with higher risk of relapse [111–113]. The chance of mixed chimerism is increased following RIC regimens and with use of a BM graft; however, mixed chimerism at early time points is also common following MAC regimens [112]. Risk of relapse appears to be lower in patients achieving full lymphoid chimerism at early time points following both RIC and MAC regimens in patients with AML [114].

It is important to emphasize that mixed or increasing chimerism is not however synonymous with disease relapse, and the timing and threshold determine test characteristics [115]. This is relevant as treatment of mixed chimerism with rapid taper of immunosuppression or donor-lymphocyte infusion (DLI) can have significant toxicity by provoking severe GVHD. A study from by Wong et al. highlights the tradeoff of chimerism testing and reported a 100-day T lymphoid chimerism threshold of 85% had a high specificity (87.5%) but low sensitivity (46.7%) for relapse, with positive and negative predictive values of 38.9% and 90.6%, respectively. Withdrawal of immunosuppression and pre-emptive DLI are commonly used for mixed chimerism, and there is evidence that this reduces relapse. A prospective study of pre-emptive DLI for mixed chimerism in pediatric AML patients found that patients with mixed chimerism had lower EFS than those with full chimerism (80% vs. 30%, $p < 0.001$) [116]. EFS was improved

better in patients with mixed chimerism that received pre-emptive treatment with withdrawal of immunosuppression or DLI ($n = 13$) than those that did not ($n = 7$), and the 3-year EFS was 46% and 0%, respectively.

Treatment with azacitidine may also be effective in treating mixed chimerism and preventing relapse. The RELAZA trial used pre-emptive treatment with azacitidine for mixed chimerism or detectable MRD post-HSCT in patients with AML and MDS ($n = 59$) [107]. In this study, chimerism was measured using STR in CD34+ sorted cells at intervals of 3–4 weeks during the first 8 months after HSCT and every 7–8 weeks from months 8 to 24. Patients with chimerism <80% ($n = 20$) were eligible for treatment with azacitidine and withdrawal of immunosuppression, and in this group, 50% had an increase of chimerism >80%. Nevertheless, 65% ($n = 13$) of patients entering the treatment phase ultimately relapsed with a median time of 231 days from the detection of MC <80%. In the RELAZA-2 trial by the same group, MRD testing was incorporated to identify AML patients at high risk for relapse following HSCT. Detection of $NPM1^{mut}$ and core-binding factor transcripts by RT-qPCR following HSCT has been shown to predict relapse [108, 117]. In this trial, CD34+ chimerism testing ($n = 108$) was performed in addition to MRD testing using RT-qPCR for $NPM1^{mut}$ ($n = 77$) or fusion transcripts DEK-NUP214 ($n = 1$), RUNX1-RUNX1T1 ($n = 9$), or CBFB1-MYH11 ($n = 10$). Patients were offered treatment with azacitidine on the basis of chimerism <80% ($n = 19$) or MRD positivity ($n = 34$), and 40% had an MRD or chimerism response and 19% had stable MRD or chimerism. The 1-year OS and PFS was 76 and 42%, respectively.

The exact benefit of interventions based on chimerism and MRD monitoring is difficult to determine given the non-randomized nature of these studies. Despite these limitations, pre-emptive treatment with taper of IST and DLI should be considered in patients with mixed or increasing chimerism or detectable MRD. In patients deemed to be at high risk of severe GVHD with this approach, treatment with azacitidine could be an alternative approach to prevent or delay relapse.

7.8 Conclusion

In conclusion, alloHSCT has an important role in the upfront treatment of intermediate and high-risk AML patients. Patients determined to have a high risk for relapse based on MRD testing may also benefit from alloHSCT in first remission. AutoHSCT is a potential option in patients with intermediate-risk AML without detectable MRD. Patients with R/R AML should be targeted to receive alloHSCT if eligible as this remains the primary curative option. Selection of conditioning regimens should be individualized; however, myeloablative regimens are preferred in fitter, younger patients. A formalized assessment of a patient's comorbidities should be performed prior to HSCT using a tool such as the HCT-CI or augmented HCT-CI to estimate the risk of NRM and guide patient discussions and inform management. Significant work remains to reduce NRM with HSCT, and a major obstacle to this remains GVHD which appears to be reduced with lymphodepletion using ATG and PT-Cy. Relapse remains the major cause of death following HSCT, and randomized prospective trials are needed to investigate the role of maintenance and pre-emptive therapy for MRD.

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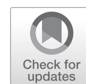

8 Novel and Investigational Therapies in Acute Myeloid Leukemia

Yazan F. Madanat and Aziz Nazha

8.1 Introduction

Acute myeloid leukemia encompasses a heterogeneous group of blood and bone marrow neoplasms that arise from the expansion of hematopoietic cell clones carrying recurrent cytogenetic and/or molecular abnormalities [1]. Overall outcomes of AML patients are generally poor with only 28.3% of patients alive at 5 years, though outcomes are very heterogeneous depending on the disease subtype and cytogenetic/molecular risk [2]. Cytogenetic risk stratification remains one of the most important prognostic factors in AML though approximately 40–50% of AML patients have a normal karyotype, but these patients' overall outcomes vary depending on their molecular profile. The 2017 European Leukemia Net (ELN) risk stratification for AML incorporated a few molecular aberrations that may improve risk stratification of AML [3]. Until recently, little has changed in the management and treatment options for patients with AML. The standard intensive chemotherapy for "medically fit" AML patients remained mainly dependent on the backbone of the combination of cytarabine and anthracycline; however, several novel drugs have received regulatory approval in AML in the last couple of years, and many novel therapeutic targets and strategies are currently under development. Thus, the landscape of therapy has evolved to include targeted therapies (midostaurin, enasidenib, ivosidenib, and venetoclax), liposomal encapsulated cytarabine plus daunorubicin (CPX-351), and monoclonal antibodies/antibody–drug conjugates (gemtuzumab ozogamicin and others) [4–9]. This chapter summarizes the data published on these novel approved agents and highlights some of the investigational agents that are currently in development.

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8.2 The Molecular Landscape of AML

Genome sequencing efforts of samples from AML patients have highlighted the genomic landscape of AML and recognized its complex structure, and some of these mutations have a significant independent impact on AML survival and response to therapy [10–12]. More importantly, these studies have led the way to develop effective targeted therapies for certain mutations. Obtaining a genomic panel of several genes using next-generation targeted deep sequencing became routine practice for AML at diagnosis and after their relapse. Such a panel carries significant information that can alter patients' prognosis and treatment recommendations.

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The Cancer Genome Atlas Project evaluated the genomic alterations in 200 patients with de novo AML and noted the presence of one or more driver mutations in each AML sample. Genomic abnormalities were classified into various functional groups. Genes affecting signaling pathways were most common constituting 59% (e.g., *FLT3*, *KIT*, *KRAS/NARS*, and *PTPN11*); other common functional groups include DNA-methylation genes (44%) (e.g., *DNMT3A* or *DNMT3B*, *DNMT1*, *TET1*, *IDH1/2*) and chromatin modifying genes (30%) (e.g., *KMT2A* fusions, *ASXL1*, *EZH2*, *KDM6A*). Nucleophosmin gene (*NPM1*) mutations occurred in 27% of patients. Other less common genomic functional groups include transcription-factor gene mutations (22%), tumor-suppressor genes (16%), spliceosome-complex genes (14%), and cohesin-complex gene mutations (13%) [13].

Other studies have focused their efforts on investigating the genomic landscape in therapy-related and secondary AML. The presence of spliceosome gene mutations such as *SRSF2*, *SF3B1*, *U2AF1*, and *ZRSR2* and mutations in *ASXL1*, *EZH2*, *BCOR*, or *STAG2* was >95% specific for secondary AML when compared to de novo disease [14]. Adjusting for clinical variables such as age, karyotype, and white blood cell count was shown to change the specificity of some of these mutations in another study of secondary AML [15].

Importantly, the genomic landscape of AML demonstrates some of the disease heterogeneity and the co-occurrence of some of these mutations could have implications on response to therapy and patient outcomes.

8.3 Novel Therapies Receiving Regulatory Approval

In the last 2 years, a total of eight new drugs have been FDA approved for the treatment of AML patients. In April 2017, the U.S. Food and Drug Administration (FDA) approved midostaurin, a multi-kinase *FLT3* inhibitor, for newly diagnosed patients with *FLT3*-positive AML. This was the first targeted therapy approved in AML demonstrating a clear survival benefit when combined with induction chemotherapy vs. chemotherapy alone [9]. About 4 months later, single-agent enasidenib, a first in class oral inhibitor of *IDH2* was approved for patients with relapsed/refractory AML carrying an *IDH2* mutation [8]. In September 2017, the monoclonal antibody drug conjugate targeting CD33, gemtuzumab ozogamicin was re-approved, after having been taken off the market in 2010, in combination with induction chemotherapy for newly diagnosed patients with AML or as a single agent in the relapsed/refractory setting [6, 16]. The liposomal encapsulated combination of cytarabine plus daunorubicin (CPX-351) was approved for newly diagnosed therapy-related AML or AML with myelodysplasia-related changes (AML-MRC) where it demonstrated a survival advantage over standard 7+3 induction chemotherapy [7]. In 2018, a few additional AML drugs received FDA approval, these include ivosidenib (*IDH1* inhibitor) single-agent oral therapy in relapse/refractory IDH1 mutated AML [4], gilteritinib (*FLT3* inhibitor) as single-agent oral therapy for relapsed/refractory FLT3 mutated AML [17], and Venetoclax, an oral BCL-2 inhibitor, which received accelerated approval for newly diagnosed older adults unfit for standard induction chemotherapy in combination with low-dose cytarabine or hypomethylating agents (azacitidine or decitabine) after showing promising results in early phase clinical trials [5, 18]. Finally, glasdegib, a smoothened hedgehog inhibitor, received approval in combination with low-dose cytarabine (LDAC) after showing a survival advantage when compared to cytarabine alone [17]. In the next few sections of this chapter, the specific outcomes and results of these trials will be summarized and some of the investigational agents being evaluated in clinical trials highlighted (Table 8.1).

Table 8.1 Selected clinical trials using investigational agents in AML

Investigational agents	Upfront vs. R/R	Single agent vs. combination therapy	Trial phase	Clinical trial identifier
Hypomethylating agents or HDAC inhibitors				
Guadecitabine	Upfront	Single agent	III	NCT02348489
Pracinostat	Upfront	Combined with azacitidine	III	NCT03151408
Monoclonal antibodies				
Vadastuximab talirine	Upfront	Combined with azacitidine	III	NCT02785900
Talacotuzumab	Upfront	Combined with decitabine	II/III	NCT02472145
AMG-330	R/R	Single agent	I	NCT02520427
ARGX-110	Upfront	Combined with azacitidine	I/II	NCT03030612
IMGN632	R/R	Single agent	I	NCT03386513
Hu5F9-G4	R/R	Single agent and in combination with azacitidine	I	NCT03248479
FLT3 inhibitors				
Gilteritinib	Upfront	Alone or combined with azacitidine (two arms)	II/III	NCT02752035
Gilteritinib	R/R	Single agent	III	NCT02421939
Crenolanib	Upfront	Combined with chemotherapy	III	NCT03258931
Crenolanib	R/R	Combined with chemotherapy	III	NCT03250338
Quizartinib	R/R	Single agent	III	NCT02039726
IDH1/2 inhibitors				
Ivosidenib	Upfront	Combined with azacitidine	III	NCT03173248
Enasidenib	R/R	Single agent	III	NCT02577406
BCL-2 inhibitor				
Venetoclax	Upfront	Combined with azacitidine	III	NCT02993523
Venetoclax	Upfront	Combined with low dose cytarabine	III	NCT03069352

8.4 Novel Cytotoxic Therapy Formulations

CPX-351 is a novel liposomal encapsulated formulation of cytarabine and daunorubicin (anthracycline) that maintains a fixed synergistic molar ratio of 5:1 between the molecules. Early preclinical development of CPX-351 showed high efficacy of this novel formulation before moving it into the clinical setting [19].

Following the phase I study, CPX-351 was tested in two randomized, multicenter, phase II clinical trials. The first clinical trial compared CPX-351 as a salvage chemotherapy for AML patients age 18–65 years in the first relapse. Response rates were similar, and there was no improvement in overall survival (OS) (hazard ratio (HR) for death, 0.75; $P = 0.19$) or event-free survival (EFS) (HR, 0.66; $P = 0.08$). One year OS was 36% vs. 27% for CPX-351 vs salvage therapy, respectively ($P = 0.33$). Subset analysis of poor risk patients with a high European Prognostic Index (EPI) demonstrated an overall survival advantage for CPX-351 vs salvage chemotherapy ($P = 0.02$, HR = 0.55) [20]. The second randomized phase II trial compared CPX-351 to standard 7+3 induction chemotherapy in newly diagnosed patients with AML age 60–75 years. Results demonstrated an improvement in overall response rate (66.7% vs 51.2%, $P = 0.07$) with no difference in EFS or OS. A planned analysis of the high-risk AML cohort (secondary AML, complex cytogenetics, or age 70–75 years) however, showed a significant improvement in response rates (57.6% vs 31.6%, $P = 0.06$), as well as prolongation of EFS (HR 0.59, $P = 0.08$) and OS (HR = 0.46, $P = 0.01$) [21]. Given the promising outcomes in patients with secondary or therapy-related AML (t-AML), this provided the rationale for the design of the randomized phase III clinical trial that led to its regulatory approval in the frontline setting. In this landmark trial, 309 patients (age 60–75 years) with t-AML or AML-MRC

based on the WHO 2008 definition, were randomized to receive CPX-351 vs 7+3 (cytarabine + daunorubicin) induction chemotherapy. CPX-351 was dosed at 100 U/m^2 (100 mg/m^2 cytarabine and 44 mg/m^2 daunorubicin) on days 1, 3, and 5 of the induction cycle. The trial allowed for another induction cycle for patients with no response to the first induction. Response rates were higher in CPX-351 vs. 7+3 (47.7% complete remission/CR with incomplete count recovery vs. 33.3% respectively, $P = 0.016$). Importantly, overall survival was improved by about 4 months (9.6 vs. 5.9 months respectively, the hazard ratio (HR) for death 0.69, $P = 0.005$). Toxicities were similar in both groups, and both 30- and 60-day mortality were lower using CPX-351 (5.9% vs. 10.6% and 13.7% vs. 21.2%, respectively), but not statistically significant between treatment arms [7]. The FDA and the European Medicines Agency (EMA) have approved CPX-351 based on these results for patients with t-AML or AML-MRC. There are, however, some limitations for the widespread use of CPX-351. These include its higher cost compared to 7+3 and the delay in obtaining cytogenetic results (therefore missing a portion of patients with AML-MRC), a group which constituted 26.9% in this trial. It is worth noting that in a post hoc exploratory analysis landmarked at the time of transplant, patients receiving induction with CPX-351 had better overall survival than was observed in the control arm (not reached vs. 10.8 months for 7+3, HR 0.46, $P = 0.009$).

8.5 Novel Hypomethylating Agents and HDAC Inhibitors

Treatment for older adults with AML unfit to receive intensive chemotherapy remains a challenge; historically, options for those patients have included hypomethylating agents (azacitidine and decitabine), low-dose cytarabine (LDAC), or best supportive care (BSC). The median survival for patients treated with azacitidine was 10.4 months compared to 6.5 months for 7+3/LDAC/BSC arm, $P = 0.1$ and median OS for decitabine (in patients with poor/intermediate risk cytogenetics) was 7.7 months compared to 5.0 months for 7+3/LDAC/BSC arm, HR 0.85, $P = 0.11$ [22, 23]. Based on these data, treatment with hypomethylating agents has been widely adopted as a standard of care for newly diagnosed patients unfit to receive standard induction chemotherapy given the safety and tolerability profile. Guadecitabine (SGI-110), a second-generation hypomethylating agent, is currently under development. Guadecitabine is a dinucleotide of decitabine and deoxyguanosine and has a longer half-life than azacitidine or decitabine. Guadicitabine has been studied in early phase clinical trials in both newly diagnosed and relapsed/refractory AML [24, 25]. Patients were treated using three different dosing schedules. The first group received guadecitabine 60 mg/m^2 for 5 days, the second group received 90 mg/m^2 for 5 days, and the third group received 60 mg/m^2 for 10 days. Cycles were repeated every 28 days. Response rates and tolerability were compared in each treatment arm. There were no significant differences in the composite complete remission (CRc) rates between the arms for newly diagnosed, treatment naïve patients (CRc 50–59%). In the relapsed/refractory setting, CRc was higher using a 10- vs. 5-day schedule. A phase III randomized controlled trial (ASTRAL-1) of guadecitabine vs. physician's choice of standard therapy (azacitidine, decitabine, or LDAC) in treatment naïve adult patients with AML did not meet the co-primary endpoints based on failure to improve complete response rate ($P > 0.04$) and overall survival ($P > 0.01$). Currently, ASTRAL-2 is an ongoing phase III trial examining guadecitabine in relapsed/refractory AML (NCT02920008).

Histone deacetylase (HDAC) inhibitors have been investigated in myeloid malignancies and were shown to exert their effects by the regulation of histone and non-histone protein acetylation [26, 27]. While most HDAC inhibitors have modest activity as single agents, pracinostat, an oral HDAC inhibitor, has shown promising results when used in combination with HMA therapy [28, 29]. Pracinostat plus azacitidine was used upfront in a cohort of 50 patients and showed a complete remission rate of 42%. The primary composite endpoint included CR, CR

with incomplete count recovery (CRi), and morphologic leukemia-free state was 54% [30]. The 1 year overall survival rate was 62% [31]. The combination is currently being evaluated compared to azacitidine plus placebo in a randomized double-blinded phase III trial for newly diagnosed older adults with AML unfit for induction chemotherapy (NCT03151408) (Table 8.1).

8.6 Monoclonal Antibodies in AML

Monoclonal antibody target cell surface antigens that are preferentially expressed on myeloid cells or blast populations. Multiple targets are currently being investigated such as antibodies targeting CD33, CD123, and CD47 among others [32]. The mechanism of actions of monoclonal antibodies may vary to include cell-mediated cytotoxicity or in an antibody–drug conjugate (ADC) mechanism, where cytotoxic compounds are introduced to cells via target antigen. Gemtuzumab ozogamicin (GO) is an ADC that targets CD33-positive cells. The anti-CD33 antibody is linked to *N*-acetyl gamma calicheamicin. Once bound to CD33, the antigen/ADC is internalized and calicheamicin released intracellularly leading to direct DNA damage and cell death (Fig. 8.1). GO was initially approved by the FDA in 2000 and was then taken off the market in 2010 when the confirmatory phase III clinical trial (SWOG S0106) failed to show clinical benefit. GO has

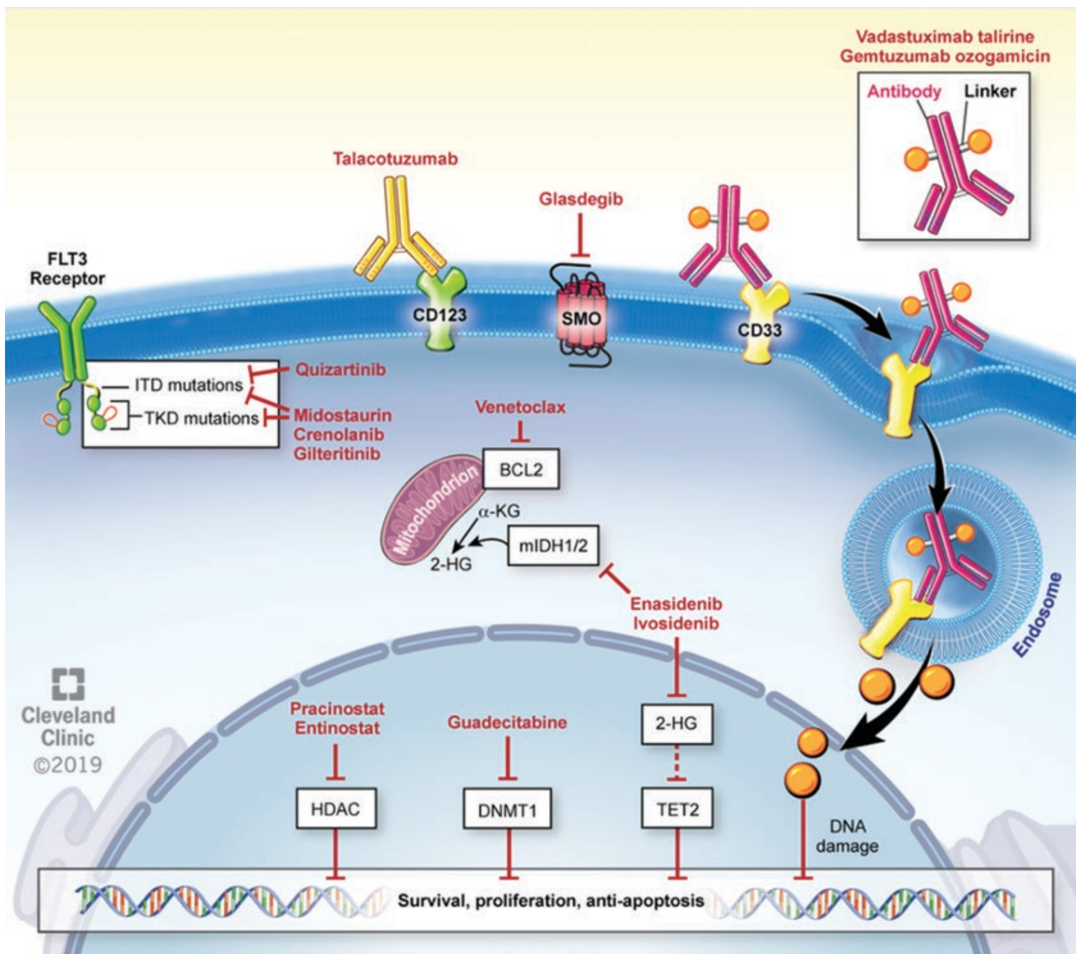

Fig. 8.1 Novel and investigational therapies in AML

been re-approved by the FDA for use in newly diagnosed CD33-positive AML in combination with chemotherapy (7+3) or as a single agent in the relapsed/refractory setting. The approval was based on a meta-analysis of 3325 patients treated with GO [6]. The pivotal trial, ALFA 0701, was a phase III randomized clinical trial of 271 patients with newly diagnosed AML (aged 50–70 years), patients received standard induction therapy (cytarabine + daunorubicin) alone or in combination with gemtuzumab. GO was given in fractionated dosing 3 mg/m^2 on days 1, 4 and 7 of the induction chemotherapy cycle. Patients achieving a CR/CRp went on to receive 5+2 consolidation plus GO at 3 mg/m^2 on day 1 of each of the two consolidation cycles.

The event-free survival (EFS) was 17.1% in the control group vs. 40.8% for GO at 2 years, HR 0.58, 0.43–0.78; $P = 0.0003$), overall survival was 41.9% vs. 53.2%, respectively (0.69, 0.49–0.98; $P = 0.0368$); however, the survival advantage was no longer significant in the long-term follow-up [33, 34]. In the large meta-analysis using data from five randomized trials (3325 patients), the addition of GO did not affect CR/CRi rate; however, reduced risk of relapse (OR 0.81, 0.73–0.90; $P = 0.0001$) and showed a 5-year OS advantage (OR 0.90, 0.82–0.98; $P = 0.01$). However, the survival advantage was largely driven by patients with good risk cytogenetics where the OS at 6 years was improved by 20·7%; OR for survival = 0·47, $P = 0.0006$. Given those results, the addition of GO has been largely adopted in favorable risk AML (i.e., core binding factor leukemia or AML with t(8;21) and inv16/t(16;16) in the upfront setting [6].

Another antibody–drug conjugate that targets CD33 is vadastuximab talirine (SGN-CD33A). The antibody is attached to a DNA binding agent, a pyrrolobenzodiazepine (PBD) dimer via a cleavable linker. The ADC showed promising activity in early phase clinical trials; however, the phase III CASCADE trial randomizing patients to hypomethylating therapy with or without SGN-CD33A was terminated early due to an increased number of deaths using the combination arm in newly diagnosed patients with AML. The pharmaceutical company has suspended patient enrollment and treatment in all of its vadastuximab talirine clinical trials including the ongoing phase I/II clinical trial in frontline high-risk myelodysplastic syndromes [35–38].

CD123 is a cell surface marker that is highly and preferentially expressed on leukemic blast cells and leukemic stem cells. Talacotuzumab is an Fc engineered anti-CD123 monoclonal antibody that activates antibody-dependent cellular cytotoxicity (ADCC) mediated by natural killer cells (NKs). It was used as a single agent after hypomethylating therapy failure with limited efficacy as a single agent. A phase II/III study of talacotuzumab in combination with decitabine vs. decitabine alone in older patients with AML ineligible for intensive chemotherapy has completed accrual, and the results of the trial are still pending. Talacotuzumab is administered by intravenous infusion at 9 mg/kg on days 8 and 22 of a 28-day cycle in combination with decitabine at the standard approved dose of 20 mg/m^2 on days 1–5 each cycle (NCT02472145).

IMGN632 is another CD123-targeting antibody–drug conjugate with a novel humanized anti-CD123 antibody joined, via a peptide linker, to a unique DNA-alkylating payload of IGN (indolinobenzodiazepine pseudodimer). In a phase I trial (NCT03386513) in patients with R/R AML, 12 patients received dose escalation of the drug. No DLTs have been observed at doses up to 0.18 mg/kg (cohort 4), and no discontinuations due to an AE have occurred. The most commonly observed treatment-emergent AEs of any grade were primarily gastrointestinal (decreased appetite, diarrhea, nausea; 25–42%), hematologic (febrile neutropenia; 42%), or vascular (peripheral edema, hypotension, sinus tachycardia; 25–33%). The most frequent grade 3+ AEs were febrile neutropenia (five patients; 42%) and lung infection (three patients; 25%); none of these events were considered related to IMGN632. In 12 evaluable patients, four (33%) achieved an objective response, including one complete remission (CR) and three complete remissions with incomplete recovery (CRi). The trial is still ongoing and further analyses will be presented in the future.

Other antibodies include AMG 330, an anti-CD33/CD3 bispecific T-cell engaging (BiTE) antibody composed of two single-chain variable fragments, where one is directed against CD33-positive leukemia cells and the other directed against CD3 found on cytotoxic T-lymphocytes (CTL) leading to CTL-mediated cell death of CD33-positive cells. A Phase I dose escalation study of AMG 330 in patients with relapsed/refractory AML enrolled 35 patients in 12 dose cohorts. The median number of cycles of AMG 330 was 1 (range, 1–6), and 89% of patients discontinued treatment. The most common reason for treatment discontinuation was disease progression ($n = 24$). The most common serious adverse event was cytokine release syndrome (CRS) occurring in 11 patients. Dose-limiting toxicities with the initial target dose of 480 μg/day were grade 2 CRS and grade 4 ventricular fibrillation, leading to a decrease in target dose to 240 μg/day. A total of four patients achieved CR/CRi at target doses of 120–240 μg/day [39].

CD70 is a cell surface marker that was noted to be upregulated with HMA therapy which could potentially contribute to HMA resistance. Based on preclinical work, a phase I/II trial combining azacitidine with ARGX-110, an anti-CD70 monoclonal antibody, in newly diagnosed AML patients unfit for intensive chemotherapy was designed (NCT03030612). The preliminary data of the first 12 patients treated showed no dose-limiting toxicity and high overall response rates (combining CR, CRi, PR, and MLFS) of 92% with 9/11 patients (82%) achieving CR/CRi [40]. Thus, monoclonal antibodies this far are showing clinical efficacy in combination therapy and not as single agents.

Another important new target in AML is CD47. CD47, also known as integrin-associated protein, is a ubiquitously expressed 50 kDa cell surface transmembrane Ig superfamily member. CD47 interacts with integrins (e.g., αvβ3, αIIbβ3, and α2β1) and thrombospondin-1 and serves as a ligand for signal regulatory protein alpha (SIRPα). CD47 is overexpressed in several solid tumors as well as hematologic malignancies such as AML. Several agents such as Hu5F9-G4 (Magrolimab), CC-90002, and others are currently in the development of AML as single agents or in combination with azacyitidine, but preliminary results are promising.

8.7 Targeted Therapies

8.7.1 Fms-Like Tyrosine Kinase 3 (*FLT3*) Inhibitors

FLT3 mutations are among the most common mutations occurring in about one-third of patients with AML. There are two well-characterized *FLT3* mutations, the first is *FLT3*-internal tandem duplications (*FLT3-ITD*) occurring in the juxtamembrane domain which are more prevalent (~25%) and point mutations in the tyrosine kinase domain (*FLT3-TKD*) which occur in 5–7% (Fig. 8.1). Multiple *FLT3* inhibitors have been used in clinical trials, some of which are now FDA approved. *FLT3-ITD* allelic ratio plays a role in prognostication, where the allelic ratio of >0.5 in the absence of *NPM1* mutations, stratifies patients in “adverse-risk” AML per ELN classification. On the contrary, the prognostic relevance of *FLT-TKD* mutations is somewhat controversial [3].

Multi-targeted kinase inhibitors such as sorafenib and midostaurin (first-generation FLT3 inhibitors) have shown in vitro inhibition of FLT3; however, single-agent activity was limited. A randomized phase II placebo-controlled clinical trial in younger adults (age < 60 yeas) with AML which assigned patients to receive sorafenib 400 mg BID in combination with induction chemotherapy showed improvement in 3-year event-free survival (EFS) (22%, 95% CI 13–32) in the placebo group vs. 40% (29–51) in the sorafenib arm (hazard ratio 0.64, 95% CI 0.45–0.91; $P = 0.013$) without an overall survival benefit. The combination with sorafenib was associated with increased toxicity (grade ≥ 3 adverse events such as fever, diarrhea, bleeding, cardiac events, hand–foot–skin reaction, and rash) [41]. Improvement in OS or EFS was not seen in older adults (age > 60 years) with FLT3-positive AML [42].

Midostaurin is now approved for the treatment of newly diagnosed AML patients in combination with induction and consolidation chemotherapy for all age groups. In early phase trials, midostaurin was used at 50 mg BID vs. 100 mg BID. The higher dose did not move forward largely due to gastrointestinal side effects [43], whereas the 50 mg dosing was well tolerated and showed high efficacy, which led to the design of the landmark trial that led to its approval; the RATIFY trial is a phase III randomized, double-blind, placebo-controlled trial of 717 patients age 18–59 years with newly diagnosed *FLT3*-positive AML which assigned patients to receive induction chemotherapy (daunorubicin plus cytarabine) followed by consolidation chemotherapy with high-dose cytarabine plus midostaurin at 50 mg twice/day or placebo added for 14 days (days 8–21) to induction and each of the consolidation cycles. Patients in remission received midostaurin/placebo maintenance for up to 12 months. Overall survival was significantly longer with midostaurin vs. placebo with a 22% reduction in risk of death (HR = 0.78; one-sided $P = 0.009$). Grade ≥ 3 adverse events were similar between treatment arms; however, nausea ($P = 0.05$), anemia ($P = 0.03$) and rash or desquamation ($P = 0.008$) were more common with midostaurin [9].

More selective second-generation *FLT3* inhibitors have reached later stages (phase III trials) in drug development and include gilteritinib, quizartinib, and crenolanib.

Quizartinib is a very selective FLT3-ITD inhibitor that was tested in a randomized controlled trial vs. salvage chemotherapy for patients with relapsed/refractory *FLT3-ITD*-positive AML. Quizartinib was dosed at 60 mg daily. Prior therapy with midostaurin was allowed. Of 367 patients ≥18 years of age randomized in 2:1 fashion, 245 received quizartinib and 122 patients received salvage chemotherapy. The overall survival was improved by 24% (HR for death = 0.76; 95% CI 0.58–0.98; stratified log-rank test, one-sided $P = 0.018$). Median overall survival was increased by about 7 weeks (OS 27 weeks vs. 20.4 weeks for quizartinib vs. chemotherapy) [44]. Despite this result, quizartinib did not receive FDA approval in the United States and currently is only approved for AML treatment in Japan. Gilteritinib is a selective FLT3 inhibitor that received FDA approval for the treatment of adults with relapsed/refractory AML with *FLT3* mutations. The phase I/II dose-escalation/expansion study demonstrated a maximum tolerated a dose of 300 mg daily due to the development of grade 3 diarrhea and elevated AST with higher doses. Grade 3 or 4 adverse events in that trial included febrile neutropenia (39%), anemia (24%), thrombocytopenia (13%), sepsis (11%), and pneumonia (11%) [17, 45]. In a phase III randomized controlled trial, 138 patients with R/R FLT3-positive AML were enrolled. Patients were treated with gilteritinib 120 mg/day oral continuous dosing. The complete remission (CR) or CR with partial hematologic recovery (CRh) rate was 21% after a median follow-up of 4.6 months. The median overall survival for patients who received gilteritinib was 9.3 months vs. 5.6 months for salvage chemotherapy (HR 0.637 (95% CI 0.490, 0.830), $P = 0.0007$) [46].

Patients treated with *FLT3* inhibitors may develop secondary mutations in D835 or F691 residues which are some of the described mechanisms of resistance [47]. Crenolanib is a potent and selective inhibitor of wild-type and mutant class III receptor tyrosine kinases *FLT3* and *PDGFRα/β*, particularly of D835 mutations [48]. Crenolanib was studied in the upfront setting in combination with chemotherapy with promising results. The preliminary analysis of 26 patients ≥18 years old enrolled in a phase II trial, crenolanib 100 mg TID was administered continuously starting on day 8 until 72 h prior to the next chemotherapy cycle. Consolidation consisted of up to four cycles of high-dose cytarabine with crenolanib starting on day 7 in each cycle. Maintenance crenolanib after consolidation or transplant was given for up to 12 cycles. Out of 25 patients evaluable for response, CR/CRi rate was 96% [49]. Thus, a phase III, randomized, multicenter trial is designed to compare the efficacy of crenolanib vs. midostaurin combined with standard chemotherapy for patients with FLT3-positive newly diagnosed AML (NCT03258931).

8.7.2 Isocitrate Dehydrogenase (*IDH*) 1/2 Inhibitors

Isocitrate dehydrogenases are enzymes that catalyze the conversion of isocitrate to alpha-ketoglutarate (αKG) in the cytoplasm (*IDH1*) or mitochondria (*IDH2*). Mutations in those enzymes are estimated to occur in up to 20% of patients with AML, in which case citrate is catalyzed into an oncometabolite (2-hydroxyglutarate, 2-HG), which leads to a hypermethylated state and a block in cellular differentiation [50–52]. *IDH1* and *IDH2* mutations are not considered prognostic at the time of AML diagnosis and current drug approvals for *IDH1/2* inhibitors (ivosidenib/enasidenib) are for use in the relapsed/refractory setting. However, it is worth noting that in a large study of >1500 patients with AML, *IDH2*^{R172} mutations were found in 1% of the cohort, patient outcomes with this gene abnormality were more favorable and similar to NPM1-mutated AML [53].

Enasidenib is an oral, first in class, FDA-approved *IDH2* inhibitor. In a phase I/II clinical trial of 239 patients with IDH2-mutated AML, a dose of 100 mg PO daily was given in the expansion phase to 176 patients with relapsed/refractory AML with an overall response rate of 40.3% (19.3% complete remission rate). The median overall survival was 9.3 months in all patients; however, that increased to 19.7 months in 19.3% of patients achieving a CR, which led to regulatory approval [8]. The IDENTIFY trial is a randomized phase III clinical trial investigating its use vs. conventional chemotherapy in older patients with *IDH2*-positive AML (NCT02577406).

Ivosidenib is an oral inhibitor of *IDH1* and was also evaluated in patients with relapsed/refractory AML. In early studies which enrolled 78 patients, the overall response rates were 38.5% with 17.9% of patients achieving a CR. Mutation clearance was observed in 27% of patients in CR [54]. In a larger phase Ib dose escalation/dose expansion trial, 258 patients were enrolled (safety cohort) and 179 of those had relapsed/refractory AML. Grade ≥ 3 adverse events included QT prolongation, *IDH* differentiation syndrome and cytopenia (anemia/thrombocytopenia). A dose of 500 mg daily dosing was chosen for the expansion cohort. Complete remission rate was 21.6% (95% CI 14.7–29.8), ORR 41.6% (95% CI 32.9–50.8), and the MRD negativity in those achieving complete remission or CR with partial hematologic recovery was 21% [4]. *IDH* inhibitors are now being evaluated in combination with induction chemotherapy for newly diagnosed patients with AML.

8.7.3 B-Cell Lymphoma 2 (BCL-2) Antagonists

Cellular death or apoptosis can be induced intrinsically or extrinsically. Intrinsic apoptosis is primarily regulated by BCL2 proteins. BCL-2 is an anti-apoptotic protein that prevents the expression of pro-apoptotic factors such as BCL-2 homology 3 (BH3) domain proteins and is overexpressed in AML. BH3 mimetics, such as venetoclax, inhibit BCL-2 and lead to apoptosis [55, 56].

Venetoclax was used as monotherapy in a phase I trial in patients with relapsed/refractory AML with an overall response rate of 19% [57]. More recently, FDA granted accelerated approval for venetoclax in combination with hypomethylating agents (HMA; azacitidine or decitabine) or low-dose cytarabine (LDAC) for newly diagnosed older adults with AML who are unfit to receive induction chemotherapy. Venetoclax was used at a dose of 600 mg PO in combination with LDAC and led to a CR/CRi rate of 62% with a median OS of 10.1 months [58]. Similarly, in a phase Ib dose-escalation and expansion study, 145 patients were assigned to receive oral venetoclax at 400, 800, or 1200 mg daily in combination with either HMA. In the expansion, 400 mg vs. 800 mg venetoclax was given. The CR/CRi was 73% in the venetoclax 400 mg—cohort with a median OS of 17.5 months (not reached for the azacitidine arm). Adverse events included nausea, diarrhea/constipation, leukopenia, febrile neutropenia, hypokalemia, fatigue, and decreased appetite [59]. Based on the results of this trial, a randomized, placebo-controlled phase III clinical trial of venetoclax

400 mg vs. placebo in combination with azacitidine (NCT02993523 was recently completed confirming the phase II results).

8.8 Summary

The treatment paradigm for acute myeloid leukemia has changed with eight new drug approvals since 2017 and will continue to evolve as many novel agents are in development. Currently, molecularly targeted therapies are limited to inhibitors of FLT3, IDH1, and two mutations. It is important to test for these mutations in the upfront setting, but to also repeat testing for these mutations as they may be acquired at relapse. Monoclonal antibodies targeting cell surface markers include anti-CD33, anti-CD123, and anti-CD70 antibodies among others. Currently, bi-specific T-cell engaging antibodies are being tested in phase I clinical trials. Lastly, BCL-2 inhibition with venetoclax has an important role in the upfront treatment setting of older patients unfit for intensive chemotherapy, in combination with low-dose cytarabine or hypomethylating agents.

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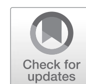

9 Immune-Based Approaches in AML

Ahmad S. Alotaibi and Naval Daver

9.1 Introduction

The applicability of immunotherapy in AML has long been appreciated, most notably highlighted by the established benefit of donor immune cells in controlling leukemia through graft versus leukemia effect post-allogeneic stem cell transplant (ASCT) and/or donor lymphocyte infusion [1]. Despite significant progress in AML therapeutics in recent years and the approval of several novel therapies in the frontline and relapsed setting, there remains a significant unmet need for more effective and more tolerable therapies, especially in relapsed AML with non-targetable mutations, post-ASCT relapsed AML, frontline older AML, and for MRD eradication [2]. Immunotherapy has revolutionized the treatment of a variety of solid and hematological malignancies [3]. In AML, the FDA approved the CD33-targeted conjugated antibody gemtuzumab ozogamicin in frontline AML in combination with anthracycline- and cytarabine-based induction, and as a single-agent therapy in relapsed AML [4, 5]. Several additional immune-based therapies that harness T and NK cells against leukemia, including immune checkpoint antibodies, bispecific and dual antigen receptor targeting (DART) antibodies, and chimeric antigen receptor (CAR) T- and NK-cell therapies are in various stages of clinical development for the therapy of AML. Development of effective and safe immune therapies will likely complement and further enhance the efficacy of AML therapy.

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9.2 Immune Checkpoint Inhibitors

Immune checkpoints (ICP) play an important role in the regulation of immune homeostasis by optimally balancing the stimulatory and inhibitory signals that mediate the T-cell immune response [6, 7]. ICP inhibitors have been widely studied and are FDA approved for several solid tumors [8, 9]. In hematological malignancies, ICP inhibitors are not as widely developed or approved; however, clinical benefits have been observed most strikingly in Hodgkin lymphoma [10, 11]. In AML, bone marrow infiltrating T-cell populations are preserved and may even be increased compared with bone marrows from healthy individuals, with an increased frequency of immune inhibitory and activating co-receptor expression (especially in relapsed AML) including PD-1, OX40, TIM3, and LAG3, suggesting a potential role for T-cell-harnessing therapies in AML [12–14]. In the last 4–5 years, several ICP inhibitors have been evaluated in clinical trials in patients with AML and will be discussed [6, 12].

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9.2.1 Single-Agent Immune Checkpoint Inhibitors

Pidilizumab is a humanized monoclonal IgG1 antibody to immune co-inhibitory receptor PD-1. It was investigated in a phase I study in patients with advanced hematologic malignancies, including eight patients with AML. This was one of the first experiences of ICP inhibitor based therapy in patients with AML. Pidilizumab was shown to be safe and well tolerated with a best response in AML of decreased peripheral blasts from 50% to 5% in one patient [15].

Ipilimumab, an anti-CTLA4 antibody, demonstrated encouraging activity in a phase I/IB study in patients with relapsed AML following ASCT [16]. In the study by Davids et al., 5 of 12 (42%) patients with relapsed AML post-ASCT achieved complete remission including three patients with extramedullary disease. Four of the five patients had a durable response which was maintained beyond 1 year. All responses were achieved at the higher dose of ipilimumab (10 mg/kg Q3 weeks for a maximum of four doses). Among the 22 patients who received the 10 mg/kg dose of ipilimumab, two patients developed chronic graft versus host disease (GVHD) of the liver and one patient developed grade II acute GVHD of the gut, which resolved with steroid administration but precluded further administration of ipilimumab. Of note, a majority of the patients treated on this trial were >1 year post-ASCT, with preserved donor chimerism, and had minimal to no GVHD post-ASCT indicating a niche post-ASCT population that demonstrated high sensitivity to ipilimumab.

9.2.2 Immune Checkpoint Inhibitors in Combination with Hypomethylating Agents

Investigators have demonstrated that patients with AML/MDS treated with hypomethylating agents (HMA) had dose-dependent upregulation of checkpoint molecules PD-L1, PD-L2, PD-1, and CTLA4 expression [17, 18]. Patients who had the highest degree of PD-L1 upregulation had the shortest duration of response to hypomethylating agent therapy and a trend to inferior overall survival with hypomethylating agent therapy. This led to the hypothesis that the activation and upregulation of immune checkpoints during HMA therapy may be a possible mechanism of resistance to HMA therapy, which may be overcome by combining HMA therapy with checkpoint inhibitor/s [19]. Nivolumab, an anti-PD-1 antibody, was combined with azacytidine in a phase II trial for patients with relapsed/refractory AML [20]. Among 70 patients treated, the overall response rate (ORR) was 33%, including 22% CR/CRi, 1 partial response (PR), and 7 patients with hematologic improvement maintained >6 months. Grade 3–4 immune-related adverse events occurred in 11% of the patients, the most frequent being pneumonitis. The median overall survival (OS) for the 70 patients was 6.3 months, and among salvage 1 patients ($n = 32$), the median OS was 10.5 months. Patients who achieved any response (CR/CRi/PR/HI) or had stable disease ($n = 29$; 42%) had significantly improved OS compared with non-responders ($n = 41$; 58%), without censoring for ASCT (16.2 vs. 4.1 months; $P < 0.0001$) and also after censoring for ASCT ($P < 0.001$). Notably, this was a high-risk population with 44% of the patients with secondary AML with poor risk cytogenetics and median number of prior therapies for AML was 2 (range, 1–7). A significant increase in the BM CD4+T effector subset expressing CTLA4 was noted in post-therapy samples, as compared with pre-therapy samples in non-responders to azacitidine with nivolumab, whereas responders did not demonstrate these changes. This observation suggested that CTLA-4 upregulation may be a mechanism of resistance to PD-1-based therapies in AML, similar to what has been shown in multiple solid tumors.

To overcome this possible mechanism of resistance, anit-CTLA-4 antibody ipilimumab was added to azacytidine and nivolumab in a phase II trial [21]. Thirty-one R/R AML patients were treated with the combination of azacytidine, nivolumab, and ipilimumab, 24 patients were evaluable at the time of last report. ORR was 44% including 36% CR/CRi and 8% who had a

hematologic improvement maintained for ≥6 months. The 1-year OS in this R/R AML cohort was 45%. The median OS in azacitidine + nivolumab + ipilimumab versus azacitidine + nivolumab versus contemporary historical HMA-based clinical trial controls in R/R AML at the same institution were 10.5, 6.4, and 4.6 months, respectively ($P = 0.0025$). Grade 3/4 immune-mediated toxicities were observed in 6 patients (25%), including rash, pneumonitis, and colitis.

In a recently presented phase II study, another anti-PD-1 antibody pembrolizumab was given in combination with azacitidine in a cohort of patients with R/R AML and newly diagnosed AML [22]. In the R/R AML cohort, 37 patients were enrolled, of whom 29 (78%) completed at least two cycles and were evaluable for response. Fourteen percent achieved CR/CRi, 1 (4%) PR, and 14% hematologic improvement. The median number of cycles to response was 4 (range, 2–6). With a median follow-up of 14.9 months, the median OS for the R/R AML cohort was 10.8 months with 40% one-year survival. The median OS for patients who achieved CR/CRi/PR was 17.2 months with 75% 1-year survival. In the second cohort, 22 newly diagnosed AML patients were enrolled, they were older patients, who were either not candidates or unwilling to receive intensive chemotherapy. Among 17 evaluable patients, 47% achieved CR/CRi, 12% PR, and 12% hematologic improvement. The median number of cycles to response was 2 (range, 2–15). With a median follow-up of 19 months, the median OS for the frontline cohort was 13.1 months. For patients who achieved CR/CRi/PR, the median OS was not reached with a 79% one-year survival. Grade 3/4 immune-related adverse events were observed in 9 (24%) patients in Cohort 1, and 3 (14%) patients in Cohort 2.

The final results of a phase II randomized, international, multicenter study (NCT02775903) of azacitidine with or without a PDL1-antibody durvalumab were reported at ASH 2019 [23]. Azacitidine with or without durvalumab was given as frontline therapy for high-risk MDS (cohort 1) or AML (cohort 2). Cohort 2 randomized 129 AML (1:1 randomization) patients ≥65 years old who were ineligible for intensive chemotherapy. One hundred eleven AML patients discontinued trial treatment. Median number of treatment cycles for the AML cohort azacitidne with durvalumab vs. azacitidine was 6.5 vs. 6.7 months. There were no statistically significant differences in ORR between azacitidne with durvalumab vs. azacitidine (31.3% vs. 35.4%) or CR rate (17.2 vs. 21.5). The median OS for azacitidne with durvalumab vs. azacitidine was 13.0 vs. 14.4 months. Notably, more than 50% of the patients discontinued the trial medications and were censored for survival analysis which may impact result interpretation.

9.2.3 Immune Checkpoint Inhibitors in Combination with Cytotoxic Chemotherapy

Chemotherapy has been demonstrated to augment the immune response against cancer [24]. Several chemotherapeutic agents reinstate immunosurveillance by influencing the tumor–host equilibrium at multiple levels [25]. In vivo experiments in mouse models have shown that the injection of cytosine arabinoside induced the expression of CD80 and CD86 and reduced the expression of PD-1 on leukemic cells, making them more susceptible to cytotoxic T-lymphocyte-mediated killing [26]. Exposure of calreticulin on the surface of dying leukemic cells after exposure to chemotherapy has been shown to enhance cellular antitumor immune responses in AML patients [27]. Clinical trials are examining the combination of immune checkpoint inhibition with cytotoxic chemotherapy in patients with AML.

In a phase II study, nivolumab was combined with idarubicin and cytarabine in patients with newly diagnosed AML or high-risk MDS (>10% blasts) [28]. Forty-four patients were enrolled of whom 42 had AML and 2 had MDS. ORR was 78%, including 64% complete responses and 14% CRi. Of these 34 responders, 18 proceeded to ASCT. At a median follow-up of 17.3 months, the median overall survival for all patients was 18.5 months, whereas the median event-free

survival was not reached. Six patients had grade 3–4 immune-related adverse events. No treatment-related deaths were attributed to nivolumab, and the combination was deemed safe with no concerning toxicities pre- or post-ASCT.

In the relapsed/refractory setting, a phase II trial examined high-dose cytarabine followed by pembrolizumab [29]. Thirty-seven patients with relapsed/refractory AML received age-adjusted high-dose cytarabine followed by pembrolizumab 200 mg IV administered on day 14 of the induction. If they achieved a response, they went on to receive maintenance therapy with pembrolizumab 200 mg IV Q3 weeks for up to 2 years, until relapse or progression of disease. The overall response (ORR: CR + CRi + PR + MLFS) rate was 46% and composite CR (CR + CRi) rate was 38%. Nine (24%) patients proceeded to ASCT. There were no instances of grade > 3 acute GVHD or veno-occlusive disease post-ASCT. With a median follow-up of 7.8 months, the median OS was 8.9 months, while event-free survival was 6.9 months.

9.2.4 Immune Checkpoint Inhibitors and Allogenic Hematopoietic Stem Cell Transplant

ASCT remains the most frequent curative approach for many patients with AML. Considering the encouraging response rates observed in patients with AML who are treated with various ICP inhibitors as discussed previously, it is likely that several patients will be considered for curative intent ASCT. There have been concerns regarding increased peri-transplantation complications, especially severe acute graft-versus-host disease (aGVHD) and veno-occlussive disease (VOD) [30]. The incidence of grade 3–4 aGVHD in a group of patients with NHL who underwent ASCT after being treated with PD-1 monoclonal antibodies was reported to be 23%, which was higher than would be expected [31]. However, the incidence of GVHD is known to vary based on many variables, including the allograft donor source, the type of post-ASCT GVHD prophylaxis, prior history of GVHD, immunosuppression at time of ICP inhibitors administration, and dosing and duration of ICP inhibitors used [32]. In a retrospective study by Oran et al., 43 patients with AML/MDS who were treated with ICP inhibitors prior to ASCT were evaluated for the incidence of aGVHD. Outcome analyses were stratified by post-ASCT GVHD prophylaxis according to the use of post-ASCT cyclophosphamide (PTCy) (22 patients) or not (non-PTCy) (21 patients). The PTCy group demonstrated a trend toward lower grade 3–4 aGVHD when compared with the non-PTCy group (5% vs. 22%). In a matched control analysis using patients with no prior use of ICP inhibitors, the risk of grade 3–4 aGVHD was increased in patients with prior ICP inhibitor exposure who did not receive PTCy (HR 8.5; 95% CI 0.9–79 [$P = 0.06$]) but was not increased in patients treated with PTCy for GVHD prophylaxis (HR 2; 95% CI 0.1–31 [$P = 0.6$]) [33].

Several ongoing, prospective, phase II/III trials will likely provide more information on this aspect. Meanwhile, considering the use of PTCy as a part of the GVHD prophylaxis in patients who received ICP inhibitors before undergoing ASCT may improve transplantation outcomes.

9.2.5 Anti-CD47 (Macrophage Checkpoint)

CD47, an integrin-associated cell surface protein, inhibits cell phagocytosis via interaction with phagocyte-expressed signal regulatory protein alpha (SIRPα) [34]. Increased expression of CD47 has been shown in a variety of solid and hematological malignancies [34, 35]. It functions as a macrophage checkpoint, providing a potent “do not eat me” signal that allows for tumor cell evasion of immune destruction by macrophages [35]. In AML, CD47 was shown to be upregulated and independently associated with a poor prognosis [36,

37]. Hu5F9-G4 (magrolimab) is a humanized monoclonal antibody that binds CD47 and blocks it from interacting with its ligand SIRPα, on phagocytic cells, leading to phagocytic elimination of cancer cells [38].

The toxicity profile of this agent in other hematologic malignancies appears favorable compared to T-cell checkpoint inhibitors [39]. An ongoing phase I clinical study (NCT03248479) is evaluating magrolimab alone or in combination with azacitidine in patients with AML and MDS [40]. At the last update (ASH 2019), 62 patients (35 MDS and 27 AML) were treated with magrolimab plus azacytidine. The majority of patients had poor cytogenetic risk, 66% and 67% for MDS and AML patients, respectively. Forty-one percent of the AML patients harbored a TP53 mutation. Magrolimab in combination with azacitidine was well tolerated with a safety profile similar to azacitidine monotherapy. No significant cytopenias, infections, or autoimmune adverse events were observed. The majority of patients had significant hemoglobin improvement and decrease in transfusion frequency with therapy. Treatment discontinuation due to adverse events occurred in only 1 of 62 (1.6%) treated patients. In the MDS cohort, 24 patients were evaluable for efficacy in whom ORR was 92% with a CR rate of 50%. In the AML cohort, 22 patients were evaluable with ORR 64% including CR/CRi in 55%. Median time to response was 1.9 months, more rapid than would be expected with azacitidine alone. No median duration of response or overall survival has been reached for either MDS or AML patients with a median follow-up of 6.4 months (range 2.0–14.4 months) for MDS and 8.8 months (range 1.9–16.5 months) for AML. A particular efficacy of magrolimab in combination with azacitidine was noticed in TP53 mutant AML patients (nine evaluable patients), with 78% CR/CRi rate and 57% MRD negativity. The study is ongoing in a potentially registrational, frontline single-arm non-blinded study. A phase III randomized study in MDS is planned.

9.3 Bispecific Antibodies

Bispecific T-cell engager (BiTE) antibodies include two single-chain variable fragments, which simultaneously juxtapose the epsilon subunit of polyclonal cytotoxic CD3+ T cells to the selected target tumor antigen on malignant cells. Thereby T cells can be recruited against the tumor in an antigen-independent manner [12, 41, 42]. Bispecific antibodies targeting CD33 and CD123 are currently being evaluated in AML.

Flotetuzumab (FLZ) is a CD123/CD3 bispecific dual-affinity retargeting (DART) molecule, administered as a 7-day/week continuous infusion. Thirty patients with R/R AML were treated in a phase I study [43]. Infusion-related reactions/cytokine release syndrome (IRR/CRS) occurred in all patients, including grade ≥3 in 4/30 (13.3%) patients. Most IRR/CRS events were of short duration and reversible with protocol-specified supportive care. Antileukemic activity was reported in 18/27 (67%) response-evaluable patients, with an overall response rate (ORR) of 22% (6/27) and a CR/CRi rate of 19% (5/27). In an updated analysis presented at ASH 2019 [44], 50 patients with R/R AML received FLZ at the phase II recommended dose. Thirty (60%) patients had primary refractory AML, which was defined as patients who failed ≥2 induction attempts (24 patients) or patients who had an early relapse (<6 months) post remission (6 patients) with no prior ASCT. This population was heavily pretreated with a median of four prior lines of therapy (range 2–9), with 40% having secondary AML and many having non-favorable cytogenetic risk (60% adverse and 23% intermediate per ELN 2017 risk category). Among 28 evaluable primary refractory patients, 32.1% achieved composite CR (CR + CRh + CRi). This is better than the expected response rate with conventional salvage therapy in this heavily pretreated, primary refractory AML population. FLZ was well tolerated, with no increased cytokine release syndrome events in primary refractory patients compared with relapsed AML patients, with 3% grade 3 and no grade 4 CRS.

AMG 330 is a BiTE that binds CD33 and CD3. In a phase I dose escalation study (NCT02520427) of AMG 330 in R/R AML [45], 35 patients were enrolled in 12 dose cohorts with a target dose range of 0.5–480 μg/day. Patients received a median of 1 (range: 1–6) cycle with AMG 330, 31/35 (89%) patients discontinued treatment for disease progression (*n* = 24), adverse events (*n* = 5, 2 treatment-related), or patient request (*n* = 2). Serious AEs were seen in 23/35 (66%) patients and included CRS (*n* = 11), febrile neutropenia (*n* = 6), and pneumonia (*n* = 4). One patient died on study due to AML progression with no treatment-related death. Two patients achieved CR and two patients achieved CRi. The study is ongoing and updated results are awaited.

AMG 673 is a half-life extended BiTE that binds both CD33 and CD3 and is genetically fused to the N-terminus of a single-chain IgG Fc region, thereby potentially increasing the half-life of the molecule. In a phase I, sequential dose escalation study (NCT03224819) [46], 30 patients with relapsed/refractory AML were enrolled in 10 cohorts and were treated with AMG 673 (dose range, 0.05–72 μg IV per dose). About 27/30 (90%) patients discontinued treatment due to disease progression (*n* = 21), patient request (*n* = 2), protocol-specified criteria (*n* = 2), or adverse events (*n* = 2). Assessment of bone marrow showed a decrease in blasts in 12/27 (44%) evaluable patients, of which 6 experienced a ≥50% reduction in blasts compared with baseline. One patient achieved complete remission with incomplete hematologic recovery (CRi). The most common treatment-related AE was cytokine release syndrome (CRS) reported in 15/30 (50%) patients (grade 1, *n* = 6; grade 2, *n* = 5; grade 3, *n* = 4; no grade 4 CRS).

AMV564 is a bivalent, bispecific CD33/CD3 BiTE. In a phase I, dose escalation study (NCT03144245) [47], 36 relapsed/refractory AML patients were enrolled in 10 dose escalation cohorts from 0.5 to 300 μg/day. No dose-limiting toxicities were reported. Median duration of treatment was 20 days (range 3–204 days). Using a lead-in intrapatient dose ramp up schedule, no grade 3 or higher cytokine release syndrome was observed. The most common grade ≥3 treatment-emergent AE were anemia, reported in 4 (11%) patients. Bone marrow blast reductions were observed in 17 (49%) of 35 efficacy evaluable patients.

XmAb14045 is a bispecific antibody targeting both CD123 and CD3. Initial results of the phase I study were presented at ASH 2018 [48]. Sixty-four patients were treated, 63 with relapsed/refractory AML. Treatment was administered weekly in 28-day cycles and continued for as long as tolerated with continuing evidence of therapeutic benefit. CRS occurred in 49 of 64 patients (77%). Seven patients (11%) developed grade ≥3 CRS, the majority of these on the first dose. There were no CRS-related deaths. No myelosuppression requiring dose modification or evidence of tumor lysis syndrome was seen. Single-agent anti-leukemic activity was documented in heavily pretreated patients with relapsed/refractory AML with a best response of CR in two patients and CRi in one patient.

The clinical experience with BiTEs in AML is still in development, with numerous clinical trials ongoing. However, the response rates have generally been lower and less robust than were achieved with CD3–CD19 bispecific T-cell engager antibody blinatumomab in B-cell acute lymphoblastic leukemia. Evaluating BiTEs in low burden disease or MRD settings in AML may yield higher efficacy given the known mechanism of action of BiTEs. Combining BiTE with immune check point inhibitors may be a potential therapeutic strategy to overcome resistance to either agent alone in patients with AML [49], and such combinations are expected to enter into clinical trials in the near future.

9.4 Chimeric Antigen Receptor T-Cell Therapy

A chimeric antigen receptor, CAR, is composed of an antigen-recognition domain linked to costimulatory molecules, which are then transduced into autologous or allogeneic T cells [50]. The binding between CAR and its antigen on tumor cells triggers a signal transduction cas-

cade through signaling domains that then activates T cells to kill the target either directly or by harnessing other components of the immune system [51]. CAR bind to their tumor antigen in an MHC-independent manner, which is their main advantage over regular T-cell receptors (TCR) [52].

Anti-CD19 CAR T-cell therapies against B-lineage malignancies have been successfully used in clinical practice and are FDA approved [53]. In contrast to lymphoid malignancies, AML is a more heterogeneous disease, lacking highly differentiated and optimally targetable tumor-specific surface antigens. Most of the AML "target" antigens being used in antibody drug conjugates, bispecific antibodies, and CAR T-cell therapies are frequently expressed in normal hematopoietic stem/progenitor cells or organ tissues, which potentially could increase the "on-target, off-tumor" toxicity.

Early phase AML CAR T and CAR NK clinical trials are ongoing, targeting CD33, CD123, and NKG2D. In a phase I study (NCT03018405, still recruiting) [54], 12 patients with hematological malignancies (8 AML, 3 MM, and 1 MDS) had received CYAD-0, a CAR product based on the receptor NKG2D with specificity for a broad range of ligands (MICA, MICB, and ULBP1–6) expressed on most tumors. CYAD-01 was administered without prior preconditioning therapy. CRS occurred in five patients, three grade 1/2 and two grade 3, with rapid resolution with appropriate therapies such as tocilizumab. No neurotoxicity was observed. Out of the eight R/R AML patients enrolled, seven were evaluable for response. The overall response rate was 42% (3/7 patients) with one complete remission with partial hematologic recovery (CR_h) and two CR with incomplete marrow recovery (CR_i). One patient proceeded to ASCT and has been in durable response for more than 1 year.

CD123-specific CAR T cells are under investigation in a phase I study (NCT02159495 still recruiting) for patients with relapsed or refractory AML (cohort 1) and BPDCN (cohort 2). Prior to T-cell infusion, all patients undergo a lymphodepleting regimen including fludarabine 25–30 mg/m^2 daily for 3 days and cyclophosphamide 300 mg/m^2 daily for 3 days. Patients receive a single dose of CD123-CAR T cells with an option for a second infusion if they continue to meet safety and eligibility criteria and still have CD123+ disease. At last data update (ASH 2017) [55], seven patients (six AML and one BPDCN) had received CD123-CAR T cells. All six patients in the AML cohort had refractory AML following ASCT, and a median of 4 (range: 4–7) prior lines of therapy. One patient achieved CR and was able to proceed for second ASCT. Another patient with CR prior to treatment remained in CR post therapy and proceeded for ASCT. Two patients had blast reduction, and one patient achieved a morphologic leukemic-free state. CRS occurred in five patients (four grade 1 and one grade 2). All toxicities were reversible and manageable. There were no dose-limiting toxicities and no treatment-related cytopenias. In the BPDCN cohort, one patient who failed a CD123 antibody–drug conjugate with a bulky subcutaneous mass achieved CR after a single dose of CD123-CAR T cells and continued to be in CR at 60 days post-infusion. He tolerated the treatment well with no CRS or neurologic toxicity.

In spite of limitations in the form of lack of an ideal AML antigen, concern over CRS, potential for prolonged myelosuppression, the field of CAR T cell as a therapeutic option in AML is progressing, both preclinically and clinically. Several strategies, like gene-editing technology, combination therapies, targeting low burden disease, or MRD, are under early investigations to optimize the CAR T-cell therapy outcome in AML.

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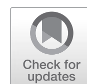
Part II

Acute Lymphoblastic Leukemia



10 Acute Lymphoblastic Leukemia: Clinical Presentation, Diagnosis, and Classification

Sandeep Gurbuxani, Joseph P. Wynne, and Richard A. Larson

10.1 Introduction

Acute lymphoblastic leukemia (ALL) is a malignant neoplasm of lymphocytes characterized by the clonal accumulation of immature blood cells in the bone marrow. These abnormal cells are arrested in the lymphoblast stage of the maturation pathway. Aberrations in proliferation and differentiation of these cells are common, and normal hematopoiesis is suppressed. Symptoms result from varying degrees of anemia, neutropenia, and thrombocytopenia or from infiltration of ALL cells into tissues. Although virtually any organ system may become involved once leukemia cells enter the peripheral blood, the lymph nodes, spleen, liver, central nervous system (CNS), and skin are the most common sites detected clinically.

ALL is a heterogeneous disease with distinct biologic and prognostic groupings. Treatment strategies tailored to specific prognostic groups have already yielded dramatic improvements in the outcomes for children with ALL, and similar risk-adapted strategies based on the biological heterogeneity of the disease are now being applied to adults. Moreover, increasing knowledge of the prognostic significance of recurrent cytogenetic abnormities plays an important role in the current WHO classification of precursor lymphoid neoplasms, and thus, cytogenetics will be emphasized in this review [1].

10.2 Clinical Presentation

10.2.1 Epidemiology

ALL is the most common malignant disease in childhood, peaking in incidence between ages 2 and 5 years [2]. In contrast, ALL only accounts for approximately 20% of acute leukemia in adults. Despite this early peak incidence in childhood, nearly 45% of all new cases are diagnosed in adults (age > 20 years). This is due to a combination of ALL developing in all age groups and a steadily increasing incidence rate above the age of 50 years [3].

The worldwide incidence of ALL is estimated to be 1–5/100,000 [4]. ALL is slightly more common among males than females (1.3:1). Geographic variations with higher incidence rates in Spain and in Latin American countries are likely related to a number of factors including socioeconomics, ethnicity, and an urban or rural setting. A higher frequency of ALL has been reported in industrialized countries and in urban

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areas. The incidence of ALL is more common in Caucasians compared with African-Americans with an age-adjusted overall incidence in the United States of 1.9/100,000 in whites and 1/100,000 in blacks. Americans of Hispanic ethnicity have the highest incidence rate at 2.5/100,000 compared to 1.6/100,000 for non-Hispanic whites [3].

10.2.2 Presentation

The clinical presentation of ALL is often sudden. Patients commonly present with a recent history of fatigue or spontaneous bleeding. Malaise, lethargy, weight loss, fevers, and night sweats are often present but typically are not severe [5]. Compared to AML, patients with ALL experience more bone and joint pain. Rarely, they may present with asymmetric arthritis, low back pain, diffuse osteopenia, or lytic bone lesions [6]. Children experience these symptoms more frequently than adults with young children often presenting with difficulty walking due to bone pain. Lymphadenopathy, splenomegaly, and hepatomegaly are more common than in AML and affect half of adults with ALL. CNS involvement is also more common in ALL compared to AML [7]. Patients may present with cranial neuropathies, most commonly involving the sixth and seventh cranial nerves. Nausea, vomiting, headache, or papilledema may result from meningeal infiltration and obstruction of the outflow of CSF leading to raised intracranial pressure. Patients may also present with a symptomatic mediastinal mass with symptoms of cough, dyspnea, and/or superior vena cava syndrome if it impinges on the great vessels. Testicular involvement, which presents as a painless unilateral mass, is noted at diagnosis in approximately 2% of boys. It is associated with infant or adolescent age, hyperleukocytosis, splenomegaly, and a mediastinal mass. The diagnosis of testicular involvement can be confirmed by wedge biopsy but is often not needed unless physical examination and imaging studies are equivocal. Bilateral biopsies have previously demonstrated the high incidence of contralateral testicular disease, necessitating radiation treatment to both testicles if testicular involvement persists after induction therapy [8, 9].

The physical examination is often notable for pallor, generalized lymphadenopathy, hepatosplenomegaly, and signs associated with thrombocytopenia, such as gingival bleeding, epistaxis, petechiae/ecchymoses, or fundal hemorrhages. Dermal involvement, known as leukemia cutis, may also be noted.

10.3 Diagnosis

10.3.1 Initial Laboratory Evaluation

The morphologic recognition and phenotypic characterization of lymphoblasts in the blood and bone marrow are of major importance in the correct diagnosis and classification of ALL. These require careful evaluation of well-prepared peripheral blood and bone marrow aspirate smears and phenotypic analysis of the blasts by flow cytometry and immunohistochemistry with an appropriate panel of surface and cytoplasmic markers.

The settings in which lymphoblasts are seen in the peripheral blood and bone marrow aspirate can vary significantly. In the majority of cases, the counts and cellularity are high, but in some, there can be pancytopenia and hypocellularity, which make the recognition of the blasts more critical. A leukoerythroblastic picture can be seen in some cases, and in rare T-cell ALL cases, there may be dysplasia in the granulocytic elements. One unusual morphologic presentation of ALL is that of precursor-B ALL with eosinophilia. This entity can show eosinophilia preceding, concurrent with, or following ALL at either diagnosis or relapse. Sometimes the eosinophilia can be so extreme as to obscure the blasts. This entity is associated with the specific cytogenetic abnormality, t(5;14)(q31;q32) [10]. In other cases, eosinophilia can be indicative of underlying rearrangements of *PDGFRA*, *PDGFRB*, *FGFR1*, and *PCM-JAK2* rearrangements. Despite initial presentation as lymphoblastic leukemia, these disorders arise from a pluripotent stem cell and are

classified as myeloid/lymphoid neoplasms with eosinophilia indicating propensity for presentation with many different morphologic features including myeloproliferative neoplasms and acute leukemia [1].

In addition to the peripheral blood and bone marrow, extramedullary involvement with predilection for involvement of the central nervous system (CNS) is common. The evaluation of cytospin slides made from the cerebrospinal fluid (CSF) may indicate CNS involvement. The current approach used to determine CNS involvement by the Children's Oncology Group (COG) has been to classify CNS leukemia into three groups: CNS 1 (<5 WBC/μL of CSF and no blasts), CNS 2 (<5 WBC/μL of CSF with blasts), and CNS 3 (≥5 WBC/μL of CSF with blasts, or cranial nerve findings) [11]. Moreover, some studies advocate using flow cytometry in addition to cytology to identify blasts in the CSF more accurately [12]. Whether this level of detection would affect outcomes is unclear.

In cases with a high cell turnover, the evaluation of blood chemistries may reflect the evidence of tumor cell lysis, such as hypocalcemia, hyperkalemia, hyperphosphatemia, elevated LDH, hyperuricemia, and elevated creatinine.

10.3.2 Cytomorphology

The cytomorphologic characteristics of lymphoblasts are varied, but are usually sufficient to suggest a blastic or neoplastic process for which phenotyping can confirm and further characterize the process. The most typical lymphoblast is a small- to intermediate-sized cell with round or oval nucleus that has a smudgy nuclear chromatin, absent or small nucleoli, and scanty cytoplasm. Comparison to normal appearing "mature" lymphocytes in the blood or marrow aspirate is useful for the assessment of size and degree of chromatin condensation. The scant cytoplasm is quite dramatic in many cells as the nucleus has an appearance of bulging out of the cell cytoplasm. The cytoplasm is pale blue and not intensely stained. In other cases, lymphoblasts exhibit significant morphologic variation. Such lymphoblasts are larger and have oval or irregular nuclear outlines and less homogeneous chromatin. Nuclei are variable but frequently prominent, and sometimes multiple. The cytoplasm is more abundant but still pale blue. The earlier FAB classification into L1 and L2 morphology is poorly reproducible and has little clinical significance. Therefore, the 2008 revision of the WHO classification of ALL adopted a classification based on immunophenotype and genotype [13].

Similarly, while Burkitt lymphoma can have a leukemic presentation, it is now recognized as a malignancy of mature B cells to distinguish it from lymphoblastic leukemia/lymphoma that arises from precursor lymphoid cells. The so-called L3 blasts (referred to as Burkitt leukemia for the remainder of this chapter) are usually quite distinctive. The blasts are large and homogeneous and have distinctive deep blue cytoplasm, which commonly contains sharply defined vacuoles. The nuclei of Burkitt cells are large and round or oval. They have a finely stippled chromatin and variable nucleoli, which sometimes are quite prominent. The larger size and intense cytoplasmic basophilia with vacuolization are decidedly the most distinctive features but are not entirely specific. Vacuoles can be seen in monoblastic and erythroid leukemia, and together with the deep blue cytoplasm, can be seen in other cases of ALL as well as in some cases of AML [14, 15]. Conversely, some cases of Burkitt leukemia with the characteristic chromosomal translocations lack the usual "L3" morphology [16].

A number of additional cytologic variants of lymphoblasts deserve mention. Although there are no particular clinical, phenotypic, or genetic correlates with these variant blasts, their recognition will help avoid exclusion of ALL from diagnostic consideration in cases where they are seen. Small lymphoblasts can be seen in rare cases of ALL [17]. These blasts are closer in size to small "mature" lymphocytes, making them difficult to distinguish from the small lymphoid cells of chronic lymphocytic leukemia (CLL). The small lymphoblasts also have more condensed chromatin making the distinction difficult further. Lymphoblasts with cytoplasmic granulation can be seen in a small percentage of ALL cases

[18, 19]. The granules are usually present in the larger blasts; they are azurophilic and usually not numerous. Nuclear clefts can be seen in some lymphoblasts and are present as deep nuclear groves. The so-called hand mirror cell is probably not a defining characteristic for a distinct entity [20]. Whether such cells are due to an artifact of the preparation is debatable. Different lymphoblasts are illustrated in Fig. 10.1.

10.3.3 Histology

Evaluation of the histology of ALL from biopsy sections becomes important when there are few circulating blasts in the blood and when the bone marrow is inaspirable. It is also critical in evaluating extramedullary sites of involvement such as lymph nodes, testes, or skin. Whether bone marrow biopsies are necessary in the typical patient with a high number of blasts in the circulation and bone marrow aspirate is disputable. However, the biopsy may provide a baseline for cellularity, degree of residual normal hematopoiesis, and the presence of necrosis or other associated features.

In typical cases, the marrow cellularity is markedly increased due to the infiltration by the densely packed blastic elements with no particular pattern of involvement. Rare cases have a predilection for paratrabecular growth, but this is very unusual. On H&E-stained sections, the blastic morphology is not easily distinguishable from myeloblasts. Burkitt leukemia does, however, have a particular histologic pattern. The features are similar to the lymph node involvement by Burkitt lymphoma.

Hypocellular presentations of ALL are relatively rare, but can present a diagnostic challenge due to the paucity of cells and thus limited material for immunophenotyping [21]. Some cases of ALL can present with frank fibrosis [22]. Inability to aspirate could be due to the fibrosis, or in some cases, due to the dense packing of the marrow by lymphoblasts. Necrosis is present in a small number of cases and can complicate the diagnosis, due to the lack of viable cells for either morphologic evaluation or for immunophenotyping [23]. Necrosis can be focal or widespread and can recur with relapsed disease. Occasional cases can show bone changes, which include osteoporosis or osteopenia [24].

In some cases of ALL, the principle manifestation of disease is extramedullary [25]. This is not uncommon in precursor T-cell ALL/lymphoma which can present with a mediastinal mass and lymphadenopathy. Other sites that may be identified prior to blood and bone marrow disease include lymph node, skin, testes, and CNS. Whenever there is concern for a lymphoblastic process in an extramedullary location, careful review of the blood and evaluation of the marrow is imperative.

Differential diagnostic considerations are based on clinical presentation as well as cytomorphologic and histologic features of blasts in the peripheral blood and marrow. Reactive causes of lymphocytosis should be excluded particularly in the pediatric age group where the morphology of the peripheral blasts can be difficult to distinguish from mature lymphocytes. In pediatric patients with high peripheral blood counts, pertussis must be considered. Pertussis can result in lymphocytosis of 20–30,000/μL, and the lymphocytes can sometimes appear atypical, although they should have mature-appearing chromatin. Furthermore, unlike in B-ALL, the hemoglobin and peripheral platelet counts are usually preserved when the lymphocytosis is reactive. In the bone marrow, hematogones or normal B-cell precursors can be increased in number in regenerative situations (Fig. 10.2). These require careful evaluation, as they closely resemble malignant lymphoblasts [26]. Small round blue cell tumors seen in pediatric patients can also mimic ALL in the marrow, but immunohistochemical studies can usually resolve any diagnostic concerns. In adults, leukemic manifestations of mature B-cell lymphoma, particularly the blastic variant of mantle cell lymphoma [27], can mimic ALL. Additionally, high-grade B-cell lymphomas can have a blast-like morphology. Immunophenotyping is needed to resolve the diagnosis in such cases. Mature B-cell lymphomas lack expression of precursor cell markers such as CD34 and terminal deoxynucleotidyl transferase (TdT), whereas they express surface

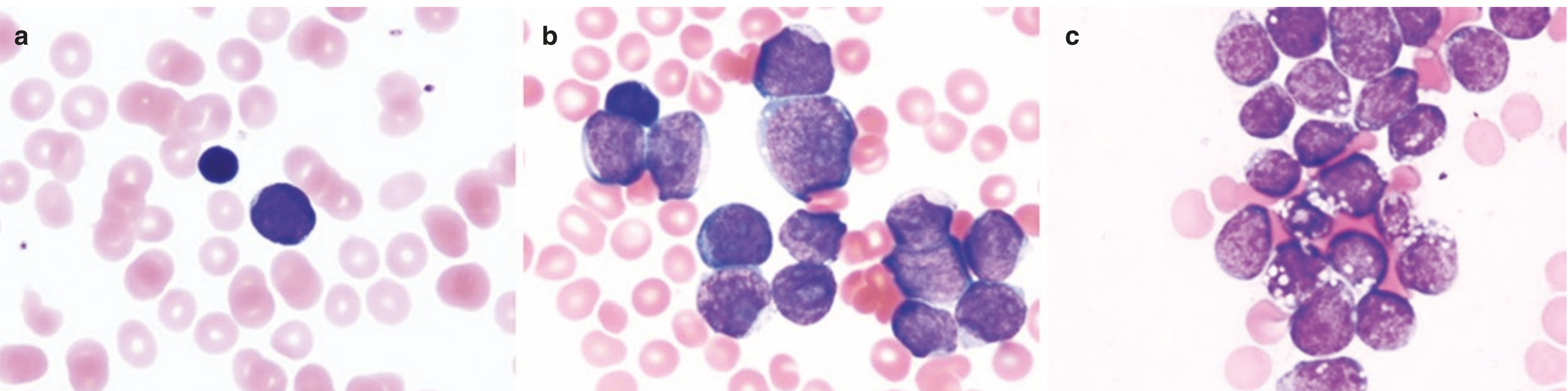

Fig. 10.1 Cytomorphology of lymphoblasts in comparison to Burkitt lymphoma (Wright-stained blood and bone marrow aspirate smears). (**a**) Small uniform blasts are about two times the size of erythrocytes and have a smudgy homogenous chromatin without prominent nucleoli. Comparison to a small lymphocyte (left) is always helpful. (**b**) Varied lymphoblasts, including numerous larger blasts with more open chromatin, prominent nucleoli, and abundant cytoplasm. (**c**) Burkitt lymphoma cells (previously called “L3” blasts) are usually distinctive with homogeneous large size and deep blue cytoplasm with prominent vacuoles. Vacuoles can, however, be seen in some cases of AML and ALL

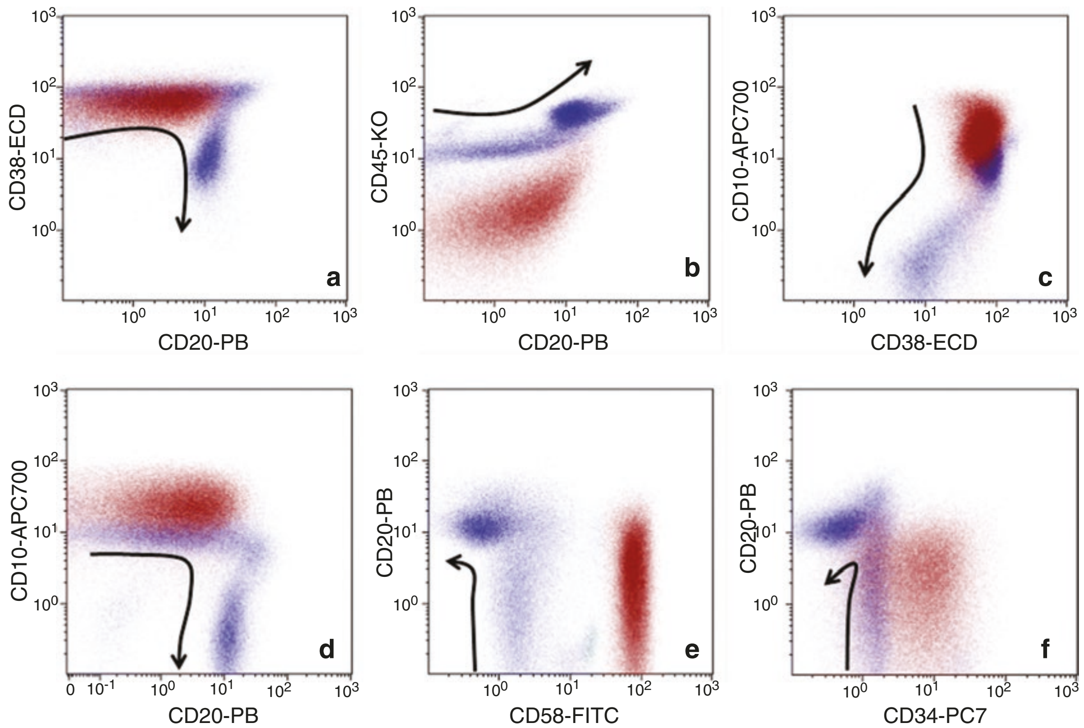

Fig. 10.2 Distinguishing malignant B-lymphoblasts from hematogones by flow cytometry. Hematogones are normal B-cell precursors that can be morphologically difficult to distinguish in regenerative marrow aspirates from blasts. However, by flow cytometry, hematogones follow a distinct and predictable pattern of antigen expression (blue dots and black arrows) (**a**, **c**). In contrast, ALL blasts (red dots) show maturation arrest and differences in intensity of the antigens normally expressed on hematogones. In the example shown here, when compared to hematogones (blue), residual ALL blasts (red) express asynchronous CD20 with weak CD45 (**b**), bright CD10 (**d**), bright CD58 (**e**), and bright CD34 (**f**)

immunoglobulin (sIg) light chains (see discussion on immunophenotyping below). In both children and adults, the differential also includes AML, mixed phenotype leukemia, and chronic myeloid leukemia (CML) presenting in lymphoid blast phase. In all except the latter, immunophenotyping by flow or by immunohistochemistry can resolve the diagnostic dilemma. These differential diagnostic considerations and a list of non-hematologic processes that may resemble ALL are summarized in Table 10.1.

10.3.4 Immunophenotype

Enzyme cytochemistry, particularly the demonstration of reactivity to myeloperoxidase (MPO), can exclude the diagnosis of ALL. Other cytochemical reactions used in the past have been largely replaced by flow cytometry. Multicolor flow cytometry allows for simultaneous detection of multiple antigens on the surface of the leukemic blasts, allowing not simply the correct diagnosis, but also providing a footprint for monitoring for residual disease post-therapy. Flow cytometry can be performed from the bone marrow aspirate or peripheral blood if adequate blasts were present. One should be careful when interpreting the immunophenotype of peripheral blood blasts particularly when the fraction of blasts is low and present with a left shift. The peripheral blood blasts could represent part of the left shift resulting from a leukoerythroblastic phenomenon and may not be representative of bone marrow leukemic blasts. Immunohistochemistry on biopsy sections should

Table 10.1 Differential diagnosis of ALL

Non-hematologic processes:
Tuberculosis
Heavy metals
Human immunodeficiency virus (HIV)
Infectious mononucleosis
Autoimmune diseases
Juvenile rheumatoid arthritis
Osteomyelitis
Hematologic processes: differential diagnostic considerations from cytomorphology and histology
In children
Pertussis
Hematogones
Small round blue cell tumors
In adults
Chronic lymphocytic leukemia (CLL)
Prolymphocytic leukemia (PLL)
Lymphoma, especially blastic variant of mantle cell
Plasmablastic myeloma
Blastic plasmacytoid dendritic cell neoplasm (BPDCN)
In children and adults
Reactive lymphocytosis (mononucleosis)
Thymoma
AML with minimal differentiation (M0) and without maturation (M1)
Mixed phenotype acute leukemia
CML presenting in lymphoid blast phase

Table 10.2 Immunophenotype

(A) Pertinent markers available for immunohistochemical studies
General: TdT, CD34
B-cell markers: CD20, CD79A
T-cell markers: CD3, CD4, CD8, CD5, CD45RO
Myeloid: MPO, CD68, lysozyme, glycophorin A, Factor VIII, CD61
Other: keratin, NSE, myogenin, CD99
(B) Commonly used markers for flow immunophenotyping in acute leukemia
General: CD34, HLA-DR, TdT, CD45
B-cell markers: CD10, CD19, cCD22, CD20, cCD79A, CD24, cytoplasmic μ, sIg
T-cell markers: CD1a, CD2, cCD3, CD4, CD8, CD5, CD7
Myeloid: cMPO, CD117, CD13, CD33, CD11c, CD14, CD15
(C) B-lineage ALL phenotypes
Pro-B: TdT+, CD19/22/79A+, CD10-, cytoplasmic μ−, sIg−
Common precursor-B: TdT+, CD19/22/79A+, CD10+, cytoplasmic μ−, sIg−
Pre-B: TdT+, CD19/22/79A+, CD10+, cytoplasmic μ+, sIg−
(D) T-lineage ALL phenotypes
Pro/immaturethymocyte:TdT+,cCD3+,CD2/5/7+/−
Common thymocyte: TdT+, cCD3+, CD2/5/7+, CD4+/CD8+, CD1a+
Mature thymocyte: TdT+/−, CD3+, CD2/5/7+, CD4+ or CD8+, CD1a−

not be considered as an alternative to multicolor flow cytometry but can be used when a bone marrow aspirate is not available and peripheral blood shows no circulating blasts.

Consensus guidelines for immunophenotyping of leukemia have been proposed by several cooperative groups [28, 29]. While there is no consensus on a single panel, the initial flow cytometry should allow for distinction of leukemic blasts from normal regenerative precursors in the bone marrow or thymus and correctly classify B, T, myeloid, acute leukemias of ambiguous lineage, and blastic plasmacytoid dendritic cell neoplasms. Commonly used markers are listed in Table 10.2B.

The large majority of cases of ALL (~85%) are of B-lineage. B-lineage assignment is based on expression of CD19, CD79a, and CD22. CD45 expression is characteristically weak or absent. In addition, TdT, CD34, and CD10 are variably expressed. Aberrant expression of CD13 and CD33 is common, and in the absence of MPO expression, the expression of these antigens does not preclude a diagnosis of ALL. Combinations of antigens expressed by the leukemic blasts correspond to the degree of maturation and associate with genetic features. The earliest stage of maturation (early precursor-B or pro-B) is characterized by the expression of CD19, cCD79a, and nuclear TdT. This subset is enriched for B-ALL with chromosome 11q23.3 rearrangements. The intermediate stage or common B-ALL is characterized by the coexpression of CD10 along with the above B-cell antigens and is enriched for *BCR-ABL1* and *ETV6-RUNX1* rearranged cases. Finally, the most mature precursor-B cells are characterized by expression of cytoplasmic immunoglobulin

mu chains and enriched for B-ALL with a *TCF3-PBX1* fusion.

While Burkitt lymphoma may have a leukemic presentation, it is a malignancy of mature B cells that express CD19 and CD10. However, in contrast to B-ALL, the malignant cells also express bright CD45, CD20, and monotypic sIg light chains. Rare B-ALL cases can express surface heavy chains (so-called transitional pre-B ALL) while others can show the expression of surface light chains without heavy chains. When seen in the context of appropriate morphology and markers of immaturity such as CD34 and TdT, the expression of surface heavy or light chains does not preclude the diagnosis of precursor B-ALL [30]. B-ALL immunophenotypes are listed in Table 10.2C.

T-ALL accounts for only 15–20% of cases. While CD3 expression is lineage specific, surface CD3 is rarely present. The use of a cytoplasmic tube for flow cytometry to determine the expression of cytoplasmic CD3 is therefore required for definitive diagnosis of T-ALL. Bright surface CD7 expression is invariable but is not T-lineage specific. Other markers that are expressed with variable frequency include CD1a, CD2, CD4, CD5, and CD8. In addition to CD1a, expression of TdT and CD34 are helpful in demonstrating the precursor cell origin of this T-cell malignancy. Similar to B-ALL, a characteristic combination of antigens expressed is indicative of the stage of maturation [31]. These stages are pro-T/T-I, pre--T/T-II, cortical T/T-III, and medullary T/T-IV (Table 10.2D). Expression of myeloid antigens can also be seen in T-ALL. CD13 and CD33 expression has been described in 19–32% of cases [32]. CD117 expression is uncommon and appears to be associated with *FLT3* mutations [33]. It is likely that the CD117-positive T-ALL cases described in the older literature represent mostly what is now recognized as early T-cell precursor ALL (ETP ALL). Initially identified by a distinct gene expression signature [34], this leukemia can be diagnosed based on immunophenotype with the lack of expression of CD1a and CD8, usually negative for CD5, as well as strong positive expression of at least one of CD34, CD117, HLADR, CD13, CD33, CD11b, or CD65. If bright CD5 expression is noted in a CD1a-negative, CD8-negative T-ALL, the diagnosis of ETP ALL can still be made based on bright expression of at least two of the antigens associated with myeloid differentiation (CD13, CD33, CD11b, CD65) or with immaturity (CD34, HLADR, CD117) [29].

Differential diagnostic issues that have to be considered in immunophenotyping include hematogones, thymoma, mixed phenotype leukemia, and CML presenting in lymphoid blast phase. Hematogones have the same immunophenotype as common precursor B-ALL cells, but the hematogones exhibit a spectrum of maturation with a continuum of cells from immature to mature showing loss of CD34 and gain of CD20 and sIg [35, 36].

Thymoma cells have the phenotype of common thymocytes and cannot be distinguished from common T-ALL/lymphoblastic lymphoma by immunophenotype alone. Correlation with clinical presentation and histology is important for the correct interpretation. When CML presents in lymphoid blast crisis, distinction from Philadelphia (Ph) chromosome-positive ALL cannot be made based on immunophenotype, as the blasts are frequently precursor B lymphoblasts. In most Ph+ cases, the presence of a concurrent myeloid component to the leukemia will alert one to the correct diagnosis. If this were not present, lineage analysis showing the BCR/ABL1 fusion in myeloid as well as lymphoid cells has been suggested as a means to differentiate the stem cell process, CML, from the lymphoid-restricted process, ALL [37]. In some cases, only the emergence of a myeloid component after treatment can indicate the correct diagnosis.

10.3.5 Cytogenetic Evaluation

Specific and well-characterized recurring chromosomal abnormalities facilitate diagnosis, confirm subtype classification, and have major prognostic value for treatment planning. Abnormalities in chromosome number or structure are found in approximately 90% of children and 70% of adult ALL patients [38]. These cyto-

genetic abnormalities are acquired somatic (rather than germline) mutations that frequently result from translocations of chromosomal DNA, resulting in new (abnormal) protein products from the resultant fusion genes. It is assumed that the protein products from these fusion genes are responsible for the cellular dysregulation that leads to the malignant state. Deletions or loss of DNA may eliminate genes that have tumor suppressor functions. Gains of additional chromosomes may lead to gene dosage effects that provide transformed cells with survival advantages.

Conventional cytogenetic analysis requires dividing cells, is technically difficult, and can be time consuming due to the presence of multiple abnormal cell lines and complex chromosomal banding patterns. Therefore, alternative diagnostic methods have been sought, including fluorescence in situ hybridization (FISH), in which labeled probes are hybridized to either metaphase chromosomes or interphase nuclei and then detected with fluorochromes. This method of analysis is more rapid, and in some cases more sensitive, than conventional cytogenetic analysis. Additionally, FISH can be used to study differentiated or nondividing cells. In B-ALL, most pediatric and adult patients can be assigned to a genetic-based classification. The most recent revision of the WHO classification recognizes specific B-ALL subtypes with recurrent cytogenetic abnormalities. The inclusion of a given cytogenetic abnormality as a specific entity is based on distinctive clinical or phenotypic properties, prognostic implications, or evidence for biology that is exclusive of other entities. For example, B-ALL with iAMP21 has been added to the entities included in the fourth edition [39]. In addition, *BCR-ABL1*-like ALL has been included as a provisional entity to include a heterogenous group of B-ALL characterized by gene expression profiles indistinguishable from B-ALL with *BCR-ABL1*, but the former lacks the *BCR-ABL1* translocation [40]. B-ALL with this gene expression profile is associated with cryptic translocations involving *CRLF2* or a multitude of tyrosine kinases [41].

10.3.6 Molecular Evaluation

Polymerase chain reaction (PCR) is an enzyme assay that provides a more sensitive and rapid method to detect clonal gene rearrangements. Translocations that result in fusion genes are especially suited for analysis with reverse transcriptase PCR (RT-PCR), a technique in which the fusion mRNA is reverse transcribed into cDNA, and then amplified by PCR using gene-specific primers. Quantitative RT-PCR allows for quantification of measurable (minimal) residual disease (MRD). A number of large prospective studies in pediatric ALL have demonstrated the independent prognostic significance of MRD detection [42, 43]; less is known about the significance of MRD detection in adult ALL [44].

10.4 Conclusion

In summary, the initial approach to the diagnosis of ALL still involves evaluation of the peripheral blood smear and bone marrow specimens with cytomorphology, immunohistochemistry, and cytogenetic analysis. Cytogenetic analysis and molecular methods are used to establish prognostically distinct subgroups of ALL. Through enhanced knowledge of the leukemogenic pathways involved in the different ALL subgroups, one can anticipate improved accuracy in diagnosis and prognosis, and ultimately, improved disease outcomes for these patients.

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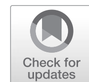

11 Frontline Therapy of Newly Diagnosed Acute Lymphoblastic Leukemia

Melissa C. Mackey, Serena Chew, Hagop M. Kantarjian, and Elias Jabbour

Acute lymphoblastic leukemia (ALL) is an aggressive malignancy of lymphoid progenitor cell lines with an annual incidence of 1.7 cases per 100,000 patients in the United States. For the year 2019, an estimate of 5930 new cases was predicted along with 1500 deaths [1]. The disease has a bimodal age distribution with 80% occurring in children (age < 5 years) and less frequently in adults (age > 50 years), with a median age at diagnosis of 15 years. Due to the implementation of intensive, multiagent chemotherapy regimens, pediatric patients have long-term survival rates above 90%, and the majority of patients are cured [2]. In contrast, despite tremendous improvements in understanding the biology and molecular mechanisms of adult ALL, long-term follow-up studies suggest a much lower long-term survival rate of 35–45% [3, 4]. This notable discrepancy in clinical outcomes has prompted further research, focusing on differences in the biology of disease and identification of high-risk features seen predominantly in adult ALL patients.

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11.1 Diagnosis and Prognostication

The diagnosis of ALL is based on bone marrow aspiration/biopsy (presence of ≥20% lymphoblasts) and flow cytometry immunophenotyping identifying malignant clones and distinguishing between B- or T-lineage diseases. B-cell ALL (B-ALL) makes up nearly 75–80% of all cases and is characterized by the expression of CD19, CD22, and CD79a [5]. The presence of CD20 expression is seen in 30–50% of cases and is correlated with poorer outcomes prior to the use of CD20-targeted therapy [6, 7]. Burkitt leukemia/lymphoma (BL) is a highly aggressive B-cell neoplasm that originates from the germinal center and expresses the B-cell markers CD19, CD20, CD22, and CD79a, along with membrane IgM with light chain restriction, as well as germinal cell markers such as CD10 and BCL6 [8]. This aggressive subtype is associated with high survival rates of 80–90% with chemoimmunotherapy [8, 9]. T-cell ALL (T-ALL) is characterized by terminal deoxynucleotidyl transferase (TdT) and cytoplasmic CD3 positivity, along with variable expression of CD1a, CD2, CD4, CD5, CD7, and CD8 [10, 11]. Early T-cell precursor ALL (ETP-ALL) is a disease recently defined as a subgroup of T-ALL arising from immature cells with the potential to differentiate into myeloid and T lineage and accounts for about 20% of T-ALL cases [12, 13]. ETP-ALL

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blasts are positive for cytoplasmic CD3, CD7, and one or more myeloid markers (such as CD33); weak for CD5 expression (<75% positive); and negative for CD8, CD4, sCD3, and CD1a expression. ETP-ALL cases have overall poor clinical outcomes characterized by primary induction failure and early relapses [14].

Abnormal karyotype occurs in about 75% of adult ALL cases [11, 15]. The most common cytogenetic abnormality observed in approximately 20–30% of B-ALL patients, with increasing frequency in older patients, is Philadelphia (Ph) chromosome resulting from the t(9;22)(q34;q11) translocation. This translocation creates the BCR-ABL fusion gene which is the main driver of Ph-positive disease and has been associated with higher relapses and poor survival in the pre-tyrosine kinase inhibitor (TKI) era. In addition, near-haploid and low-hypodiploid ALL are two separate subtypes associated with very poor outcomes [16]. Near-haploid ALL cases harbor alterations targeting the RAS signaling (71%) and *IKZF3* gene (13%), while the majority of low-hypodiploid ALL cases harbors TP53 alterations (>90%) [17]. Both near-haploid and low-hypodiploid ALL subtypes activate both RAS and PI3K signaling pathways, providing potential means for targeted therapy to inhibit those pathways. Cytogenetic abnormalities associated with negative impact on clinical outcomes in adults with Ph-negative B-ALL are t(4;11)(q21;q23)/KMT2A-AFF1 (9–13% cases), IGH-r (2–5% cases), and low hypodiploidy/near triploidy (4–6% cases) [18–21]. In adults with Ph-like ALL, high CRLF2 expression is seen in 50% of cases which is associated with reduced survival due to the activation of the JAK-STAT pathway, leading to leukemic cell proliferation and differentiation. Approximately half of patients with CRLF2 rearrangement harbor concomitant JAK2 mutations. Another major subgroup, encompassing of 15% of Ph-like ALL, involves rearrangements of ABL-class genes (*ABL1*, *ABL2*, *PDGFRB*, and *CSF1R*). ABL-class rearrangements are mutually exclusive with JAK-STAT and CRLF2 and are often present with IKZF1 alterations. IKZF1 mutations/deletions were reported in 68–73% of all Ph-like ALL cases and usually do not respond to therapy and remain minimal residual disease (MRD) positive at the end of induction therapy [22, 23]. Of note, IKZF1 mutations/deletions can lead to overexpression of CRLF2 [24]. In addition, JAK2 and EPOR rearrangements together are seen in 10% of Ph-like ALL and are associated with a poor prognosis. In patients with BL, concurrent IGH-BCL2 and MYC rearrangements are commonly seen. Patients with complex cytogenetics (≥5 chromosomal abnormalities) were previously thought to have poorer prognosis, but more recent studies have been inconsistent [15, 20, 25]. T-ALL patients with complex karyotype or chromosome 17p deletion carry a very poor prognosis due to the lack of response to standard treatment [8, 10, 11]. On the other hand, mutations in NOTCH1 and/or FBXW7 confer overall favorable prognosis in the absence of abnormalities in RAS/PTEN, and this designation applies to approximately 50% of patients with T-ALL [26].

11.2 Treatment of Burkitt Leukemia (BL)/Mature B-Cell ALL

BL is a rare type of ALL that occurs in children and adults (1–5%) with high tendency for central nervous system (CNS) involvement and is associated with high survival rates (80–90%) with the use of chemoimmunotherapy [27]. In a randomized, open-label, phase III trial, 260 patients with newly diagnosed BL received intensive chemotherapy plus rituximab or placebo. The 3-year event-free survival (EFS) and overall survival (OS) rates were higher in the chemotherapy plus rituximab arm (75% vs. 62%; $p = 0.024$ and 70% vs. 83%; $p = 0.11$, respectively). Adverse events were similar in both groups [28]. Other trials have also shown that the addition of 4–8 doses of rituximab combined with chemotherapy increases both the complete remission (CR) and OS rates from approximately 70% to 85% and 40–50% to 80%, respectively [8, 28–30].

Low-intensity chemotherapy in patients with BL has also been evaluated in the frontline setting. In a single-center, uncontrolled, prospective

study, 30 patients (median age 33 years) with untreated BL received either standard DA-EPOCH-R (dose-adjusted regimen containing etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and rituximab) or SC-EPOCH-RR (short course regimen with a double dose of rituximab). DA-EPOCH-R was dose adjusted based on the neutrophil nadir. Patients who received DA-EPOCH-R demonstrated promising results with PFS and OS ≥95% and ≥90%, respectively [31]. One potential area of concern with DA-EPOCH-R regimen variations is the lack of CNS-penetrating systemic chemotherapy such as high-dose methotrexate and cytarabine, which are essential components of high-intensity BL therapy. A recent analysis of modern BL therapy amplified this concern, with significantly higher rates of CNS recurrence at 3 years with DA-EPOCH compared to high-intensity chemotherapy (12% with DA-EPOCH, 3% and 4% with hyper-CVAD and CODO-M/IVAC, respectively; hazard ratio 3.50 with DA-EPOCH) [32]. This increased CNS recurrence was despite utilizing intrathecal CNS prophylaxis with DA-EPOCH. Despite the high response rate in the frontline setting, patients with R/R continue to have poor outcomes. A phase III clinical trial comparing the R-CODOX--M/R-IVAC (cyclophosphamide, doxorubicin, vincristine, methotrexate/ifosfamide, etoposide, high-dose cytarabine) versus DA-EPOCH-R in patients with untreated BL is underway (EudraCT Number: 2013-004394-27).

11.3 Treatment of Ph-Negative ALL

The treatment of ALL is complex and often involves multiple cycles of chemotherapy during induction, consolidation, and maintenance phases. Intensive induction chemotherapy regimens are modeled after either the pediatric-inspired roadmap regimens or the hyper-CVAD (hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone alternating with methotrexate and cytarabine) regimen developed at MD Anderson Cancer Center [4, 33–37]. Pediatric-inspired regimens have previously been studied in patients of all ages, but their use in patients >40 years has largely fallen out of favor due to higher rates of toxicity and treatment-related mortality in this patient population and is generally reserved for use in the adolescents and young adults (AYA) population. See Table 11.1 for more information. Hyper-CVAD is a dose-intensive chemotherapy regimen used in adult ALL patients. Between 1992 and 1998, 204 patients (median age 39.5 years) were treated with four cycles of hyper-CVAD with intrathecal CNS prophylaxis and growth factor support [34]. Overall, 91% of patients treated achieved a CR with an induction mortality of 6%, which was a significant improvement compared to historical data utilizing the VAD (vincristine, doxorubicin, and dexamethasone) regimen [34, 38]. The estimated 5-year OS and CR rates were 39% and 38%, respectively [34]. The long-term follow-up (median 63 months) confirmed these results, reporting achievement of overall CR in 92% and 5-year OS and CR rates of 38% in both [3].

11.3.1 Addition of CD20 Targeted Monoclonal Antibodies

CD20 expression (≥20% of cells) is found in 30–50% of precursor B-ALL leukemia blasts and in >90% of mature B-ALL [5]. CR rates in patients who received conventional chemotherapy (e.g., hyper-CVAD regimens) were similar regardless of CD20 expression; however, patients with CD20 expression had significantly higher relapse and lower OS rate [5]. The poor outcomes of CD20 expression in patients with precursor B-ALL led to the incorporation of CD20 monoclonal antibodies to improve survival outcomes [5, 7]. Rituximab is a humanized chimeric anti-CD20 monoclonal antibody that mediates antibody- and complement-dependent cellular cytotoxicity. The addition of rituximab to chemotherapy for ALL was first evaluated in 2010 and was found to improve survival in patients with Ph-negative B-ALL. Patients with untreated B-ALL with CD20 expression in ≥20% cells

Table 11.1 Treatment of ALL in the frontline setting at MD Anderson Cancer Center

Type	Subtypes	Management	Outcomes	
B-cell ALL	BL	Hyper-CVAD + rituximab + 16 intrathecal chemotherapy EPOCH + rituximab or ofatumumab	OS (3 years): 80–90%	
	Ph-negative	Hyper-CVAD ± rituximab + 8 intrathecal chemotherapy	**Age, years**	**OS (5 years)**
			≤30	64%
			31–59	50%
			≥60	21%
		Hyper-CVAD ± ofatumumab + 8 intrathecal chemotherapy	**Age, years**	**OS (5 years)**
			<40	75%
			≥40	60%
		Hyper-CVAD + blinatumomab + 8 intrathecal chemotherapy	OS (1 year): 90%	
	Ph-positive	Hyper-CVAD + ponatinib + 12 intrathecal chemotherapy	OS (5 years): 73%	
	Ph-like	Hyper-CVAD-based regimens ± TKIs ± blinatumomab[a]		**Median OS**
			CRLF2+	23 months
			Non-CRLF2	49 months
T-cell ALL	All subtypes	HyperCVAD + nelarabine + pegasparginase[a]	OS (3 years): 65%	
AYA	–	Augmented BFM HyperCVAD ± rituximab or ofatumumab	OS (5 years): 60%	
Elderly	–	Mini-hyper-CVD + inotuzumab ± blinatumomab[a]	**Age, years**	**OS (3 years)**
			60–69	63%
			≥70	44%

[a]Ongoing clinical trials at MD Anderson Cancer Center

received 12 doses of rituximab plus hyper-CVAD. The rates of CR duration (70% vs. 38%, $p < 0.001$) and OS (75% vs 47%, $p = 0.003$) were superior with the modified hyper-CVAD plus rituximab regimens compared to historical controls with standard hyper-CVAD in patients aged <60 years [39]. These improved outcomes with rituximab were also seen when added to the pediatric-inspired GRAALL-2005 regimen in a phase III randomized trial [40]. Patients aged <60 years with CD20-positive, Ph-negative B-ALL were randomized to receive 16–18 doses of rituximab plus standard intensive chemotherapy. The 2-year EFS was statistically improved with the addition of rituximab compared to without (65% vs. 52%; $p = 0.04$). Although the 2-year OS was not statistically improved with rituximab (71% vs. 64%, respectively; $p = 0.10$), improvement in OS with rituximab was seen in a sensitivity analysis censoring for allogeneic hematopoietic stem cell transplant (HSCT) ($p = 0.02$) [40].

Ofatumumab is a second-generation CD20-directed monoclonal antibody that binds to the juxtamembrane small-loop epitope of CD20, a different binding site than rituximab. It has increased complement-dependent cytotoxicity compared to rituximab and is more potent as a result of slower dissociation from the binding site [41, 42]. Given that ofatumumab binds more strongly to extracellular epitope of CD20, it remains effective even in the setting of lower CD20 expression (<20% of cells). In a phase II study, the addition of ofatumumab to hyper-CVAD for patients with newly diagnosed Ph-negative, CD20-positive ALL demonstrated high CR/CR with incomplete platelet recovery (CRp) and MRD-negativity rates of 98% and 93%, respectively. The estimated 2-year OS rate was 81%, and the benefit of adding ofatumumab

was seen in both high (≥20% of cells) and low (<20% of cells) CD20 expression [43].

11.3.2 Addition of CD19 Targeted Antibody

CD19 expression is seen in 90% of cases of B-ALL, and the addition of CD19 antibody to chemotherapy has shown promising results in the frontline setting. Blinatumomab is a bispecific T-cell engager (BiTE) with dual affinity for CD19 on B cells and CD3 on T cells which exerts an immune response by activating/engaging T cells to eradicate leukemic cells [44]. Given that blinatumomab has demonstrated encouraging response rates in the relapsed/refractory (R/R) setting, the use of blinatumomab given sequentially in combination with hyper-CVAD in younger patients (<60 years) was evaluated in the frontline setting [45–47]. Patients received four cycles of hyper-CVAD followed by four cycles of blinatumomab and then maintenance therapy with POMP (6-mercaptopurine, vincristine, methotrexate, and prednisone). Nineteen patients (median age 42 years) have been treated thus far, and the ORR was 100%. MRD negativity was achieved in 93% of patients after one cycle of chemotherapy. The 1-year OS and RFS rates were 93% and 75%, respectively. This trial is currently ongoing, and follow-up data are needed to validate these findings [46].

11.3.3 Addition of CD22 Targeted Antibody

CD22 is expressed in 90% of cases of B-ALL [48]. Inotuzumab ozogamicin (INO) is a humanized antibody–drug conjugate (ADC) consisting of an anti-CD22 monoclonal antibody bound to calicheamicin, a potent alkylating agent [5]. Due to the success of INO in treatment of R/R ALL, it was also evaluated in the frontline setting in combination with low-dose chemotherapy in older patients (median age 68 years) with Ph-negative ALL [49–52]. Further details regarding INO will be discussed in the "elderly" section.

11.4 Treatment of ALL in AYA Population

The treatment of ALL in AYA (age 15–39 years) has been a challenge, and research has focused on the genetic and molecular features of the disease, as well as optimizing therapeutic regimens for these patients [53]. Intensive chemotherapy based on pediatric protocols remains the standard of care for AYA, and results comparing pediatrics versus adult regimens are developing. AYA patients receiving pediatric intensive chemotherapy regimens have demonstrated remarkable improvement in long-term outcomes with OS of 60–70% [54–60]. Pediatric regimens contain higher cumulative doses of non-myelosuppressive chemotherapy (corticosteroids, vincristine, and PEG-asparaginase), and subsequently carry more adverse events related to hyperbilirubinemia, pancreatitis, and avascular bone necrosis. Relative to pediatric regimens, adult regimens consist of more myelosuppressive chemotherapy (daunorubicin, cytarabine, cyclophosphamide), leading to higher incidences of neutropenia and infections [61]. The efficacy of the pediatric regimen augmented Berlin-Frankfurt-Münster (BFM) in 106 patients (median age 22 years) was compared to 102 patients treated with hyper-CVAD (median age 27 years) in a single institution study [37]. The CR rate with augmented BFM was comparable to that seen with hyper-CVAD (93% vs. 98%, respectively), and the 5-year duration of CR (53% vs. 55%) and OS (60% vs. 60%) were comparable with both regimens. Toxicity profiles between regimens differed significantly, with hepatotoxicity (41%), thrombosis (19%), pancreatitis (11%), and osteonecrosis (9%) being most commonly reported with augmented BFM, likely related to asparaginase use. With hyper-CVAD, myelosuppression and associated complications were most frequent [37].

A prospective CALGB 10403 study, which mirrored the previously published pediatric AALL0232 regimen, evaluated 295 AYA patients (median age 24 years; range 17–39 years) [61]. The median EFS was 78.1 months, more than double that of the historical control of 30 months.

The 3-year EFS and OS rates were 59% and 73%, respectively, and the median OS was not reached. The overall regimen was well-tolerated with treatment-related mortality of 3%. The most common asparaginase-related toxicities were low fibrinogen (42%), elevated transaminases (28%), bilirubin (18%), and hyperglycemia (30%), comparable to those seen with augmented BFM. Interestingly, this study found that pre-treatment risk factors such as obesity and Ph-like phenotype were associated with poorer response [61]. Although improvements in the outcome of AYA population have been made, survival outcome remains poor when compared to other age groups with ALL. Other factors that have played a negative role on clinical outcomes of AYA patients include noncompliance to therapy, lack of follow-up, no insurance, and psychosocial issues [61].

11.5 Elderly Patients

Elderly patients (age > 60 years) with ALL are known to have poor outcomes (including low CR rates, increased mortality, short remission duration) and are often unable to tolerate intensive chemotherapy. Although elderly patients receiving intensive chemotherapy (e.g., hyper-CVAD) were able to achieve similar CR rates than that seen in younger patients (age < 60 years) of 84% vs. 92%, respectively, high mortality rate of 34% was seen in elderly patients, mainly due to infections. The 5-year OS was low of 20% [62]. Given the high mortality and intolerance of intensive therapy in this patient population, novel therapeutic agents including targeted therapies and dose modification to the hyper-CVAD regimen were developed.

In a phase II study, 135 patients (median age 68 years) were treated with either hyper-CVAD ± rituximab (n = 77) or mini-hyperCVD (an attenuated regimen of hyper-CVAD without an anthracycline) in combination with INO (1.8 and 1.3 mg/m^2 during induction and consolidation, respectively) ± blinatumomab (n = 58) as frontline treatment. Due to the high rates of veno-occlusive disease (8%) likely related to INO with the first 47 patients treated, the protocol was amended to reduce the dose of INO (1.8 and 1.3 mg/m^2 during induction and consolidation, respectively) thereafter to minimize the risk. The combination of mini-hyperCVD plus INO ± blinatumomab was associated with higher ORR (98% vs. 88%) and fewer rates of early death (0% vs. 8%) compared to standard hyper-CVAD ± rituximab. The 3-year EFS (64% vs. 34%; P = 0.003) and OS rates (63% vs. 34%; P = 0.004) were superior with the combination of mini-hyperCVD plus INO ± blinatumomab compared to hyper-CVAD ± rituximab [63]. The most frequent grade 3–4 adverse events were prolonged thrombocytopenia (81%), infections (52–69%), hyperglycemia (54%), hypokalemia (31%), increased aminotransferases (19%), and bilirubin (17%); only one case of veno-occlusive disease was reported after the amendment [63]. The lower dose of INO improved tolerability without compromising efficacy. In addition, the use of sequential addition of blinatumomab may increase the depth of response and reduce the risk of veno-occlusive disease by distancing the INO from subsequent HSCT. Through this ongoing study, attenuated chemotherapy doses combined with sequential targeted therapy have shown improved OS compared to historical controls utilizing full-dose chemotherapy in the elderly population.

In addition, blinatumomab has also shown promise as a single agent in the frontline setting for elderly patients. In a phase II trial, 31 newly diagnosed Ph-negative B-ALL patients (median age 75 years) received blinatumomab followed by POMP maintenance [64]. The 1-year OS and DFS rates were 65% and 56%, respectively. MRD negativity was achieved in 12 of 13 (92%) responders. Importantly, this regimen was well-tolerated with only one patient who developed grade 3 cytokine release syndrome [64].

11.6 Treatment of Ph-Positive ALL

TKIs in combination with multi-agent cytotoxic chemotherapy is the standard of care for frontline Ph-positive ALL. Imatinib is a first-generation

TKI that was studied in combination with chemotherapy in Ph-positive patients and showed promising results in the frontline setting. The addition of imatinib to intensive and non-intensive chemotherapy resulted in CR rates >90% and OS rates ranging from 33% to 76% [65–79]. Continuous imatinib therapy resulted in lower relapse rates compared to intermittent dosing. Despite better outcomes with the addition of imatinib to chemotherapy, the high incidences of imatinib resistance and relapses led to the evaluation of more potent TKIs for the treatment of frontline Ph-positive ALL patients.

Nilotinib is a second-generation TKI which has more potent activity against BCR-ABL1 than imatinib and has activity against all known imatinib-resistant BCR-ABL mutants except for T3151 [80, 81]. It has shown high rates of efficacy in combination with intensive chemotherapy in newly diagnosed Ph + ALL patients in two studies, with CR rates in over 90% of patients and complete molecular responses (CMR) over 80% reported in both studies [82, 83]. Nilotinib combined with low-intensity chemotherapy in elderly patients demonstrated CR rate of 87% and 2-year OS rate of 67% [84]. Although nilotinib-based regimens have shown promising results, resistance mutations with Y253H, E255V, and T315I may develop after nilotinib initiation. Multiple clinical trials are still evaluating the role of nilotinib in Ph-positive ALL, and it is currently not approved for this indication.

Dasatinib is a second-generation TKI, with greater selectivity and potency against BCR-ABL1 than imatinib, and has greater penetration into the CNS. Similar to nilotinib, dasatinib is active against most ABL1 kinase domain mutations noted with imatinib, except for T315I mutation. In a phase II study of dasatinib plus hyper-CVAD for the treatment of newly diagnosed Ph-positive ALL, 72 patients (median age 55 years) were treated; 69 patients (96%) achieved CR [85]. The 5-year OS rate was 46%. Forty-five patients (65%) achieved CMR at 4 weeks after treatment initiation. A total of 12 patients (17%) underwent HSCT in first CR, and 7 deaths occurred post HSCT. The high rate of treatment-related mortality was likely due to advanced age. In patients who achieved CMR, durable remissions and long-term survival were demonstrated even without the use of HSCT. Notably, 22 patients (31%) had relapsed disease, 8 (36%) of whom had isolated CNS relapse. Seven patients (54%) developed ABL mutations at relapse: four with T315I, two with V299L, and one with F359V [85]. These results were validated by a subsequent multicenter trial that evaluated the same regimen in younger patients ≤60 years (median age 44 years) with newly diagnosed Ph-positive ALL. Of the 97 patients treated, 83 (88%) achieved CR/CRi and 41 (49%) underwent HSCT in first CR. The 3-year OS and RFS rates were 69% and 62%, respectively. A landmark analysis showed a statistically significant survival benefit with the use of HSCT as consolidation therapy in younger Ph-positive ALL patients [86].

Several studies have also evaluated dasatinib plus low-intensity chemotherapy in elderly patients with Ph-positive ALL [87–89]. In the GIMEMA study, 53 patients (median age 69 years) with newly diagnosed Ph-positive ALL received dasatinib plus corticosteroids. All patients achieved hematologic CR. The OS and DFS rates at 20 months were 69% and 51%, respectively. Twelve patients with relapsed disease (71%) developed a T315I mutation [88]. In a more recent update from GIMEMA investigators, 60 patients (median age 42 years) treated with the same regimen had a 3-year OS rate of 58%. Only 19% achieved CMR by day 85, which is significantly lower than CMR rates reported with dasatinib plus intensive chemotherapy [87]. In another study, 71 patients (median age 69 years) with newly diagnosed Ph-positive ALL received induction therapy with dasatinib, dexamethasone, and vincristine. Sixty-seven (96%) patients achieved CR. The 5-year OS and EFS rates were 28% and 36%, respectively. CMR was achieved in 24% of patients and 10% underwent HSCT. Thirty-six patients had relapsed disease, most of which occurred during maintenance therapy. Mutation analysis was conducted in 21 relapsed patients and found 18 with T315I, 1 with F317L, 1 with V299L, and 1 with a compound mutation (F137I + F359I + F359C) [89].

Lastly, the GIMEMA LAL2116 D-Abla trial confirmed the effectiveness of dasatinib plus chemotherapy-free regimens in inducing high rates of CR and CMR. This multicenter phase II study included 63 patients (median age 54.5 years) who received dasatinib plus corticosteroids as induction followed by blinatumomab post-induction consolidation treatment for at least two cycles with up to three additional cycles based on response and the discretion of the treating physician [90]. Fifty-four percent of patients had IKZF1 deletion and 24% of those patients also had PAX5 and/or CDKN2A/B deletion. The primary endpoint was to determine the rate of CMR or positive non-quantifiable (PNQ) disease after two cycles of blinatumomab. After induction therapy with dasatinib, 29% had a molecular response (6 CMR and 11 PNQ). The rates of molecular responses further increased after subsequent cycles of blinatumomab (56.3% after second cycle, 65.6% after third cycle, and 80% after fourth cycle). The 12-month OS and DFS rates were 94.2% and 87.8%, respectively. Patients with IKZF1 deletion plus PAX5 and/or CDKN2A/B deletion had significantly inferior DFS of 61.4% ($p = 0.01$). So far, 12 patients underwent HSCT, and no transplant-related mortality has been noted. Overall, dasatinib in addition to low- or high-intensity chemotherapy and chemotherapy-free regimens have shown to be effective in the frontline setting; however, T315I mutation and patients harboring IKZF1 and/or PAX5 and/or CDKN2A/B deletions remain a clinical challenge.

Ponatinib is a third-generation TKI with potent activity against BCR-ABL1 and T315I mutation. It was rationally designed to overcome the resistance of the T315I mutation seen with first- and second-generation TKIs. Ponatinib is 520 times more potent than imatinib and inhibits both wild-type and mutant BCR-ABL1 [91–93]. A single-center phase II study investigated the safety and efficacy of ponatinib (45 mg/day for 14 days in cycle 1 then continuously in subsequent cycles) plus hyper-CVAD in 37 patients (median age 51 years) with newly diagnosed Ph-positive ALL [94]. All patients achieved complete cytogenetic response (CCyR), 95% achieved major molecular response (MMR), and 78% achieved CMR. The 2-year EFS and OS rates were 81% and 80%, respectively. Notable grade 3–4 adverse events were infections (54%), elevated transaminases (38%), skin rash (22%), hypertension (16%), pancreatitis (16%), thrombotic events (8%), and myocardial infarction (8%). Given that two deaths from myocardial infarction were reported, potentially related to ponatinib treatment, the protocol was amended to reduce the dose of ponatinib to 30 mg/day starting with cycle 2, then 15 mg/day upon achievement of a CMR [95, 96]. In the long-term follow-up of the same trial, 76 patients (median age 47 years) were treated; all patients achieved CR, and 77% achieved CMR [95]. The 5-year EFS and OS rates were 68% and 74%, respectively. A landmark analysis at 6 months demonstrated similar CR duration and OS in patients who achieved CMR, with or without allogeneic SCT. In addition, no CNS relapses reported in patients who received at least 12 prophylactic doses of intrathecal chemotherapy. The lower doses of ponatinib resulted in no further cases of myocardial infarctions and were successfully utilized without compromising efficacy [95, 96].

Ponatinib-based regimens were also evaluated as frontline therapy in older or unfit patient who are unable to tolerate intensive therapies. In the GIMEMA phase II prospective study, 42 patients (median age 68 years) with newly diagnosed Ph-positive ALL were treated with ponatinib (45 mg/day) plus corticosteroids [97]. The 2-year OS rate was 66%. CMR was achieved in 46% of patients, which is 20–25% higher than the CMR rate reported with dasatinib and corticosteroids, suggesting that ponatinib induces a persistent deep molecular response. Overall, 36 adverse events were reported to be related to ponatinib, with 64% of patients requiring dose reduction. These adverse events may be due to the high dose of ponatinib used. Lower doses of ponatinib like those utilized in the ponatinib plus hyper-CVAD study may improve tolerability without compromising efficacy. In addition, a small retrospective study found that the combination of ponatinib with blinatumomab is safe and effective in patients with R/R Ph-positive ALL [98]. However,

prospective studies with a larger sample size are warranted. A phase II study of the sequential combination of low-intensity chemotherapy and ponatinib followed by blinatumomab and ponatinib in patients with newly diagnosed Ph-positive ALL is currently ongoing (ClinicalTrials.gov identifier: NCT03147612).

11.7 Ph-Like ALL

Ph-like ALL is a high-risk ALL that occurs in 20–30% in young adults (age 20–40 years) and 78% in patients of Hispanic ethnicity [99, 100]. This subtype of ALL is associated with adverse clinical features and patients typically presents with high white blood cell (WBC) count >100,000/mm^3. A paucity of evidence has reported significantly reduced survival outcomes in patients with Ph-like ALL, with a 5-year EFS and OS rates of 23.2% and 26.5%, respectively. In a retrospective study, 148 patients with untreated Ph-like ALL received hyper-CVAD (for patients aged ≥18 years) or augmented BFM (for patients aged <40 years) [23]. Of the 148 patients, 56 patients (median age 34 years) had Ph-like ALL, and of these patients, 37 (61%) had CRLF2 overexpression. The majority of patients with CRLF2 rearrangements (84%) had concurrent IKZF1 deletion. The CR/CRp rate in Ph-like ALL group was 89%, similar to the other B-cell ALL groups (94%). MRD status at the time of CR showed no difference in median OS in patients with Ph-like ALL (MRD-negative group 26.2 months vs. MRD-positive group 23 months; $P = 0.138$). The 5-year OS was significantly worse in patients with Ph-like ALL compared to other patients (23% vs. 59%; $P = 0.006$) [99]. Another study evaluated the use of hyper-CVAD plus ofatumumab in Ph-like ALL patients. The 4-year EFS (56% vs. 58%) and OS (40% vs. 69%) rates were lower in patients with Ph-like ALL. Given that patients are more likely to remain MRD-positive after induction therapy, the utilization of blinatumomab as frontline or for MRD in first CR may improve outcomes. Further studies are needed to determine the role of blinatumomab, other immunotherapies, and allogeneic HSCT for the treatment of Ph-like ALL in the frontline setting. Ongoing clinical trials are evaluating the benefit of targeted therapies such as JAK2 inhibitors (e.g., ruxolitinib) or TKIs (e.g., dasatinib and ponatinib) in combination with chemotherapy in the frontline setting.

11.8 Treatment of T-Cell ALL

T-ALL is characterized by male sex predominance, older age at onset, and overall poor outcomes. It comprises about 25% of adult cases and is phenotypically distinct from B-ALL [101–103]. ETP-ALL accounts for up to 20% of T-ALL cases and has especially overall poor clinical outcomes characterized by primary induction failure and early relapses [10–14]. Due to the lack of targetable markers and mutations, treatment of T-ALL had been challenging prior to the development of nelarabine. Nelarabine is a water-soluble T-cell-specific purine analog prodrug that is converted to 9-β-D-arabinofuranosylguanine triphosphate (ara-GTP). Ara-GTP is incorporated into the cell and inhibits DNA synthesis resulting in apoptosis [101]. Two independent studies laid the foundation for utilizing nelarabine for the treatment of T-ALL, with CR rates of 31–36% in the R/R setting [102, 104].

In a single-arm phase II study, nelarabine was added to hyper-CVAD for the treatment of newly diagnosed adult T-ALL ($n = 40$) and T-lymphoblastic lymphoma (T-LBL; $n = 26$). Patients received eight cycles of hyper-CVAD plus two cycles of nelarabine (650 mg/m^2/day for 5 days), followed by 30 months of maintenance therapy, consisting of POMP and an additional two cycles of nelarabine. CR rates in T-ALL and T-LBL were 87% and 100%, respectively. With a median of 42.5 months follow-up, the rates of CR and OS at 3-years were 66% and 65%, respectively, which were not significantly improved compared to historical data with the hyper-CVAD regimen alone [105]. Nelarabine was also added to standard frontline treatment in the COG AALL0434 trial. Intermediate and high-risk patients with T-ALL were randomized to standard therapy alone or with addition of nelarabine

(650 mg/m^2/day for five of six total courses). The 4-year DFS was improved from 83.3% to 89.9% with the addition of nelarabine ($p = 0.0332$), supporting the addition of this agent to frontline T-ALL therapy [106].

ETP-ALL is a subgroup of T-ALL and has an overall poor prognosis related to high rates of treatment resistance [14]. However, one study suggested that those with ETP-ALL who receive asparaginase-based therapy with early intensification may achieve similar outcomes to other patients with T-ALL, especially in patients who underwent allogeneic HSCT [107]. A study evaluated 47 ETP-ALL patients treated with GRAALL regimens and found that despite expected high levels of early chemotherapy resistance, the 5-year OS was not inferior to patients with non-ETP-ALL (59.6% vs. 66.5%; $p = 0.33$) [107]. Finally, preclinical studies show that ETP-ALL is highly BCL-2 dependent and is highly sensitive to venetoclax, a BCL-2 inhibitor [108, 109]. The use of venetoclax has demonstrated excellent response in case reports in the R/R ETP-ALL setting [110]. In a small phase I study, elderly patients with newly diagnosed or R/R ALL, including ETP-ALL were treated with venetoclax plus mini-hyperCVD. In patients with untreated ALL, the ORR and CR rates were 100% and 90%, respectively [111]. Importantly, all newly diagnosed ALL patients who achieved CR were also MRD negative by flow cytometry. Further investigation on the benefit of venetoclax in this patient population is underway.

11.9 Role of Allogeneic Stem Cell Transplantation

ALL patients with high-risk features (e.g., Ph-positive, hypodiploidy, complex karyotype, t(4;11) translocations, translocations involving 11q23, and elevated WBC) at diagnosis are commonly recommended to undergo allogeneic HSCT in first CR if a donor is available. However, a newer, risk-adapted approach, which takes into consideration patients' risk status over time, has been incorporated into this decision. This approach helps to identify patients most likely to benefit from allogeneic HSCT.

The prognostic utility of day 14 bone marrow in ALL was evaluated, and it was found that patients with poor cytologic bone marrow response at day 14 had lower CR rates and poorer EFS and OS [112]. The day 14 bone marrow blast percentage was the only factor predictive for the achievement of CR following induction chemotherapy. Further, the presence of day 14 bone marrow blasts was independently prognostic for EFS and OS when only pretreatment characteristics were considered. However, they did not provide additional prognostic information when MRD information was available. Thus, the presence of MRD is the most important adverse prognostic marker in ALL, a topic more fully addressed in another chapter.

The impact of the type of donor and intensity of conditioning regimens was evaluated in 282 patients with Ph-negative ALL and high-risk disease who were all transplanted in first CR [113]. The overall 3-year post-transplant RFS and OS were estimated to be 65% and 69%, respectively. No difference in outcomes was observed between B-cell and T-cell ALL patients, nor was there a difference based on donor type. In a second study of 161 patients with Ph-positive ALL transplanted in first CR, the 5-year overall RFS and OS rates were 48% and 58%, respectively [114]. Again, no differences in outcome were seen when evaluating based on donor type or intensity of conditioning regimen. Notably, only a minority of patients in either study received reduced intensity conditioning, so myeloablative regimens should be preferentially offered whenever feasible [113, 114].

Cytogenetic analysis and immunophenotyping at diagnosis should be utilized to provide early identification of those patients with poor prognosis (such as ETP-ALL and Ph-like ALL) in whom allogeneic HSCT should be consid-

Table 11.2 Indications for HSCT in first CR

Type	Subtype	MRD status[a]	HSCT
B-ALL	Ph-negative + ≥1 high-risk features[b]	Negative	After CR1
	Ph-negative	Positive	After blinatumomab for 2–4 cycles[c]
	Ph-positive	Positive	After blinatumomab + TKI × 2–4 cycles (MRD > 3 logs)[d]
T-ALL	All subtypes + high-risk features[b] All subtypes	Negative Positive	After CR1 After venetoclax-based regimen

[a]MRD assessment at 3 months
[b]CRLF2, MLL rearrangement (11q23), ETP-ALL, t(4,11), low hypodiploidy, complex CG ≥ 5 abnormalities, TP53 mutation
[c]Consider inotuzumab in CD22 positive patients
[d]If MRD ≤3 logs, then continue blinatumomab + TKI (HSCT is not warranted)

ered in first CR [115]. The decision to offer allogeneic HSCT in first CR should be individualized to each patient based on a number of factors. For high risk patients, allogeneic HSCT in first CR should be offered if there is an available donor. For standard risk patients, patients with MRD-positive disease in CR may benefit most from allogeneic HSCT in first CR rather than MRD-directed therapy. See Table 11.2 for the indications of allogeneic HSCT for ALL patients in first CR.

11.10 Conclusion

Treatment of ALL in adults has previously been considered to be challenging, with poor long-term survival rates of 35–45% compared to high long-term survival and cure rates (>90%) in pediatric patients. More recent data on novel therapies such as the addition of monoclonal antibodies and other targeted agents to standard, and also attenuated doses of, chemotherapy backbones has demonstrated significant improvements in survival rates in untreated ALL patients. In addition, recent data has further narrowed indications for allogeneic HSCT in first CR based on a number of factors including cytogenetics and immunophenotyping at diagnosis and MRD status after initial therapy. Ongoing studies are evaluating the safety and efficacy of chemotherapy-free regimens, which are designed to improve toxicity profiles, especially in those older patients who otherwise would not tolerate intensive chemotherapy.

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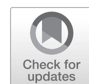

12 Acute Lymphoblastic Leukemia (ALL) in Children and Adolescents

Michael Rytting

12.1 Introduction

In the pediatric population, acute lymphoblastic leukemia (ALL) is the most common cancer diagnosis [1, 2]. The incidence of ALL peaks in Caucasian males in the pre-school period and then gradually decreases. In the adolescent and young adult population, up to about 25 years of age, ALL remains an oncologic problem, but is no longer the most commonly encountered malignancy [3]. In general, about 80% of newly diagnosed leukemia patients in the pediatric population have pre-B ALL. About 15% of young patients will have T-cell ALL, and the remaining patients will be diagnosed with mature B-cell ALL. In economically developed countries, almost all pediatric and younger adolescent patients with leukemia are treated in a designated children's hospital or in a large cancer center with pediatric expertise. Since the disease is the most commonly encountered malignancy in the pediatric age group, pediatric hematologists/oncologists tend to be familiar with current protocols and clinical management of these young patients. The majority of pediatric patients with leukemia who are treated in developed countries are placed on protocol therapy, which has led to steady and significant improvements in outcome over the last four decades [1]. In the United States, disparities in outcome have varied in minority populations [4, 5]. Decreased survival rates persist in the Hispanic and African American population. Such disparities may be secondary to underlying molecular abnormalities that are just now being discovered, such as mutations in CRLF2 in Hispanic patients. On the other hand, some of the differences in outcome can still be attributed to economic factors [6]. Outcomes also vary by age, leukemic blast phenotype, leukemia cytogenetics, and presenting features such as initial white blood cell count. Classically, low-risk ALL is defined by patient age less than 10 years at diagnosis along with a white blood cell count under 50,000/μl with no central nervous system or testicular involvement. Children over age 10 years or with a white blood cell count over 50,000/μl or with T-cell phenotype ALL are defined as high-risk patients. In the last decade, response to therapy measured by minimal residual disease at set time points and cytogenetic and molecular features found in leukemic cells have been used to further define risk groups and to determine therapy.

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12.2 Workup, Diagnosis, and Initial Management

The clinical evaluation and laboratory evaluation of the suspected pediatric leukemia patient are very similar to the evaluation of an older leukemia patient. Basic laboratory studies including uric acid are drawn as well as clotting times and various immune titers. The value of HIV testing in this population, as compared to the adult population, is debatable. All pediatric patients who are suspected to have an acute leukemia and are scheduled to undergo anesthesia should have a chest X-ray to eliminate the possibility of a significant mediastinal mass. Other imaging studies can be tailored to the patient's presentation. In boys, the testicular examination is essential. In the event of abnormalities on the testicular examination, an ultrasound of the testis is warranted. An initial testicular biopsy is usually not required. Occasionally, pediatric patients with acute lymphoblastic leukemia will have unusual presentations and normal initial blood counts. An isolated tumor that is diagnosed as a lymphoblastic lymphoma should always be followed up with a bone marrow evaluation, even in the setting of completely normal blood counts. An evaluation of the peripheral blood smear is invaluable. An experienced hematologist is frequently able to diagnose a leukemia before the patient undergoes procedures and anesthesia, which will allow administration of the first intrathecal therapy with the first bone marrow aspiration. While the diagnosis of ALL by peripheral blood morphology may appear certain, it is usual to wait for confirmation with flow cytometry or immunochemistry prior to declaring the diagnosis and starting therapy. The flow cytometry antigen panels used for the diagnosis of adult ALL are almost always adequate for pediatric patients. The DNA index by flow cytometry can be very helpful in pediatric cases. More and more flow cytometry markers are entering practice, such as CRLF2, and close collaboration with a hematopathologist is required to determine which antigens should be included in the evaluation of the newly diagnosed ALL patient. Various guidelines, such as those noted by the Euroflow group, provide an excellent framework for designing antigen panels for patients with suspected ALL [7].

Pediatric patients who are suspected to have leukemia should start intravenous fluids at above the maintenance rate in order to maintain good urine output. Antibiotics do not need to start immediately unless there is a fever or the patient appears infected. Many pediatric leukemia doctors do not regard the patient's presenting neutrophil count as protective in the initial treatment of these children. Pediatric patients with either clearly diagnosed circulating blasts or with multiple cytopenias are then scheduled for bone marrow evaluation. If there are clear circulating blasts, then it is routine to administer the first intrathecal chemotherapy while the patient is receiving anesthesia for the bone marrow procedure. If the patient cannot undergo anesthesia safely, as in the case of a large mediastinal mass, procedures may need to be performed with local anesthesia in an awake child. In the setting of a significant number of circulating blasts, most testing can be performed on the blood, and the initial bone marrow may be foregone. A delay in the lumbar puncture might be considered if the patient cannot undergo anesthesia and is incapable of holding still during the procedure since the initial lumbar puncture should be performed in a situation that is least likely to produce a traumatic puncture. There is some evidence that a delay in the timing of the initial lumbar puncture probably does not affect outcome significantly [8, 9]. Prior studies have indicated that introduction of circulating blasts into the spinal fluid via traumatic lumbar puncture can lower overall event-free survival rates, and this decrease is statistically significant [10]. For this reason, it is recommended that the initial lumbar puncture be performed by the most experienced practitioner available in a controlled environment where the patient is not likely to move.

As in adult patients with leukemia, children with ALL are at risk of the tumor lysis syndrome once treatment starts. Patients with particularly high white count, mainly over 200,000 cells/μl, may need urgent intervention to prevent complications of leukostasis [11]. The initiation of therapy with corticosteroids can rapidly decrease the

white blood cell count and is not likely to obscure the diagnosis in the first few days of treatment. Delay of therapy while waiting for diagnostic confirmation, on the other hand, can result in significant morbidity. Most patients can be successfully managed with increased intravenous fluids as well as allopurinol. In cases with very high white blood cell counts or marked organ infiltration or in the setting of impaired renal function, rasburicase may be considered to help prevent complications of tumor lysis. The vast majority of patients, however, do not require rasburicase in their initial therapy. Close monitoring of electrolytes, urine output, daily weight, and peripheral blood counts are required. Almost all patients will require transfusions during the initial part of intensive therapy.

The standard evaluation of a newly diagnosed pediatric patient with leukemia includes the above evaluation of spinal fluid and bone marrow as well as examination of peripheral blood. This basic assessment is accompanied by a rapidly expanding number of molecular and cytogenetic tests. All patients should be tested for the presence of the Philadelphia chromosome, and many institutions offer very rapid screening by fluorescent in situ hybridization (FISH) or polymerase chain reaction (PCR) testing that is available within 24–48 h. Standard cytogenetics to determine leukemic cell ploidy have been offered for decades. Molecular testing for common translocations and deletions that can be used for risk stratification is now also standard in most patients. The distribution of important cytogenetic alterations in various age groups is described in several recent publications [12–14]. High-risk lesions become more prominent with age, while favorable cytogenetic findings are more likely to be encountered in the younger population. Particularly favorable findings such as trisomies of chromosomes 4, 10, or 17 as well as the ETV6/RUNX1 translocation are much more common in younger patients [15–17]. Internal amplification of genetic material on chromosome 21, an unfavorable finding, is now becoming a standard test [18]. Similarly, and especially in Hispanics and children with trisomy 21, testing for CRLF2 mutations or for overexpression is recommended [19]. See Table 12.1 for commonly requested cytogenetic and molecular testing for newly diagnosed patients.

Table 12.1 Standard cytogenetic/molecular testing

Ploidy or DNA index
t(9;22); MLL; ETV6-RUNX1; iAMP21; TCF-PBX3
Philadelphia-like kinase expression (low-density array)
CRLF2 expression/mutation; Ikaros; TP53

As the above molecular and cytogenetic testing returns on each patient and prognosis changes, therapy may be adjusted in an attempt to improve outcome [20, 21]. Participation in clinical trials that specifically evaluate therapy changes in patients with both high risk and favorable risk disease features is recommended.

The response to therapy, as would be expected, is an excellent predictor of outcome in pediatric acute lymphoblastic leukemia. The Berlin–Frankfurt–Muenster (BFM) study group has emphasized the response to 7 days of prednisone therapy plus one intrathecal treatment as an indicator of prognosis even in Philadelphia chromosome positive cases [22, 23]. Recently, testing for minimal residual disease early in treatment, and particularly at the end of induction, has been shown to be the most predictive factor in forecasting prognosis of pediatric ALL patients [24–29]. Testing for MRD as early as day 8 in therapy predicts prognosis in standard risk children with pre-B ALL. European groups have tended to rely on minimal residual disease testing using polymerase chain reaction (PCR) evaluation of IGH or T-cell receptor gene rearrangements. The children's oncology group has focused on flow cytometry as the preferred method for evaluating minimal residual disease. Both methods have been shown to be feasible in cooperative group settings and accurate in predicting outcome in children [30]. Studies now are incorporating the results of minimal residual disease testing into therapy. Patients with rapid clearance of minimal residual disease are candidates for decreased intensity treatment, while patients with persistent minimal residual disease can be assigned to more intensive treatment or to treatment that investigates new agents. Interestingly, but of significant

clinical import, T-cell ALL patients may have a slower clearance of minimal residual disease but still have an excellent prognosis [31, 32]. Some of the first trials to successfully incorporate minimal residual disease testing into therapy have been published by the United Kingdom Acute Lymphoblastic Leukemia (UKALL) consortium and the Dutch Children's Oncology Group (DCOG) consortium [20, 21, 33]. Failure to clear minimal residual disease in the bone marrow or any other site predicts a very poor prognosis, and treatment of such patients on clinical trials is paramount [27, 34, 35].

12.3 Therapy

Induction chemotherapy for newly diagnosed patients is relatively standard in developed countries. Treatment for standard-risk patients with ALL frequently consists of oral corticosteroids such as dexamethasone combined with vincristine, asparaginase, and intrathecal therapy. Anthracycline is admitted for high-risk patients in many instances. Variations to this standard induction are used in most pediatric treatment groups, and the outcomes are similar for lower and higher risk patients (Tables 12.2 and 12.3). In geographic locations with more limited resources, intensification of therapy that entails high levels of risk may not be effective in increasing survival due to treatment related mortality [40]. As noted above, alterations to therapy based on response to induction and later phases of treatment can improve cure rate and decrease exposure to some chemotherapeutic agents for subsets of patients who have an excellent prognosis.

T-cell ALL patients are regarded as high-risk patients from the date of diagnosis. With intensification of therapy, pediatric and adolescent T-cell ALL patients have quite good outcomes (Table 12.4). The addition of nelarabine to therapy of T-cell ALL is currently being evaluated [41]. One interesting outcome from the addition of nelarabine to pediatric T-cell ALL ther-

Table 12.2 Standard-risk B-ALL studies

Study group	*N*	EFS (%)	OS (%)	Reference
COG	5311	89	96	Devidas et al. [36]
DFCI	460	91	94	Vrooman et al. [37]
UKALL	521	94	98	Vora et al. [21]
AIEOP/BFM	1007	94	–	Conter et al. [28]
SJCRH	258	89.7	97.7	Pui et al. [38]

AIEOP Associazione Italiana Ematologia Oncologia Pediatrica, *BFM* Berlin–Frankfurt–Munster, *COG* Children's Oncology Group, *DFCI* Dana Farber Cancer Institute, *SJCRH* St. Jude's Children's Research Hospital, *UKALL* United Kingdom Acute Lymphoblastic Leukemia

Table 12.3 High-risk B-ALL studies

Study group	*N*	EFS (%)	DFS (%)	Reference
COG	3154	75.3	85	Larsen et al. [39]
DFCI	218 (DFCI high risk)	77	84	Vrooman et al. [37]
UKALL	386 (MRD low risk)	94.7	–	Vora et al. [20]
AIEOP/BFM	341 (MRD low risk)	86.9	–	Conter et al. [28]
SJCRH	164	82	89.7	Pui et al. [38]

AIEOP Associazione Italiana Ematologia Oncologia Pediatrica, *BFM* Berlin–Frankfurt–Munster, *COG* Children's Oncology Group, *DFCI* Dana Farber Cancer Institute, *SJCRH* St. Jude's Children's Research Hospital, *UKALL* United Kingdom Acute Lymphoblastic Leukemia, *MRD* minimal residual disease

Table 12.4 T-cell studies

Group	*N*	EFS (%)	DFS (%)	Reference
COG	1562	83.8	89.5	Winter et al. [42]
DFCI	125	75	78	Goldberg et al. [43]
UKALL	187	82	91	Patrick et al. [44]
AIEOP/BFM	464	76	81	Schrappe et al. [32]
SJCRH	76	78	87	Pui et al. [38]

AIEOP Associazione Italiana Ematologia Oncologia Pediatrica, *BFM* Berlin–Frankfurt–Munster, *COG* Children's Oncology Group, *DFCI* Dana Farber Cancer Institute, *SJCRH* St. Jude's Children's Research Hospital, *UKALL* United Kingdom Acute Lymphoblastic Leukemia

apy is a decrease in central nervous system relapse.

In the past, many pediatric patients with low-level involvement of the spinal fluid with blasts were treated with radiation therapy. The current consensus, however, has changed. At the present time, only patients with frank leukemic involvement of the spinal fluid (CNS 3) receive radiation [45]. Likewise, not all patients with testicular involvement are treated with radiation on current treatment protocols [46]. Instead, response to chemotherapy with normalization of the testicular examination can be used to determine whether patients should proceed to radiation.

12.4 Relapse

Pediatric ALL patients, as reviewed above, have excellent cure rates. Nevertheless, some patients do relapse. Isolated central nervous system relapse has been well studied in pediatric groups. In general, isolated CNS relapses that occur late in treatment are very amenable to further therapy with relatively high cure rates [47, 48]. Early central nervous system relapse and bone marrow relapse are more difficult to treat. Recently, the addition of mitoxantrone to relapse therapy has had a surprising and impressive impact on cure rates [49].

12.5 New Therapies

Patients with refractory ALL or early relapse of ALL may benefit from therapies that enlist the immune system. Antibodies that target tumor antigens have been shown to improve survival in adult patients and are entering pediatric studies. Blinatumomab, a bi-specific antibody that targets CD 19 and CD3, may improve outcomes in patients with persistent minimal residual disease [50]. Inotuzumab, an anti-CD 22 antibody, has shown activity in patients with relapsed ALL [51, 52]. At present, these antibodies are being incorporated into therapy of very high risk pediatric patients as well as into relapse regimens.

Chimeric antigen receptor (CAR) T cells have been an exciting addition to therapy as well. While early in evaluation, the high therapeutic potential of CARs is evident [53]. Whether CAR T-cell therapy may be curative alone or should be followed up with transplantation is a current, unanswered question [54]. CAR T-cell therapy does have significant toxicities, including severe systemic inflammatory reactions as well as central nervous system toxicity [55]. In addition, due to B-cell aplasia that occurs after CARs, these patients often have a long-term immunodeficiency that requires immunoglobulin infusions.

12.6 Conclusion

Pediatric ALL, either of the pre-B phenotype or the T-cell phenotype, is a highly curable disease using multiagent chemotherapy regimens. Cure rates are high enough in some low-risk ALL children that therapy is now being reduced in some trials. There remain subgroups of children with ALL who have a high risk for relapse and thus with suboptimal cure rates. These very high-risk subgroups are actively sought for enrollment on clinical trials. New therapies, particularly antibodies that enlist T cells, have provided hopeful results for patients who relapse or have refractory disease.

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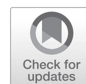

13 Hematopoietic Cell Transplantation (HCT) for Acute Lymphoblastic Leukemia (ALL)

Limei Michelle Poon and Partow Kebriaei

13.1 Introduction

Acute lymphoblastic leukemia (ALL) is a malignant neoplasm derived from T or B lymphoid precursors. While there have been marked advances in the treatment and outcomes of children with a 5-year survival at more than 90%, therapeutic progress of adult ALL has until recently, remained inferior, with only 30–40% of patients achieving long-term leukemia-free survival [1–3]. Outcomes of patients with relapsed, refractory ALL has been dismal, with poor responses to salvage chemotherapy (40–45% complete remission (CR) rates) and guarded overall survival (OS) of <10% [4–7]. Treatment-related toxicities of salvage chemotherapy in this setting are common and may cause organ-related toxicities which preclude allogeneic transplantation. Together with the potent antileukemic activity of alloHCT in ALL, this has led to a compelling argument to consider alloHCT in CR1, even among standard risk patients, despite the acute and long-term toxicities.

In the recent decade, however, there have been significant advances in the field of ALL. In addition to the development of minimal residual disease (MRD) monitoring strategies and oncogenomics which has revolutionized prognostication for ALL, the use of pediatric-type regimens for ALL as well as the advent of novel therapeutic agents which have been incorporated in both the upfront and salvage setting has improved outcomes for adult ALL patients. Importantly, however, accessibility of alloHCT has also improved with the development of novel conditioning regimens and increasing experience with the use of alternative donor transplantation. This has led to a difficult conundrum as to which ALL patients would benefit from alloHCT in CR1.

In this chapter, we aim to re-examine the role of alloHCT for adult Ph-positive and -negative patients in CR1 in the era of key advances, including MRD, pediatric-based ALL treatment protocol, oncogenomics, and novel therapeutic agents. We also explore the latest updates in the field of alloHCT for ALL, including the use of alternative donors and the development of reduced intensity conditioning regimens and their impact on the ALL treatment paradigm.

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13.2 AlloHCT in CR1 for Ph-Negative ALL

13.2.1 Historical Data

In the pre-MRD era, conventional prognostic factors have included age (>35–40 years), white blood cell (WBC) count in relation to B subtype, immunophenotype, cytogenetics, and time to complete remission (CR) [8, 9] (see Table 13.1A). These conventional risk factors have been used in several large prospective studies, looking at the role of alloHCT based on genetic randomization. Despite differences in study design and factors used for risk stratification, several of these early studies have shown benefit of allogeneic transplantation in high-risk patients. In the French LALA 94 trial [2], patients with high-risk disease, who had achieved a CR and had a matched related donor (MSD), were assigned to alloHCT, while those with no donors were assigned to chemotherapy alone or autologous transplantation (autoHCT). In an intention to treat analysis, high-risk patients who had a donor were found to have a significant survival advantage compared to the no-donor group (5-year leukemia-free survival 45% vs. 23%). Similarly, in the GOELALA02 trial [10], which compared alloHCT to autoHCT for high-risk ALL patients, the 6-year OS was significantly improved in the alloHCT arm 75% versus 40% after autoHCT, $p = 0.0027$.

Among patients with standard-risk ALL, recent studies have also suggested a benefit for alloHCT. In the UKALL XIIMRC/ ECOG2993 trial [11], which was the largest study for ALL alloHCT based on "genetic randomization," standard-risk Ph-negative ALL patients in the donor arm had a statistical benefit in OS compared to the no-donor group (53% vs. 45% respectively, $p < 0.05$). Similar findings were seen in the HOVON-18/37 trial [12] which allocated standard- and high-risk patients with MSD to alloHCT and those without to auto-HCT. Disease-free survival at 5 years in the donor group was improved compared to the no-donor group (60% vs. 42%, $p < 0.05$), but these improved outcomes were most pro-

Table 13.1 Prognostic factors for ALL

(A) Traditional risk factors for ALL	
Age	Higher risks associated with older age groups While there is no clear definitive age cutoff, age >35–40 years is normally used to delineate between standard and high risk groups
Cytogenetics	Good risk cytogenetics: • Hyperdiploidy (51–65 chromosomes) • t(12;21)(p13;q22) Poor risk cytogenetics: • t(v;11q23) (MLL rearrangement) or t(4:11)/MLL-AF4 • t(9;22)(q34;q11.2) • Low hypodiploidy (<40 chromosomes) or near-tetraploidy (>80 chromosomes) • Complex cytogenetics (≥5 chromosomal abnormalities)
WBC counts	B-ALL: WBC $\geq 30 \times 10^9$/L T-ALL: WBC $\geq 100 \times 10^9$/L
Disease response	Induction failure defined as failure to achieve complete remission within 4 weeks of induction treatment
(B) Novel risk factors for ALL	
Immunophenotyping	Good prognostic marker: • Cortical T phenotype (CD1a positive) Poor prognostic marker: • Early T precursor ALL (Prognostic significance appears to be dependent on treatment protocol)
Oncogenetics	• IKAROS deletions in B-ALL • Absence of NOTCH1/ FBXW7 mutation and/ or the presence of N/K-RAS mutation and/ or PTEN alteration in T ALL • Ph-like B-ALL
Novel cytogenetic subgroup	Intrachromosomal amplification of chromosome 21 (iAMP21) in B-ALL
Minimal residual disease	• MRD > 10^{-3} post induction • MRD detectable post consolidation

nounced in the standard-risk compared to the high-risk group.

Of note, even among the trials which could not demonstrate overall survival benefit for the alloHCT group (e.g., in the high-risk group within the MRC/ECOG population) [11, 13], careful review of the data showed that the relapse rates in the alloHCT arms were consistently lower compared to the chemotherapy or auto-HCT arm, suggesting that the conflicting results were due to the high non-relapse mortality of alloHCT, abrogating the overall survival benefits rather than the lack of efficacy of the graft versus leukemia effect.

Overall, the results of these trials provide the initial rationale for consideration of alloHCT in the first-line treatment of ALL. Given that non-relapse mortality remains a significant concern with alloHCT, there remains a clear need for refinement of the selection criteria for alloHCT in order to identify specific subpopulations who would benefit most from alloHCT. This has been made possible in recent times due to increasing trials reviewing the significance of MRD and advances in the genomics field in ALL.

13.2.2 Role for AlloHCT for ALL in CR1 Following Current Advances in the Field

13.2.2.1 Minimal Residual Disease and Impact on AlloHCT in CR1

Minimal residual disease refers to a level of disease that is undetectable by morphological studies and is not associated with symptomatic disease. Methods of MRD monitoring for ALL include multiparameter flow cytometry, real-time quantitative polymerase chain reaction (RT-PCR), and in recent years, next-generation sequencing. Using these methods, residual disease can be diagnosed with a sensitivity of up to 0.001% (10^{-5}).

In adults, despite the use of different methods of MRD quantification and different chemotherapy regimens, several large cooperative groups including the German Multicenter Study Group for Adult ALL (GMALL), UK MRC group, the North Italy Leukemia group (NILG), and the Spanish PETHEMA group (Programa para el Estudio de la Terapéutica en Hemopatía Maligna) [14–16] have shown that early MRD response (in the context of intensive pediatric inspired regimens) is highly prognostic and predictive for relapse. In addition, in recent years, results of MRD assessment have been incorporated into the risk stratification for Ph-negative ALL in many of these cooperative groups, allowing identification of standard-risk patients who would benefit from alloHCT and high-risk patients (based on conventional criteria) for whom alloHCT might be avoided. In one of the first prospective studies using MRD for decisions regarding alloHCT, Bassan et al. measured MRD at weeks 10, 16, and 22 using real-time quantitative PCR in Ph-negative patients (excluding MLL-positive patients). Allogeneic transplant was omitted in patients who had no MRD detectable, even if they had conventional high-risk factors. MRD level was found to be the most important prognostic factor for LFS (72% for MRD-negative patients vs. 14% for MRD-positive patients at 5 years, $p = 0.001$). Similarly, the PETHEMA group showed that high-risk patients (based on pretreatment conventional high-risk characteristics) with rapid MRD clearance could be treated with chemotherapy alone, with 5-year DFS and OS of 55% and 59% in this patient population. Of note, in both the studies, MRD clearance was found to be the most significant prognostic factor for OS and duration of CR, as compared to conventional risk factors.

As a result of these consistent findings of the prognostic significance of MRD, the European Working Group for Adult Acute Lymphoblastic Leukemia (EWALL) and the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation (EBMT) jointly issued a recommendation supporting the persistence of MRD as the strongest predictor of relapse, trumping conventional risk factors, especially in patients treated with intensive pediatric-like regimens [17]. Based on this guideline, alloHCT was recommended in CR1 for patients with MRD >1:10^{-3} post induction and detectable

post consolidation. In contrast, in patients with conventional risk factors who achieved MRD negativity, the use of alloHCT in CR1 may not be required.

There remains however a number of unanswered questions with regard to MRD status and the optimal strategy for alloHCT. First, it is unclear whether the achievement of an MRD-negative state should be the goal for all patients. While achieving MRD negativity is associated with best outcomes, the additional treatment to try to render patients into a minimal disease-free state has historically been associated with infectious complications or organ toxicities that might preclude transplantation or increase treatment-related mortality (TRM) and offset the potential benefits of improved disease control. In recent years, studies have shown that the use of blinatumomab, a bispecific T cell–engager antibody construct that directs T cells to CD19 cells, has allowed a majority of patients (78% of evaluable patients) with persistent residual disease to achieve MRD negativity with acceptable toxicity [18]. This has led to the FDA approval of blinatumomab for patients with MRD of >0.1% or greater in the first or second CR and suggests blinatumomab as a feasible option for rendering patients MRD-negative prior to transplantation. The efficacy of blinatumomab in eradicating MRD has led to a second treatment controversy, about the role for alloHCT for patients who fail MRD timepoints but who are rendered into MRD negativity by blinatumomab. The currently published trials are probably not powered or designed to answer these questions, and more prospective studies are needed to address these pertinent clinical conundrums.

13.2.2.2 Novel High-Risk ALL Subtypes Based on Immunophenotype, Molecular Markers, and Significance

Apart from the use of MRD for risk prognostication, a number of novel high-risk ALL subtypes based on immunophenotype, molecular markers, and cytogenetics have been identified in recent years (Table 13.1B). These include (1) Ph-like ALL, (2) B-ALL associated with intrachromosomal Amplification of Chromosome 21 (iAMP21), (3) early T-precursor ALL, and (4) T-ALL associated with N/K *RAS* gene mutation or *PTEN* gene alterations.

Ph-like ALL has a gene expression profile similar to Ph-positive ALL, but without the BCR–ABL fusion gene. First described by the Children's Oncology group and the St Judes children's hospital [19], as well as den Boer et al. from the Netherlands [20], this entity has been identified as a new provisional entity in the WHO 2016, based on defined gene expression profile and not by a single molecular aberration. Apart from the various genomic alterations that activate kinase and cytokine receptor signaling, such as the overexpression of cytokine receptor-like factor 2 (CRLF2), JAK/STAT signaling, and fusions involving JAK2, ABL1, ABL2, and many other tyrosine kinases, this entity is also associated with a high frequency of deletions of the IKZF1 which encodes IKAROS, the lymphoid transcription factor. Clinically, Ph-like ALL has been associated with high non-response and relapse rates, as well as poor long-term survival in both children and adults. The prognostic significance of Ph-like ALL in relation to conventional risk factors and novel prognostic markers such as early MRD responses has been a key question. Several pediatric studies, including a large study from the St Judes group, have suggested that the adverse prognostic impact of Ph-like ALL appeared to be eliminated by MRD-based treatment stratification [21], suggesting that an MRD-based treatment strategy alone may be sufficient to guide transplant decisions. In adults, there has been limited studies. The GRAALL group reviewed the prognostic significance of conventional and new risk factors including MRD and oncogenetics in 423 adult patients enrolled in their two pediatric-inspired trials (GRAALL-2003 and GRAALL-2005) [22]. ALL high-risk genetic subsets assessed in this study included IKAROS gene deletion for B-ALL, while a high-risk profile which included the absence of NOTCH1/FBXW7 mutation and/or the presence of N/K-RAS mutation and/or PTEN alteration was used for T-ALL patients. In this study, with the use of

more intensive pediatric-like protocol, most conventional risk factors with the sole exception of the MLL rearrangement lost prognostic significance. In contrast, novel prognostic markers including high-risk genetics and poor MRD response showed statistical independence on multivariate analysis in predicting relapse. For adults with Ph-like ALL, the GMALL, HOVON, MD Anderson group, and St Judes group [23–26] have looked at the prognostic significance of this entity with adult patients. While Ph-like ALL was associated with poor MRD responses, and inferior DFS and OS in these studies, the limited patients in these studies and heterogeneity of treatment protocols used make it difficult to tell if the Ph-like subtypes and MRD response had independent prognostic significance.

iAMP21 defines a distinct cytogenetic subgroup of childhood B-cell precursor acute lymphoblastic leukemia, associated with the finding of three or more extra copies of RUNX1 on a single abnormal chromosome 21. It comprises about 2% of all childhood B-ALL, while being extremely rare in adults. iAMP21 has been associated with poor EFS and higher risks of relapse, especially when patients are treated on standard risks arms [27, 28]. In contrast, when these patients are treated on high-risk arms, findings from the Children Oncology group trials [28] as well as from results from the International Childhood ALL Working Group (Ponte di Legno group) [29] and UK ALL-2003 [30] studies demonstrate that highly statistically significant improvements in EFS, OS, and relapse rates have been noted. The role of MRD for iAMP21 patients remains unclear, with conflicting results between the different studies, largely due to the different treatment strategies in the studies [28, 31]. Nevertheless, both the children Oncology and UK groups agree that these patients should be treated on the high-risk arms of the protocols. Allogenic transplantation should be considered in the upfront setting for these patients.

Early T-precursor ALL (ETP ALL) is a leukemia derived from thymic cells at the early T-precursor differentiation stage and appears to have multilineage pleuripotency [32]. In the WHO 2016, ETP ALL is a novel provisional entity, defined based on immunophenotype, with immature T-cell markers as well as positivity for one or more of the myeloid/stem cell markers [33]. ETP ALL appears to have differing prognostic significance depending on the treatment provided. The use of pediatric, response-based protocols appeared to abrogate the poor prognosis of ETP ALL in pediatric, adolescent, and young adult (AYA) patients, as seen by the results from the Children's Oncology Group and Medical Research Council Working Party on Leukaemia in Children UK National Acute Lymphoblastic Leukaemia 2003 study [34]. In contrast, outcomes of adult ETP patients appear to be poor especially if treated with an adult protocol. The MD Anderson reported their results treating ETP ALL (n = 19) with HyperCVAD, an adult-type chemotherapy protocol and showed poorer outcomes compared to patients with other T-ALL subtypes [35]. Importantly, however, in the largest report of uniformly diagnosed and treated adults with ETP ALL, the GRAALL group showed that risk stratification and therapy intensification with alloHCT based on early treatment response appeared to negate the poor risks of ETP ALL, leading to similar outcomes compared to other T-ALL subtypes [36].

Currently while we recognize these unique and novel ALL subtypes which are associated with adverse prognosis, it remains unclear if all patients should be offered alloHCT, independent of the treatment regimen or MRD response. More studies in this field are needed to help us to better determine the optimal management of this ALL subtype and whether there is a role for upfront allogeneic transplantation vs. using a pediatric protocol with MRD-guided therapy in these disease entities.

13.3 AlloHCT Beyond CR1

13.3.1 Pre-Novel Agent Era

The outcome of patients with relapsed refractory ALL has been historically poor as demonstrated by several multicenter trials which have shown that complete remission rates to salvage therapy

usually range from 40% to 45% with overall survival rates of less than 10% [4–6, 14]. These studies have also confirmed that alloHCT offers the best chances for potential cure for patients in CR2. In data from one of the largest studies, the UKALLXII/ECOG2993 trial, patients with relapsed disease had a 5-year survival of 15–23% following alloHCT (with outcomes depending on the donor type) which was markedly improved compared to chemotherapy alone (4%) ($p < 0.05$). The GMALL group reported outcomes of 547 ALL patients following relapse. Patients who managed to proceed to alloHCT had a 3-year OS of 38% while none of the non-transplantation patients had long-term remission. Similar findings were also reported by the French LALA and Spanish PETHEMA groups.

The poor outcomes for patients with relapsed/refractory disease largely results from the difficulty in attaining a second remission, and the TRM related to salvage therapy, which may be as high as 20% in some studies [37, 38]. Given these risks, some have argued for a potential role for immediate alloHCT for patients with low tumor burden and immediate donor availability. The data on this practice has been conflicting. In one of the largest studies addressing this issue, Terwey et al. showed a 5-year OS rate of 47% in 19 patients with relapsed or refractory disease who received an alloHCT without prior reinduction chemotherapy [39]. These encouraging results were in contrast to the group that had received prior reinduction chemotherapy where the 5-year OS was 18% [39]. These findings have however not been reproduced in other studies which suggest that alloHCT in CR2 is associated with better outcomes compared to alloHCT in active disease [40, 41]. In recent years, the EBMT has developed a prognostic score for ALL patients transplanted with refractory disease following two courses of chemotherapy [42]. Use of TBI and use of female donors for male recipients were associated with improved outcomes, and patients without these factors had an OS of 8% compared to 22% and 57% in those with 1 or 2 factors, respectively. While these studies suggest that there are selected refractory patients who might benefit from transplantation without further salvage therapy, with the advent of novel therapeutic agents with high response rates and MRD negativity, the paradigm has shifted away from immediate alloHCT for patients with refractory disease.

13.4 Role of AlloHCT Beyond CR1 in the Era of Novel Salvage Regimens

After many decades of limited progress of new drugs, in recent years, there has been a number of major advances which has significantly reshaped the treatment landscape for relapsed refractory ALL. In the last 5 years, three novel agents, including inotuzumab, an anti-CD22 monoclonal antibody linked to calicheamicin, blinatumomab (discussed above), a CD3-CD19 bispecific antibody construct, and tisagenlecleucel, a CD19-directed genetically modified autologous T-cell immunotherapy, has been approved by the U.S. Food and Drug Administration as ALL salvage therapies.

Inotuzumab was approved for the treatment for ALL following results from the INO-VATE trial, a randomized controlled trial (RCT) comparing inotuzumab against physician's choice (Standard of care [SOC]) for the first- or second-line salvage for relapsed-refractory ALL [43]. Responses were significantly higher in the inotuzumab group (CR rates 81% vs. 29% for SOC, $p < 0.001$). Among the responders, MRD-negativity rates were also markedly better in the inotuzumab group (78% vs. 28%, $p < 0.001$). Despite these impressive responses, however, the median PFS and OS were short lived in both groups (median PFS 5 months vs. 1.8 months, $p < 0.001$, and median OS 7.7 vs. 6.7 months, $p = 0.02$), and alloHCT was still needed to consolidate clinical responses. Importantly, however, the use of inotuzumab has been found to be associated with hepatotoxicity including veno-occlusive disease (VOD) in patients undergoing alloHCT, especially in patients receiving two alkylating agents or who underwent transplantation with bilirubin levels which were higher than the upper limit of normal [44]. These findings are

a stark reminder that novel agents may impact on alloHCT outcomes and that understanding of drug toxicities, careful selection of preparative regimens, and avoidance of other concomitant hepatotoxic drugs need to be considered to reduce post-transplantation toxicities. Blinatumomab has also been compared to SOC in a phase 3 RCT (the Tower study) in patients with relapsed-refractory ALL. Of the 405 randomized patients, the overall response rates were higher in the blinatumomab compared to the SOC arm (45% vs. 30%, $p = 0.007$) [45]. Among the responders, MRD-negative rates were also higher in the blinatumomab arm (75% vs. 48%). Blinatumomab also showed prolonged median survival of 7.7 months compared to SOC 4.0 months for all patients, and with best results seen when blinatumomab was used in first-line salvage (11.1 months vs. 5.5 months for SOC). Despite these impressive results, alloHCT appears to still be needed for prolonged disease remission.

While the high rates of MRD negativity associated with the use of single-agent blinatumomab and inotuzumab have helped disease control and PFS, the remission durations remain short, and alloHCT has remained relevant and important for prolonged disease-free survival. The development of chimeric antigen receptor T cells however may change this treatment paradigm.

Currently, there are more than 240 trials of CAR-T cells worldwide. To date, however, only one CD19 CAR-T cells from Novartis targeting CR19 antigen on B cells, tisagenlecleucel, has been approved for treatment of ALL (a second CD19-directed CAR-T-cell product, axicabtagene, is commercially available for patients with relapsed/refractory large B-cell lymphoma). With response rates of more than 80% among relapsed-refractory ALL patients treated with the CD19 CAR-T, there is a significant potential of this treatment. However, before this treatment can be applied broadly worldwide, clear guidelines to manage the potentially fatal complications such as cytokine release syndrome and neurotoxicity associated with this treatment are crucial. More work is required to ensure reproducibility and feasibility of central manufacture and to overcome the prohibitive costs of this therapy. In addition, whether CAR-T will obviate the need for alloHCT will likely also depend on the persistence of these cells. The persistence of CAR-T cells appears to depend on the costimulatory domain. Tisagenlecleucel, for example, contains the 4-1BB domain, which seems to improve the persistence of the CAR-T cells through amelioration of T-cell exhaustion. In the phase 2 global studies of tisagenlecleucel in relapsed-refractory ALL, the median persistence of the cells was 168 days at data cutoff, with ongoing persistence for as long as 20 months. In addition, despite only 9% of patients in this high-risk population undergoing alloHCT, the relapse-free survival was an impressive 80% at 6 months and 59% at 12 months. In contrast, in a phase 1 study of axicabtagene (which contains a CD28 costimulatory domain) involving 21 pediatrics and young ALL patients, CAR-T cells were not detected beyond 68 days; therefore, axicabtagene was used as a bridge to alloHCT rather than as the sole treatment [46].

More data and results from the ongoing clinical trials are necessary to determine the optimal patient population, sequence in treatment, and optimal role of these agents in ALL treatment. For now, alloHCT continues to retain relevance in the management of patients with ALL beyond CR1. It is plausible however that with increasing understanding of these different treatment modalities and rational combination between them in both the first- and second-line setting, these may eventually obviate the need for alloHCT for adult ALL patients.

13.5 Optimal Conditioning and Donor Selection for AlloHCT for ALL

From the data discussed above, it is evident that alloHCT remains a crucial modality in the treatment armamentarium for ALL and provides the best chances for long-term disease control in specific patient populations. Advances in reduced intensity conditioning regimens and use of alternative donor alloHCTs have helped increase the

accessibility of alloHCT to patients who might benefit most from it.

13.5.1 Choice of Myeloablative Conditioning

Myeloablative (MA) conditioning for ALL has traditionally included total body irradiation (TBI) or busulfan. Despite the lack of randomized studies, TBI was generally preferred historically due to its ability to eradicate leukemia cells in sanctuary sites, usually in combination with cyclophosphamide or etoposide. In many centers, however, there has been a shift toward using TBI-free myeloablative regimens due to the concerns of the long-term complications of TBI such as infertility, cardiovascular complications, as well as secondary malignancies. Busulfan and thiotepa have been the backbone of these conditioning regimens due to their ability to cross the blood–brain barrier. In a large retrospective analysis by the CIBMTR, Kebriaei et al. compared the outcomes of 819 patients who received an MA conditioning with TBI and cyclophosphamide or etoposide with those in 299 patients treated with i.v. busulfan combined with cyclophosphamide, fludarabine, clofarabine, or melphalan [47]. Despite a higher relapse rates compared to TBI-based conditioning (relative risk, 1.46; 95% confidence interval, 1.15–1.85; $p = 0.002$), there was an equivalent 3-year disease-free survival (Bu 45% vs. TBI 48%, $p = 0.35$) and OS (Bu 57% vs. TBI 53%, $p = 0.35$). Similarly, the EBMT recently performed a matched pair analysis [48] and found that thiotepa-based conditioning was associated with higher relapse rates compared to TBI conditioning but with equivalent 2-year PFS and OS. The encouraging disease-free and overall survival in these studies and the acceptable toxicity profiles support the use of busulfan- and thiotepa-based conditioning as viable alternatives to TBI-based regimens.

13.5.2 Choice of Reduced Intensity Conditioning (RIC)

RIC has allowed alloHCT to be accessible to older patients as well as patients with comorbidities which would preclude an MA alloHCT. The optimal RIC regimen as yet remains unclear, with mainly phase 2 studies reported from various centers, and there has not been one regimen which has been proven to be superior [49–52]. Observational retrospective studies from the databases of the EBMT and CIBMTR [53, 54] have demonstrated the feasibility and efficacy of RIC for ALL but have also suggested that the benefits of RIC are best seen in those transplanted in CR1 compared to those transplanted beyond CR1 (51% vs. 33% in the EBMT group and 45% vs. 28% in CIBMTR study). Among patients treated in CR1, results from the prospective UK NCRI UKALL14 study suggested that MRD positivity after induction was associated with significantly lower 2-year OS, EFS, and a higher risk of relapse (40.6%, 28.4%, and 57.2% respectively) [55]. These findings suggest that for patients with CR2 and beyond or those with persistent MRD post induction, outcomes of RIC alloHCT remains suboptimal, and the use of reduced toxicity, MA conditioning regimens, or post-transplant measures should still be considered to reduce risks of relapse.

13.5.3 Donor Choice

Given that a significant proportion of patients who need a transplant do not have a sibling donor, alternative donors including matched unrelated (MUD), cord blood, and haploidentical donors have been investigated. A recent EBMT report which looked at donor types in adults with ALL transplanted between the years 2010–2012 and 2001–2003 [56] showed an increase in unrelated donor transplants, accounting for the majority of all transplants in 2010–2012 (52%) followed by matched related donor transplants (37%) and mismatched related donors (5%).

13.5.4 MUD Transplants

Several studies have confirmed that results from MSD and MUD transplants are comparable. These include (1) Studies from Germany and Sweden that showed no differences in DFS in patients with high-risk ALL that underwent MSD vs. MUD transplants (42% vs. 45% at 5 years for patients in CR1) [57] (2) Japanese registry data that showed similar OS between MSD vs. MUD transplants (65% vs. 62% at 4 years, $p = 0.19$) and (3) a recent registry study from the CIBMTR in 672 ALL patients which showed no differences in leukemia-free survival between MUD and MSD transplants [58]. While MSD and MUD transplants are currently preferred options for alloHCT for ALL patients, there remains a significant minority of patients who lack a HLA-matched donor and for whom alternative approaches are required. These alternatives include cord blood (UCBT) and haploidentical transplants.

13.5.5 Haploidentical Transplants

Haploidentical transplants involve the use of mismatched related donors including siblings, children, or parents. There has been an increased use of haploidentical transplants (over the use of UCBT) likely due to the introduction of post-transplant cyclophosphamide immunosuppressive protocols which allows for these transplants to be done without costly ex-vivo T-cell depletion [59]. The EBMT recently reviewed the outcomes of haploidentical transplants in 208 patients with ALL transplanted between 2007 and 2014 [60]. In this series, patients transplanted in CR1 ($N = 91$) had a 3-year OS of 52%, which was comparable to the results of MSD and MUD transplants, while patients transplanted with active disease had poor outcomes, with an OS of 5% at 3 years. Similar findings were found in a recent multicenter retrospective study by Srour et al. [61], where patients transplanted in CR1 had a 3-year DFS of 52%. Overall, these findings suggest that for adult patients with high-risk ALL in CR1, haploidentical transplants are an acceptable alternative with comparable outcomes to a well-matched donor. For patients with active disease, however, efforts should be made to improve disease remission prior to alloHCT, given the poor outcomes.

13.5.6 Umbilical Cord Blood (UCB) Transplants

Similar to haploidentical transplants, registry data from the CIBMTR and EBMT [62] has confirmed that LFS for UCB transplants was comparable with that from a 8/8 or 7/8 allele-matched peripheral blood stem cells (PBSC) or bone marrow transplantation. Recent publications from the University of Minnesota [63, 64] and Fred Hutchinson Cancer Research Center (FHCRC) [65] have also compared UCB transplants with alternative donor sources for ALL patients and found similar PFS and OS among these patients. Unlike haploidentical transplants, UCB transplants were associated with lower relapse rates compared to MUD transplants, when done in patients with MRD positivity.

Despite these encouraging findings, in recent years, there has been an increasing use of haploidentical transplants over UCBT, likely due to the costs and higher NRM with cord blood transplants.

13.6 Conclusion

AlloHCT is associated with a potent graft vs. leukemia effect and remains an essential modality in the treatment of adult ALL, despite the development of novel agents as well as pediatric styled protocols for adult patients. The development of MRD-based risk stratification has allowed more accurate prognostication for ALL compared to conventional risk factors, while the development of RIC protocols and increasing use of alternative donors have improved accessibility for allo-HCT. With the development of new targeted immunotherapies, and studies looking at the best combinations for these new modalities, it remains to be seen how these will change the role of allo-HCT in the future.

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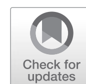

14 Minimal or Measurable Residual Disease in Acute Lymphoblastic Leukemia

Shilpa Paul, Caitlin Rausch, Hagop M. Kantarjian, and Elias Jabbour

14.1 Introduction

Acute lymphoblastic leukemia (ALL) is a hematologic malignancy characterized by clonal expansion of immature lymphoid progenitor cells that with other predisposing factors lead to leukemogenesis. Diagnosis is based on ≥20% lymphoid blasts in the bone marrow (BM) or biopsy material, and treatment is divided into three phases known as induction, consolidation, and maintenance, aimed to first achieve complete remission (CR) and then to eradicate minimal or measurable disease (MRD) and prevent relapse. Assessment of disease response has historically been based on the achievement of morphologic remission with <5% lymphoid blasts in the BM, which was thought to be a reliable indicator of prolonged survival and decreased relapse. Although most patients achieve a CR post induction treatment, relapses still occur, proving the inability of such an assessment to detect low levels of residual leukemic clones. For the past decade, more precise and sensitive assays have been developed to detect MRD to the 10^{-4} or 10^{-5} limit. MRD, in this context, refers to remaining malignant cells that share the same genotype and phenotype as the originating leukemia. Several studies have associated MRD positivity with early relapse and decreased survival. The presence of MRD is a strong independent risk factor for poor prognosis; therefore, its assessment post induction or at additional time points during therapy is not only important for prognostic implication but can also help tailor therapy. Development of therapies targeting MRD in ALL, such as blinatumomab, inotuzumab ozogamicin, and chimeric antigen receptor T-cell therapy (CAR T), has provided better therapeutic options and improved survival. Herein we discuss the different techniques to assess MRD, its prognostic implication, and therapies directed to eradicate MRD.

14.2 MRD Assays

There are several methods that can be utilized to detect MRD which have their own advantages and disadvantages (Table 14.1). For it to be a reliable assay, it must uphold the following standards: high level of sensitivity and specificity, rapidly available and reproducible, and ideally, standardized. Ultimately, each technique analyzes a large number of cells to detect a small residual leukemic population among normal cells. This can be done by using FC to identify leukemia-associated immune phenotypes (LAIP), quantitative polymerase chain reaction (qPCR),

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Table 14.1 Assays for the detection of minimal residual disease

Assay	Sensitivity	Advantages	Limitations
Multiparameter flow cytometry LAIPs	10^{-4} (0.01%)	• Quick • Sensitive • Inexpensive	• Limited standardization • Interlaboratory variability (preparation of sample, interpretation of result) • Requires technician expertise • May not detect immature blasts
Polymerase chain reaction RQ-PCR for IG/TCR gene rearrangement	10^{-4}–10^{-5} (0.01–0.001%)	• Standardized (European Consortium) • Sensitive	• Labor intensive • Time consuming • Requires individual reagent • Requires technician expertise • Relatively expensive
Polymerase chain reaction RQ-PCR for fusion genes	10^{-4}–10^{-5} (0.01–0.001%)	• Sensitive • Easy	• Applies to limited number of ALL cases
Next-generation sequencing	10^{-6} (0.0001%)	• Quick • Highly sensitive • Detect clonal evolution (new and sub-clones)	• Relatively expensive • Not standardized • Requires large data set management • Requires biostatistician

IG immunoglobulin, *LAIP* leukemia-associated immunophenotypes, *RQ-PCR* real-time quantitative polymerase chain reaction, *TCR* T-cell receptor

or NGS to detect immunoglobulin (*IG*) and/or T-cell receptor (*TCR*) gene rearrangement, or real-time quantitative PCR (RQ-PCR) to find pathogenic fusion genes like *BCR-ABL1*. MRD assessment should ideally be done on a remission bone marrow instead of peripheral blood for higher sensitivity. Selection of MRD assay ultimately depends on the cost and availability of the test as well as the experience and expertise with the method.

14.2.1 Flow Cytometry

MRD by multiparameter flow cytometry (MFC) is the most commonly used method to detect MRD in the United States (US) for its rapid turnaround time and accessibility. It can provide detection of 1 leukemic blasts in 10,000 cells (0.01% or 10^{-4}), similar to the PCR assays. MFC allows for the simultaneous detection of multiple altered or absent protein markers on the cell surface. The test has evolved from 4- to 6-plus color to ≥12-plus color immunostaining to quantify LAIP, thereby increasing sensitivity of the test to ≤0.0002% [1–3]. The addition of markers and expanding panel has significantly improved the capability of this assay to detect malignant cells. Precision and sensitivity of MFC can also be improved by using different fluorochromes that help differentiate malignant cells from regenerating blasts, if available [4]. Concordance between MFC and RQ-PCR to detect MRD is comparable; therefore, selection between these two methods depends on its availability and expertise on conducting these assays [5]. Some of the limitations and disadvantages of MFC is that it lacks standardization and is unable to detect MRD at levels lower than 10^{-4} or detect clonal evolution like the NGS assay. There can also be interlaboratory variability due to different techniques, method of sample preparation, and interpretation of results.

14.2.2 Polymerase Chain Reaction (PCR)

Quantitative PCR can be utilized in Ph-negative B-cell ALL and T-cell ALL to detect *IG* or *TCR* gene rearrangements, and reverse transcriptase PCR can be used in Ph-positive ALL to detect *BCR-ABL1*, the pathogenic driver fusion gene.

During normal lymphocyte maturation in both B- and T-cells, discontinuous somatic recombination of variable (V), diversification (D), and junction (J) gene segments occur, but crossover, insertion, and deletion at the N-region lead to *IG* and *TCR* rearrangements that become the basis of malignant lymphocytes. Since leukemic cells originate from a mother lymphocyte, identical *IG* or *TCR* gene rearrangements allow for the detection of malignant lymphocytes from normal cells. Through amplifying DNA or RNA and creating an allele-specific oligonucleotide (ASO) primer that is compatible to the junction regions along with reverse primers and fluorescent probes, these rearrangements can be detected. This method has high sensitivity with detection at 10^{-5} level (0.001%) [5–7]. Concerted effort by the EuroMRD Consortium has made the ASO-based qPCR, the most standardized and reproducible assay to detect MRD [8]. This method can be laborious and time-consuming and requires expertise as technical failures can occur. Additionally, this assay may not be able to detect immature cells or there may be false negatives if clonal evolution of lymphoid cells occurs. A new technique, droplet digital PCR, uses water-oil emulsion droplet and requires smaller sample and reagent volume, thereby reducing cost compared to standard qPCR. Few studies suggest that this method may be more sensitive than qPCR [9, 10].

Gene translocations such as t(9;22)(a34;q11) *BCR-ABL1,* t(12;21)(p12;q23) *ETV6-RUNX1*, t(4;11)(q21;q22) *MLL-AFF1* can be detected by RQ-PCR, although *BCR-ABL1* translocation, an important driver mutation in Ph-positive ALL, is the most established and stable target gene. RQ-PCR quantifies the fusion gene by amplifying DNA or RNA and comparing it to a standard curve of sequenced BCR-ABL1 dilution. Commonly, RQ-PCR using mRNA is done because not only is it highly sensitive (10^{-4} or 10^{-5} level), but the test is also quick and simple. However, results can be negatively impacted by the instability or contamination of RNA, and this assay is also limited to the small percentage of ALL patients carrying these fusion genes.

14.2.3 Next-Generation Sequencing (NGS)

NGS produces high-throughput sequencing of immune receptor genes. It can rapidly assess genetic abnormalities and mutations in large segments of DNA or RNA. Although it focuses on *IG/TCR* gene rearrangements similar to PCR-based assays, this technique is less laborious and quicker than PCR method because it does not require individual reagents [11–13]. It is also highly sensitive detecting 10^{-6} level (0.0001%), which is 1 to 2 logs better than the PCR and MFC assays [12]. Patients with MRD negativity by MFC and PCR have shown persistent low-level disease by NGS [1, 14]. In a large study with pediatric B-cell ALL, NGS assay detected 38.7% more MRD-positive disease and had a lower false-negative rate than the MFC assay [15], which significantly impacts outcomes. Therefore, NGS assay to detect MRD is preferred, if feasible. A higher sensitivity to detect MRD is clinically advantageous for earlier therapeutic interventions and/or planning for allogeneic stem cell transplant (alloHSCT) resulting in better outcomes. Unlike the RQ-PCR, it can detect leukemic rearrangements irrespective of their presence at diagnosis [12]. NGS provides comprehensive information about the clonal evolution of the leukemic cells including the detection of new and sub-clones, thereby preventing false-negative results. This assay can be costly and requires construction and management of a large data set for which expertise in interpretation and biostatistics is needed.

14.3 Prognostic Impact of MRD

MRD has become an important surrogate marker for outcomes in ALL. Several studies have shown that persistence of MRD at the end of induction or its reappearance signifies chemotherapy resistance, early relapse, and poor prognosis [16–18]. Relapse from time of treatment initiation is about 8 months in MRD-positive ($>10^{-4}$) patients receiving chemotherapy [17]. Risk of relapse has shown to be proportional to the degree of MRD

burden [17, 19], which may be influenced by the sensitivity of the assay to detect low-level disease. Molecular relapse has also been noted to precede hematologic relapse by about 2.6 months. A recent study demonstrated shorter overall survival (OS), relapse-free survival (RFS), and duration of complete remission (DoR) in patients who had high baseline MRD $\geq 10^{-1}$ compared to those with MRD between 10^{-4} and 10^{-3} [20]. Due to the prognostic impact of MRD, it has superseded historical ALL risk stratification involving patient-related factors, cytogenetics, and immunophenotype. Therefore, evaluation of MRD should be done at CR, then at 3 months, followed by every 3–6 months thereafter to help tailor therapy and optimize outcomes.

14.3.1 Ph-Negative B-Cell and T-Cell ALL

A meta-analysis of 13,637 children and adults with ALL of B-cell and T-cell origin, including Ph-positive disease, confirmed that achievement of MRD-negativity early in treatment was significant for better outcomes in both patient populations [19]. In pediatric patients, MRD-negative disease prolonged 10-year event-free survival (EFS) compared to MRD-positive disease (77% vs 32%, respectively). Similarly, adults who achieved MRD-negativity also had higher 10-year EFS than those who did not (64% vs. 21%, respectively). The OS among MRD-negative pediatrics (HR 0.28; 95% CI: 0.19–0.41) and adults (HR 0.28; 95% CI: 0.20–0.39) was the same. Of note, the risk of relapse associated with MRD positivity post CR was predicted to be highest for the first 3 years and then equivalent, or if not lower, in pediatrics thereafter. Several other studies have confirmed impact of early MRD clearance (<0.01%) on outcomes [18, 21–24]. Ribera and colleagues showed that HR ALL patients who achieved MRD-negativity (<0.01% by MFC) after first induction had better OS rate than those who achieved it after consolidation (90% ± 19% vs. 66% ± 10%) [18]. In another study, early MRD responders (<0.01% by MFC at CR) had better 3-year OS than later MRD responders (76% vs. 58%, respectively) [24]. As of now, both cytogenetic and molecular abnormalities along with MRD status play an important role in predicting risk of relapse; hence, the attainment of MRD negativity (<0.01%) at CR can help improve outcomes. Studies have shown that early clearance of MRD can impact survival, thus current treatment strategies are incorporating novel agents early in the course of treatment.

Recent studies have also demonstrated that combining ALL-specific molecular markers with MRD evaluation can improve risk stratification and prevent early relapse [21]. In the GRAAL trial, cumulative incidence of relapse (CIR) was higher among patients with MRD $\geq 10^{-4}$ by PCR and the presence of *MLL* gene rearrangement or *IZKF1* gene deletion in B-cell ALL and *PTEN* alteration, *NRAS/KRAS,* and *NOTCH1/FBXW7* mutations in T-cell ALL [21]. Additionally, complex karyotype (≥5 chromosome abnormalities) and low hypodiploidy/near triploidy remain strong prognostic factors for poor outcomes despite MRD status [25]. Further studies are needed to determine how best to incorporate cytogenetic and molecular abnormalities with MRD status to tailor therapy.

14.3.2 Ph-Positive ALL

Among patients with Ph-positive ALL, measurement of MRD status by quantifying *BCR-ABL1* transcript through RQ-PCR holds prognostic value [26–28]. In a study of adult patients with Ph-positive ALL who received chemotherapy plus a tyrosine kinase inhibitor (TKI) without subsequent alloHSCT, *BCR-ABL1* gene translocation measured by qPCR at CR and then every 3 months was a strong predictor for survival. Achievement of complete molecular response (CMR) at 3 months, defined by the absence of quantifiable *BCR-ABL1* transcript, resulted in 4-year OS of 66% [29]. Among all the TKIs, ponatinib has demonstrated superiority in inducing and maintaining CMR [30]. In addition, alloHSCT after achievement of CMR has not shown to improve outcomes in Ph-positive ALL patients [30]. If a patient does not achieve CMR

at 3 months, then changing treatment to a more MRD-directed therapy like blinatumomab plus TKI, if not used initially, or clinical trial with InO plus a TKI should be considered with re-evaluation of MRD status (Fig. 14.1). The benefit of TKI maintenance post alloHSCT in Ph-positive ALL is also being studied to prevent relapse.

14.3.3 Allogenic Stem Cell Transplant

MRD-positivity prior to and post alloHSCT is a strong predictor for relapse. In adult ALL patients, detection of MRD $\geq 10^{-4}$ by NGS pre-alloHSCT predicted for post-transplant relapse (HR 7.7; 95% CI: 2.0–30, $p = 0.003$) [31]. Similarly, in children with relapsed ALL, MRD-positivity compared to MRD-negativity, detected at $\geq 10^{-4}$ by PCR, prior to alloSHCT was prognostic for higher 5-year CIR (57% vs. 13%, $p < 0.001$) and shorter 5-year EFS rate (27% vs. 60%, $p = 0.004$).

Earlier studies have demonstrated that MRD-negative disease preceding alloHSCT, particularly in first complete response (CR1), results in better outcomes than being MRD-positive [32,

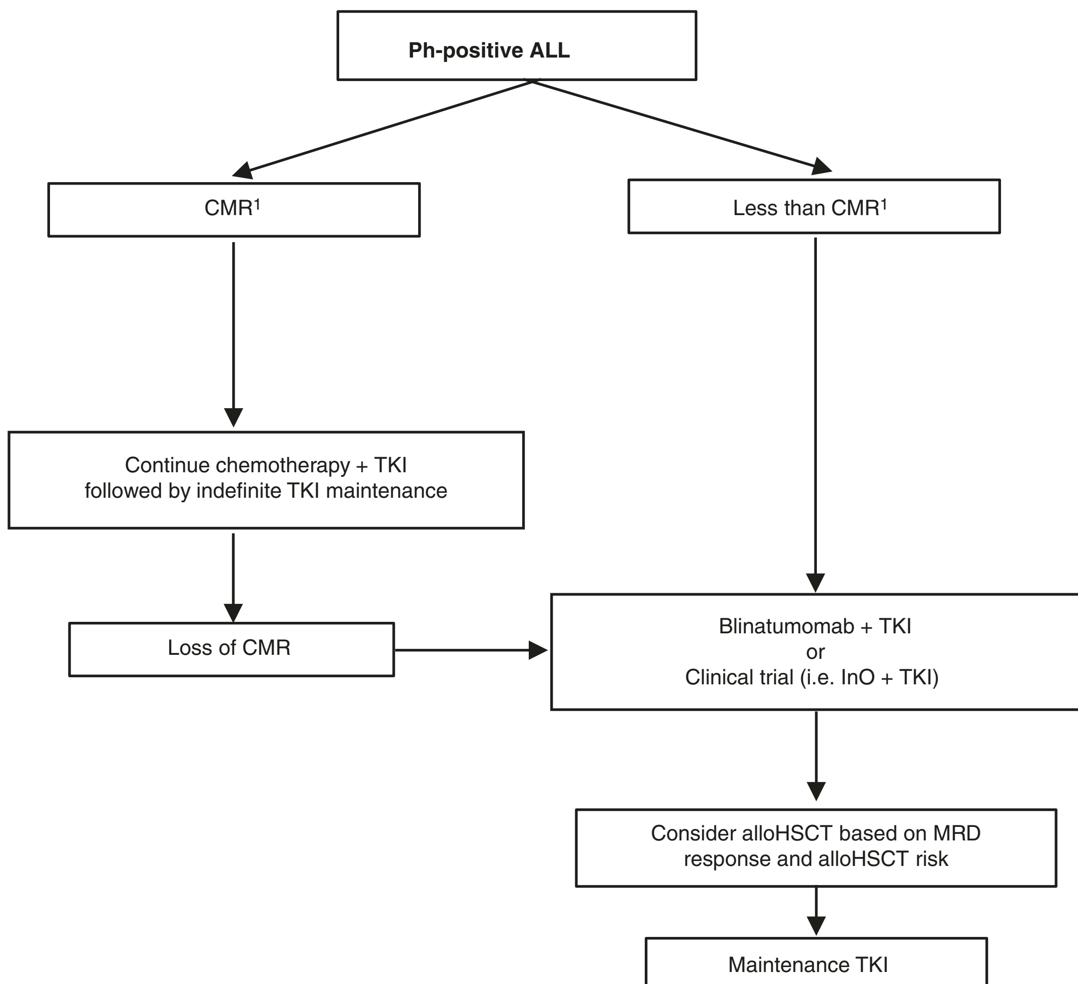

Fig. 14.1 Minimal residual disease-directed treatment algorithm in Ph-positive ALL

33]. Prior to the era of MRD-directed therapies, alloHSCT was the only option to improve outcomes in MRD-positive patients. In the two German Multicenter ALL (GMALL) studies (06/99 and 07/03), adult ALL patients with persistent MRD, defined as $\geq 1 \times 10^{-4}$ by RQ-PCR, after induction (day 71) or first consolidation (week 16), were stratified to high-risk category and allocated to alloHSCT [16, 17]. Those with molecular failure who underwent alloHSCT in CR1 had better survival than those who did not (54% vs. 32%). However, only 47% of patients with molecular failure were able to receive alloHSCT due to aggressive disease. MRD positivity preceding alloHSCT results in higher risk of relapse and may lead to more morbidity and mortality due to potential alloHSCT related complications. Nowadays highly effective and tolerable MRD-directed therapies, such as blinatumomab or clinical trial with InO or CAR T-cells discussed below, should be used to eradicate MRD and maintain MRD-negativity, as opposed to alloHSCT.

14.4 MRD-Directed Therapies

14.4.1 Blinatumomab

Blinatumomab, a bispecific T-cell engager antibody targeting CD3 and CD19 was initially studied in patients with relapsed or refractory (R-R) B-cell ALL. Initial phase II studies were challenging due to high rates of toxicity including cytokine release syndrome (CRS) and neurotoxicity, as well as limited activity in patients with high burdens of disease (>50% bone marrow blasts) [34]. Despite these initial shortcomings, when compared to standard-of-care (SOC) in an analysis of the phase III TOWER trial, blinatumomab treatment resulted in significantly higher rates of CR/CRi (44% vs. 25%, $p < 0.001$) and MRD-negativity (76% vs. 48%, $p = 0.004$) in patients with R-R Ph-negative ALL [35]. Moreover, blinatumomab monotherapy also resulted in 88% of responders achieving MRD-negativity (undetectable *BCR-ABL1* by qPCR, sensitivity $\leq 10^{-5}$) in patients with R-R Ph-positive ALL [36]. The association between tumor burden and toxicity as well as the high rates of MRD-negativity achieved with blinatumomab led to its investigation in patients with persistent or recurrent MRD.

After promising results with an initial phase II pilot study in patients with MRD-positive disease demonstrating long-term RFS, the phase II BLAST trial enrolled 116 adult patients with persistent or recurrent MRD $\geq 10^{-3}$ after at least three courses of intensive chemotherapy [37–39]. Complete MRD response, defined as MRD $<10^{-4}$ as detected by RT-PCR, was achieved in 78% ($n = 88$) of evaluable patients after cycle 1, and in an additional two patients after cycle 2. Most patients evaluable for MRD response had a baseline MRD $\geq 10^{-3}$ to $<10^{-1}$ ($n = 94$; 91%) and were in second CR (CR2) or later ($n = 37$; 36%). The ability to achieve a complete MRD response was independent of baseline MRD level, age, or line of therapy. Patients in CR1 had significantly improved RFS (24.6 vs. 11 months; HR 2.09; 95%CI: 1.26–3.48, $p = 0.004$) and trend for improved OS (36.5 months vs. 19.1 months, $p = 0.084$) compared to those treated in CR2 or later [37]. Achieving an MRD response conferred a significant improvement in OS regardless of receiving alloHSCT. Patients in CR1 who achieved MRD-negativity with blinatumomab did not confer a benefit from alloHSCT, likely related to the high rate of morbidity and mortality associated with alloHSCT. Among responders ($n = 85$), median OS was not reached (95% CI: 27.3 months to not estimable) compared to 12.5 months (95% CI: 3.2–39.7 months) for non-responders [40]. At a median follow-up of 53 months, 40.5% ($n = 30/74$) patients who proceeded to alloHSCT in continuous complete remission after blinatumomab were alive, compared to 33.3% ($n = 12/36$) patients who did not [40].

A retrospective comparison of blinatumomab to SOC for MRD in adult patients with Ph-negative B-cell ALL in CR1 demonstrated a significant advantage in RFS and trend for improvement in OS for patients treated with blinatumomab [41]. Median RFS (unadjusted for alloHSCT) for patients in CR1 with quantifiable

MRD $\geq 10^{-3}$ by any detection method treated with blinatumomab was 35.2 months, compared to 8.3 months for patients treated with SOC (p = 0.002). Median OS (unadjusted for alloHSCT) for patients treated with blinatumomab was 36.5 months compared to 27.2 months for patients treated with SOC (p = 0.27); however, estimated probability of OS at 18 months was significantly greater for blinatumomab (71% vs. 55%, p = 0.019) [41]. Propensity score matching was conducted in this retrospective analysis to account for differences in the treatment arms; however, every covariate could not be accounted for robustly, including alloHSCT. Despite the limitations of this retrospective review, this analysis demonstrates the benefit of blinatumomab therapy compared to SOC for patients in CR1 with MRD-positivity (Fig. 14.2).

Long-term follow-up of a phase II study of R-R ALL patients treated with blinatumomab [34] has demonstrated continued benefit of achieving MRD negativity with blinatumomab even in the relapsed setting. Ninety patients with R-R Ph-negative ALL who achieved a CR/CRi within the first two cycles of therapy had MRD assessment by qPCR at a sensitivity of $\leq 10^{-4}$ and were included in this analysis [42]. Overall, 75 patients (83.3%) achieved an MRD response (qPCR $<10^{-4}$) which was independent of prior line of therapy including prior alloHSCT. Achieving MRD-negativity translated into significantly longer RFS, DOR, and OS compared to those who did not achieve MRD-negativity. Patients achieving MRD-negativity had a median RFS and OS of 9 months and 20.6 months, respectively, compared to 2.3 months and 12.5 months in MRD non-responders ($p < 0.05$) [42]. Similarly, retrospective analyses of patients with R-R ALL treated with blinatumomab as part of the TOWER study and as standard of care indicated the greatest outcomes in patients treated in the first salvage setting who achieve MRD negativity and proceed to alloHSCT [43, 44]. While prognosis of MRD-positivity is most robust in the frontline treatment setting, this data provides insight into the value of MRD negativity in the R-R setting, demonstrating

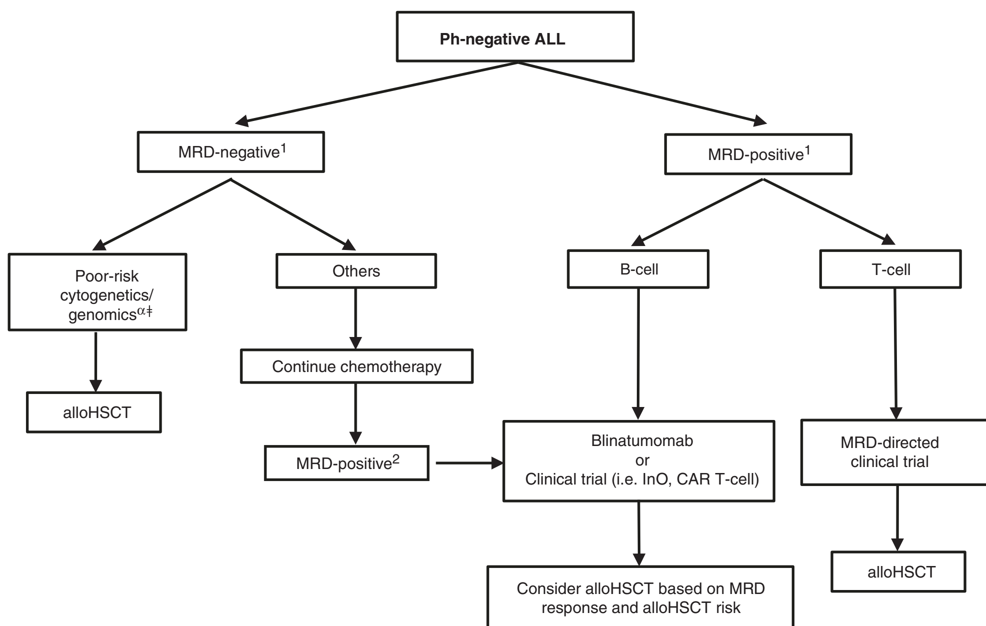

Fig. 14.2 Minimal residual disease-directed treatment algorithm in Ph-negative ALL

the need to further understand the prognosis and role of MRD monitoring in patients with relapsed ALL.

Incorporation of blinatumomab earlier into therapy is a strategy currently being evaluated in order to improve rates of MRD-negativity in the frontline setting with the hope to improve long-term outcomes, particularly in patients with high rates of MRD-positivity and relapse with traditional chemotherapy alone. Ph-like ALL is one high-risk subgroup that is characterized by low rates of MRD-negativity after induction, high rates of relapse, and poor overall survival [45, 46]. Incorporating novel therapies such as blinatumomab or InO earlier in therapy for patients with Ph-like ALL may increase the rate of MRD-negativity and hopefully improve overall outcomes. An ongoing phase II study incorporating blinatumomab into the HyperCVAD (cyclophosphamide, vincristine, doxorubicin, dexamethasone alternating with high-dose methotrexate and cytarabine) chemotherapy regimen for patients <60 years of age with newly diagnosed Ph-negative B-cell ALL has resulted in MRD-negativity in 96% of patients and 2-year OS of 90% [Richard-Carpentier; ASH 2019] (NCT02877303). This clinical trial also incorporates blinatumomab into the maintenance courses of frontline therapy in an effort to sustain deep remissions achieved during induction and consolidation. The addition of blinatumomab to a lower intensity treatment program composed of reduced doses of cyclophosphamide, vincristine, and dexamethasone alternating with methotrexate and cytarabine (mini-HCVD) with InO for newly diagnosed patients ≥60 years of age is also ongoing (NCT01371630). Furthermore, utilization of blinatumomab as a maintenance strategy post-alloHSCT is also being evaluated for patients with high risk of relapse (NCT02807883).

14.4.2 Inotuzumab

Inotuzumab ozogamicin (InO), an antibody drug conjugate bound to calicheamicin that targets CD22, leads to high rates of MRD-negativity and therefore is being investigated for MRD-directed therapy (NCT03441061). In the phase III INO-VATE trial, 80.7% of patients with R-R B-cell ALL treated with InO achieved a CR/CRi of whom 78.4% achieved MRD-negativity ($<10^{-4}$ by MFC), compared to 29.4% of patients treated with SOC of whom 50% achieved MRD negativity ($p < 0.001$ for CR/CRi and MRD-negativity comparisons) [47]. Similar to blinatumomab, therapy with InO can result in deep remissions, as evidenced by high rates of MRD-negativity in a population with relapsed disease.

Post hoc analysis of 121 patients in the InO arm who achieved CR/CRi found that the 76 patients who achieved CR/CRi and were MRD-negative at end of therapy had significantly longer PFS and OS versus those who were MRD-positive [48]. Proceeding to alloHSCT benefitted all patients regardless of MRD or salvage status. As anticipated, patients who achieved MRD-negativity with InO in the first salvage setting then proceeded to alloHSCT experienced the greatest benefit, consistent with prior retrospective analyses [43]. Patients should proceed to alloHSCT as soon as MRD-negativity is achieved to gain the most benefit from InO while reducing the risk of sinusoidal obstructive syndrome (SOS) that may occur with prolonged exposure followed by alloHSCT [49, 50]. Similar to what was observed with blinatumomab therapy in this setting, this study demonstrates the significance of MRD status in the salvage setting and also emphasizes the need for alloHSCT in patients with R-R ALL.

Integrating InO earlier into therapy and in combination with other agents can lead to higher rates of MRD-negativity in the frontline setting and better outcomes overall. The combination of the low-intensity, mini-HCVD program, with InO has demonstrated efficacy and safety in patients with R-R as well as patients ≥60 years of age with newly diagnosed Ph-negative B-cell ALL [51, 52]. Notably, of 48 newly diagnosed patients ≥60 years old treated with this combination, 98% of patients achieved a response, including MRD-negativity in 78% of evaluable patients [52]. When using propensity score matching to compare this therapy to a similar cohort of patients treated with intensive chemotherapy

without InO, the mini-HCVD plus InO program resulted in higher 3-year EFS (64%) and OS (63%) compared to intensive chemotherapy alone (34% for both EFS and OS, $p < 0.05$) [53]. An updated analysis of 64 patients treated with the mini-HCVD with Ino program, which added blinatumomab for four courses following the initial four courses of mini-HCVD plus InO, has consistently resulted in an overall response rate of 98% and MRD-negativity of 77% after cycle 1 and 94% overall. At median follow-up of 37 months, the 3-year OS of 54% still compares favorably to the historic 3-year OS of 34% observed in a similar population treated with intensive chemotherapy (Nicholas J. [54]). These data demonstrate the long-term impact of adding InO to frontline therapy, likely driven by a higher rate of MRD negativity.

14.4.3 Chimeric Antigen Receptor Therapy

Chimeric antigen receptor (CAR) T-cell therapy has been extensively studied in the R-R Ph-negative B-cell ALL setting, but therapy is complicated by significant toxicity, and efficacy is greatest in patients with low burdens of disease. Tisagenlecleucel is a CD19-directed CAR T-cell attached to a 4-1BB signaling domain that gained FDA approval for the treatment of patients aged ≤25 years of age with R-R B-cell ALL after early phase clinical trials resulted in high overall response and MRD-negativity rates [55, 56]. Treatment with tisagenlecleucel in a multicenter phase II study in 75 heavily pretreated patients aged 3–23 years of age with R-R B-cell ALL resulted in an overall response rate of 81% (66% of all screened patients, $n = 92$) and an MRD-negativity rate of 100% among responders [56]. Although patients were required to have at least 5% bone marrow blasts at the time of screening, at least 15 patients were in MRD-negative CR at the time of CAR T-cell infusion due to the receipt of interim chemotherapy [57]. A phase I trial of 53 adult patients with R-R B-cell ALL using another CD-19 directed CAR product attached to a CD28 costimulatory domain demonstrated a response rate of 83% and MRD-negativity in 67% of patients [58]. At the time of infusion, 40% of patients had <5% bone marrow blasts, of which 11% were MRD-negative. As anticipated, patients achieving MRD-negativity had prolonged survival compared to those who did not achieve MRD-negativity. Greatest benefit was seen in patients with a low burden of disease, defined as <5% bone marrow blasts at baseline. Median OS for these patients was 20.1 months compared to 12.4 months ($p = 0.02$) for those with a higher burden of disease. In addition, lower rates of CRS and neurotoxicity were observed in patients with low burden of disease [58]. Similarly, a retrospective analysis of 39 patients with R-R B-cell ALL treated with CD19 directed CAR T-cells attached to a 4-1BB signaling domain found that baseline bone marrow blasts of ≥5% prior to CAR T-cell infusion were associated with worse EFS [59]. High rates of MRD-negativity are possible with CAR T-cell therapy; however, greatest efficacy is observed in patients with low burden of disease. Due to their optimal efficacy and safety profile in patients with minimal disease, CAR T-cells are an option for MRD eradication and bridge to alloHSCT in the relapsed population. Clinical trials investigating tisagenlecleucel and other CAR T products for patients with MRD positivity are ongoing (NCT03876769; NCT02935543).

Studies investigating alternate CAR T-cell targets including CD22 [60] as well as bispecific CAR T-cells targeting CD19 and CD22 are ongoing with early results indicating promise in terms of high rates of MRD-negativity [61]. Further investigation will determine whether these products continue to show highest activity in patients with low levels of disease.

14.4.4 Allogeneic Stem Cell Transplantation

While alloHSCT is considered the standard, yet most intensive, approach for patients with MRD-positivity, this concept is being strongly challenged with the availability and integration of modern therapies in the frontline setting. The

importance of alloHSCT for patients in CR1 with MRD-positivity was demonstrated when 580 patients who were treated as part of the GMALL trials 06/99 and 07/03 protocols were analyzed [62]. Patients with persistent MRD ($\geq 10^{-4}$ by qRT-PCR) after first consolidation who proceeded to alloHSCT had significantly higher probability of continuous complete remission at 5 years compared to those who did not proceed to alloHSCT (66% vs. 12%, $p < 0.0001$) and increased 5-year OS of 54% versus 33%, $p = 0.06$. Estimated 5-year OS for patients with MRD recurrence was 80% for patients who underwent alloHSCT in CR1 versus 15% for those who did not ($p = 0.02$) [62]. Similarly, the GRAALL group also demonstrated a significant benefit of alloHSCT in CR1 on RFS and OS for patients with poor MRD response, defined as MRD $\geq 10^{-3}$ after induction therapy [63]. Furthermore, a retrospective review of 272 patients aged ≥15 years old with newly diagnosed Ph-negative B-cell ALL who were MRD-positive ($\geq 10^{-4}$ by RQ-PCR or $\geq 10^{-3}$ by MFC) after ≥3 intensive chemotherapy courses demonstrated improvement in DOR (OR: 0.52; 95% CI: 0.351–0.761, $p = 0.0009$), RFS (OR: 0.686; 95% CI: 0.487–0.966, $p = 0.03$), and OS (OR: 0.738; 95% CI: 0.526–1.036, $p = 0.08$) for patients who underwent alloHSCT in CR1 versus those who did not [20]. Median DOR and RFS were 68.4 months and 34.4 months, respectively, for patients who underwent alloHSCT versus 7.4 months and 6.7 months. Median OS was 76.1 months versus 20.4 months. A trend for improved outcomes was seen based on lower baseline MRD [20]. These analyses provide evidence for the need for patients with MRD-positivity to proceed with alloHSCT in first CR. Similarly, a prospective study in 307 patients with high-risk Ph-negative B-cell and T-cell ALL, as defined by baseline disease features, found that those who achieved MRD <0.01% (by MFC) at the end of induction and <0.01% (by MFC) after consolidation therapy (week 17) did not benefit from alloHSCT [18], demonstrating the ability to tailor therapy based upon MRD response, even in patients with high-risk disease.

As new therapies are available and incorporated into the overall treatment of patients with ALL, the role of alloHSCT may begin to change. The prior analyses did not include patients treated with blinatumomab or CAR T-cell therapy, both novel treatment options for MRD eradication. A post hoc analysis of the BLAST trial which evaluated blinatumomab for MRD was conducted to evaluate the benefit of alloHSCT after blinatumomab therapy [37]. This analysis did not demonstrate an OS benefit of alloHSCT for patients treated with blinatumomab in CR1 (OR: 1.83; 95% CI: 0.69–4.9; $p = 0.24$); however, the benefit of alloHSCT was observed for patients treated in CR2 or later (OR: 0.31; 95% CI: 0.12–0.83; $p = 0.02$) [37]. This demonstrates the effectiveness of blinatumomab at eliminating MRD and improving long-term outcomes. Achieving MRD-negativity with blinatumomab in newly diagnosed patients may preclude the need for alloHSCT in CR1; however, prospective analyses comparing alloHSCT to blinatumomab is necessary to make this determination, specifically for high-risk cytogenetic subgroups.

14.5 Conclusion

MRD remains the most significant prognostic factor for patients with ALL, particularly for patients in the frontline setting. Progress in the development of these sensitive assays has allowed for a variety of assays to be commercially available, however an international standard should be established. While the prognosis of patients with MRD-positive disease remains poor, novel treatments including blinatumomab are effective at eradicating MRD-positivity and reducing the need of subsequent alloHSCT if possible. Inotuzumab and CAR T-cell products are currently being evaluated specifically for this indication. AlloHSCT continues to play an important role in the management of patients with MRD-positive disease, however as effective therapeutics are developed, the treatment paradigm may shift away from alloHSCT toward novel targeted therapies.

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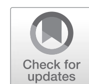

15 Management of Philadelphia Chromosome-Positive Acute Lymphoblastic Leukemia

Serena Chew, Nicholas J. Short, Hagop M. Kantarjian, and Elias Jabbour

15.1 Introduction

Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph-positive ALL) comprises approximately 30% of ALL cases in adults, with a progressively increasing incidence with age. The Philadelphia chromosome is derived from a translocation between *ABL1* on chromosome 9 and a breakpoint cluster region (BCR) on chromosome 22, resulting in a BCR-ABL1 fusion gene [1]. The two most prominent BCR-ABL1 transcripts in ALL are the p190 and p210 transcripts, which are seen in approximately 75% and 25% of cases, respectively [2]. Ph-positive ALL is an aggressive disease with increased risk for central nervous system (CNS) involvement. [2] Prior to the advent of tyrosine kinase inhibitors (TKIs), treatment with standard chemotherapy resulted in complete remission (CR) rates of 50–60%, with long-term survival rate of less than 20% [2, 3]. Allogeneic hematopoietic stem cell transplant (HSCT) was the only potentially curative modality. Despite a potential for cure with HSCT, non-relapse mortality and relapse rates after transplant remain high, ranging from 30% to 50% [4]. Furthermore, a large proportion of patients with Ph-positive ALL are older and have significant comorbidities that further limit their ability to tolerate an intensive approach.

In recent years, the development and incorporation of TKIs through all stages of therapy have significantly improved survival outcomes in patients with Ph-positive ALL, resulting in 5-year survival rates as high as 70–80%. As TKIs have revolutionized the treatment of Ph-positive ALL by producing deep molecular remissions, the role of allogeneic HSCT has become less clear [5]. Novel treatment modalities including more potent TKIs, bispecific T-cell engagers (e.g., blinatumomab), drug conjugate monoclonal antibodies (e.g., inotuzumab ozogamicin), and chimeric antigen receptor T-cell (CAR-T) therapies have provided more options for patients with Ph-positive ALL. In this chapter, we will provide an overview of the current and future paradigms in the treatment of patients with Ph-positive ALL and discuss ongoing challenges that need to be addressed in order to further optimize clinical outcomes.

15.2 Prognostic Significance of Genomic and Chromosomal Aberrations

Genomic alterations are frequently seen in patients with Ph-positive ALL. The most common genomic aberration is deletion of *IKZF1*,

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which is seen in up to 84% of patients [6]. The *IKZF1* gene is located on chromosome 7q12 and encodes for the Ikaros transcription factor [6]. Deletion of exons 4–7 is the most frequent deletion that occurs along the *IKZF1* gene. Loss of exons 4–7 leads to the expression of dominant-negative Ikaros isoform, resulting in reduced tumor suppression function, and confers poor outcomes [7–9]. *CDKN2A/B* is located on chromosome 9p21, and deletions of these genes are present in approximately 40% of Ph-positive ALL cases [8]. Approximately 50% of patients with *IKZF1* deletion will have co-occurrence of *CDKN2A/B* and/or *PAX5 gene deletions.* Together, this genotype is often referred to as "IKZF1-plus" and *has* been associated with worse disease-free survival (DFS) and overall survival (OS) [9]. Another genomic aberration observed in approximately 10–20% of patients is the *BTG1* deletion*,* which has been associated with inferior DFS and remission duration [10]. *BTG1* is located on chromosome 12q21 and plays a role in apoptosis and glucocorticoid response [11]. Additionally, poor prognosis has been observed in patients with two or more deletions, irrespective of the gene involved [10]. Other less common aberrations, including *MEF2C* and *KRAS*, have been associated with a favorable prognosis [9]. It is important to note that most genotypic prognostic studies have been performed in patients who received first- or second-generation TKIs. It remains to be determined whether more potent later-generation TKIs such as ponatinib may be able to overcome the prognostic impact of some of these alterations.

Approximately 60–80% of patients with Ph-positive ALL will harbor additional chromosomal abnormalities (ACAs), most frequently with alterations chromosomes 7, 9, and 14. The presence of −9/9p and/or +der(22)t(9;22) has both been associated with poorer outcomes [12, 13]. In one analysis, patients with one or more these poor-risk ACAs in the absence of hyperdiploidy had significantly shorter 5-year relapse-free survival (RFS; 33% vs. 59%, $P = 0.01$) and OS (24% vs. 63%, $P = 0.003$). Interestingly, adverse outcomes were seen in patients treated with imatinib or dasatinib but not in those treated with ponatinib, suggesting that ponatinib may overcome the negative prognostic impact of poor risk ACAs.

15.3 Tyrosine Kinase Inhibitors

The *BCR-ABL1* fusion gene affects multiple tyrosine kinase signaling pathways leading to leukemic cell proliferation, differentiation arrest, and resistance to apoptosis [6]. BCR-ABL TKIs prevent adenosine triphosphate (ATP) from binding to the BCR-ABL1 oncoprotein, thereby inhibiting hyperactive downstream signaling [6]. The introduction of BCR-ABL TKIs in the treatment of Ph-positive ALL has improved survival outcomes. Imatinib was the first TKI to be discovered with high specificity for BCR-ABL1 oncoprotein. However, high rates of resistance to imatinib have been reported. This is thought to be secondary to point mutations within the *ABL1* domain. Point mutations that can occur within the *ABL1* kinase domain may be at the contact site (e.g., T315I, F317L), SH2 binding site (e.g., M351T), the ATP binding loop (e.g., Y253 and E255), or activating loop [1, 14]. Another mechanism of resistance is the overexpression of SRC family kinases (e.g., LYN, HCK), which leads to stabilization of activated conformation of BCR-ABL1, resulting in decreased drug binding and increased leukemic cell proliferation [15].

The established mechanisms of resistance to imatinib therapy subsequently led to the development of the second-generation TKIs (e.g., dasatinib, nilotinib, and bosutinib), which were designed to potentially overcome these resistance mechanisms. Second-generation TKIs have shown activity against most known imatinib-resistant *ABL1* mutations, with the notable exception of T315I. T315I is commonly known as a "gatekeeper mutation" and is extremely resistant to all first- and second-generation TKIs [2, 12, 16]. Approximately 75% of the patients treated with a first- or second-generation TKI will develop T315I mutation at relapse, leading to treatment failure [17, 18]. As a result, a third-generation TKI known as ponatinib was developed, with potent activity against both wild-type

and mutant BCR-ABL1, including T315I. Because of its broader spectrum of activity and promising clinical data, ponatinib is currently the preferred TKI inhibitor for the treatment of Ph-positive ALL at our institution.

While TKIs are an integral component to the treatment of Ph-positive ALL, the depth and duration of response with single-agent TKIs is suboptimal. Survival outcomes are significantly improved when combined with multi-agent chemotherapy regimens. A summary of TKI-based regimens in the frontline and relapsed and refractory (R/R) settings are shown in Tables 15.1 and 15.2, respectively.

15.3.1 Imatinib

Improvement in outcomes was seen when imatinib was combined with intensive chemotherapy in the frontline setting, demonstrating high CR rates greater than 90%, with OS ranging from 30% to 50%. [19–27, 37, 51] In a 13-year follow-up of a phase II study from MD Anderson Cancer Center, 54 patients with newly diagnosed Ph-positive ALL were treated with imatinib in combination with hyper-CVAD (hyperfractionated cyclophosphamide, vincristine, and doxorubicin dexamethasone alternating with high-dose cytarabine and methotrexate) chemotherapy [25].

Table 15.1 Treatment of Ph-positive ALL with TKI-based regimens in the frontline setting

	Reference	*N*	Median age, years (range)	CR, %	Overall CMR, %	OS, %	HSCT, %
Intensive chemotherapy							
Imatinib	Lee et al. [19]	20	41 (16–71)	95	45	33 (5 years)	85
	Yanada et al. [20]	80	48 (15–63)	96	38	76 (1 year)	61
	de Labarthe et al. [21]	45	45 (16–59)	96	29	65 (1.5 years)	51
	Bassan et al. [22]	59	45 (30–66)	92	–	38 (5 years)	72
	Tanguy-Schmidt et al. [23]	45	45 (16–59)	96	61	52 (4 years)	76
	Fielding et al. [24]	169	42 (16–64)	92	–	38 (4 years)	72
	Daver et al. [25]	54	51 (17–84)	93	45	43 (5 years)	30
	Chalandon et al. [26]	133	45(21–59)	91	23	46 (5 years)	65
	Lim et al. [27]	87	41 (16–71)	94	89	33 (5 years)	64
Dasatinib	Ravandi et al. [28]	72	55 (21–80)	96	60	46 (5 years)	17
	Ravandi et al. [17]	97	44 (20–60)	88	–	69 (3 years)	42
Nilotinib	Kim et al. [29]	90	47 (17–71)	91	86	72 (2 years)	70
	Liu et al. [30]	30	40 (21–59)	100	83	45 (4 years)	53
Ponatinib	Jabbour et al. [31, 32]	76	46 (21–80)	100	86	78 (3 years)	24
Low-intensity chemotherapy							
Imatinib	Chalandon et al. [26]	135	49 (18–59)	98	28	46 (5 years)	62
Dasatinib	Rousselot et al. [18]	71	69 (59–83)	96	24	36 (5 years)	19
	Chiaretti et al. [33]	60	42 (19–60)	97	19	58 (3 years)	42
Nilotinib	Ottmann et al. [34, 35]	79	65 (55–85)	94	42	47 (4 years)	16
	Chalandon et al. [36]	60	47 (18–59)	98	–	96 (1 year)	52
Chemotherapy-free							
Imatinib	Vignetti et al. [37][a]	29	69 (61–83)	100	4	74 (1 year)	–
Dasatinib	Foà et al. [38][a]	53	54 (24–77)	100	15	69 (2 years)	34
	Fedullo et al. [9][a]	63	55 (24–82)	97	36	94 (1 years)	19
Ponatinib	Martinelli et al. [39][a]	42	69 (27–85)	93	46	83 (3 years)	–

Abbreviations: *CMR* complete molecular response, *CR* complete remission, *N* number of patients, *HSCT* hematopoietic stem cell transplantation, *OS* overall survival, *TKI* tyrosine kinase inhibitors

[a]TKI plus corticosteroids

[b]Dasatinib plus corticosteroids followed by blinatumomab consolidation for at least two cycles

Table 15.2 Treatment of Ph-positive ALL with TKI-based regimens in the relapsed and refractory setting

	Reference	*N*	Median age, years (range)	CCyR, %	MCyR, %	Median OS, months
Monotherapy						
Imatinib	Ottmann et al. [40]	56[a]	50 (22–78)	–	–	4.9
Dasatinib	Porkka et al. [41]	46	48	54	57	8.0
	Ottmann et al. [42]	36	46 (15–85)	58	42	–
	Lilly et al. [43]	84	52 (21–77)	–	70 vs. 52[b]	9.1 vs. 6.5[b]
Nilotonib	Ottmann et al. [44]	41	46 (18–75)	32	50	5.2
Ponatinib	Cortes et al. [45, 46]	449[c]	62 (20–80)	38	47	–
Combination therapy						
Dasatinib + hyper-CVAD	Benjamini et al. [47]	34[d]	52 (21–77)	42	35	–
Dasatinib + hyper-CVAD	Ravandi et al. [48]	23[e]	49 (21–69)	43	65	–
Bosutinib + INO	Jain et al. [49]	14[f]	62 (19–74)	73	–	–
TKI + blinatumomab	Assi et al. [50]	20	65 (30–77)	71	75	Not reached

Abbreviations: *BP-CML* blast phase chronic myeloid leukemia, *CCyR* complete cytogenetic response, *INO* inotuzumab-ozogamicin, *MCyR* major cytogenetic response, *N* number of patients, *OS* overall survival, *TKI* tyrosine kinase inhibitors, Hyper-CVAD (hyperfractionated cyclophosphamide, vincristine, doxorubicin dexamethasone alternating with cytarabine and methotrexate)

[a]48 patients with Ph-positive ALL and 8 patients with BP-CML

[b]Dasatinib 140 mg daily was associated with higher MCyR and median OS than patients receiving dasatinib 70 mg twice daily

[c]32 patients with Ph-positive ALL and 417 patients with CML. The results provided are only in patient with ALL

[d]19 patients with Ph-positive ALL and 15 patients with BP-CML

[e]14 patients with Ph-positive ALL and 9 patients with BP-CML

[f]12 patients with Ph-positive ALL and 2 patients with BP-CML

CR was achieved in 93%, and among those who achieved CR, 87% achieved complete cytogenetic response (CCyR), 45% achieved complete molecular response (CMR), 38% achieved major molecular response (MMR, defined as BCR-ABL/ABL <0.1%). The 5-year OS and DFS rates were both 43%. Sixteen patients (30%) underwent allogeneic HSCT in first remission. No difference in DFS were observed in transplanted and non-transplanted patients (43% vs. 63%, $p = 0.52$). However, a trend toward improved outcomes (5-year DFS 82% vs. 33%, $p = 0.16$), although not statistically significant was seen in young patients <40 years, treated with imatinib plus hyper-CVAD followed by HSCT. The insignificant finding is most likely due to limited sample size in this subset of patients.

In a large prospective study, the outcome of newly diagnosed Ph-positive ALL patients treated with imatinib-based therapy ($n = 175$) was compared with the outcome of patients treated with chemotherapy alone ($n = 266$) [24]. Patients in the imatinib cohort were stratified to receive either imatinib in conjunction with the second cycle of induction therapy ($n = 89$, referred as "early imatinib") or imatinib as monotherapy after completing two cycles of induction therapy ($n = 86$, referred to as "late imatinib"). The CR and 4-year OS rates were significantly higher with imatinib plus chemotherapy compared to chemotherapy alone (CR: 92% vs. 82%, $p = 0.004$; OS: 38% vs. 22%, $p = 0.003$). When comparing the outcomes of early and late imatinib cohorts, a trend toward improved survival was seen with earlier exposure of imatinib ($p = 0.1$). By multivariate analysis, imatinib benefitted both transplanted and non-transplanted patients.

Despite improved outcomes with imatinib, several limitations exist, including poor CNS penetration and high incidences of secondary resistance and relapse, mainly driven by development of the T315I mutation. The suboptimal outcomes with imatinib-based therapy have subsequently led to the development of more potent second- and third-generation TKIs.

15.3.2 Dasatinib

Dasatinib is a multi-targeted kinase inhibitor of BCR-ABL1 and SRC-family kinases. Dasatinib inhibits BCR-ABL1 with binding affinity approximately 325 times more compared to imatinib [52]. Furthermore, the fact that dasatinib inhibits SRC-family kinases is important, as it is a known mechanism of resistance in downstream pathways that has been observed in imatinib-treated patients [15]. Another potential advantage of dasatinib is its enhanced penetration of the CNS compared to imatinib [1, 15]. Dasatinib has shown promising results both as a single agent and when combined with chemotherapy in the R/R setting, providing a rationale for utilizing dasatinib in the frontline setting. In a phase II study, the combination of dasatinib plus hyper-CVAD was studied in 72 patients (median age of 55 years) with untreated Ph-positive ALL [28]. The CR rate was 96%, and among those who achieved CR, 57 (83%) achieved CCyR after one cycle and 64 (93%) achieved MMR. Twenty-two patients (31%) relapsed, including 8 (36%) with CNS relapse despite receiving 8 prophylactic doses of intrathecal (IT) chemotherapy. Thirteen relapsed patients underwent a mutational analysis, and 7 patients (54%) developed *ABL* mutations: 4 with T315I, 2 with V299L, and 1 with F359V [28]. The 5-year OS rate was 46%. A subsequent multicenter trial that evaluated the same regimen in 97 younger patients (median age 44 years) with newly diagnosed Ph-positive ALL, similarly demonstrated a high combined CR and complete remission with incomplete hematologic recovery (CRi) rate of 88%. The 3-year OS and RFS rates were 69% and 62%, respectively [17]. A landmark analysis showed a statistically significant improvement in the 3-year RFS and OS (p = 0.038 and 0.037, respectively) in patients who underwent HSCT in first remission [17]. However, data are not available regarding the molecular response of patients who did or did not receive transplant. We therefore do not know whether the HSCT benefit was seen in all subgroups or whether HSCT selectively benefited those with suboptimal early molecular response.

Recent studies have shown that dasatinib plus low-intensity or chemotherapy-free regimens appear to be an effective option, particularly in untreated older or unfit Ph-positive ALL patients [18, 33, 38]. One study evaluated 71 older patients (median age 69 years) with untreated Ph-positive ALL who were treated with dasatinib, dexamethasone, and vincristine. In this study, CR was achieved in 96% of patients, and CMR was achieved in 24%. The 5-year OS and EFS rates were 28% and 36%, respectively. A mutation analysis was conducted in 21 relapsed patients and 18 (75%) acquired T315I mutation and one acquired F317L [18]. Risk-adapted lower-intensity regimens have also been studied in younger populations. In the GIMEMA LAL 1509 study, 60 younger patients (median age 42 years) were treated with dasatinib plus corticosteroids [33]. Patients who did not achieve CMR by the end of induction therapy (day 85) subsequently received chemotherapy and/or allogeneic HSCT. Fifty-eight patients (97%) achieved CR and 11 (19%) achieved CMR at day 85. Importantly, the CMR rate was significantly lower in this study than CMR rates seen with dasatinib and intensive chemotherapy, suggesting that chemotherapy may contribute to deeper responses. Patients harboring both *IKZF1 deletion* plus *CDKN2A/B* and *PAX5* deletions had inferior DFS and higher incidence of relapse of 40% vs. 65% and 40% vs. 14% at 18 months, respectively. For the entire population, the 3-year OS rate was 58%. Interestingly, superior DFS was observed in patients who achieved CMR compared to those who did not, despite these patients receiving only dasatinib and corticosteroids (day 85 DFS rates: 75% vs. 44%, $p = 0.06$) [33]. These results suggest that early, deep response to therapy may identify patients who may have good options with minimal therapy. With this, chemotherapy-free treatment has become an attractive option that has opened new avenues, with investigators exploring other combinations including dasatinib plus blinatumomab.

15.3.3 Nilotinib

Ongoing studies have demonstrated promising results when combining nilotinib with intensive chemotherapy in patients with newly diagnosed Ph-positive ALL. A phase II study evaluating nilotinib plus chemotherapy has found an overall CR of 91%, CMR of 86%, and a 2-year OS of 72%. Notably, patients who achieved deep molecular remissions had favorable survival outcomes, regardless of whether or not they underwent allogenic HSCT in first remission [29]. Another study showed similar results with CR achieved in 100% of patients, and CMR achieved in 83.3% [30]. Nilotinib was also studied in combination with low-intensity chemotherapy in older patients, resulting in CR and 2-year OS rates of 87% and 67%, respectively [34, 44]. Although the published data on nilotinib combinations are encouraging, the follow-up is generally short. Additionally, like imatinib and dasatinib, nilotinib does not have activity against the T315I mutation, likely limiting its potential to lead to durable remissions and cure in the absence of HSCT.

15.3.4 Ponatinib

Ponatinib has shown substantial anti-leukemic activity as monotherapy in a pivotal phase II (PACE) trial, demonstrating major cytogenetic response (MCyR) and CCyR rates of 47% and 38%, respectively. Despite this, the long-term survival outcomes remain low, with 3-year OS of 18% [45, 46]. In a single-center phase II study, 76 patients (median age 47 years) with newly diagnosed Ph-positive ALL received ponatinib (45 mg daily for 14 days during the first cycle, then continuously in subsequent cycles) plus hyper-CVAD [31, 53]. Two deaths from myocardial infarction potentially related to ponatinib treatment were noted in an initial report. Therefore, the protocol was amended to reduce the dose of ponatinib to 30 mg daily starting the second cycle, with further reduction to 15 mg daily once CMR was achieved. Thirty-five patients (46%) had at least one underlying CV risk factor including hypertension, hyperlipidemia, diabetes, coronary artery disease, and peripheral artery disease. All patients achieved CR, with 83% achieving CMR and 97% achieving MMR. No early mortality during induction therapy was noted. The 5-year continuous CR, EFS, and OS rates were 83%, 67%, and 71%, respectively. A landmark analysis at 6 months demonstrated a favorable trend toward improved OS in patients who did not undergo HSCT compared with those who did (87% vs. 70%, $p = 0.32$). In addition, no CNS relapses were observed in patients who received 12 prophylactic doses of IT chemotherapy. The most common grade 3–4 adverse events were transaminase (32%), increased bilirubin (17%), pancreatitis (17%), hypertension (16%), bleeding (13%), and skin rash (12%). After protocol amendment using lower ponatinib doses and better control of cardiovascular risk factors, no further significant vascular toxicities were encountered.

Ponatinib at a daily dose of 45 mg in combination with corticosteroids was evaluated in a phase II (GIMEMA) study in 42 older or unfit patients (median age 68 years) with newly diagnosed Ph-positive ALL [39]. The CR and CMR rates were 75% and 46% at 24 weeks, respectively; the estimated 2-year OS was 66%. The incidence of CMR with ponatinib was 20–25% higher than that of the combination of dasatinib and corticosteroids. Dose reductions were frequent, as only 15 patients (36%) were able to tolerate the initial dose of 45 mg daily at 24 weeks. During the study, 13 serious adverse events were reported including two deaths suspected to be related to ponatinib. It is possible that lower doses of ponatinib in elderly patients may improve tolerability without compromising efficacy. Given the lower rate of CMR with corticosteroids compared to intensive chemotherapy (46% vs. 83%), there is interest in evaluating the use of ponatinib with inotuzumab ozogamicin and/or blinatumomab, in hopes of reducing treatment-related mortality, achieving deeper responses and further improving outcomes.

With the positive outcomes seen with newer TKIs, the selection of the best TKI in the front-

line setting has been increasingly questioned. There are no randomized head-to-head trials comparing the different TKIs. However, one multicenter meta-analysis and a propensity analysis have demonstrated improved response rates with ponatinib [54, 55]. When compared to hyper-CVAD plus dasatinib, a propensity score analysis showed improved OS with hyper-CVAD plus ponatinib (2-year: 83% vs. 61%; $p = 0.03$), which was likely driven by deeper remissions obtained with ponatinib (82% CMR rate) compared with dasatinib (65% CMR rate) and lower resistance rate driven by the acquisition of T315I mutation [55]. Some authors have considered whether baseline sequencing could identify patients who are most likely to benefit from later-generation TKIs, perhaps due to the presence of a pre-existing *ABL1* resistance mutation. In the study by Rousselot and colleagues of dasatinib plus low-intensity chemotherapy, 36 patients were tested for *ABL1* mutations by polymerase chain reaction (PCR) at the time of relapse, and 75% of patients were positive for the T315I mutation. The detection of this mutation was associated with early relapses compared to patients without it (median of 7 months vs. not reached, $p < 0.001$) [18]. While these findings suggested that perhaps baseline sequencing could help to select the optimal TKI, another study from MD Anderson Cancer Center using highly sensitive and specific Duplex Sequencing in 63 patients with untreated Ph-positive ALL prior to TKI initiation and during treatment showed that the very low-level *ABL1* mutations present at baseline do not contribute to relapse [53]. Of note, using this highly accurate sequencing method, a pretreatment T315I mutation was only identified in 1 of 63 tested patients. Additional studies confirmed the superior accuracy of Duplex Sequencing compared to PCR for detection of *ABL1* mutations. Thus, the practice at our institution is not to use baseline sequencing to select TKI therapy; rather, we use ponatinib as the frontline TKI for all patients without an absolute contraindication (e.g., active, severe cardiovascular disease).

15.4 CNS Prophylaxis

CNS relapse remains a significant therapeutic challenge in patients with Ph-positive ALL. The incidence of CNS relapse ranges from 8% to 17%, despite the use of TKI combination therapies and prophylactic IT chemotherapy [56]. This has led to the investigation of identifying the adequate number of IT chemotherapy needed to prevent CNS relapses. A retrospective review conducted at our institution compared the rate of CNS relapse in patients with newly diagnosed Ph-positive ALL treated with ≤8 or >8 prophylactic ITs plus hyper-CVAD and a TKI (mainly dasatinib) [57]. Higher incidence of CNS relapse was observed in those treated with ≤8 prophylactic ITs compared to those treated with >8 prophylactic ITs (10% vs. 0%, $p = 0.23$). The 3- and 6-year CNS RFS was 89% and 88%, respectively, in patients receiving ≤8 prophylactic ITs and 100% in patients receiving >8 ITs, respectively ($p = 0.041$). In a multivariate analysis, use of more prophylactic ITs (median of 12 treatments) was associated with decrease rate of CNS relapses ($p = 0.03$) [57]. As a result, the protocols at our institution were amended to increase the number of IT chemotherapy administrations to 12 for the treatment of newly diagnosed Ph-positive ALL. The optimal number of prophylactic IT chemotherapy needed in patients with R/R Ph-positive ALL is currently unknown, although we routinely repeat 6–8 doses of IT chemotherapy at the time of relapse.

15.5 Monoclonal Antibodies and Immunotherapy

15.5.1 CD19 Targeted Antibody

Blinatumomab is a bispecific T-cell engager (BiTE) that binds to CD3-positive cytotoxic T cells and CD19-positive B cells, resulting in apoptosis [58]. A small retrospective study showed that the combination of blinatumomab with a TKI, mainly ponatinib, was efficacious in 20 patients, with R/R Ph-positive ALL. Of the 20 patients included, 6 patients had posi-

tive MRD prior to the initiation of therapy. Overall, 50% achieved CR, 71% achieved CCyR, 75% achieved MMR, and all MRD-positive patients achieved MRD negativity [50]. In another small retrospective study, evaluating the combination of TKIs and blinatumomab found that 8 out of 9 patients with R/R Ph-positive ALL achieved MRD negativity after a median of one cycle, suggesting that this combination is an effective consolidation method for Ph-positive ALL patients to achieve or maintain CMR [59].

The GIMEMA LAL2116 D-Abla trial is the first trial that evaluated the sequential use of dasatinib plus corticosteroids as induction followed by consolidation with blinatumomab for at least two cycles in 63 patients with a median age of 54 years (range, 24–82 years) [60]. After the second cycle of blinatumomab, 32 patients (60%) achieved a molecular response, with 22 patients (41%) achieving CMR and 10 patients (19%) with positive non-quantifiable *BCR-ABL1* transcripts. The rates of molecular responses further increased after subsequent cycles of blinatumomab, with rates of 69.2% after the third cycle and 79.4% after the fourth cycle. At the 12-month follow-up, the OS and DFS rates were 94.2% and 87.8%, respectively. In a mutation analysis conducted in 15 patients with positive MRD before the administration of blinatumomab, 6 patients acquired T315I mutation and 1 acquired E255K; however, these mutations cleared after initiation of blinatumomab [60]. Ongoing studies are evaluating the benefit of ponatinib plus blinatumomab, and the sequential use of ponatinib plus low-intensity chemotherapy as induction followed by blinatumomab (ClinicalTrials.gov Identifier: NCT03263572, NCT03147612). These combination therapies may provide safer and effective treatment modalities in the older population that is unsuitable for intensive chemotherapy or allogeneic HSCT. Early data suggest that both treatment options appear to be beneficial in preventing the emergence of T315Im, decreasing treatment-related mortality, deepening molecular responses, and, in turn, leading to more durable remissions.

15.5.2 CD22 Targeted Antibody

Inotuzumab ozogamicin (INO) is a CD22-directed antibody that is bound to calicheamicin, a potent alkylating agent [61]. The outcomes of INO in patients with R/R Ph-positive ALL were evaluated in a phase I/II (INO-1010) and a phase III (INO-1022) study [61, 62]. A total of 38 patients (16 from INO-1010 and 22 from INO-1022) with Ph-positive ALL were treated with INO compared to 27 patients treated with standard chemotherapy. Of note, 19 patients (86%) in the INO group and 26 patients (96%) in the standard chemotherapy group had prior treatment with one or more TKIs. Higher rates of CR/CRi and MRD negativity were achieved in patients receiving INO compared to standard chemotherapy; the CR/CRi rates were 56–73% and 56%, respectively, and the MRD negativity rates were 63% and 19%, respectively. However, no difference in OS and progression-free survival (PFS) were observed [61].

In a phase I/II study, 12 patients with R/R Ph-positive ALL and 2 patients with blast phase chronic myeloid leukemia were treated with bosutinib (300–500 mg/day) plus INO at a weekly dose of 0.8 mg/m^2 on day 1 and 0.5 mg/m^2 on day 8 and 15 followed by 1 mg/m^2 once every 4 weeks for patients who achieved a response. All but one patient (92%) achieved CR/CRi. Of the 11 patients (79%) who achieved CR/CRi, 91% (10/11) achieved CCyR, 79% (8/11) achieved MRD negativity, and 55% (6/11) achieved CMR. The median OS and EFS were 8.2 and 8.1 months, respectively [49]. The most common adverse event was elevated alanine transaminase, and no cases of veno-occlusive disease reported. Given the promising activity of INO, continued studies are underway to fully assess the safety and efficacy in patients with Ph-positive ALL (ClinicalTrials.gov Identifier: NCT01363297, NCT02311998).

15.5.3 Chimeric Antigen Receptor T-Cell Therapy

CAR-T-cell immunotherapy involves T cells that have been modified genetically to effec-

tively target cell surface and exert cytotoxic effects, in this case, against CD19-positive B cells [63]. Currently, tisagenlecleucel (KYMRIAH®, Novartis), a CD19-directed autologous CAR-T-cell therapy, has been approved for the treatment of relapsed B-cell ALL in patients younger than 26 years. A paucity of literature suggests that the utilization of anti-CD19 CAR-T therapy for the treatment of relapsed Ph-positive ALL after standard chemotherapy or allogeneic HSCT may be a viable option [63]. Well-designed studies are warranted to fully assess the clinical outcomes of CAR-T patients with Ph-positive ALL and to determine who is most suitable for this approach.

15.6 Venetoclax

Venetoclax is a BCL2 inhibitor that has activity in hematological malignancies that express high levels of BCL-2, including Ph-positive ALL. However, in preclinical models, when venetoclax is given as monotherapy, resistance due to upregulation of MCL1, an antiapoptotic BCL2 family member, has been observed [64]. The combination of TKIs and venetoclax have shown to have synergistic activity in preclinical studies. Evidence has suggested that venetoclax plus a BCR-ABL1 TKI may potentially overcome MCL1-mediated resistance seen with venetoclax therapy. The highest degree of synergy was observed with dasatinib and ponatinib. This is due to the inhibition of LYN kinase, known to play a major role in cell proliferation and apoptosis. Inhibiting LYN activity leads to reduced STAT5 phosphorylation, thereby preventing MCL1 upregulation [65]. Preclinical studies evaluating dasatinib plus venetoclax demonstrated superior cytotoxic effects when compared to either agents alone [66]. Given the promising results in preclinical trials, the combination of venetoclax plus ponatinib in R/R Ph-positive ALL is presently being studied at our institution (ClinicalTrials.gov Identifier: NCT03576547).

15.7 Role of Allogeneic Hematopoietic Stem Cell Transplant

Historically, the standard of care in adults with Ph-positive ALL was combination chemotherapy followed by allogeneic HSCT in all eligible patients. Allogeneic HSCT has improved survival outcomes; however, high incidences of relapse and non-relapse mortality are limiting factors. As TKIs have revolutionized the treatment of Ph-positive ALL, the role of allogeneic HSCT has become less clear. Evidence has shown a trend toward improved outcomes with earlier generation TKIs (e.g., imatinib) followed by HSCT compared to chemotherapy alone. However, this benefit was generally not seen in patients who achieved deep molecular responses with TKI and intensive chemotherapy [24–26, 28]. However, among patients who do undergo HSCT, outcomes are better for patients with deeper pre-HSCT molecular response. For example, in one large study, including 441 patients treated with TKI followed by HSCT, OS was significantly better in patients who achieved CMR at the time of HSCT [67]. Deeper molecular responses may also identify patients who do not need to undergo HSCT in the first remission. One study evaluated the impact of CMR in 85 patients with Ph-positive ALL who received TKI plus hyper-CVAD without subsequent allogenic HSCT [68]. The 4-year OS and RFS rates in patients who achieved CMR at 3 months were 66% and 61%, respectively. Overall, these excellent long-term survival results for patients who did not undergo HSCT suggest that HSCT may be safely deferred in first remission for patients who achieve CMR by 3 months of therapy. Thus, our preference is to use the TKI and regimen associated with the highest rate of early CMR.

For patients who remain MRD-positive at 3 months after induction therapy, the historical standard of care has been allogeneic HSCT. However, in the blinatumomab era, it has becomes less clear whether allogenic HSCT is needed to further improve survival outcomes in patients who achieved negative MRD after blina-

tumomab initiation. In the BLAST trial, patients with MRD-positive B-cell ALL (mainly Ph-negative) received blinatumomab (15 μg/m^2 per day by continuous IV infusion) for up to four cycles [69]. After the first cycle, complete MRD response was achieved in 80% of patients (75% in patients with Ph-positive ALL). A post hoc analysis found no significant difference in OS in patients who underwent allogenic HSCT compared with those who continued to receive chemotherapy after MRD negativity was achieved (p = 0.24). As a result, data from this trial was extrapolated to guide treatment decision in patients with Ph-positive disease at our institution. Our preference is to enroll patients in a clinical trial with blinatumomab or INO, with the goal of achieving MRD clearance, which may translate to durable remissions. Given the high risk of transplant-related morbidity and mortality, the decision for HSCT should be individualized, accounting for underlying comorbidities and risk factors [69].

Several studies have been conducted evaluating the clinical outcomes of maintenance therapy after allogeneic HSCT. In the largest retrospective study including 473 transplanted patients, 157 patients received a TKI (mainly imatinib) for primary prophylaxis against relapse. Primary prophylaxis with TKIs was associated with an improved OS ($p = 0.002$), as well as reduced risk of relapsed (p = 0.01) and non-relapse mortality ($p = 0.01$) [70]. In another study, imatinib (400 mg daily) was administered to patients with MRD positivity after allogeneic (n = 25) or autologous HSCT (n = 2). MRD negativity was achieved in 14 patients (52%) after a median duration of 1.5 months. A high relapse rate of 92% was observed in patients who failed to achieve MRD negativity. Additionally, a study reported by Pfeifer and colleagues showed that the use of imatinib both prophylactically and pre-emptively was associated with lower relapse rate and durable remissions in Ph-positive ALL [71]. Published studies thus far have demonstrated the rationale of utilizing TKIs as maintenance therapy post allogenic HSCT [72]. To date, there is no consensus on which TKI is best for maintenance therapy, and future studies are needed to evaluate ponatinib in this setting. Our approach is to give post-HSCT TKIs for at least 2–3 years, usually with ponatinib, given its broader range of activity.

15.8 Conclusion

The incorporation of TKIs into induction, consolidation, and maintenance therapy has greatly improved clinical outcomes and is considered the standard of care for adults with Ph-positive ALL. Despite the significant progress in generating deep molecular remission, frequent relapses remain to be a challenge. There has been significant advancement in understanding the biology of the disease and prognostic impact of genomic and chromosomal abnormalities. This led to the development of novel treatment modalities, including more potent TKIs (e.g., ponatinib), bispecific T-cell engager (e.g., blinatumomab), drug conjugate monoclonal antibodies (e.g., inotuzumab ozogamicin), and CAR-T therapies all propagating the hope to further improve clinical outcomes. Figure 15.1 illustrates the proposed treatment algorithm for patients with Ph-positive ALL. Further studies are needed to shed light on the role of reduced or chemotherapy-free induction therapy, the benefit of allogenic HSCT in first remission, and the effect of prophylactic TKI post-HSCT.

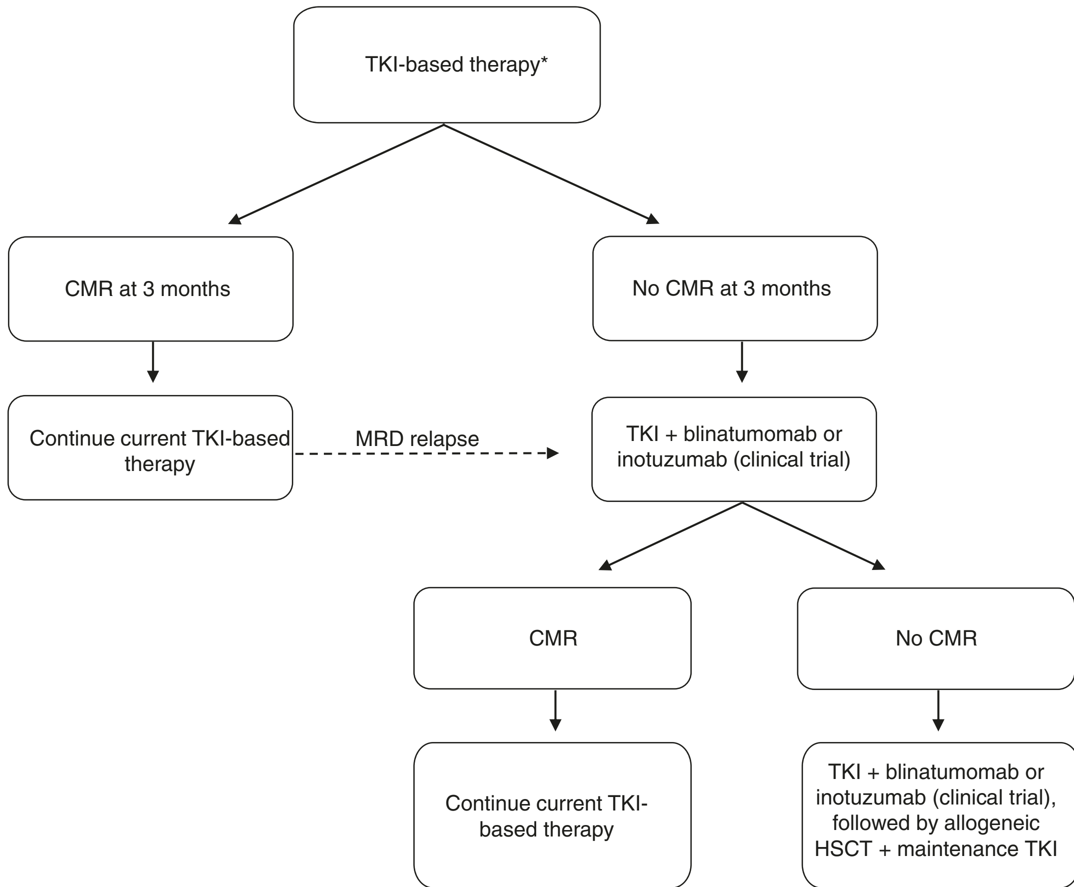

Fig. 15.1 Treatment algorithm of newly diagnosed Ph-positive ALL at MD Anderson Cancer Center. Abbreviations: *CMR* complete molecular response, *HSCT* hematopoietic stem cell transplant, *MRD* minimal residual disease, *TKI* tyrosine kinase inhibitor

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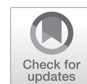

16 Ph-Like ALL: Diagnosis and Management

Emily K. Curran and Sarah K. Tasian

16.1 Clinical Characteristics

Ph-like ALL is now known to be a common subset of B-ALL across the age spectrum [1, 2], occurring in approximately 8% of children with National Cancer Institute (NCI) standard risk (SR) [3–7] and 15% of children with NCI high-risk (HR) B-ALL [8–10]. Ph-like ALL is even more prevalent with increasing age, occurring in up to 30% of adolescents and young adults (AYAs) 16–39 years old and in 20–40% of adults 40 years and older [8, 11–16]. Patients with Ph-like ALL often present with hyperleukocytosis at diagnosis with white blood cell (WBC) counts >50,000 cells/mL [8, 9, 14] and are twice as likely to be male than female [8, 13, 14]. The Ph-like subgroup has also been strongly associated with Hispanic/Latino and Native American ancestry [13, 17], likely in part related to the inherited susceptibility locus in *GATA3* that is more prevalent in this population [18–20]. Ph-like ALL-associated *cytokine receptor-like factor 2 (CRLF2)* rearrangements (described in detail below) are also particularly common in patients of Hispanic/Latino and Native American ethnicity.

Ph-like ALL is associated with inferior clinical outcomes in both children and adults with >60% relapse risk identified in numerous retrospective analyses [8, 9, 13–15, 17, 21]. Most patients with Ph-like ALL have minimal residual disease (MRD) after induction therapy [9, 12–15, 21], but their ability to achieve MRD negativity by end of consolidation therapy, a now-known extremely important prognostic time point [22], has not been systematically studied. While one study reported that risk-directed treatment based upon end-of-induction (EOI) MRD status could abrogate the adverse prognosis of Ph-like ALL [23], other larger cooperative group studies have not confirmed this observation [21]. Current and planned clinical trials of targeted treatment strategies aimed to reduce relapse risk and improve long-term survival are discussed later in this chapter.

16.2 Genetics and Biology

To date, more than 70 discrete Ph-like ALL-associated kinase fusions and mutations have been identified with incidence that varies

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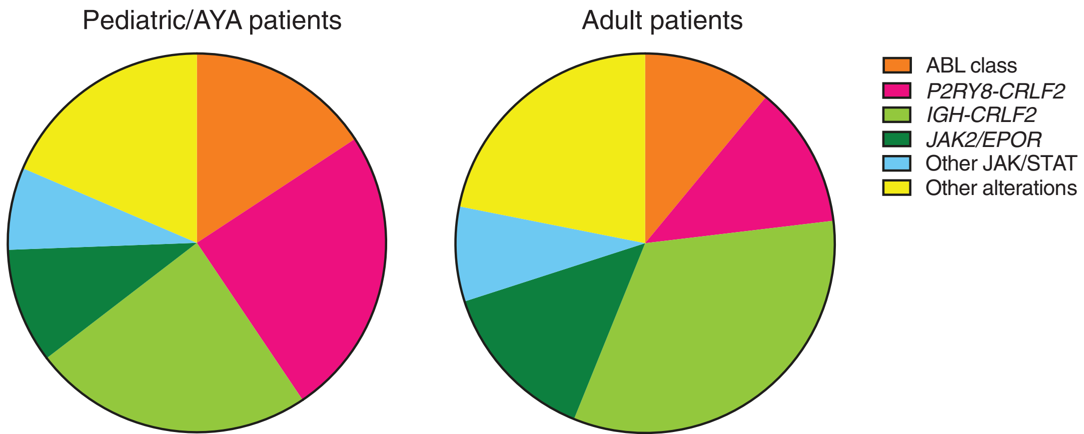

Fig. 16.1 Incidence of Ph-like genetic alterations in children, adolescents/young adults, and older adults with B-ALL. (*Data are summarized from Reshmi et al. Blood 2017* (*n* = 284 patients) [9] *and Roberts et al. JCO 2017* (*n* = 194 patients) [14])

slightly among younger versus older patients (Fig. 16.1) [1]. Despite this genetic heterogeneity, all Ph-like ALL cases are characterized by constitutive cytokine receptor and kinase signaling [24–26], the majority of which can be "binned" into (1) JAK/STAT pathway-activated or (2) ABL/PDGFR pathway-activated subtypes [8, 11, 27, 28]. JAK-STAT signaling activation in Ph-like ALL is driven by *CRLF2* (*cytokine receptor-like factor 2*) rearrangements, *JAK2* (*Janus kinase 2*) or *EPOR* (*erythropoietin receptor*) translocations, and mutations in JAK-STAT pathway-associated genes, including *SH2B3* deletions and *IL7R* insertions/deletions (indels) and other rare point mutations. ABL/PDGFR pathway activation occurs in "ABL class" Ph-like ALL harboring rearrangements of *ABL1* (*Abelson kinase 1*), *ABL2* (*Abelson kinase 2*), *CSF1R* (*colony-stimulating factor-1 receptor*), or *PDGFRB* (*platelet-derived growth factor receptor beta*). These Ph-like ALL-associated alterations are described in greater detail below.

16.2.1 *CRLF2* Rearrangements and Overexpression

Rearrangements of *CRLF2* resulting in its overexpression and constitutive signaling activation are the most common genetic lesions and occur in approximately 50–60% of children and adults with Ph-like ALL [8, 14, 29, 30]. In normal B-cell development, CRLF2 and interleukin receptor alpha (IL7Rα) proteins form the heterodimeric thymic stromal lymphopoietin receptor (TSLPR) complex that induces B-cell proliferation and differentiation and STAT5 phosphorylation upon binding to its ligand, thymic stromal lymphopoeitin (TSLP) [31–33]. In Ph-like ALL, *CRLF2* alterations result in TSLPR surface antigen overexpression readily detectable by flow cytometry and activation of JAK2/STAT5 and PI3K/mTOR intracellular signaling [11, 27, 34]. Numerous preclinical studies have demonstrated the in vitro and in vivo therapeutic potential of JAK and PI3K pathway inhibitors in *CRLF2*-rearranged ALL [11, 27, 34–38].

There are two major mechanisms of *CRLF2* rearrangement that result in *CRLF2* overexpression. One involves translocation of *CRLF2* (located on the pseudoautosomal region [PAR1] of chromosome X or Y) to the immunoglobulin heavy chain transcriptional enhancer on chromosome 14 (*IGH-CRLF2*), which is the more common rearrangement in AYA patients and is more frequent in Hispanic patients [13, 17, 29, 30]. The second alteration involves fusion of *P2RY8* to *CRLF2* via focal interstitial deletion of PAR1 located at chromosome Xp22/Yp11 [24,

29]. *P2YR8-CRLF2* fusions are two- to fivefold more common than *IGH-CRLF2* rearrangements in younger children [9, 29, 39] and also occur in 60% of children with trisomy 21-associated ALL [4, 29, 40]. While virtually all Ph-like cases harboring *IGH-CRLF2* translocations have the classic kinase-activated gene expression signature detectable by a Ph-like low-density microarray assay [41] (LDA; described in detail below), not all cases with *P2RY8-CRLF2* fusions have the LDA signature and are thus not considered Ph-like, particularly in children with SR B-ALL [7] or trisomy 21/Down syndrome-associated B-ALL [17, 29, 40, 42]. Rare activating point mutations in *CRLF2* (usually F232C) that also result in TSLPR surface overexpression have also been described in patients with Ph-like ALL [5, 11, 27], but these are significantly less common than the *IGH-CRLF2* and *P2YR8-CRLF2* rearrangements.

Approximately half of patients with *CRLF2*-rearranged Ph-like ALL have concomitant *Janus kinase 2* (*JAK2*) or *JAK1* mutations, with the most common being R683G in the JAK2 pseudokinase domain [9, 11, 14, 17, 29, 40, 43, 44]. These JAK mutations appear to cooperate with *CRLF2* rearrangements to promote leukemogenesis and induce constitutive kinase signaling that is sensitive in vitro and in vivo to JAK inhibitors, such as ruxolitinib and CHZ868 [27, 29, 35–38]. Patients with *CRLF2*-rearranged Ph-like ALL may also have mutations in other components of the JAK-STAT pathway, such as *IL7R* indels [8, 9, 45], leading to similarly activated signaling that is also sensitive to JAK inhibition [37, 46, 47].

16.2.2 JAK2 and EPOR Rearrangements and Other JAK Pathway Alterations

Often cytogenetically cryptic *JAK2* and *EPOR* rearrangements are also relatively common, each occurring in approximately 8–10% of NCI high-risk children and AYAs with Ph-like ALL, but virtually never in younger children with SR B-ALL [7]. *JAK2* fusions appear to increase in frequency with increasing age with a peak incidence in young adults [8, 9, 14]. As with *CRLF2* rearrangements, *JAK2* and *EPOR* fusions also result in constitutive activation of JAK/STAT signaling and are quite sensitive to JAK inhibitors in vitro and in vivo [8, 15, 28, 34, 37, 48]. More than ten unique *JAK2* fusion partners have been reported to date [1]. At least four *EPOR* fusion partners have been described, all of which result in a truncation of the cytoplasmic tail of *EPOR* and lead to deregulated expression [28]. Finally, rare alterations in other JAK/STAT pathway genes occur in approximately 6–7% of patients with Ph-like ALL, including activating mutations in *IL7Rα*, *JAK1*, *JAK3*, *TYK2*, and *TSLP* and deletions of JAK/STAT-negative regulatory genes, such as *SH2B3* (Table 16.1) [9, 14].

Table 16.1 Currently identified JAK/STAT and ABL class lesions and fusion partners in Ph-like ALL

Subtype	3′ kinase gene	5′ fusion partners	# Fusion partners
JAK/STAT	*CRLF2*	*CSF2RA*, *IGH*, *P2RY8*	3
	JAK2	*ATF7IP*, *BCR*, *EBF1*, *ETV6*, *GOLGA5*, *HMBOX1*, *OFD1*, *PAX5*, *PCM1*, *PPFIBP1*, *RFX3*, *SMU1*, *SNX29*, *SSBP2*, *STRN3*, *TERF2*, *TPR*, *USP25*, *ZBTB46*, *ZNF274*, *ZNF340*	21
	EPOR	*IGH*, *IGK*, *LAIR1*, *THADA*	4
	TSLP	*IQGAP2*	1
ABL class	*ABL1*	*CENPC*, *ETV6*, *FOXP1*, *LSM14A*, *NUP153*, *NUP214*, *RANBP2*, *RCSD1*, *SFPQ*, *SNX1*, *SNX2*, *SPTNA1*, *ZMIZ1*	13
	ABL2	*PAG1*, *RCSD1*, *ZC3HAV1*	3
	CSF1R	*MEF2D*, *SSBP2*, *TBL1XR1*	3
	PDGFRA	*FIP1L1*	1
	PDGFRB	*ATF7IP*, *EBF1*, *ETV6*, *SNX29*, *SSBP2*, *TNIP1*, *ZEB2*, *ZMYND8*	8
	LYN	*GATAD2A*, *NCOR1*	2

16.2.3 ABL Class Rearrangements

Rearrangements of ABL class genes (*ABL1*, *ABL2*, *CSF1R*, *PDGFRA*, *PDGFRB*) occur in 10–15% of children and adults with Ph-like ALL and are mutually exclusive from *CRLF2* rearrangements and other JAK pathway alterations [8, 9, 14]. Similarly to *JAK2* and *EPOR* rearrangements, *ABL* class alterations are extremely uncommon in children with SR B-ALL [7]. Numerous 3′ fusion partners with the 5′ ABL class genes have also been reported (Table 16.1). Of note, patients with *PDGFRB* fusions (most commonly *EBF1-PDGFRB*) frequently present with WBC > 100,000 cells/mL and fail induction therapy [49–51]. Preclinical studies demonstrate appreciable sensitivity of Ph-like leukemias with ABL class alterations to SRC/ABL inhibitors [15, 44]. Anecdotal clinical reports have also noted excellent responses of patients with therapy-resistant ABL class-mutant Ph-like ALL when imatinib or dasatinib was added to chemotherapy [8, 49, 50, 52].

16.2.4 Other Kinase Alterations

Rare fusions involving other kinases have also been reported in patients with Ph-like ALL, some occurring as $n = 1$ cases. Involved fusion genes include *NTRK3*, *BLNK*, *DGKH*, *FGFR1*, *IL2RB*, *LYN*, *PTK2B*, *TYK2* [6, 8, 9, 14, 53–55]. Ras pathway mutations (e.g., in *KRAS*, *PTPN11*, *NF1*, and *NRAS* genes) also occur in approximately 3–6% of patients with Ph-like ALL but are often subclonal and typically occur in association with *CRLF2* overexpression [8, 13]. Preclinical studies in Ph-like ALL and other malignancies suggest that some of these fusions and mutations may be therapeutically targetable with kinase inhibitors or other small molecule inhibitors [56].

16.3 Diagnosis of Ph-Like ALL

Identification of Ph-like ALL-associated alterations has been challenging given the tremendous genetic heterogeneity of the disease and continued discovery of new fusion partners or mutations. While unbiased RNA sequencing (RNAseq) of every patient with B-ALL provides the most comprehensive genetic characterization and allows identification of previously unidentified genetic lesions, this approach is expensive and labor intensive (particularly for downstream informatics analyses), making this modality cost-prohibitive for many large clinical trials and clinical testing. Instead, targeted genetic testing algorithms have been developed that are capable of identifying the most common Ph-like ALL kinase fusions and mutations and are most cost-effective. In addition, routine clinical immunophenotyping and cytogenetics/fluorescence in situ hybridization (FISH) analyses can be adapted for rapid initial identification of Ph-like ALL cases to allocate for subsequent specific genetic testing and are discussed below in more detail.

16.3.1 Targeted Screening Approaches

To minimize cost and time needed to identify patients with Ph-like ALL who require detailed molecular characterization, several clinical screening assays and rapid diagnostic testing have been developed. One of the most widely used screening tests involves the optimized LDA analysis to quantify expression of an 8- or 15-gene signature (*IGJ*, *SPATS2L*, *MUC4*, *CRLF2*, *CA6*, *NRXN3*, *BMPR1B*, *GPR110*, *CHN2*, *SEMA6A*, *PON2*, *SLC2A5*, *S100Z*, *TP53INP1*, *IFITM1*) that is highly predictive of Ph-like ALL [57]. The LDA card also includes *BCR-ABL1* and *ETV6-RUNX1* detection, as patients with these lesions also have activated kinase expression signatures, but are not Ph-like and thus excluded from further Ph-like ALL testing. Leukemia samples with LDA scores of ≥0.5 (range 0–1) are considered positive for the Ph-like ALL gene expression signature and subjected to additional genetic analysis for complete molecular characterization based upon level of *CRLF2* expression (high versus low) that is also included on the LDA. Leukemia samples with high *CRLF2* expression by LDA are assessed for

P2RY8-CRLF2 fusions on the LDA itself or via reverse transcriptase polymerase chain reaction (RT-PCR). Ph-like samples with high *CRLF2* expression, but without *P2RY8-CRLF2*, are then tested by FISH for *IGH-CRLF2* translocations. *CRLF2*-rearranged samples are further assessed for *JAK1*, *JAK2*, and *IL7RA* mutations via PCR. Those with the Ph-like signature and normal *CRLF2* expression are subjected to kinase fusion testing to assess for *JAK2*, *EPOR*, and ABL class alterations [1].

Flow cytometric detection of increased TSLPR surface antigen staining of diagnostic bone marrow or peripheral blood Ph-like ALL samples is extremely predictive of *CRLF2* rearrangements [27]. TSLPR staining is now being incorporated into routine clinical immunophenotyping by several groups given its cost efficacy and results return within 24–48 h [13]. Leukemia specimens with increased TSLPR staining can then be sent for specific genetic testing for *P2RY8-CRLF2* fusions or *IGH-CRLF2* translocations and for *JAK1/2* and *IL7RA* mutation analyses. These and other Ph-like ALL-associated point mutations and insertions/deletions (indels) can also be identified by DNA-based next-generation sequencing platforms.

Routine clinical FISH analysis can identify some of the more common Ph-like ALL fusions and is also an advantageous modality given usual rapidity of test resulting. Clinically available break-apart probes can assess for rearrangement of known kinases frequently involved in Ph-like ALL (e.g., 3′ *ABL1*, *ABL2*, *CRLF2*, *CSF1R*, *JAK2*, *PDGFRB*). While FISH testing can quickly identify potential Ph-like ALL cases with aberrant signals at these genes, this modality may not provide information regarding the specific 5′ fusion partner for which further genetic analysis will likely be necessary for complete genetic characterization. However, FISH testing may be adequate to identify Ph-like ALL cases as either CRLF2/JAK pathway- or ABL class-mutant for the addition of relevant TKI therapy. Targeted RT-PCR analysis for known Ph-like kinase fusions with confirmatory sequencing (initially implemented by the Children's Oncology Group [COG]) has been a valuable testing resource to date but has the disadvantage of potential inability to identify fusions with previously unknown 5′ partners [48].

16.3.2 Unbiased Testing Approaches

As RNA-based testing platforms capable of new fusion discovery have become more cost-effective and clinically available, many cooperative groups and academic institutions are currently using these more comprehensive diagnostic modalities to streamline the identification of patients with Ph-like ALL. Digital molecular barcoding platforms such as targeted NanoString analysis can identify over 200 different genetic alterations, but currently is capable of identifying only known oncogenic fusions. Capture-based RNA sequencing such as the ArcherDX FusionPlex panel with anchored multiplex PCR chemistry is agnostic of the 5′ fusion partner, thus allowing for potential identification of novel fusions. Finally, FoundationOne Heme Panel is a targeted DNA and RNA sequencing method that is able to detect translocations and fusions in over 400 cancer-related genes [58]. These diagnostic approaches are being increasingly integrated into clinical practice and for identification of appropriate subjects for clinical trials.

16.3.3 Tiered Testing Approaches Used for Current Clinical Trials

In practice, many groups utilize a step-wise testing approach by which patients first undergo cost-effective, rapid screening by LDA to identify those with the Ph-like signature (and to exclude the majority of patients with B-ALL who have a negative Ph-like signature who do not require additional molecular testing). Patients with positive Ph-like screening results are then allocated for appropriate downstream genetic testing. An example of this approach is the diagnostic algorithm currently being used by COG to identify patients with Ph-like ALL or potential participation in the AALL1131 (NCT02883049) and AALL1521

(NCT02723994) [59] clinical trials testing chemotherapy with the ABL/PDGFRB inhibitor dasatinib or the JAK inhibitor ruxolitinib (Fig. 16.2) [1]. In this approach, diagnostic leukemia specimens from children and AYAs with HR B-ALL are analysed by LDA to identify patients with the Ph-like signature. Those with evidence of *CRLF2* overexpression on the LDA assay, but without detected *P2RY8-CRLF2* fusions, are then allocated to FISH testing for *IGH-CRLF2* translocations. Patients with identified *CRLF2* rearrangements are also assessed for *JAK1* and *JAK2* point mutations and IL7R indels in known exons by PCR. Specimens identified as Ph-like with normal *CRLF2* expression are allocated for capture-based kinase fusion analysis (via customized ArcherDX FusionPlex methodologies) to assess for ABL class and JAK pathway-associated kinase fusions. Research-level RNA sequencing can be performed on specimens from remaining patients with evidence of the Ph-like signature on LDA testing who do not have an identifiable kinase alteration via current testing modalities.

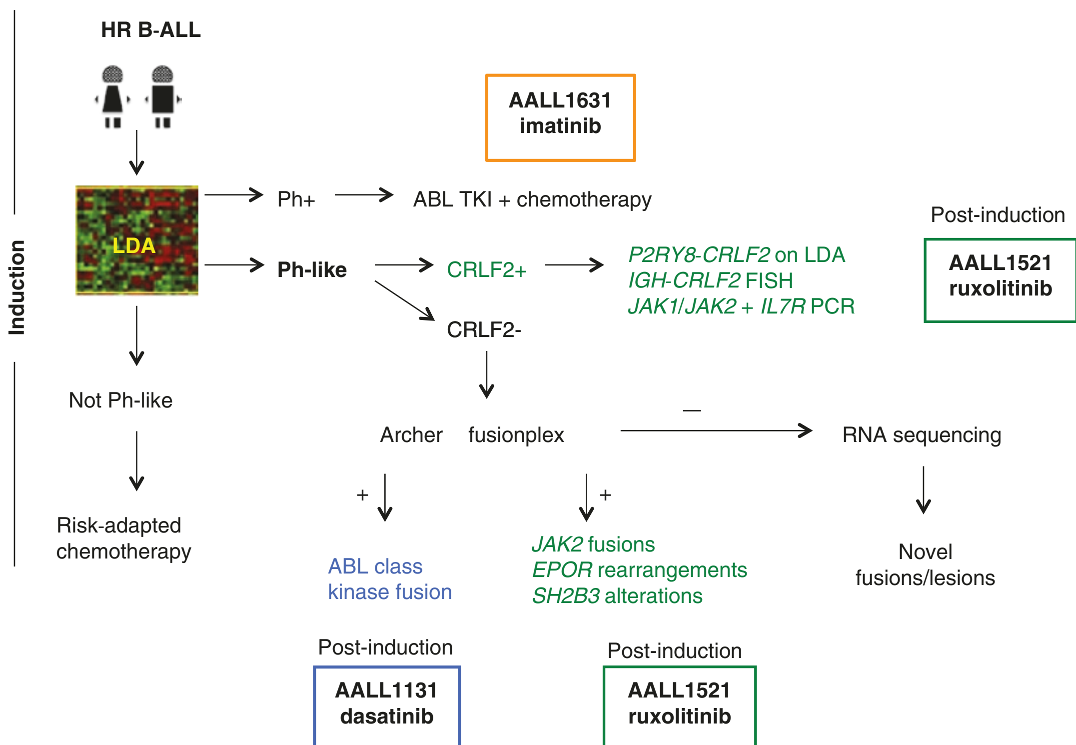

Fig. 16.2 Tiered genetic screening approach to diagnose Ph-like ALL utilized in Children's Oncology Group trials AALL1131 (NCT02883049) and AALL1521 (NCT02723994). Diagnostic leukemia samples from children, adolescents, and young adults with HR B-ALL are analyzed by the low-density microarray (LDA) assay, and those with a score of 0.5–1 are considered positive for the Ph-like signature. Patients with Ph+ ALL are also identifiable by LDA and are allocated to AALL1631 (NCT03007147). As part of the LDA assay, *CRLF2* overexpression that is suggestive of *CRLF2* rearrangements can be assessed and with direct detection of the *P2RY8-CRLF2* fusion. Ph-like specimens with *CRLF2* overexpression, but no evidence of *P2RY8-CRLF2*, are then allocated to FISH testing to identify *IGH-CRLF2* translocations. Patients with identified *CRLF2* rearrangements are also assessed for *JAK1* and *JAK2* mutations and *IL7RA* insertions/deletions by PCR testing. Specimens identified as Ph-like with normal *CRLF2* expression are allocated for capture-based kinase fusion analysis (via customized ArcherDX FusionPlex methodologies) to assess for ABL class and JAK pathway-associated alterations. RNA sequencing can be performed for remaining cases with evidence of the Ph-like signature by LDA, but without an identifiable kinase alteration

Table 16.2 Current clinical trials of TKI-based therapy in patients with Ph-like ALL

Ph-like alterations	TKI	Disease status	Ages	Clinical trial
ABL class	Dasatinib	Newly diagnosed	1–30 years	NCT01406756 (COG AALL1131)
ABL class	Dasatinib	Newly diagnosed	1–18 years	NCT03117751 (SJCRH Total XVII)
ABL class	Dasatinib	Relapsed	≥10 years	NCT02420717 (MDACC)
CRLF2/JAK pathway	Ruxolitinib	Newly diagnosed	1–21 years	NCT02723994 (COG AALL1521)
CRLF2/JAK pathway	Ruxolitinib	Newly diagnosed	1–18 years	NCT03117751 (SJCRH Total XVII)
CRLF2/JAK pathway	Ruxolitinib	Newly diagnosed	18–39 years	NCT03571321
CRLF2/JAK pathway	Ruxolitinib	Relapsed	≥10 years	NCT02420717 (MDACC)

16.4 Management

As aforementioned, most Ph-like ALL cases can be broadly categorized as JAK inhibitor-targetable (including *CRLF2*, *JAK2*, or *EPOR* rearrangements, *SH2B3* deletions, and *IL7R* indels) or ABL/PDGFR inhibitor-targetable kinase fusions (*ABL1*, *ABL2*, *CSF1R*, and *PDGFRB*). Although TKI-based therapies are not yet the standard of care for patients with Ph-like ALL, several ongoing clinical trials are investigating whether addition of ruxolitinib or dasatinib to multi-agent backbone chemotherapy can improve the known poor clinical outcomes of this population (Table 16.2).

16.4.1 Targeting JAK Pathway Alterations in Ph-Like ALL

Preclinical studies have demonstrated in vitro and in vivo activities of several JAK inhibitors in Ph-like ALL with *CRLF2* and other JAK pathway alterations [28, 34, 37, 38, 60]. While several JAK inhibitors have been developed, the most widely used in both preclinical and ongoing clinical trials for Ph-like ALL is the selective JAK1/JAK2 inhibitor ruxolitinib [61], which is approved by the US Food and Drug Administration and by the European Medicines Agency for the treatment of adult patients with myelofibrosis and polycythemia vera (usually harboring somatic *JAK2* V617F mutations). The recent COG ADVL1011 phase 1 trial of ruxolitinib in children and AYAs aged 1–21 years with relapsed or refractory cancers demonstrated safety and tolerability in the pediatric population and identified the recommended phase 2 dose of ruxolitinib (RP2D) without identification of a maximally tolerated dose for further evaluation [62]. An ongoing phase 1/2 trial conducted at the MD Anderson Cancer Center (MDACC) is currently assessing the safety and potential efficacy of ruxolitinib in addition to hyper-CVAD backbone chemotherapy for AYA and older adult patients with relapsed/refractory *CRLF2*-rearranged/JAK pathway-mutant ALL (NCT02420717). Combination therapy to date has been well tolerated with no dose-limiting toxicities (DLTs) identified, but the potential efficacy of this approach in the relapsed setting has not yet been determined [63].

Based on ruxolitinib safety from the ADVL1011 trial and improved efficacy in the AALL0031 and AALL0622 trials with imatinib or dasatinib in addition to chemotherapy for pediatric patients with Ph+ ALL [62, 64–66], the COG is now non-randomly integrating TKIs into chemotherapy for children and AYAs with newly diagnosed Ph-like ALL. These approaches aim to target critical oncogenic kinase pathways with goals of improving MRD-negative remission rates and decreasing relapse risk in these high-risk patients, which may thereby ultimately improve disease-free and overall survival. The AALL1521 phase 1/2 trial is currently testing the safety and efficacy of ruxolitinib in combination with augmented Berlin-Frankfurt-Munster

(BFM)-based post-induction chemotherapy (NCT02723994). Patients aged 1–21 years with *CRLF2*-rearranged or other JAK pathway-mutant Ph-like ALL are identified during induction therapy using the tiered approach described above and shown in Fig. 16.2. Eligible patients are stratified into four cohorts based upon underlying Ph-like genetic alterations and end-of-induction MRD status to elucidate potential differences in therapeutic efficacy by subgroup. Results of the trial's Part 1 safety phase demonstrated tolerability of ruxolitinib in combination with intensive multi-agent chemotherapy without dose-limiting toxicities and identified the RP2D [59]. Part 2 of the trial is currently assessing the efficacy of combination therapy in patients with *CRLF2*-rearranged Ph-like ALL with and without concomitant JAK point mutations with a primary endpoint of 3-year event-free survival (EFS) in comparison to historic control patients treated with chemotherapy alone. An arm of the St. Jude Children's Research Hospital (SJCRH) Total XVII trial is also piloting the safety and potential efficacy of ruxolitinib in addition to chemotherapy in children with JAK pathway-mutant Ph-like ALL (NCT03117751). A recently opened multi-institutional phase 1 trial (NCT03571321) will also establish the safety of ruxolitinib in combination with the pediatric-based CALGB10403 chemotherapy regimen (NCT00558519) [16, 67] in AYAs 18–39 years of age. The study will expand to a larger phase 2 trial if initial safety of the combination therapy is demonstrated in this population.

16.4.2 Targeting ABL Class Alterations in Ph-Like ALL

Preclinical studies have also demonstrated potent in vitro and in vivo activities of ABL/PDGFR TKIs in Ph-like ALL harboring ABL class alterations [8, 15, 44]. In addition, anecdotal case reports have demonstrated potential clinical efficacy of imatinib and dasatinib with chemotherapy in patients with relapsed or chemotherapy-refractory Ph-like ALL [8, 49, 50, 52]. As aforementioned, numerous trials have now demonstrated the safety and remarkable efficacy of TKI in addition to intensive chemotherapy in children and adults with Ph+ ALL [64, 65, 68–70]. Ongoing studies are now investigating similar use of combination TKI approaches for patients with Ph-like ALL and ABL class fusions.

The MDACC trial described above (NCT02420717) is also testing the safety and potential efficacy of dasatinib in combination with hyper-CVAD chemotherapy in AYAs and older adults with relapsed/refractory Ph-like ALL and ABL class kinase fusions [63]. Arms of the COG AALL1131 (NCT01406756) and SJCRH Total XVII (NCT03117751) trials are also investigating the potential efficacy of dasatinib in addition to chemotherapy in children and AYAs with HR ABL class-mutant Ph-like ALL. Definitive results from these studies have not yet been reported.

16.4.3 Remaining Questions in Ph-Like ALL

Whether patients with Ph-like ALL should undergo consolidative hematopoietic stem cell transplant (HSCT) in first remission is still unknown. The majority of children and adults with Ph-like ALL have EOI MRD positivity, which adds incrementally to the inferior prognosis already conferred from the Ph-like signature and associated kinase fusions. While patients with Ph-like ALL who achieve EOI MRD negativity have improved disease-free survival compared to those with MRD positivity, outcomes remain inferior to those of patients with EOI MRD-negative non-Ph-like ALL [13, 21]. It is plausible that up-front intervention with the addition of TKI to chemotherapy for patients with newly diagnosed Ph-like ALL could decrease the rates of end-of-consolidation MRD positivity and indication for HSCT. Data from the aforementioned ongoing clinical trials may help to answer this question.

Over the past several years, new antibody-based immunotherapies such as the CD19×CD3 bispecific T-cell-engager blinatumomab and the CD22 antibody–drug conjugate inotuzumab ozo-

gamicin have demonstrated impressive responses in patients with relapsed/refractory B-ALL with >70% remission reinduction rates [71–75]. A recent study also reported particular success of blinatumomab in converting adult patients with MRD-positive level disease to MRD negativity as a bridge to HSCT, leading to FDA approval for both multiply relapsed and primary chemotherapy-refractory MRD+ indications [76]. A randomized clinical trial by the Alliance cooperative group is currently investigating whether incorporation of inotuzumab ozogamicin followed by blinatumomab into frontline treatment can improve outcomes for AYAs with CD22+ B-ALL versus standard-of-care chemotherapy (NCT03739814). In addition, chimeric antigen receptor (CAR)-redirected T-cell immunotherapies targeting CD19 or CD22 have reported initial remission reinduction rates of 80–95% in patients with multiply relapsed/refractory B-ALL, although immunotherapeutic resistance has emerged as a major source of treatment failure with approximately 50% of patients remaining in remission at 1–2 years after CAR T cells [77–81]. While blinatumomab, inotuzumab, and CAR T-cell therapies are broadly applicable to all subtypes of B-ALL expressing surface CD19 and/or CD22, it is likely that these immunotherapies can also specifically benefit patients with Ph-like ALL and relapsed or residual disease.

16.5 Conclusions

Ph-like ALL is a common and high-risk B-ALL subtype associated with chemotherapy resistance and high rates of relapse across the age spectrum. It is hoped that addition of TKIs to chemotherapy will decrease relapse and improve survival in patients with newly diagnosed Ph-like ALL similarly to the remarkable outcomes now achievable with combined TKI and chemotherapy for patients with Ph+ ALL. Clinical trials of ruxolitinib and dasatinib addition for patients with *CRLF2*-rearranged/JAK pathway-mutant and ABL class-mutant Ph-like ALL, respectively, are underway. However, the potential rates of response and long-term efficacy of these approaches are not yet known. Recent preclinical studies have also demonstrated improved antileukemia activity of dual TKI therapy specifically in Ph-like ALL [34], although the potential clinical tolerability or toxicity of multi-kinase inhibition in combination with chemotherapy has not been well-explored in patients with acute leukemia. Finally, given the remarkable recent success of CD19- and CD22-targeted antibody-based and cellular immunotherapies in patients with relapsed/refractory B-ALL, integration of TKIs with immunotherapy for patients with Ph-like ALL may be an attractive future strategy for further augmentation of outcomes.

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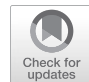

17 Adult Burkitt Leukemia/ Lymphoma

Pamela Abdayem, Jean-Marie Michot, Valérie Camara-Clayette, Yegor Vassetsky, and Vincent Ribrag

17.1 Introduction

Initially described by Dennis Burkitt in 1958, Burkitt lymphoma (BL) is a highly aggressive B-cell non-Hodgkin lymphoma (NHL). Originating from mature, germinal, or post-germinal center B cells, it often manifests as extra nodal disease or as an acute leukemia. Being the first cancer described containing viral particles (Epstein–Barr virus) and pathologically driven by a genetic translocation, BL largely contributed to the field of tumor immunology and molecular genetics. Despite the recent improvement in disease outcomes with pediatric-inspired chemotherapy protocols, data about the optimal management of elderly or other specific patient groups and relapsed BL remain scarce.

According to the revised 2016 World Health Organization (WHO) classification of hematologic malignancies, BL and Burkitt cell acute lymphoblastic leukemia (L3ALL) are a single entity, a mature B-cell neoplasm with c-MYC overexpression. The 2016 WHO classification also proposes three aggressive B cell neoplasms that resemble BL: "Burkitt-like lymphoma with 11q aberration"; "High-grade B cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangement," and "High-grade B cell lymphoma, not otherwise specified" [1].

Accounting for 1–3% of all cases of ALL, L3ALL rather than BL is usually considered when patients present with extensive marrow infiltration (greater than 25% blasts) and a low tumor burden disease. Central nervous system (CNS) involvement is equally prevalent in both forms of the disease. L3ALL predominates in children and adolescents and is less common in adults. Unlike the other types of ALL, it is treated similarly to BL with short and intensive chemotherapy protocols.

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17.2 Forms: Endemic, Sporadic, and Immunodeficiency-Related

Three distinct clinical forms of BL/L3ALL have been individualized. With similar histological characteristics and comparable outcomes, each form has specific epidemiologic, genetic, and clinical features.

17.2.1 Endemic Form

In 1958, Dennis Burkitt reported 38 cases of "sarcomas" affecting the jaw and abdomen in children at Mulago hospital in Uganda. This endemic variant accounts for 30–50% of all childhood tumors in equatorial Africa with an estimated incidence of 3–6 cases per 100,000 children per year [2]. It occurs mainly in males (male to female ratio is approximately 2:1) aged 4–7 years.

The prevalence of this disease in tropical regions suggested the involvement of an infectious agent. Yellow fever was initially suspected; Epstein–Barr virus (EBV) was later identified and became the first example of an oncogenic pathogen. In addition, the geographic distribution of BL corresponded with areas holo- or hyperendemic for *Plasmodium falciparum* malaria in the "lymphoma belt of Africa" and in Papua New Guinea/Irianjaya in Asia, making it a polymicrobial disease [3]. The increased EBV load observed during acute malaria infection seems to result not only from an impairment of the EBV-specific T-cell response and polyclonal B-cell activation but also from viral reactivation directly driven by malarial antigens. Chene et al. [4] identified a cysteine-rich inter-domain region 1α (CIDR1α) of the *Plasmodium falciparum* membrane protein 1 that increases B-cell survival and revives the memory compartment where EBV is known to persist, therefore triggering viral replication. *Euphorbia tirucalli*, a herbal remedy used in the "lymphoma belt," might reactivate EBV leading to c-MYC altered expression and increased occurrence of endemic BL, according to preliminary results by Manucci et al. [5]. Similarly, sub-Saharan populations are highly exposed to aflatoxin B1 which acts as a cofactor in EBV-mediated lymphomagenesis [6].

17.2.2 Sporadic Form

The sporadic form is observed in the United States (US) and Western Europe. In the US, it includes 30% of pediatric lymphomas and less than 1% of adult NHLs, with an estimated incidence of three cases per million persons per year in both children and adults [7]. In Europe, the incidence is approximately 2.2 cases per million persons per year [8]. Median age at diagnosis is 11 years and 30 years, among children and adults, respectively [9]. Sporadic BL is most common among Caucasian males with a 3 or 4:1 male to female ratio [7, 10, 11]. Contrary to the endemic form where EBV genome is ubiquitous, EBV infection is detected in approximately 20% of sporadic cases only.

17.2.3 Immunodeficiency-Related Form

This variant is mainly observed in patients with human immunodeficiency virus (HIV) infection and CD4 count higher than 200 cells/μL [12]. The relative risk of developing BL in HIV-positive compared to HIV-negative individuals is 50. BLs represent 30% of HIV-associated lymphomas. Thus, the lifetime risk of developing BL in HIV-positive patients is between 10% and 20%. In contrast with most other HIV-related lymphomas that develop at a stage of profound immunodeficiency, its incidence has not decreased with the use of antiretroviral therapy (ART). Increased aggressiveness of the disease combined with poor health status of HIV-infected patients are responsible for the dismal prognosis of this variant compared to HIV-negative BL [13].

17.3 Pathogenesis

17.3.1 c-MYC

17.3.1.1 Functions of c-MYC

MYC is one of the first described oncogenes; its expression is associated with independent cell growth [14]. MYC acts both as a positive and as a negative regulator of gene transcription [15–17]. It therefore regulates cell cycle transition, cell differentiation, growth, metabolism, protein synthesis, adhesion, migration, and angiogenesis. MYC contributes to genomic instability, triggers telomere aggregation, and controls the balance between stem cell self-renewal and differentiation. It can also drive focus formation and anchorage-independent growth in vitro as well as full tumorigenesis in vivo. Deregulation of *MYC* expression can occur by many mechanisms: retroviral transduction, retroviral promoter or enhancer insertion, chromosomal translocation, gene amplification, and activation of hormones or growth factors, their receptors, second messengers, or transcriptional effectors that converge on MYC expression. Alterations in mechanisms that directly or indirectly stabilize *MYC* mRNA and/or protein can also deregulate expression of this potent oncogene [18].

17.3.1.2 c-MYC in BL

The development of BL relies on the constitutive expression of the *MYC* proto-oncogene located at chromosome 8q24, which encodes the MYC protein transcription factor. The dysregulation of c-MYC, a genetic hallmark of BL, is a consequence of a chromosomal translocation between chromosome 8 at the locus q24 and either chromosome 14 (t(8;14) (q24;q32), 70–80% of cases) or chromosome 22 (t(8;22)(q24;q11), 10–20% of cases), or chromosome 2 (t(2;8)(p12;q24), 2–5% of cases) [19]. *MYC* gene at 8q24 is therefore juxtaposed with one of the immunoglobulin (Ig) loci on chromosomes 14, 22, or 2. Transgenic mice that expressed the MYC gene under the control of the Ig heavy-chain intronic enhancer (Em), emulating the chromosomal translocation found in BL, developed B-cell lymphomas with a latency of 4–6 months [20]. BL cells express activation-induced cytidine deaminase (AID), which mediates both Ig somatic hypermutation and Ig class switch recombination (CSR). Thus, human BLs have somatically mutated Ig variable regions, and *IG/MYC* translocations typically involve Ig switch regions, suggesting that they arise by aberrant CSR [21].

DNA breaks in the involved Ig genes occur through processes that are normal for B cells, namely either attempted Ig V(D)J recombination or Ig class switch recombination. DNA breaks near *MYC* might result from the recruitment of activation-induced cytosine deaminase (AID) [22, 23]. DNA breaks in *MYC* may come to be spatially close to DNA breaks in the IG gene loci in the interphase nucleus, which is a prerequisite for these DNA ends to be joined to form *MYC* translocations. This was observed upon B-cell activation in mice or in HIV-infected individuals where HIV-1 Tat protein induced spatial proximity between the *MYC* and *IGH* loci [24]. Identification of MYC translocation by Polymerase Chain Reaction (PCR) is often impossible due to the variable DNA break sites although a method using the long-range PCR was proposed. Fluorescence in situ hybridization (FISH), standard cytogenetic techniques, and more recently immune-FISH are more accurate diagnostic tools.

DNA break sites on both chromosomes differ between endemic and sporadic BL [25]. In endemic cases, the breakpoint on chromosome 14 involves the heavy chain joining region, while in non-endemic cases, it involves the heavy chain switch region. In endemic cases, the break site on chromosome 8 is usually adjacent to MYC, while in sporadic cases, it often lies in intron 1 within the gene [26].

17.3.2 Beyond c-MYC

High-throughput sequencing approaches identified additional cytogenetic and molecular events cooperating with MYC to induce BL [27–29].

Cytogenetic aberrations include gains in 1q, 13q, 7q, 8q as well as losses in 3, 4p, 4q, 9p and 13q [30]. Poirel et al. [31] showed that +7q and del(13q) were independently associated with a significant inferior event free survival (EFS) in children and adolescents with BL.

Overexpression of MYC likely triggers TP 53-dependent apoptotic pathways, thus increasing the selection for TP 53 inactivating mutations (35% of cases) [32, 33].

Mutations in the CCND3 gene encoding cyclin D3, a D-type cyclin that regulates the G1–S cell-cycle transition, explain the rapidly proliferative character of BL [34]. They are present in 38% and 2.6% of sporadic and endemic tumors, respectively.

All three variants of BL express highly recurrent mutations in the transcription factor TCF-3 gene (10–25%) and/or in its negative regulator ID3 (35–58%) [27–29]. Identification of these mutations, typically absent in diffuse large B-cell lymphoma (DLBCL), might offer a diagnostic solution when DLBCL and BL are difficult to distinguish. TCF-3 has a central role in survival and proliferation [28, 29]. It directly transactivates CCND3, thereby promoting cell-cycle progression. It also transactivates ID3 as well as the related family members ID1 and ID2, thus inducing expression of its own negative regulators.

As for signaling pathways, BL relies on BCR signaling which is mostly "tonic" and antigen-independent. PI3K is the main pathway in opposite to other antigen-dependent pathways such as NF-KB [35, 36]. PI3K activity in BL is also dependent on TCF-3, suggesting a connection between oncogenic activation of this transcription factor in BL and tonic BCR signaling. First, TCF-3 directly upregulates BCR expression. Second, TCF-3 increases BCR signaling by negatively regulating PTPN6, encoding the SHP-1 phosphatase. SHP-1 attenuates BCR signaling by dephosphorylating the ITAM motifs of the CD79A and CD79B signaling subunits of the BCR [29]. Other PI3K signaling triggers include inactivating mutations of PTEN.

17.3.3 EBV

Discovered in the 1960s, EBV is the first recognized human cancer virus. It is present in all cases of endemic BLs and in a minority of sporadic BLs. About 25–40% of BL occurring in HIV-positive patients are EBV-associated. EBV-transformed lymphoblastoid cell lines (LCLs) express several proteins involved in the modulation of oncogenesis pathways such as PI3K and NF-KB [37]. However, a significantly restricted pattern of viral gene products, primarily EBNA1, which is involved in the replication of the EBV genome, is expressed in the BL cells. This may be the result of vigorous selective pressure by T cells that are specific for latent antigens. In the absence of functional T cells, EBV-induced LCLs grow unimpeded, as in the case of posttransplant lymphoproliferative disorder [38].

The adepts of the "hit-and-run" hypothesis argue that EBV contributes to the pathogenesis of most BLs, but malignant cells are obliged to either repress most latent viral gene expression or lose the viral genome completely, because of the incompatibility between c-MYC and EBNA2/LMP1 expression [39] and the immune-selection against EBV transformation–associated proteins [40, 41]. Nevertheless, available evidence suggests that EBV-negative BLs arise independently of EBV involvement. In addition, EBV-positive and EBV-negative cases of BL differ in the number of somatic mutations in their immunoglobulin heavy chain (V_H) genes, in the involvement of antigen selection, as well as in the translocation breakpoints in the *MYC* locus, suggesting distinct cell origins and pathogenesis [42, 43].

17.3.4 HIV

Although HIV does not infect B cells, the increased incidence of BL in HIV-positive individuals could result from both underlying immunodeficiency and direct viral-induced lymphomagenesis. HIV may trigger chronic B-cell activation and dysregulated monoclonal expansion [44]. As mentioned previously,

overexpression of AID in activated B cells triggers DNA breaks leading to the *MYC-IgH* translocation. Another potential mechanism is that HIV-encoded Tat protein induces a sustained *MYC* relocalization next to *IGH* [24] and induces aberrant expression of AID in circulating B cells.

17.4 Clinical Features

17.4.1 Clinical Presentation

The disease is predominant in males, with a median age ranging between 25 and 35 years. Nearly 25% of patients are older than 50. BL often presents as a rapidly growing tumor, with a very short doubling time (24 h) and a quick dissemination to extranodal sites including the bone marrow and the central nervous system (CNS). Primary tumor sites vary between endemic and sporadic forms of the disease. Seventy percent of patients present with advanced stage III or IV disease [45]. Spontaneous tumor lysis syndrome (TLS), with high lactate dehydrogenase (LDH) and uric acid levels, is frequent at diagnosis and often requires early admission into the intensive care unit and potential hemodialysis upon initiation of treatment. Mental neuropathy, resulting from infiltration of inferior dental nerves, is frequently found in BL and L3ALL and generally indicates CNS involvement [46]. Cervical lymphadenopathy might be associated with higher rates of CNS infiltration.

17.4.1.1 Endemic Forms

The facial skeleton, mainly the jaw, is affected in 50% of the cases of endemic BL. In a Ugandan case series, Orem et al. [47] showed a decrease in mandibular presentation and an increase in abdominal presentation with advancing age. At the time of initial presentation, CNS involvement is found in 30–40% of patients, whereas bone marrow involvement is seen in less than 10% of the cases [19].

17.4.1.2 Sporadic Forms

Patients typically present with a rapidly growing abdominal mass and symptoms related to bowel obstruction, gastrointestinal bleeding, or rarely bowel perforation. Bowel intussusception is more common in children. Involvement of the jaw or facial bones occurs in 25% of cases. Lymphadenopathy, if present (10–20% of cases), is generally localized. Bone marrow and CNS involvement is detected in approximately 30% and 15% of cases, respectively, at the time of initial presentation [19].

17.4.1.3 Immunodeficiency-Related Forms

Patients often have signs and symptoms related to the underlying immunodeficiency. Lymph node, bone marrow, and CNS involvement are more common.

17.4.1.4 Leukemic Forms

In L3ALL with CNS involvement, other cranial nerve palsies were described. Blasts are not always detectable in the cerebrospinal fluid (CSF). While anemia is less frequent, thrombocytopenia is present in most patients, and leukocytosis is found in two thirds of the cases but exceeds 50×10^9/l in only 10–20% of the patients. Myelocytes and metamyelocytes are usually found alongside blasts, a rather unusual finding in most other types of acute leukemias.

17.4.2 Diagnosis

Considering the aggressiveness and rapid doubling time of BL, rapid diagnosis is crucial. The latter is based on the pathologic evaluation of involved tissue. Immunophenotyping and cytogenetic identification of c-MYC rearrangement are mandatory. Molecular diagnosis is difficult due to the diverse DNA break sites.

17.4.2.1 Histology

Macroscopically, BL consists of a whitish tumor with necrotic and hemorrhagic foci, compressing and infiltrating adjacent structures. Lymph node

involvement is less frequent and is mainly present in immunodeficiency-related forms.

Microscopically, sheets of cohesive, monomorphic, medium-sized atypical lymphoid cells with basophilic cytoplasm replace normal tissue architecture. Proliferation and apoptotic cell death rates are extremely high (Ki-67+ fraction approaching 100 percent). A classic "starry-sky" pattern is usually observed: the "sky" represented by the background of basophilic tumor cells and the "stars" being the numerous interspersed tangible body macrophages (histiocytes), with a large clear cytoplasm, that have ingested apoptotic cells. At higher power, BL cells have round nuclei with dark nucleoli and resemble the small non-cleaved cells within normal germinal centers of the secondary lymphoid follicle. Macrophages are irregularly shaped with pale nuclei and inconspicuous nucleoli.

Other morphologic variants exist. An important granulomatous reaction might mask the tumor cells in the background. A plasmacytoid appearance, with single centrally placed nucleoli and eccentric cytoplasm, is mostly described in immunodeficient individuals.

17.4.2.2 Immunophenotype

BL cells express surface immunoglobulin of the IgM type and immunoglobulin light chains (kappa more often than lambda), pan-B cell-associated antigens (CD19, CD20, CD22, CD79a), germinal center-associated markers (CD10 and BCL6), as well as HLA-DR and CD43. They lack expression of CD5, B-cell leukemia/lymphoma 2 (BCL2), TdT, and CD23. BCL6 protein staining is in a nuclear pattern and independent of BCL6 gene rearrangement [48].

CD21, the EBV/C3d receptor, is expressed in EBV-positive disease. Adhesion molecules LFA-1 (CD11a/CD18), p150/95 (CD11c), and CD44 are usually absent.

CD5-positive, CD10-negative, and BCL2-positive variants were reported. MYC and BCL6 rearrangements should be sought out in tumors with high BCL2 expression, to rule out diffuse large B-cell lymphoma (DLBCL). Moreover, DLBCLs often lack the expression of CD 10 and have a lower proliferative index (Ki-67 less than 90%).

In regard to the leukemic form, a high correlation was found between L3 morphology and the presence of surface Ig although cases of morphologically L1 or L2 ALL with surface Ig, and cases of morphologically L3ALL without surface Ig have been described, both in children and adults [49–52]. Hoelzer et al. [50] reported poor outcomes in patients with surface Ig and L1 or L2 morphology; this form is probably a subtype of ALL different from L3ALL that requires other therapeutic approaches. Kantarjian et al. [53] also described L3 morphology in only 11 of their 18 cases of mature B-cell ALL.

17.4.2.3 Cytogenetics

Translocation (8;14), between the long arm of chromosome 8, the site of the *MYC* oncogene (8q24), and the immunoglobulin heavy chain gene on chromosome 14 is the most frequent translocation observed in 80% of BLs. Translocation t(2;8) and translocation t(8;22) between 8q24 and kappa light chain on chromosome 2 and lambda light chain on chromosome 22, respectively, are much less frequent. Rearrangements involving *MYC* can be detected both by routine cytogenetics and by FISH using a *MYC* break apart probe that employs two different fluorescent colors which hybridize to both ends of the gene [35]. A few studies reported lack of *MYC* rearrangements in up to 5% of tumors with features typical of BL [54, 55]. Diagnosis of BL in the absence of *MYC* rearrangement is not recommended in the 2016 World Health Organization classification of lymphoid neoplasms [1]. Many of the cases that were previously categorized as BL in the absence of a *MYC* rearrangement are better classified as the new provisional entity "Burkitt-like lymphoma with 11q aberration."

Conventional cytogenetic analysis also revealed additional chromosomal abnormalities that lack prognostic values in 30–40% of patients [56]. Onciu et al. reported additional abnormalities in 81 and 73% of the children and adults, respectively. Of the most commonly observed abnormalities involving chromosomes 1, 6, 13, 17, and 22, only those of chromosome 17 were associated with a poor prognosis in adults [57].

Comparative genomic hybridization (CGH) analysis also showed that L3ALL had higher numbers of cytogenetic changes than BLs, including a high level of genetic amplification [58].

17.4.2.4 Molecular Biology

Two large studies using gene expression profiling (GEP) described a characteristic molecular signature discerning between BL and DLBCL [35, 55]. New approaches such as Nanostring analysis that facilitate the detection of RNA signature might render the use of GEP more attractive in the future for the diagnosis of BL [59].

Next-generation sequencing studies of BL identified mutations in the transcription factor TCF3 (10–25% of cases) or a negative regulator of TCF3, ID3 (35–58% of cases) [38]. Most TCF3 mutations are gain-of-function mutations that block ID3 binding to TCF3. They are rarely found in DLBCL and other B-cell lymphomas, suggesting that TCF3 gain of function is a defining lesion that could be used to diagnose BL in the coming years.

17.4.3 Staging and Pretreatment Evaluation

Zeigler and Magrath developed the earliest staging system for BL in 1974. Nowadays, staging is performed according to the Ann Arbor, or more often the St. Jude (Murphy) systems [19]. As per the Lugano criteria, being a 100% fluorodeoxyglucose-avid lymphoma, current staging of BL must be based on fluorodeoxyglucose positron emission tomography (FDG-PET) scans [60]. CT scans of the neck, chest, abdomen, and pelvis are an acceptable alternative. Unilateral bone marrow aspiration and biopsy are recommended. Lumbar puncture with assessment of CSF by cytology and flow cytometry is also advised.

Pretreatment evaluation should include:

- Complete blood count with differential, renal function, electrolytes, uric acid, and LDH levels.
- Hepatitis B, hepatitis C, and HIV serologies.
- Assessment of cardiac function with echocardiogram or MUGA before the administration of anthracyclines.
- Fertility counselling: while sperm banking for men is performed rapidly, options for women are limited given the urgent need to start treatment.

In case of spontaneous tumor lysis with renal failure at diagnosis, ICU admission and initiation of hemodialysis should be considered before starting treatment, to prevent the high mortality risk associated with worsening of TLS upon initiation of chemotherapy.

17.4.4 Prognosis

Two-year survival rates of 80–90% are reported in prospective trials using modern regimens. However, these excellent rates likely overestimate those achieved in clinical practice, where patients are less “fit.”

In 2013, Costa et al. published survival data from the Surveillance Epidemiology and End Results (SEER) database that included 3691 cases of BL diagnosed between 1973 and 2008. The estimated 5-year survival rate improved from 41% to 54% in patients diagnosed from 1973 to 2001, and from to 2002 to 2008, respectively. Survival decreased with age (87%, 60%, 48%, and 33% for patients aged ≤19 years, 20–39 years, 40–59 years, and ≥60 years, respectively), and advanced stage (hazard ratio 1.90; 95% CI 1.65–2.19) [61].

Other negative prognostic factors include CNS involvement, bone marrow infiltration, presence of t(14;18), del 13q, abnormalities in 1q and 7q, lack of early response to chemotherapy and absence of complete remission (CR) at the end of the treatment [58, 62–65].

HIV-infected patients carry a poorer prognosis that mostly results from their underlying fragility as well as the use of non-optimal doses of chemotherapy [66]. Several studies showed that relapse-free survival (RFS) is identical in HIV-negative and HIV-positive patients after achieving complete remission. Recent antiretroviral

therapies might improve outcomes in this population.

A fairly large number of small series of L3ALL (with 3–10 patients) treated with conventional ALL regimens have been reported, and their results have been uniformly poor with CR rates of only 30–50% and most patients subsequently relapsing in the CNS [56]. Early death from TLS was common. Outcomes in children dramatically improved with the new regimens developed by several groups, especially the St. Jude's group, the German Berlin, Frankfurt, Munster (BFM) group, and the French Société Française d'Oncologie Pédiatrique (SFOP). In L3ALL patients treated "optimally," poor prognostic factors include poor performance status at diagnosis [67], older age, high white blood cell (WBC) counts (>50 × 10^9/l) and hemoglobin <8 g/dl [50]. Elevated LDH levels had borderline significance [50]. CNS infiltration was not associated with poorer prognosis in the SFOP 86 trial, which incorporated higher dose methotrexate (MTX) (8 g/m^2), high-dose cytarabine (Ara C) 3 g/m^2, and cranial irradiation at 24 Gy [68]. In opposite to most other hematological malignancies, p53 mutations have no prognostic value in BL or L3ALL [69].

17.5 Treatment

17.5.1 BL/L3ALL in Children

Initially used in children with BL/L3ALL, classical ALL or lymphoma regimens combining moderate doses of cyclophosphamide (CPM), anthracyclines, vincristine (VCR),and prednisone with CNS prophylaxis failed to achieve CR in advanced disease [68, 70].

The St. Jude's group, French SFOP, and German BFM group succeeded in improving survival rates in children with BL. They initiated treatment with a "pre phase" combining low doses of steroids and chemotherapy (CPM, VCR) to prevent the onset of potentially lethal tumor lysis syndrome. Urate oxidase was given concomitantly. High-dose chemotherapy was started a week later. Treatment protocols consisted of fractionated high doses of CPM (or ifosfamide), intermediate or high dose (HD) MTX and Ara C, and teniposide or etoposide in addition to doxorubicin and VCR. CNS treatment was gradually intensified with HD MTX (at 5 g/m^2 in BFM trials and 8 g/m^2 in SFOP trials), a greater number of triple intrathecal injections (with Ara C, MTX, hydrocortisone), consolidation with etoposide and HD Ara C, and cranial irradiation [64, 70, 71]. These new protocols resulted in 70–75% cure rates in children with advanced disease, regardless of CNS infiltration. For instance, the overall event-free survival (EFS) was 89% at 6 years in 266 pediatric patients with BL treated per BFM protocol [71]. Cure rates exceeded 80% when CNS prophylaxis with HD intravenous MTX (8 g/m^2) and intrathecal chemotherapy was used in the LMB89 protocol [64] (Figs. 17.1, 17.2, 17.3, and 17.4).

17.5.2 General Therapeutic Strategy in Adults

BL/L3ALL is an aggressive disease requiring chemotherapy in all disease stages. Radiotherapy has a very limited role, limited mainly to cases of spinal cord compression or testicular involvement. Similarly, as response to chemotherapy is rapid and disease is inevitably disseminated, there is no role for surgery even in localized disease. Surgical resection of residual masses is not beneficial.

The approaches that showed improved results in children were rapidly proposed to adults. For instance, the SFOP and BFM protocols were applied to adults, with no or minor modifications. The standard of care in BL remains undefined; however, all groups share the same following principles:

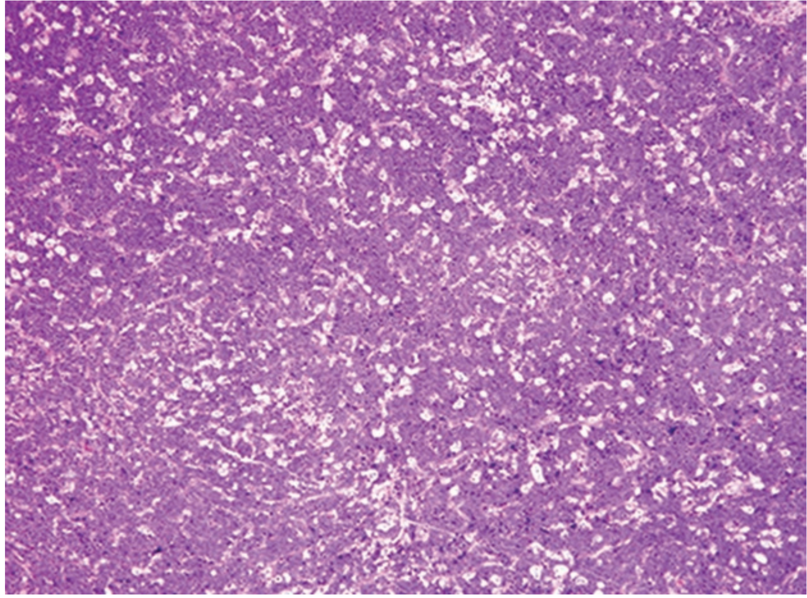

Fig. 17.1 Low-power magnification with the characteristic "starry sky" pattern. (Reproduced with permission from Ioachim HL, Medeiros LJ. Burkitt lymphoma. In: Ioachim's lymph node pathology, 4th edition, Lippincott Williams & Wilkins, Philadelphia 2009)

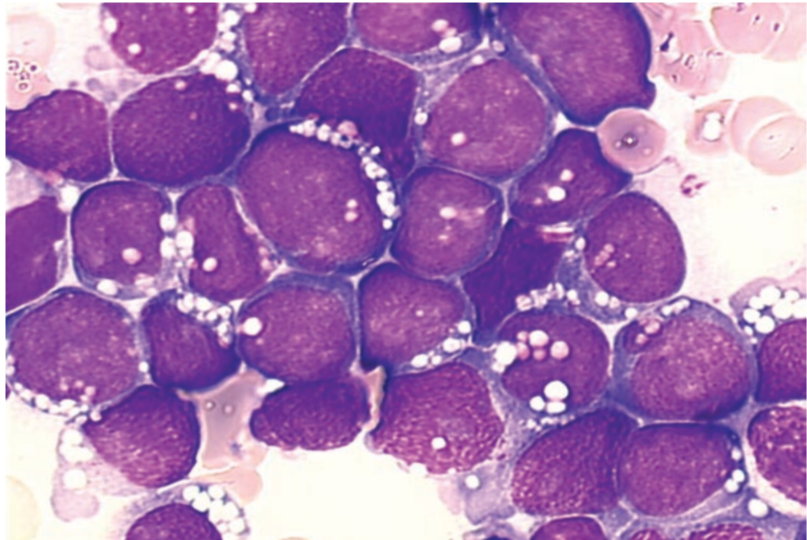

Fig. 17.2 BL cells, with basophilic cytoplasm, round nuclei with coarse chromatin, multiple nucleoli, and several cytoplasmic vacuoles. (Reproduced with permission from Ioachim HL, Medeiros LJ. Burkitt lymphoma. In: Ioachim's lymph node pathology, 4th edition, Lippincott Williams & Wilkins, Philadelphia 2009)

- Referral to expert centers increases remission rates and limits complications.
- Patients should be enrolled in clinical trials whenever possible.
- In case of high tumor burden at diagnosis, a "pre phase" induction therapy with low doses of steroids and chemotherapy reduces the risk of fatal TLS.
- Aggressive CNS-oriented treatment using intravenous (high-dose) as well as regular intrathecal MTX and cytarabine is recommended. With the incorporation of CNS prophylaxis, CNS relapse rates drop from 30–50% to approximately 6–11% [72–74]. Prophylactic whole-brain radiation is no longer used due to its long-term toxicity.
- Systemic treatment must be initiated rapidly and consists of a short intensive course of high-dose, multi-agent cytotoxic chemotherapy. Dose reductions should be avoided.
- Given the high proliferative rate of these tumors, chemotherapy must be re-

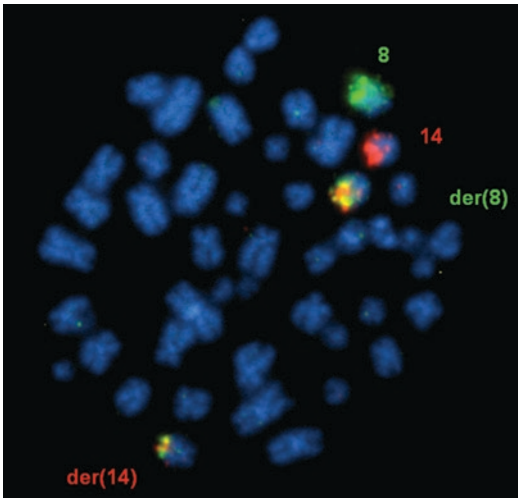

Fig. 17.3 Fluorescence in situ hybridization showing t(8;14), a hallmark of BL

administrated upon hematologic recovery rather than at a predefined schedule.

– TLS should be anticipated with ICU admission and "preventive" hemodialysis of patients presenting with renal failure and high uric acid levels. Rasburicase administration and aggressive hydration are mandatory.
– Rituximab is a standard of care and should be added to all treatment regimens [65].
– As most relapses occur within 1 year of diagnosis, prolonged maintenance treatment is not needed.

Toxicity remains one the major challenges in the treatment of BL. Unlike children and young adults who generally tolerate intensive therapy, older and/or immunosuppressed patients develop

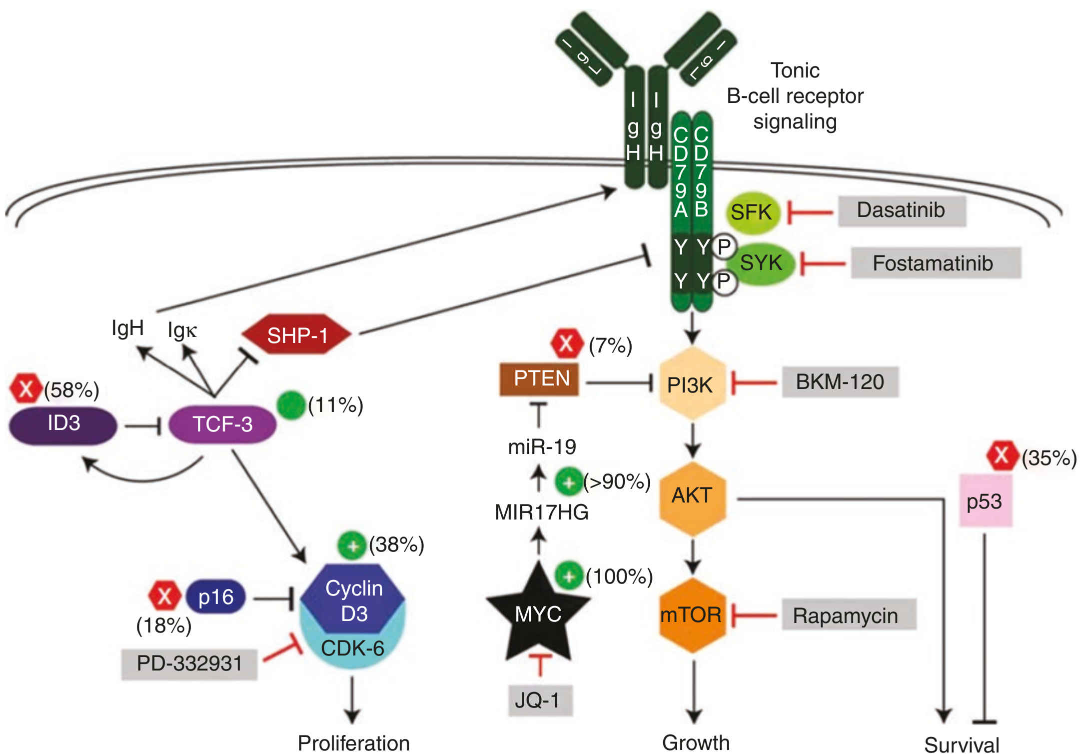

Fig. 17.4 Recurrent oncogenic pathways in BL. Gain-of-function mutations are indicated by + signs and loss-of-function mutations by X signs. Potential drugs to block these pathways are highlighted in gray. (From Schmitz et al. [29])

more treatment-related side effects. Dose reductions are often necessary and affect response rates. These patients, who are usually excluded from clinical trials, have reduced quality of life and shortened survival (Tables 17.1, 17.2, 17.3, and 17.4).

Table 17.1 Clinical presentations of BL/L3ALL

	Endemic	Sporadic	Immunodeficiency-related
Incidence	3–6 per 10^5 children/year	2–3 per 10^6 subjects/year 30% of pediatric NHLs 1% of adult NHLs	30 % of HIV associated NHL, CD4+ >0.2 × 10^9/l
Age	Children > adults Mainly males 4–7 years	Children > adults	Adults
Localization	Extranodal	Extranodal	Frequently extranodal (gut, bone marrow)
	Jaw (50%)	Mainly the gut Jaw (25%)	
Bone marrow infiltration	<10 %	30%	20–60%
CNS involvement	30–40%	15%	20–30%
EBV	>90%	20%	20–40%
c-Myc	Identical in all types	80% t(8;14),	15% t(2;8), 5% t(8;22)

Table 17.2 Diagnosis and management of laboratory and clinical tumor lysis syndrome

Metabolic abnormality	Laboratory TLS[a]	Clinical TLS[b]	Prevention	Management
Hyperuricemia	Uric acid >8.0 mg/dl (475.8 μmol/l) in adults or above the upper limit of the normal range for age in children	Acute kidney injury	(1) Intravenous hyperhydration (2500–3000 ml/m^2 per day in the patients at highest risk) (2) Loop diuretic agent (furosemide) after optimal state of hydration, to achieve a target urine output of at least 2 ml/kg/h (3) Allopurinol or rasburicase[e] (4) Avoid urinary alkalization[f]	– Hyperhydration – Rasburicase – Hemodialysis
Hyperkalemia	Potassium >6.0 mmol/l	Cardiac dysrhythmia or death		– Oral sodium polystyrene sulfonate, insulin and glucose, beta-agonists – Calcium gluconate and cardiac monitoring – Low potassium intake – Frequent monitoring – Hemodialysis
Hyper-phosphatemia	Phosphorus >4.5 mg/dl (1.5 mmol/l) in adults or >6.5 mg/dl (2.1 mmol/l) in children	Cardiac dysrhythmia or death		– Phosphate binders – Limit phosphorus intake – Hemodialysis[c]
Hypocalcemia	Corrected calcium <7.0 mg/dl (1.75 mmol/l) or ionized calcium <1.12 mg/dl (0.3 mmol/l)	Cardiac dysrhythmia or death, seizure, neuromuscular irritability		– Avoid hyperphosphatemia – If symptomatic, calcium supplementation at the lowest dose required to relieve symptoms[d]

Adapted from Cairo and Bishop [75]

[a]Laboratory TLS requires the simultaneous presence of two or more metabolic abnormalities within 3 days before initiation of therapy or up to 7 days afterward

[b]Clinical TLS is defined by the presence of laboratory TLS with clinical manifestations, increased creatinine level, seizures, cardiac dysrhythmia, or death

[c]Continuous venovenous hemodiafiltration more effectively reduces phosphate levels compared with conventional hemodialysis [76]

[d]Excessive calcium supplementation increases the calcium–phosphate product and the rate of calcium phosphate crystallization, particularly if the product is greater than 60 mg^2 per square deciliter

[e]Uric acid may take 2 days or more to decrease with allopurinol. Rasburicase is more effective than allopurinol

[f]Decreases calcium phosphate solubility

Table 17.3 Patients characteristics in selected regimens for adult Burkitt lymphoma/leukemia

Regimen	Author (year)	Number of patients	HIV status	Older patients	Median age, years (range)	Stage III/IV (%)	Risk category	Rituximab
LMBA-02	Ribrag[a] (2016)	260[b]	Negative	Age ≥ 60 years: 23%	47	62% CNS(+): 25%	Group B[c]: 48% Group C[c]: 52%	Yes
GMALL-B-ALL/ NHL2002	Hoelzer[d] (2014)	363	Unknown	Age > 55 years: 27%	42 (16–85)	71% CNS(+): 10% BM(+): 42%	CNS(+)[e]	No
CODOX-M/ IVAC UKLG LY06 Trial	Mead[f] (2002)	52	Negative	Age ≥ 60 years: 0%	35 (16–60)	61%	LR[g]: 23% HR[g]: 77%	No
CODOX-M/ IVAC MRC/NCRI LY10 Trial	Mead[h] (2008)	128 58 BL 70 DLBCL	Negative	Age ≥ 60 years: 10%	37 (17–76)	73% CNS (+): 12% BM(+): 44%	LR[g] 24% HR[g] 76%	No
R-CODOX M/ IVAC AMC 048 Trial	Noy[i] (2015)	34	Positive (100%)	–	42 (19–55)	74% CNS(+): 0%	LR[j]: 6% HR[j]: 94%	Yes
CALGB 9251	Rizzieri[k] (2004)	92[l]	Negative	Age ≥ 60 years: 21%	47 (17–78)	99% CNS(+): 5% BM(+): 63%	Cohort 1[l]: 57% Cohort 2[l]: 43% CNS(+)	No
CALGB 10 002	Rizzieri[m] (2014)	105	Negative	Age ≥ 60 years: 27%	43 (19–79)	49% CNS(+): 14%	CNS(+)[n]	Yes
Hyper-CVAD	Thomas[o] (1999)	26	?	Age ≥ 60 years: 46%	58 (17–79)	CNS(+): 21%	CNS(+)[p]	No
R-Hyper-CVAD	Thomas[q] (2006)	31	Negative[r]	Age ≥ 60 years: 29%	46 (17–77)	CNS(+):7%	CNS(+)[p]	Yes
(R)-Hyper-CVAD	Cortes[s] (2002)	13	Positive (100%)	Age ≥ 35 years: 77%	43 (32–55)	CNS(+):23%	CNS(+)[p]	Yes[s]
DA-EPOCH-R	Dunleavy[t] (2013)	30[u]	Positive (37%)	Age ≥ 40 years: 40%	33 (15–88)	67% CNS(+): 3% BM(+): 13%	LR[v] 17% IR[v] 73% HR[v] 10%	Yes

Table 17.3 (continued)

Regimen	Author (year)	Number of patients	HIV status	Older patients	Median age, years (range)	Stage III/IV (%)	Risk category	Rituximab
RA-DA-EPOCH-R	Dunleavy[w] (2015–ongoing)	77	Positive (26%)	Age ≥ 40 years: 55%	45 (19–78)	64% CNS(+): 10%	LR[x] 14% HR[x] 86%	Yes

[a]Ribrag et al. [65].
[b]Randomized phase 3 study. Patients assigned to two groups: group 1 receiving chemotherapy alone and group 2 receiving chemotherapy with rituximab
[c]Group B: absence of BM or CNS involvement. Group C: Presence of BM or CNS involvement; patients further stratified into five groups according to age and CNS status. Group C patients received MTX 8000 mg/m^2, triple IT injections and enforced consolidation with HD Ara-C and VP-16. Cranial RT was delivered to patients with CNS (+) disease
[d]Hoelzer et al. [77]
[e]CNS(+) patients received more IT injections as well as cranial irradiation. Mediastinal irradiation was recommended in patients with mediastinal tumor (<7.5 cm) at diagnosis
[f]Mead et al. [78]
[g]Low-risk patients had normal LDH, WHO performance status 0–1, Ann Arbor stage I–II and ≤1 extranodal site. All other patients were high risk. LR patients received three cycles of modified CODOX-M while HR patients were given four cycles of alternating modified CODOX-M and IVAC. In LY06 trial, CNS prophylaxis in LR patients included HD IV MTX (6.7 g/m^2) along with IT injections of MTX and Ara-C. HR patients were additionally given HD intravenous Ara-C. In LY-10 trial, MTX dose was reduced to 3 g/m^2 in all patients and 1 g/m^2 in those older than 65 years
[h]Mead et al. [79]
[i]Noy et al. [80]
[j]LR patients were those with stage I disease, <10 cm and normal LDH or intra-abdominal disease only and total resection and normal LDH after surgery. They received three cycles of rituximab and CODOX-M. All other patients were considered HR and received R-CODOX-M/IVAC in an R-CODOX-M/IVAC/R-CODOX-M/IVAC sequence for a total of four cycles
[k]Rizzieri et al. [81]
[l]Patients divided into two cohorts. Cohort 1 included 52 patients receiving IT chemotherapy and cranial irradiation. Cohort 2 included 40 patients who received less IT injections. Cranial irradiation was performed exclusively in HR cohort 2 patients. CNS(+) patients received weekly triple IT injections until CSF clearance then 4 weekly doses followed by cranial RT
[m]Rizzieri et al. [82]
[n]CNS (+) patients received additional triple IT injections twice weekly until CSF clearance then monthly for four treatments, followed by cranial radiation. Those with gonadal disease received RT to the testes during systemic therapy
[o]Thomas et al. [83]
[p]All patients received alternated IT MTX and Ara-C on days 2 and 7 of each course of HD MTX and Ara-C. If there was CNS involvement, IT therapy was increased to twice weekly until CSF clearance. The IT therapy then alternated MTX and Ara-C weekly for four doses (including planned IT days 2 and 7 if course given). The program was then resumed for prophylaxis until completion of chemotherapy. No prophylactic cranial irradiation was administered. Therapeutic RT was given if indicated, e.g., for cranial nerve palsies or intracranial mass
[q]Thomas et al. [84]
[r]Ten patients with HIV-related BL were reported separately
[s]The protocol was modified to include rituximab [85].
[t]Dunleavy et al. [86]
[u]Of the 30 patients, 19 were HIV-negative and were treated with DA-EPOCH-R. Eleven were HIV-positive and received SC-EPOCH-RR (lower-dose short-course combination with a double dose of rituximab)
[v]LR: resected stage I or abdominal stage II cancer. HR: central nervous system involvement, at least 25% blasts in bone marrow, or both characteristics. IR: not in either of the other risk groups
[w]Dunleavy et al. [87]
[x]LR: Normal LDH, ECOG P.S. 0–1, stage I or II disease and maximum tumor size <7 cm. These patients received three cycles without IT prophylaxis. HR patients received six cycles with IT prophylaxis days 1 and 5 on cycles 3–6

Table 17.4 Patient outcomes in selected regimens for adult Burkitt lymphoma/leukemia

Regimen	Author (year)	ORR (%), CR (%)	EFS or PFS (%)	OS (%)	Grade 3–5 toxicities (%)
LMBA-02	Ribrag[a] (2016)	–	75% in the rituximab arm vs. 62% in the control arm at 3 years $p = 0.024$	83% vs. 70% at 3 years $p = 0.011$	Infections: 17% vs. 15% Non-hematologic toxicity: 17% Neurotoxicity: <1% Mucositis: 9%
GMALL-B-ALL/NHL2002	Hoelzer[b] (2014)	CR: 88% 84% in patients older than 55 years	75% 60% in patients older than 55 years	80% 62% in patients older than 55 years	Neutropenia: 58% at cycle A1[c] Infections: 38% at cycle A1[c] Mucositis: 29%
CODOX-M/IVAC UKLG LY06 Trial	Mead[d] (2002)	ORR: 86.5% CR: 76.5% CR in LR: 83% CR in HR: 74%	64.6% at 2 years HR: 59.5% at 2 years	72.8% at 2 years HR: 69.9% at 2 years	Myelosuppression: 100% Mucositis: 42% Thrombocytopenia: 66% Diarrhea: 8%
CODOX-M/IVAC MRC/NCRI LY10 trial	Mead[e] (2008)	–	64% at 2 years 85% LR 49% HR	67% at 2 years 88% LR 52% HR	Neutropenia: 99% Febrile neutropenia: 80% Thrombocytopenia: 86% Mucositis: 45% Neuropathy: 8% Toxic deaths: 8%
R-CODOX-M/IVAC AMC 048	Noy[f] (2015)	–	69% at 1 year	69% at 2 years	All toxicities: 79% Hematologic: 59% Infectious: 41% Metabolic: 18% Mucositis: 0% Toxic deaths: 3%
CALGB 9251	Rizzieri[g] (2004)	ORR 85% CR 74% No significant differences between the two cohorts	52% CH1 vs. 45% CH2 at 3 years	54% vs. 50%	Myelosuppression: 100% Infection: 55% Mucositis: 51% Neuropathy Sensory neuropathy: 9% Motor neuropathy: 18% Toxic deaths: 8%
CALGB 10 002	Rizzieri[h] (2014)	CR 83% Compared to CALGB 9251 improvement in CR EFS OS with addition of Ritux and filgrastim	78% at 2 years	80% at 2 years	Febrile neutropenia or infection: 93% Mucositis: 69% Renal insufficiency: 10% Neurologic: 25% Pulmonary toxicity: 18% Toxic deaths: 7%

Table 17.4 (continued)

Regimen	Author (year)	ORR (%), CR (%)	EFS or PFS (%)	OS (%)	Grade 3–5 toxicities (%)
Hyper-CVAD	Thomas[i] (1999)	CR 81%	–	49% at 3 years	Myelosuppression: 100% Febrile neutropenia: 86% Induction deaths: 19%
R-Hyper-CVAD	Thomas[j] (2006)	CR 86%	80% at 3 years	89% at 3 years	Myelosuppression: 100% No induction deaths
(R)-Hyper-CVAD	Cortes[k] (2002)	CR 92%	–	mOS: 12 m 48% at 2 years	Myelosuppression: 100% Fever/infections: 35% of cycles
DA-EPOCH-R	Dunleavy[l] (2013)	–	95% at 86 months for DA-EPOCH-R 100% at 73 months for SC-EPOCH-RR	100% at 86 months for DA-EPOCH-R 90% at 73 months for SC-EPOCH-RR	Grade 4 neutropenia: 46% of cycles Fever and neutropenia: 19% of cycles Mucositis 5% of cycles No toxic deaths Toxicity lower in the SC-EPOCH-RR group
RA-DA-EPOCH-R	Dunleavy[m] (2015-ongoing)	–	87% at 25 months 84% over 40 No significant difference according to risk, age, and HIV status	88% at 25 months 83% over 40 No significant difference according to risk, age, and HIV status	Same as above Toxic deaths: 7% (infection)

[a] Ribrag et al. [65]
[b] Rates decrease in the following cycles
[c] Hoelzer et al. [77]
[d] Mead et al. [78]
[e] Mead et al. [79]
[f] Noy et al. [80]
[g] Rizzieri et al. [81]
[h] Rizzieri et al. [82]
[i] Thomas et al. [83]
[j] Thomas et al. [84]
[k] Cortes et al. [85]
[l] Dunleavy et al. [86]
[m] Dunleavy et al. [87]

17.5.3 Chemotherapy Protocols

In 2016, Ribrag et al. [65] published the results of a randomized, controlled, open-label, phase 3 trial, of rituximab and chemotherapy (LMB regimen) versus chemotherapy alone in 260 adult patients with BL/L3ALL. With a median follow-up of 38 months, 3-year EFS and overall survival (OS) were significantly improved in the rituximab group without added toxicities.

Apart from rituximab, there is no current standard of care in BL/L3ALL due to the lack of randomized clinical trials and the heterogeneity of single-arm trials. Three main treatment approaches are available.

17.5.3.1 Intensive Short Duration Combination Chemotherapy

Magrath Regimen CODOX-M/IVAC

CODOX-M (CPM, VCR, doxorubicin, and HD-MTX) with IVAC (ifosfamide, Ara C, etoposide, and intrathecal MTX), also called the Magrath regimen, is one of the most widely used chemotherapy protocols outside of a clinical trial [78, 79, 88]. Developed in the 1980s, this regimen uses a risk-adapted approach based on disease bulk of 10 cm or greater, elevated LDH, poor performance status, and advanced stage. One case series and three prospective trials have been reported on the use of CODOX-M/IVAC in patients with newly diagnosed BL [78, 79, 88, 89]. Chemotherapy doses were slightly different between the studies. CNS prophylaxis consisted of intrathecal cytarabine and methotrexate. Patients experienced severe toxicities that required prolonged hospitalization, antibiotics and transfusion support. Two-year OS ranged from 67% to 92%, although among adults, the rate was approximately 75%. In a retrospective analysis, addition of rituximab to CODOX-M/IVAC in 40 adult patients, most of them having high-risk disease, resulted in an overall response rate (ORR) of 90% compared to 88% in patients without rituximab (no statistically significant difference). In all patients, progression-free survival (PFS) and OS were 68% and 71%, respectively. Furthermore, significantly fewer relapses were reported among patients receiving rituximab compared with those receiving chemotherapy alone [90].

Lymphome Malin B (LMB) Protocol

The SFOP adopted the already successful risk-adapted LMB89 pediatric regimen in adults with a median age of 33 years and mostly advanced disease. EFS and OS at 2 years were 65% and 70%, respectively [91]. Toxicity was important and treatment-related mortality was significant. As mentioned previously in the only randomized trial by Ribrag et al. [65], addition of rituximab to this regimen significantly improved EFS and OS. Patients were stratified according to severity of disease into group B (no CNS or BM involvement) or group C (all other patients).

The German Adult ALL Group Experience

GMALL-B-ALL/NHL2002 remains the largest published multicenter prospective international trial for BL. Three hundred sixty three patients in 98 centers received six cycles of HD MTX, HD Ara C, CPM, etoposide, ifosfamide, steroids, and intrathecal therapy along with eight doses of rituximab [77]. Doses were reduced in patients older than 55 years. CR rates were excellent at 88%, OS and PFS were 80% and 71% at 5 years, respectively. Patients with CNS involvement received 24 Gy of radiation and those with bulky mediastinal disease received 36 Gy of mediastinal radiation. In patients older than 55 years (27%), OS and PFS were 62% and 60%, respectively.

The Cancer and Leukemia Group B (CALGB) Experience

The CALGB (study 9251) adapted the German adult ALL protocol by replacing teniposide with etoposide [81]. Subsequent studies (CALGB 10002) have evaluated this regimen in combination with rituximab and filgrastim support in 105 adults [82]. CR rates, 4-year EFS and OS were 83%, 74%, and 78%, respectively.

17.5.3.2 ALL-Like Therapy with a Stepwise Induction, Consolidation, and Maintenance Therapy Lasting at Least 2 Years from Diagnosis

Hyper CVAD

Hyper-CVAD is a regimen developed by the MD Anderson Cancer Center for ALL using fractionated CPM, VCR, doxorubicin, and dexamethasone alternating with HD MTX and HD Ara C [53]. Following successful outcomes in ALL, this protocol was tested in BL. In combination with rituximab, R-hyper-CVAD induced CR in 86% of patients. OS at 3 years was 89%, and EFS 80% [84]. No treatment-related deaths were observed despite significant toxicity.

17.5.3.3 Infusional Chemotherapy with Dose-Adjusted EPOCH Plus Rituximab

Dose-adjusted etoposide, VCR, and doxorubicin administered as a 96-h continuous infusion with oral prednisone and bolus dose-escalated CPM is mostly used in AIDS-related BL. Dose adjustments are based on nadir neutrophil counts during the previous cycle. Dunleavy et al. treated 17 patients (median age 25 years) with sporadic BL with DA EPOCH, rituximab and intrathecal chemotherapy for 6–8 cycles. After a median follow-up of 86 months, freedom from progression and OS were 95% (95% CI 75–99%) and 100% (95% CI 82–100%), respectively [86]. These results might be the consequence of prolonged exposure to anthracyclines etoposide and VCR which may inhibit DNA repair and favor apoptosis by enhancing genotoxic stress and impeding microtubule-dependent protein transport. Conversely, the small sample of adults, the small number of patients with central nervous system involvement, and the wide confidence intervals are major limitations. This regimen could be a valid option in older or less fit patients given its good toxicity profile. Nevertheless, larger studies are needed before recommending its use in all patients with sporadic BL. Indeed, the Hemato Oncology Foundation for Adults in the Netherlands (HOVON) group, the United Kingdom Cancer Research group, and the Swiss Group for Clinical Cancer Research (SAKK) are currently conducting a randomized phase 3 study (HOVON 127) comparing R-CODOX-M/IVAC to dose-adjusted R-EPOCH in high-risk BL patients.

17.5.4 Evaluation of Response

Assessment of response should be done 1 month after the completion of planned therapy (or sooner, if refractory disease is suspected). History, physical examination, laboratory studies (CBC, LDH levels, biochemical profile), and post-treatment CT scan are recommended.

17.5.5 Disease Surveillance

There are no randomized data comparing schedules of follow-up. History, physical examination, CBC, serum chemistries, and LDH are recommended every 3–4 months during the first year, every 6 months during the second year, and then annually.

Most relapses occur during the first year after treatment and are usually symptomatic. The benefit of imaging in routine surveillance is therefore uncertain. Younger patients may be at risk for second malignancies; care should be taken to limit exposure to radiation.

17.5.6 Relapsed and Refractory BL

Relapsed disease should be confirmed by biopsy. Patients have an extremely poor prognosis and must be enrolled in clinical trials whenever possible. Best supportive care is a valid option.

In pediatric series, second-line chemotherapy followed by intensification with autologous hematopoietic stem cell transplantation (HSCT) was effective in 40% of the cases. The former series are biased, since they mostly include “fit”

patients who received sub-optimal first-line treatment. Drugs used for salvage chemotherapy include HD MTX and HD Ara C, and/or etoposide and cisplatin.

Outcome of adult BL/L3ALL patients who had PR or relapsed after first-line intensive protocols (SFOP, BFM, or German ALL trials) is dismal [67, 77].

Fit patients who received suboptimal regimens in first-line may respond to dose-intensive regimens.

17.5.7 Role of Hematopoietic Stem Cell Transplantation (HSCT)

In opposite to other aggressive lymphomas with high-risk features at presentation, there is lack of solid data regarding the efficacy of high-dose chemotherapy followed by HSCT both at first CR [67] and relapse. An observational study conducted by the Center for International Blood and Marrow Research (CIBMTR), reported decreased rates of ASCT during 1985 and 2007. At the time, rituximab was not a standard of care; however, a subset of patients achieved long-term disease control with a 5-year OS of 83% and 53% for ASCT at first and second remission, respectively [92].

In patients with relapsed disease, 2-year PFS after autologous SCT is around 30–40%. These relatively good results, however, were mostly reported in selected series of patients that had received sub-optimal first-line therapy, and who were able to reach ASCT. In fact, Cremer et al. [93] showed in a single-center retrospective study that aggressive salvage therapy is ineffective in patients who relapse after induction with a short-intensive chemo-immunotherapy protocol.

Allogeneic SCT may be considered in relapsing patients with a sibling or matched related donor who may not be eligible for or may have previously received an autologous SCT. Five-year PFS is around 27% and treatment-related morbidity is high [92]. Disease status at transplant and chemosensitivity is the most significant prognostic factors [94].

17.6 Treatment for Specific Demographics

17.6.1 HIV-Infected Patients

During an 8-year period, the National Cancer Institute of Italy declared 46 (35%) cases of BL among 131 cases of HIV-associated NHL [13]. As mentioned previously, unlike other types of HIV-associated lymphomas, BL usually develops at a stage when immune functions are still preserved. It is considered an AIDS-defining malignancy. Bone marrow infiltration is found in 20–60% of cases. HIV-infected patients are often excluded from clinical trials; data regarding treatment efficacy and survival are scarce. In HIV-associated BL, chemotherapy results in CR rates of 20–45% and median survival of 3–6 months, approximately [95, 96]. Outcomes are less favorable than in HIV-negative BL, with a CR rate of 40% vs. 65% ($p = 0.03$) [13]. The difference may result from the higher incidence of deaths from opportunistic infections as well as the use of less dose-intensive regimens in immunocompromised individuals.

With the concurrent use of active antiretroviral therapy, HIV-associated BL and sporadic BL treated with dose-intensive protocols have comparable outcomes. In a retrospective study by Wang et al., 14 HIV-positive adults were treated with different protocols. Outcomes were compared with those of 24 HIV-negative patients with BL who had similar characteristics and were treated concomitantly (13 with CODOX-M/IVAC; 11 with other regimens). Of the 8 patients who received CODOX-M/IVAC (Magrath regimen), 5 achieved a CR (63%). The 2-year EFS rate was 60%. Long-term EFS was not adversely affected by HIV status ($p = 0.88$). CODOX-M/IVAC was associated with improved EFS ($p = 0.05$), in all patients, regardless of HIV status. Treatment-related adverse events were equally prevalent in HIV-positive and -negative patients [97]. These results suggest that HIV-infected patients with BL have better outcomes when treated with dose-intensive regimens. Furthermore, the addition of rituximab to modified CODOX-M/IVAC (intensified treatment of CNS disease if present at diagnosis along with

preventive measures against mucositis, hematologic and neurologic toxicities) did not increase toxicity in a population of 34 HIV-positive adults with BL. Estimated PFS at 1 year was 69% and OS at 1 and 2 years was 72% and 69%, respectively [80]. Similarly, HIV-infected patients who received DA-EPOCH-R had favorable outcomes with CR, 2-year PFS and OS rates of 82%, 66%, and 70%, respectively. Treatment-associated deaths occurred in 10% of patients and may be minimized by sequential rather than concurrent administration of rituximab in those with a CD4 count less than 50/μL [98].

As in HIV-negative patient, CNS prophylaxis is mandatory. There are possible interactions between chemotherapy agents and anti-retroviral therapy, particularly with HD methotrexate. Temporary interruption of ART is recommended in this case.

In conclusion, HIV-infected patients with acceptable CD4 counts and without advanced AIDS symptoms should be treated similarly to HIV-negative patients with dose-intensive short chemoimmunotherapy protocols.

17.6.2 The Elderly

Most clinical trials excluded older adults. Of 25 patients over the age of 60 years who received hyper CVAD with or without rituximab, those who tolerated the treatment had a survival benefit comparable to younger patients [84]. Nevertheless, most studies showed a poor tolerance of hyper CVAD in older adults [99].

A comprehensive geriatric assessment is important to assess comorbidities and functional status in the elderly patient. It leads to the elaboration of a "customized" treatment plan.

Treatment options include:

- EPOCH: Less toxic than conventional therapy with promising results in a younger population [86].
- Standard CHOP chemotherapy with rituximab and intrathecal therapy. Two-year progression-free survival is likely less than 30% with this approach [100].

17.6.3 Patients with Cardiac Dysfunction

Anthracyclines are contraindicated in patients with a baseline left ventricular ejection fraction below 30%. Non-anthracycline-containing regimens should be used in this setting. Chemotherapy protocols requiring intravenous fluid hydration may also be difficult to administer. Pegylated liposomal doxorubicin allows higher cumulative doses with equivalent efficacy and less cardiac toxicity.

17.7 Future Perspectives

Despite the improvement in CR and survival rates with the use of intensive chemotherapy, the treatment of BL remains challenging, particularly in special "vulnerable" populations (elderly, immunocompromised patients, etc.) as well as in resource-poor environments where the disease is endemic and access to adequate chemotherapy and supportive care is difficult [47]. Therefore, the development of new treatment protocols that are more affordable and better tolerated is crucial. These new protocols may target the following pathways:

17.7.1 TCF3 Pathway Activation

In opposite to other lymphoma types, mutations in TCF3/ID3 genes are detected in more than two thirds of sporadic and HIV-related BLs and in 40% of EBV-positive cases and may be used for diagnostic purposes in difficult situations. Moreover, TCF3 acts as a lineage-survival oncogene and activates the pro-survival PI3-K pathway in BL. Agents that block this transcription factor are yet to be developed [29].

17.7.2 PI3K Pathway

Preclinical studies showed a potential benefit of blocking the PI3K pathway, an essential pervasive pro-survival mechanism in BL cell lines. The

PI3K-targeting agents used in these studies have proven their efficacy and tolerability in the treatment of other neoplasms. Potential candidates for future clinical trials include:

- BKM-120 that targets all of the catalytic isoforms of PI3K [29].
- Rapamycin or its analogs that may be used to block the mTORC1 kinase complex, activated by PI3K signaling [29].
- Inhibitors of SYC (e.g., fostamatinib) or SRC-family kinases (e.g., dasatinib) that target BCR-proximal kinases and thus bloc PI3K in an important fraction of BL lines [29].

17.7.3 Cyclin-Dependent Kinase Inhibitors

In BL, pairing of cyclin D3 with CDK-6 results in an active kinase. Knockdown of either subunit is toxic for these cells [29]. PD 0332991, a CDK-4/6 inhibitor initially blocks BL cell-cycle progression at the G1–S phase transition, as expected. However, unexpectedly, significant apoptosis is induced by the continuous exposure of BL cells to this agent. The association of increased MYC expression by the t(8;14) translocation with a complete G1 block likely triggers a checkpoint response that results in apoptosis. In BL xenografts, response to PD 0332991 is spectacular with virtual disappearance of tumor cells by day 10 of treatment [29]. Targeting the cyclin D3-CDK-6 appears to be beneficial and should be evaluated in clinical trials.

17.7.4 Targeting MYC Expression

Suppression of MYC remains challenging because of both the diverse mechanisms driving its aberrant expression and the challenge of disrupting protein-DNA interactions.

Bromo and extra-terminal (BET) family inhibitors are small molecules that prevent the binding of the BET family of chromatin adaptors to chromatin, a process usually required for MYC expression. Thus, BL cell lines are killed following the downregulation of MYC expression. Significant antitumor activity of BET inhibitors was observed in xenograft models of BL and acute myeloid leukemia. These preclinical studies support further clinical investigation of BET inhibition in relapsed BL.

Antisense oligonucleotides directed at several different sites of human c-Myc mRNA reduced proliferation of HL-60 and Raji cells in vitro [101]. In a murine model of BL, antisense oligonucleotides delayed tumor onset by 3–6 days and reduced total tumor mass by 40–65%, compared with controls [102].

Human mitochondrial peptide deformylase (HsPDF) and mitochondrial sirtuin SIRT 4 are additional candidates that could be targeted in MYC-overexpressing cancers.

17.7.5 Other Potential Candidates

Additional novel therapeutic agents include:

- Monoclonal antibodies directed at common B-cell antigens such as CD22 and HLA-DR.
- Chimeric antigen receptor (CAR) T-cell therapy against B-cell antigens is particularly effective in childhood ALL and might therefore be beneficial in relapsed/refractory BL.
- Selective serotonin re-uptake inhibitors (SSRIs) cause apoptosis of lymphoma cells without affecting normal germinal center B cells. The underlying mechanism is still unclear but is probably independent of the serotonin transporter [103, 104].
- Proteasome inhibitors may result in apoptosis of BL cell lines [105].
- Blockade of EBV-related viral proteins EBNA-1 and EBNA-2, detected in 20–50% of sporadic and HIV-related BL, limits cell growth and increases apoptosis of EBV-immortalized cells in vitro [106, 107].
- Targeting epigenetic modifications with DNA methyltransferase inhibitors (decitabine or 5-azacytidine) or histone deacetylase inhibitors (depsipeptide, MS-275, or suberoylanilide hydroxamic acid) [108].

17.8 Conclusion

BL is a highly aggressive, chemo-sensitive NHL that requires diagnosis and treatment in expert centers. Nowadays, rapid establishment of a short intensive course of chemoimmunotherapy, with CNS-oriented therapy and aggressive prevention and treatment of tumor lysis syndrome yields excellent outcomes in young “fit” patients. Despite the improvement in CR and survival rates, many challenges remain, particularly in the treatment of certain patient groups such as immunocompromised and elderly individuals as well as the treatment of relapsed/refractory disease. Targeting PI3K pathway, MYC and TCF3 expression, and cell cycle kinases are among many therapeutic options to be tested in future clinical trials.

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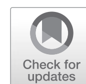

18 Management of Relapsed and Refractory ALL

Ashwin Kishtagari and Anjali S. Advani

18.1 Introduction

Acute lymphoblastic leukemia (ALL) is a malignant transformation and proliferation of lymphoid progenitor cells in the bone marrow, blood, and extramedullary sites. Treatment of ALL in children is one of the great success stories of combination chemotherapy. Unfortunately, adults fare much worse. Most current induction regimens obtain complete responses (CR) in 65–90% of newly diagnosed adult patients with ALL. However, up to 10% of patients will have disease that is refractory to initial treatment, and 40–70% of patients who do achieve CR will ultimately relapse [1]. Relapsed/refractory (R/R) ALL has been associated with a rather dismal prognosis, with 3- and 5-year overall survival (OS) historically reported to be 24% and 10%, respectively, in older studies [2–4]. The prognosis of patients with R/R ALL depends on several parameters, including duration of first remission, response to prior salvage therapy, disease burden at the time of relapse, and age of the patient [3].

Treatment of R/R ALL therefore represents a challenge. Treatment strategies such as variations of chemotherapy as a salvage therapy remain ineffective for many patients. The key therapeutic goal in treating R/R ALL is to induce a CR and for a patient to be able to proceed to hematopoietic stem cell transplant (HSCT), which ultimately remains the only known cure. Multiple advances in our understanding of biology of ALL over the past decade have led to significant breakthroughs in the development of novel immunotherapeutic approaches that hold the promise in improving the outcomes of patients. Table 18.2 and Fig. 18.1 highlight selective novel drugs and targets of interest for R/R ALL, which will be discussed throughout the course of this chapter. We will not discuss Philadelphia chromosome positive (Ph+) ALL and Ph-like ALL in this chapter as they are being discussed in Chaps. 16 and 17 in this book.

18.2 Immunotherapy

In 2017, three groundbreaking immunotherapies (blinatumomab, inotuzumab, and chimeric antigen receptor T cells) targeting various surface antigens on ALL cells for R/R B-ALL were FDA approved based on impressive outcomes observed in clinical trials. These approvals have changed the treatment paradigm for R/R ALL.

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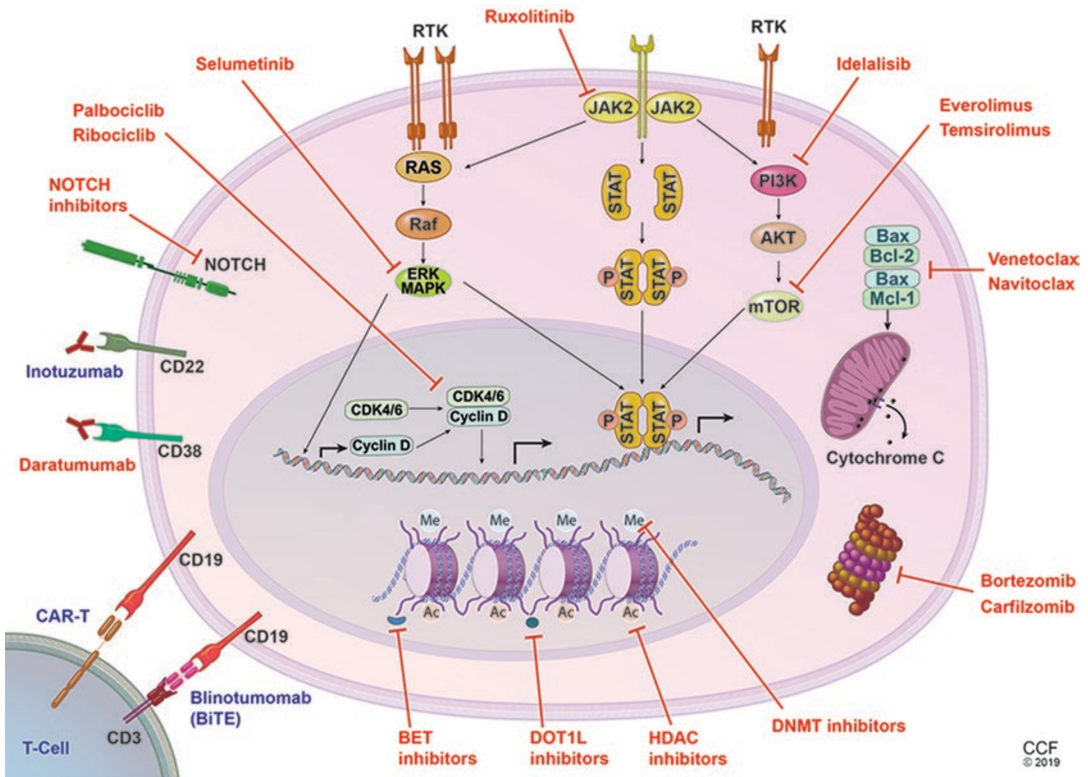

Fig. 18.1 Therapeutic targets and drugs in ALL. Nodes to attack specific cell-surface antigens such as NOTCH, CD22, CD38 and CD19 on B-lymphoblasts. Nodes to modulate B-lymphoblasts epigenetics include chromatin post-translational modifications through DNA methyltransferase (DNMT) inhibitors and histone deacetylase inhibitors (HDACs) as well as transcription factor activation via BET bromodomain inhibitors and DOT1L inhibitors. Nodes to attack protein homeostasis by increasing unfolded protein stress include direct inhibition of the proteasome. Nodes to activate apoptosis at the mitochondrion by inhibition of Bcl-2 and Mcl-1. Nodes to target signal transduction involved in the regulation of key cell proliferation and differentiation pathways such as mTOR, PI3K, JAK2, ERK/MAPK, and CDK4/6. *ALL* Acute lymphoblastic leukemia; *BET* Bromo- and extra-terminal domain; *DOT1L* Disruptor of telomeric silencing 1-like; *Bcl-2* B-cell lymphoma 2; *Mcl-1* Myeloid cell leukemia 1; *mTOR* Mechanistic target of rapamycin; *PI3K* Phosphoinositide 3-kinase; *JAK2* Janus kinase 2; *ERK* Extracellular-signal-regulated kinase; *MAPK* mitogen-activated protein kinase; *CDK* cyclin-dependent kinase

18.3 Blinatumomab

Blinatumomab is a bispecific antibody directed to CD19 (B-cell differentiation antigen) and CD3 (T-cell antigen) receptors. Bivalent binding of CD19 to B-lymphoblasts and CD3 to T cells induces a synapse which leads to release of inflammatory cytokines, production of cytolytic proteins, and proliferation of cytotoxic T cells, resulting in lysis of B-lymphoblasts. This CD19/CD3-bispecific antibody construct is the first T-cell engaging and the first CD19-specific antibody approved by the FDA.

A phase II multicenter single-arm trial of R/R ALL treated 36 patients with blinatumomab in cycles of 4-week continuous infusion followed by a 2-week treatment-free interval with a dose-finding stage and an extension stage [5]. Within two cycles, CR or CR with partial hematologic recovery (CRh) was achieved in 69% (25/36) of patients, with the majority (88%) of responders achieving minimal residual disease (MRD) negative status (<0.001%). Median OS was 9.8 months, and median relapse-free survival (RFS) was 7.6 months [5]. This led to an international, multicenter, phase II single-arm study which enrolled

189 patients with R/R Ph-negative ALL. Eighty-one patients (43%) achieved CR ($n = 63$, 33%) or CRh ($n = 18$, 10%) within two cycles of treatment, most did so after one cycle ($n = 64$) [6]. No difference in CR/CRh rates was observed based on prior salvage therapies, prior allogeneic HSCT, or age. Median OS was 6.1 months and median RFS was 5.9 months. Notably, there was a correlation between tumor burden and response rates: in patients with <50% bone marrow blasts, the rate of CR/CRh was 73% compared to 29% in patients with ≥50% bone marrow blasts [6]. This study led to accelerated FDA approval of blinatumomab for R/R Ph-negative ALL.

Blinatumomab was compared to standard chemotherapy in patients with R/R Ph-negative ALL in a randomized, multicenter phase III TOWER trial [7]. Four hundred and five patients were randomized 2:1 to blinatumomab ($n = 271$) or standard salvage chemotherapy ($n = 134$). Blinatumomab was administered at the standard dose continuously over 4 weeks for up to five cycles, followed by up to 12 months of maintenance. Remission rates favored blinatumomab within 12 weeks after initiation of treatment: CR (34% vs. 16%, $p < 0.001$), CR plus CRh (44% vs. 25%, $p < 0.001$). Median OS significantly improved with blinatumomab (7.7 vs. 4.0 months; $p = 0.01$) at a median follow-up of approximately 12 months [7]. Adverse events (grade 3 or higher) were reported in 87% of patients in the blinatumomab arm and in 92% of the patients in the chemotherapy group. Unique to the blinatumomab arm was the occurrence of the cytokine release syndrome (CRS), reported in 14.2% (≥grade 3 in 5%) of patients receiving blinatumomab [7]. The mechanism of action of blinatumomab generates its unique side effect profile: Cytokine release syndrome and neurological toxicities are thought to be the result of T-cell stimulation, proliferation, and cytokine release.

A single-arm, multicenter, phase II BLAST study evaluated blinatumomab in patients with CR with MRD positivity after intensive chemotherapy. Seventy-eight percent (88/113) of patients achieved MRD negativity after the first cycle of blinatumomab treatment and 67% of patients subsequently proceeded to allogeneic HSCT [8]. This study demonstrated the ability of blinatumomab to eradicate MRD positivity and serve as a bridge to allogeneic HSCT, leading to FDA approval in this setting. Blinatumomab was also evaluated in R/R Ph-positive ALL. A cohort of 45 patients, who were R/R to first- or later-generation tyrosine kinase inhibitors (TKI), were treated in the phase II, single-arm, multicenter ALCANTARA trial [9]. Within two cycles of treatment, 36% (16/45) achieved CR/CRh, including 10 patients with T315I mutations. The majority of responders (14/16, 88%) achieved MRD negativity. Median OS was 7.1 months [9]. Phase II studies evaluating blinatumomab in combination with TKIs, including dasatinib (NCT02143414; NCT02744768) and ponatinib (NCT03263572), are ongoing. Preliminary data demonstrate that blocking PD-1, PD-L1, or CTLA-4 enhances effector T cells, thus improving blinatumomab's activity against B-lymphoblasts. Accordingly, trials of combination immunotherapy (pembrolizumab, nivolumab ± ipilimumab) and blinatumomab are currently ongoing (NCT03160079, NCT03512405, and NCT02879695).

Blinatumomab should be considered in patients with low disease burden (<50% blasts) R/R B-ALL. Suitable candidates should proceed to consolidation with allogeneic HSCT. Blinatumomab has not been well evaluated in R/R ALL patients with active CNS disease due to concerns of neurotoxicity with concurrent intrathecal therapy.

18.4 Inotuzumab Ozogamicin

Inotuzumab ozogamicin is a humanized monoclonal antibody–drug conjugate targeting CD22. It consists of a CD22-targeting immunoglobulin G4 humanized monoclonal antibody conjugated to calicheamicin, a cytotoxic agent that cleaves double-stranded DNA [10]. This drug was initially developed for the treatment of non-Hodgkin lymphoma (NHL), but further development was focused on CD22+ ALL. CD22 is an attractive targeting molecule for an antibody–drug conjugate in ALL: (1) CD22 is a B-cell restricted type I transmembrane protein expressed in >90% of B-ALL. (2) Following ligand binding or antibody crosslink-

ing, CD22 is rapidly internalized, thus making it an ideal target for cytotoxic drug delivery by antibody–drug conjugates [10]. Inotuzumab is currently approved for the treatment of R/R B-ALL by the US FDA and the European Medicines Agency.

Based on the demonstration of inotuzumab's safety and efficacy in lymphoma, Phase I and II trials with single-agent inotuzumab were conducted in R/R ALL with overall remission rates of 58–68% (CR/CR with incomplete count recovery (CRi)) and MRD negative rates of 72–84% [11, 12]. A phase III multicenter, open-label, randomized trial (INO-VATE study) compared inotuzumab to standard of care intensive chemotherapy for R/R CD22+ B-ALL in first or second salvage [13]. Inotuzumab was administered weekly for a total dose of 1.8 mg/m^2 per cycle (0.8 mg/m^2 on day 1 and 0.5 mg/m^2 on days 8 and 15 of a 21-day cycle), reduced to 1.5 mg/m^2 once patients achieved CR or CRi. Patients were allowed to proceed to stem cell transplant at the investigator's discretion. Patients randomized to the inotuzumab treatment arm had a significantly higher CR rate compared to standard chemotherapy (80.7% vs. 29.4%, $p < 0.001$). Of the patients who achieved CR/CRi, inotuzumab had a significantly higher rate of MRD negativity (78.4% vs. 28.1%, $p < 0.001$), and more patients proceeded directly to HSCT (41% vs. 11%) [13]. Remission duration was longer in the inotuzumab arm (median, 4.6 vs. 3.1 months, $p = 0.03$). Median OS was 7.7 months in the inotuzumab group and 6.7 months in the standard chemotherapy group, although the hazards ratio suggested improved OS at 0.77 ($p = 0.04$), likely reflecting a separation of the two survival curves at later time-points (2-year OS, 23% vs. 10%). This difference is likely explained by the greater proportion of patients proceeding to stem cell transplant in the inotuzumab arm. Treatment-related neutropenia, thrombocytopenia, infusion-related reactions, hepatic toxicities, including transaminitis and hyperbilirubinemia, and veno-occlusive disease (VOD) are unique adverse events of inotuzumab. In the phase III trial, the rate of VOD, a potentially fatal condition, was higher in the inotuzumab arm (11% vs. 1%). This complication occurred mainly in patients who undergo or had received a prior stem cell transplant especially if a dual-alkylator conditioning regimen was given [13].

Inotuzumab was evaluated in combination with hyper-fractionated reduced-dose cyclophosphamide, vincristine, dexamethasone (mini-hyper-CVD) in patients with R/R ALL with a median age of 35 years (range, 18–78 years), and the combination produced a CR rate of 78% with a 1 year OS rate of 46% [14]. Multiple clinical trials are currently underway to improve our understanding of how and when to best use inotuzumab: safety and efficacy of using a TKI and inotuzumab concurrently for treatment of relapsed Ph+ ALL (NCT02311998), inotuzumab in combination with intensive chemotherapy in the frontline setting (NCT03150693, NCT03488225), and using inotuzumab to eliminate MRD (NCT03441061).

Inotuzumab is effective in patients with high disease burden (>50% blasts) and can be used in combination with intrathecal therapy for patients with CNS disease.

18.5 CAR-T-Cell Therapy

Genetically engineered T cells expressing a chimeric antigen receptor (CAR-T) targeting specific antigens (CD19) present on B-lymphoblasts have generated promising results in children and adults with R/R disease. Tisagenlecleucel (CTL019) by Novartis, an autologous anti-CD19 CAR-T cell therapy, was recently approved (2017) by the US Food and Drug Administration (FDA) for patients up to the age of 25 years with B-ALL that is refractory or in second or greater relapse.

CARs are engineered molecules which consist of an extracellular binding domain (scFv), a transmembrane domain, a costimulatory domain (either 4-1BB or CD28), and intracellular CD3-ζ signaling domain. In this treatment strategy, a patient's own T cells (autologous) are transduced to express an anti-CD19 CAR that, when reintroduced into the patient, directs specific binding and killing of CD19+ B cells. Prior to CAR-T-cell infusion, patients typically receive chemotherapy in an effort to induce lymphodepletion to enhance CAR-T-cell expansion and persistence

Table 18.1 Selected CAR-T-cell therapy studies in relapsed and/or refractory B-ALL

Group/Reference	CAR design (costimulatory domain/vector)	Median age	Number of patients	Prior Allo-HSCT (%)	CR/CRi (%)	MRD-CR (%)	Allo-HSCT post CAR-T cell therapy (%)	CRS
UPenn/CHOP Maude et al. [17]	4-1BB/ lentivirus	14 (5–60)	30	18 (60)	27 (90)	22 (73)	3 (10)	100% (27% severe)
UPenn/CHOP Global, multicenter Maude et al. [18]	4-1BB/ lentivirus	11 (3–23)	75	46 (61)	61 (81)	61 (81)	8 (11)	40% (13% severe)
MSKCC Park et al. [19]	CD28/ retrovirus	44 (23–74)	53	19 (36)	44 (83)	32 (60)	17 (32)	43% (42% severe)
NCI Lee et al. [20]	CD28/ retrovirus	13 (5–27)	21	8 (38)	14 (67)	12 (57)	10 (48)	43% (5% severe)
FHCRC Turtle et al. [21]	4-1BB/ lentivirus	40 (20–73)	30	11 (37)	29 (97)	25 (83)	13 (43)	50% (50% severe)
FHCRC Gardner et al. [22]	4-1BB/ lentivirus	12 (1–25)	43	28 (65)	41 (95)	41 (95)	11 (26)	93% (23% severe)

MRD– minimal residual disease negative by flow cytometry, *CR* complete remission, *CRi* complete remission with incomplete blood count recovery, *HSCT* hematopoietic stem cell transplant, *CRS* cytokine release syndrome, *MSKCC* Memorial Sloan Kettering Cancer Center, *UPenn* University of Pennsylvania, *CHOP* Children's Hospital of Philadelphia, *NCI* National Cancer Institute, *FHCRC* Fred Hutchinson Cancer Research Center

in vivo [15]. The major studies published on CAR-T-cell therapy in B-ALL are summarized in Table 18.1. Important differences between these studies include different transduction methods, costimulatory domains, and lymphodepleting chemotherapy regimens.

Initial CAR-T-cell clinical trials included a phase I trial in 16 adult patients with R/R B-ALL treated with a CD19 CAR-T with a CD28 costimulatory domain. The remission rate was impressive, at 88%. Some patients underwent a subsequent allogeneic HSCT after CAR-T therapy [16]. Another phase I trial of CD19 CAR-T cell with a 4-1BB costimulatory domain in 30 patients (25 pediatric and 5 adults) with R/R B-ALL reported a 90% CR rate by morphology (73% MRD-negative CR), and prolonged B-cell aplasia in some patients up to 2 years [17]. Durable remissions up to 24 months are correlated with persistence of CAR-T cells.

In a phase II, single-arm, multicenter, global ELIANA study of 75 pediatric and young adult patients with R/R B-cell ALL, tisagenlecleucel (4-1BB costimulatory domain) resulted in an overall response rate (ORR) of 81% (CR 60% and CRi 21%). MRD by flow cytometry was negative in 95% of the responders by day 28 [18]. Most relapses were CD19 negative. With a median follow-up of 13.1 months, the OS at 12 months was 76%, and the median duration of CAR-T cell persistence was 168 days (range 20–617 days). This study illustrated the feasibility of utilizing centralized manufacturing of CAR-T cells to broaden access to CAR-T-cell therapies beyond a few specialized centers [18]. Encouraging results have been obtained with CAR-T cells developed and evaluated by investigators at the Memorial Sloan Kettering Cancer Center (MSKCC) with a CD28 costimulatory domain in a phase I single-center trial in adults with ALL (median age 44 years, range 23–74). Among the 53 adult patients with R/R B-ALL who received the CAR-T cell infusion, the CR rate was 83%, and with a median follow-up of 29 months, median EFS and OS were 6 and 13 months, respectively [19]. Better outcomes were observed in patients with low disease burden (≤5% bone marrow blasts) at the time of CAR-T-cell infusion. Most importantly, CAR-T-cell therapies are effective in treating relapsed B-ALL after allogeneic HSCT, an area of unmet need. It is feasible to collect and manufacture

donor-derived T cells from the recipient and safely infuse without induction of graft versus host disease (GVHD) [17–22].

The main unique adverse events with CAR-T-cell therapy are CRS, B-cell aplasia, and neurologic toxicity. The incidence of CRS across several different CAR-T-cell products for B-ALL are summarized in Table 18.1. The frequency of these side effects correlates with the disease burden and is less likely to occur in patients with ≤5% bone marrow blasts. The assessment and management of toxicities in patient receiving CAR-T-cell therapy is reviewed in Ref. [23]. CRS can present with a variety of symptoms ranging from flu-like symptoms to high fevers which can progress to life-threatening manifestations of severe hypotension, hypoxia, and end-organ damage. Life-threatening manifestations require interventions with anti-IL6R (tocilizumab)-directed therapy; and many trials are now incorporating tocilizumab earlier in the treatment course. Neurologic toxicity associated with CAR-T-cell therapies can also vary from headache, dizziness, memory loss, impaired speech (dysarthria, aphasia), alterations in mental status, seizures, and encephalopathy to coma [24].

Despite impressive long-term data with CAR-T-cell therapies, relapses across all studies remain a limitation of this therapy. Relapse occurs because of poor persistence of CAR-T cells and loss of the targeted CD19 epitope (antigen escape). Minimizing CD19-positive relapses may result from a better understanding of the biology of persistence. To improve CAR-T-cell persistence, a number of methods are being investigated: (1) inclusion of a 4-1BB costimulatory domain as opposed to CD28, (2) selection and separate manufacturing of bulk CD4+ T cells and central memory CD8+ T cells upfront and then administered in a controlled 1:1 ratio to the patient. CARs equipped with 4-1BB costimulatory domains appear to be associated with longer persistence compared to CD28 costimulatory domain CARs [25]. However, 4-1BB containing CARs are also associated with higher rates of CD19-negative relapse. Currently, dual B-cell antigen targeting (e.g., CD19 and CD22), aimed at preventing or treating CD19 antigen escape, is being tested in clinical trials and may result in the next generation of CAR-T-cell therapies [26].

The emergence of antigen loss and escape are frequent causes of resistance to CD19-targeted CAR-T-cell therapy. This has fueled the development of CARs directing alternative B-cell antigens. A first-in-human, phase I, intent-to-treat clinical trial using CD22 targeted CAR-T-cell therapy in 21 pediatric and adult patients with R/R B-ALL, 17 of whom had relapsed after prior anti-CD19-directed immunotherapy [26]. Twelve patients (12/21; 57%) achieved a CR. Dose-dependent activity was observed with improved responses at higher doses. Eleven out of 15 (73%) patients achieved morphologic CR with a dose of $\geq 1 \times 10^6$ CD22 CAR-T cells per kg body weight [26]. The same group demonstrated important preclinical data showing efficacy of a bispecific CD19/CD22 CAR in a murine model that led to initiation of two ongoing phase 1 clinical trials (NCT03330691; NCT03233854) [26].

18.6 Other Therapies

18.6.1 Vincristine Sulfate Liposome Injection (VSLI)

Liposomal vincristine (VSLI) constitutes encapsulating vincristine in a sphingomyelin/cholesterol envelope. This process enhances drug delivery to the target tissues and decreases neurotoxicity by reducing the percentage of free drug in the plasma leading to increased efficacy with acceptable toxicity. In a phase II single-arm, open-label trial of 65 patients with B- or T-ALL with second or greater relapse, who were previously treated with standard vincristine, the CR/CRh rate with VSLI was 20%, with an overall response rate of 35% [27]. Median OS was 4.6 months. VSLI was administered at a dose of 2.25 mg/m^2. It was well tolerated with a side effect profile similar to standard-formulation vincristine. VSLI received accelerated approval from the US FDA in 2012 for the treatment of adults with Ph-ALL in second or greater relapse or whose disease has progressed following at least two or more lines of treatment.

18.6.2 BCL-2 Inhibitors

Dysregulation of the B-cell leukemia/lymphoma-2 (BCL-2) family of proteins of the intrinsic apoptotic pathway can promote cancer and impair responses of malignant cells to therapies. ALL blast cells express higher levels of BCL-2 and BCL-xL than normal B and T cells [28], and therefore, dual inhibition may be beneficial. Venetoclax is a highly selective BCL-2 inhibitor, and navitoclax is an investigational, orally bioavailable small molecule inhibitor of BCL-2, BCL-xL, and BCL-w [29].The addition of navitoclax to venetoclax has demonstrated synergistic effects in preclinical models and might mitigate the dose-limiting thrombocytopenia associated with navitoclax alone [30]. Trials have recently been launched to explore the activity of BCL-2 inhibitors in ALL. These include a phase 1, multicenter, open-label, dose escalation study of venetoclax plus navitoclax as a chemo-sensitizing agent in pediatric and adult patients (aged ≥4 years) with R/R B-and T-ALL (NCT03181126). Patients receive daily oral venetoclax on day 1 and received oral navitoclax on day 3. Treatment continued for two cycles. Investigators could administer chemotherapy (peg-asparaginase, vincristine, and dexamethasone) at their discretion. Preliminary data presented recently showed that of the nine patients treated, five patients achieved a response (CR/CRi/CRp). Of the remaining four patients, one patient had a partial response and three patients had stable disease in this heavily pretreated group [31]. The combination treatment is relatively well tolerated with no grade 4 adverse events reported. Grade 3 or less adverse events include nausea and vomiting, back pain and muscle spasms. Other trials looking at BCL-2 inhibitors include: venetoclax ± chemotherapy in pediatric and young adult patients with R/R ALL (NCT03236857).

18.7 Relapsed and Refractory T-ALL

Survival of patients with newly diagnosed T-cell ALL has significantly improved but survival remains quite poor for those patients who relapse. For adults with T-ALL treated on the E2993/UKALL12 study who achieved a CR, the incidence of relapse at 5 years was 42% [32]. Most T-ALL disease recurrences occur within the first 2 years of diagnosis, and relapsed disease remains very difficult to salvage, with survival rates <7% at 5 years [2]. There is no single standard of care salvage chemotherapy regimen used in treatment of patients with relapsed and refractory T-ALL. Nelarabine and liposomal vincristine are both US Food and Drug Administration (FDA)-approved drugs for the treatment of relapsed and/or refractory T-ALL. The notably minimal armamentarium of molecularly targeted therapies for T-ALL stands in sharp contrast to the remarkable progress that has been made in B-ALL although other avenues are being explored, as mentioned above with BCL-2 inhibitors.

Nelarabine, a purine nucleoside analog, has single-agent activity in T-ALL. It was granted accelerated approval by the US FDA in 2005 for the treatment of patients with R/R T-ALL. Today, nelarabine remains the only therapy approved specifically for R/R T-ALL. In two phase II trials of adult patients with R/R T-ALL or lymphoblastic lymphoma treated with nelarabine monotherapy, the CR rate was 31–36%, with 1-year OS rate of 24–28% [33, 34]. Neurotoxicity, including neuropathy, mental status changes, and seizures, has been reported in up to 18% of patients, but is usually mild and reversible (grade 3 and 4 in ≤5% of patients) [33, 34]. In two small retrospective series, nelarabine was studied in combination with etoposide and cyclophosphamide as a treatment in the salvage setting in R/R T-ALL [35, 36]. Seven pediatric patients (2–19 years of age) with R/R T-ALL were treated sequentially with nelarabine and etoposide/cyclophosphamide, 71% (5/7) patients achieved a CR after receiving 1–2 cycles [35]. All patients in the study experienced neurotoxicity, grade 2–3 sensory and motor neuropathies. This was reversible in most cases. In a study of five adult patients (50–63 years of age) treated with the same regimen, 60% (3/5) achieved CR after 1–2 cycles, and two of these patients were successfully bridged to allogeneic HSCT [36].

Activating mutations in *NOTCH1* were discovered in a majority (60%) of T-ALL cases

15 years ago [37, 38]. Notch signaling plays a crucial role in normal T-cell development, hematopoiesis, and cell growth and proliferation [38]. After ligand binding, Notch receptors undergo a series of cleavages, first by a metalloprotease and subsequently by the γ-secretase complex [39]. After cleavage, the intracellular domain of Notch protein translocates into the nucleus and activates transcription of a variety of genes. Given that Notch signaling is frequently activated in T-ALL, a large number of preclinical studies and clinical trials have investigated the efficacy of targeting Notch in T-ALL. γ-Secretase inhibitors (GSIs) prevent the ability of Notch signaling to activate transcription by blocking intramembrane proteolytic processing of Notch1 by the γ-secretase complex, thereby preventing translocation to the nucleus [40]. Encouraging preclinical data led to the early phase clinical trials of GSIs for R/R T-ALL. Unfortunately, this has not translated successfully into the clinic. These trials were disappointing due to limited anti-leukemic effects and systemic toxicity, namely gastrointestinal toxicity [41]. Current research aims to identify alternative approaches that prevent or overcome resistance to GSIs, inhibit downstream effectors of Notch signaling, and improve the specificity of agents targeting mutant Notch1 [42].

In a recent publication, samples collected from patients enrolled in the COG ALL1231 study of T-ALL were noted to have consistent expression of CD38 at the time of diagnosis, after completion of 1 month of induction chemotherapy, and most importantly at the time of relapse [43]. The study also reported efficacy of daratumumab (a monoclonal antibody which binds to an epitope of CD38) in 14 of 15 T-ALL patient-derived xenografts studied. An international multicenter phase II study is currently evaluating daratumumab in combination with chemotherapy for children and young adults (≤30 years) with relapsed and/or refractory T- or B-cell ALL (NCT03384654).

One member C3 (AKR1C3) of aldo-keto reductase family belongs to a superfamily of oxidoreductases that are broadly expressed in human tissues. AKR1C3 is expressed at high levels in T-ALL [44]. OBI-3424 is a first-in-class novel highly selective small-molecule prodrug converted by AKRC13 to a DNA alkylating agent. This selective mode of activation distinguishes OBI-3424 from traditional alkylating agents. OBI-3424 exerted profound in vivo efficacy against a broad range of T-ALL patient-derived xenografts (PDXs) and significantly reduced leukemia infiltration in the bone marrow [45]. OBI-3424 is being studied in a phase I/II clinical trial in patients with solid tumors, such as hepatocellular carcinoma (HCC) and castrate-resistant prostate cancer (CRPC), which has begun enrollment. A clinical trial in T-ALL is scheduled to begin soon.

The dramatic and promising results of cellular and antibody-based immunotherapies in the B-ALL have generated much interest in the development of targeted immunotherapies for the treatment of T-ALL. It is challenging to target T-cell malignancies using CAR-T cells because of the shared expression of target antigens between CAR-T cells and T-lineage tumor cells [46–50]. In this regard, CAR-Ts against pan T-cell antigens have two major drawbacks: (1) CAR-T cells self-targeting/fratricide and (2) T-cell aplasia, leading to life-threating immunodeficiency. Numerous preclinical studies demonstrated that T cells transduced with CD3, CD5, CD7 or TCR CARs, the most expressed pan-T-cell antigens, efficiently eliminate T-ALL blasts in vitro and are able to control the disease in vivo [46–50], leading to phase I clinical trials with CAR T-cells for T-ALL (NCT03081910, NCT03690011, NCT03590574). Many creative approaches are being evaluated including CRISPR/Cas9 gene editing to prevent the antigen (CD7) expression on the surface of CAR-T cells to overcome the issue of fratricide/self-targeting [48].

There are many other targets and therapies including proteasome inhibitors, CXCR4 inhibitors, CDK 4/6 inhibitors, signal transduction inhibitors, and epigenetic therapies which are currently in development for treatment of R/R B and/or T-ALL. They are not discussed in this chapter extensively given the limited space. They are outlined in Table 18.2 and illustrated in Fig. 18.1.

Table 18.2 Selected emerging and approved therapies for relapsed and/or refractory ALL

Drug class/mechanism (References)	Agent	Patient population and notes	Phase
Monoclonal antibodies			
CD19 [5–9]	Blinatumomab (bispecific T-cell engager)	FDA approved for R/R Ph-negative B-ALL and B-ALL in CR with MRD+ disease	
		R/R B-ALL (blinatumomab in combination with pembrolizumab)	I/II (NCT03160079, NCT03512405)
		R/R B-ALL (blinatumomab in combination with nivolumab ± ipilimumab)	I (NCT02879695)
		R/R B-ALL (blinatumomab in combination with ibrutinib)	II (NCT02997761)
		B-ALL (blinatumomab maintenance following allogenic-HSCT)	II (NCT02807883)
CD22 [11–14]	Inotuzumab	FDA approved for R/R Ph-negative B-ALL	
		R/R and newly diagnosed CD22+ B-ALL (inotuzumab followed by blinatumomab)	II (NCT03739814)
		R/R Ph+ B-ALL (safety and efficacy of combination of bosutinib and inotuzumab)	I/II (NCT02311998)
		B-ALL in CR with MRD+ (tolerability and efficacy if using inotuzumab to eliminate MRD+ disease)	II (NCT03441061)
CD38 [43]	Daratumumab	1–30 years old with R/R T- or B-ALL (daratumumab in combination with chemotherapy)	II (NCT03384654)
Chimeric antigen receptors T cells (CAR-T)			
CD3, CD5, CD7, and T-cell receptor beta (TCR B) [46–50]		≤75 years old with relapsed T-ALL or T-cell lymphoma	I (NCT03081910, NCT03690011, NCT03590574)
CD19 [16–22]	Tisagenlecleucel	(FDA) for patients under 25 years old with refractory or those with second or later relapsed B-ALL	
CD19/CD22 dual-targeted [26]		1–30 years old with R/R CD19+ B-ALL ≥18 years with R/R B-cell malignancies	I (NCT03241940, NCT03330691, NCT03233854)
CD38		12–70 years with relapsed B-ALL after CD19 CAR-T adoptive cellular immunotherapy with CAR-T cells targeting CD38	I/II (NCT03754764)
BH3-mimetics (targeting apoptosis)			
BCL-2 inhibitors [28, 30, 31]	Venetoclax	≥18 years old with R/R T-or B-ALL (venetoclax in combination with liposomal vincristine)	Ib/II (NCT03504644)

(continued)

Table 18.2 (continued)

Drug class/mechanism (References)	Agent	Patient population and notes	Phase
		≤25 years old with R/R malignancies, including T-or B-ALL	I (NCT03236857)
	Navitoclax	≥4 years old with R/R T-or B-ALL (navitoclax in combination with venetoclax and chemotherapy)	I (NCT03181126)
Proteasome			
Proteasome inhibitors	Bortezomib	≥18 years old with R/R B- or T-ALL (bortezomib in combination with chemotherapy)	II (NCT01769209)
	Carfilzomib	1–21 years old with R/R B-or T-ALL (carfilzomib in combination with induction chemotherapy)	Ib (NCT02303821)
Neddylation inhibitors	Pevonedistat	16–39 years old with R/R B-or T-ALL (pevonedistat in combination with induction chemotherapy)	I (NCT03349281)
Chemokine receptors			
CXCR4 inhibitors	BL-8040	≥18 years old with R/R T-ALL (BL-8040 in combination with nelarabine)	IIa (NCT02763384)
IL7-JAK-STAT-CRLF2			
JAK inhibitors	Ruxolitinib	≥10 years with R/R Ph-like ALL (combination of ruxolitinib or dasatinib with chemotherapy)	II (NCT02420717)
		13–75 years old with R/R early T-precursor ALL (ruxolitinib in combination with chemotherapy)	I/II (NCT03613428)
PI3K/AKT/mTOR			
PI3K inhibitors	Idelalisib	≥18 years old with R/R B-ALL or ≥ 65 years old with newly diagnosed B-ALL for whom standard therapies are not recommended	I/II (NCT03742323)
mTOR inhibitors	Everolimus	18 months to 21 years old with relapsed B-or T-ALL (everolimus in combination with chemotherapy)	I (NCT01523977)
	Temsirolimus	1–21 years old with R/R B-or T-ALL (temsirolimus with etoposide and cyclophosphamide)	I (NCT01614197)
MAPK-RAS			
MEK inhibitors	Selumetinib	All ages with R/R B- or T-ALL with RAS pathway mutations (selumetinib in combination with dexamethasone)	I/II (NCT03705507)
Cell cycle regulation			
CDK4/CDK6 inhibitors	Palbociclib	≤21 years old with R/R B-or T-ALL (palbociclib in combination with chemotherapy)	I (NCT03515200)
		≥15 years with R/R B-or T-ALL (palbociclib in combination with dexamethasone)	I (NCT03132454)

Table 18.2 (continued)

Drug class/mechanism (References)	Agent	Patient population and notes	Phase
	Ribociclib	1–30 years old with R/R B-or T-ALL (ribociclib in combination with everolimus and dexamethasone)	I (NCT03740334)
Epigenetic drugs			
DNA methyltransferase 1 (DNMT1) inhibitors	Decitabine and 5-azacitidine		
Histone deacetylase (HDAC) inhibitors	Vorinostat and Romidepsin		
Bromodomain-containing protein 4 (BRD4) inhibitors	Birabresib	≥18 years old with R/R B- or T-ALL	I (NCT01713582)
DOT1-like histone lysine methyltransferase inhibitors	Pinometostat	≥18 years old with R/R B- or T-ALL with rearrangement of the MLL gene	I (NCT01684150)
Cytotoxic therapies			
Antimetabolites [33–36]	Nelarabine	FDA approved for treatment of R/R T-ALL	
Alkylators [44, 45]	OBI-3424 (AKR1C3 inhibitor)	A first-in-class novel highly selective small-molecule prodrug that is converted by AKRC13 enzyme to a DNA alkylating agent	
Vinca alkaloid	Liposomal vincristine (VSLI)	FDA approved for R/R Ph-negative B-ALL	

R/R relapsed and/or refractory, *CD* cluster of differentiation, *CAR* chimeric antigen receptor, *mTOR* mammalian target of rapamycin, *CDK* cyclin-dependent kinase, *AKR1C3* aldo-keto reductase 1c3

18.8 Conclusion

The treatment of ALL is evolving rapidly owing to the increased understanding of the genetic heterogeneity of ALL, which has contributed to the development of numerous novel therapies. Monoclonal antibodies, immunomodulators, CAR-T-cell therapies, and small molecule inhibitors targeting key molecular pathways are exciting additions to the therapeutic armamentarium of ALL. Some of the active agents in the salvage setting are currently being actively investigated for frontline use. Although the efficacy of these therapies is impressive, they are not without toxicity, both physical and financial. Current active and future clinical trials will hopefully guide us in determining how to best incorporate these novel therapies into the existing treatment algorithms to improve the cure rates of R/R ALL.

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