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# Precision Molecular Pathology of Myeloid Neoplasms

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# Precision Molecular Pathology of Myeloid Neoplasms

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# Preface

Our understanding of myeloid neoplasms has been radically transformed by notable molecular developments over the past 10 years. In large part, this rapid advancement specifically has been centered on profound studies based on emerging and newer genetic technologies. Additionally, as our molecular pathology knowledge has grown, the ability to treat diseases with molecularly targeted therapies has become a simple reality. Yet, keeping abreast of all these advancements has become increasingly difficult.

The primary goal of this book is to provide the necessary and foundational molecular and diagnostic knowledge of myeloid neoplasms and further increase the readers' awareness and understanding of specific targeted therapies, where applicable. Critical myeloid neoplasms are covered here in this book and separated into well-defined and organized chapters. Authors are experts with special interest in their relative areas, and important literature and guidelines are consolidated into this comprehensive book. Figures and tables are made accessible, allowing easy access to critical information for diagnoses and understanding of prognosis and treatment.

This textbook serves as a useful resource for clinicians and pathologists who diagnose, treat, and study myeloid neoplasms. The information provided here will not only guide accurate diagnoses, appropriate ancillary molecular tests, and patient management but also vastly stimulate investigative efforts.

Orlando, FL, USA — Chung-Che (Jeff) Chang
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# Chapter 1
# Acute Myeloid Leukemia with Recurrent Genetic Abnormalities: Part I Cytogenetic Abnormalities

**Jenny Hoffmann and Dahui Qin**

## Introduction

Recurrent cytogenetic abnormalities have been used to subtype AML for many years, providing important prognostic information, as well as identifying potential molecular targets to guide therapy. The advent of new technology has generated abundant data, revealing a large number of genetic abnormalities existing in different AML cases. These findings provide ever-increasing evidence for subtyping in AML and include, but are not limited to, t(15;17)( q24.1;q21.2);*PML-RARA*, t(8;21)(q22;q22);*RUNX1-RUNXT1*, inv(16)(p13q22);*CBFB-MYH11*, t(9;11)(p22;q23);*KMT2A-MLLT3*, t(6;9)(p23;q34);*DEK-NUP214*,inv(3)(q21;q26.2);*RPN1-MECOM*,andt(1;22)(p13;q13);*RBM15-MKL1* [1–3] (please see Table 1.1 for a summary). Another example is the *BCR-ABL1* translocation, the disease-defining genetic alteration in chronic myeloid leukemia (CML), which has also been described in acute lymphocytic leukemia (ALL) and was later reported in AML. Now, the evidence indicates that de novo AML with a *BCR-ABL1* translocation should be considered as a provisional category of AML [2]. Many other translocations and inversions have been found in AML, which are sometimes recurring. These recurring cytogenetic abnormalities are less frequent, more often seen in pediatric patients with uncertain prognostic or therapeutic significance. AML with such cytogenetic abnormalities are not included in this category at this time [2]. Some translocations and inversions are seen in therapy-related myeloid neoplasms and are also excluded from this category [1]. Typically, a recurrent cytogenetic abnormality will create a fusion gene encoding a chimeric protein, with one exception that has been recognized: AML with inv(3)(q21.3;q26.2) [2, 4, 5]. This chapter will describe the recurrent genetic abnormalities in AML.

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**Table 1.1** Recurrent cytogenetic abnormalities in AML

| AML subtype | Cytogenetic abnormality | Molecular background | Clinical implications |
|---|---|---|---|
| AML with t(15;17) (q24.1;q21.2) | Forming PML-RARA fusion gene | PML-RARA may repress differentiation and apoptosis | Sensitive to all-trans-retinoic acid (ATRA) treatment |
| AML with t(8;21) (q22;q22) | Forming RUNX1-RUNXT1 fusion gene | RUNX1-RUNXT1 may inhibit transcription | Associated with favorable prognosis |
| AML with inv(16) (p13.1q22) or t(16;16) (p13.1;q22) | Forming CBFB-MYH11 fusion gene | CBFB-MYH11 may inhibit transcription | Associated with favorable prognosis |
| AML with t(9;11) (p22;q23) | Forming MLLT-MLL (KMT2A) fusion gene | MLLT-MLL may affect gene expression | Associated with unfavorable prognosis |
| AML with t(6;9) (p23;q34) | Forming DEK-NUP214 fusion gene | DEK-NUP214 may increase translation | Associated with unfavorable prognosis |
| AML with inv(3) (q21q26.2) or t(3;3) (q21;q26.2) | Bringing a GATA2 distal hematopoietic enhancer (G2DHE) to the vicinity of the EVI1 gene | Causing aberrant EVI1 expression, GATA2 haploinsufficiency, and transcription inhibition | Associated with unfavorable prognosis |
| AML with t(1;22) (p13;q13) | Forming RBM15-MKL1 fusion gene | RBM15-MKL1 may alter epigenetic regulation | Associated with unfavorable prognosis |
| AML with t(9;22) (q34;q11.2) | Forming a BCR-ABL1 fusion gene | BCR-ABL1 may increase cell proliferation | May benefit from TKI therapy |

## Acute Myeloid Leukemia with t(15;17)(q24.1;q21.2);*PML-RARA*

AML with *PML-RARA* is also called acute promyelocytic leukemia (APL) and comprises 5–8% of all AML cases. The key cytogenetic abnormality is formation of the *PML-RARA* fusion gene, most commonly caused by a t(15;17)(q24.1;q21.2), although cryptic and variant translocations also account for a minority of cases [2].

APL presents with myeloblasts and abnormal promyelocytes (which are considered blast equivalents) in the bone marrow and peripheral blood. On occasion, the blast count may be less than 20%, but the presence of a *PML-RARA* is sufficient for the diagnosis of APL. There are two morphologic variants of APL: the hypergranular (classic) and the microgranular variant. The abnormal promyelocytes in the hypergranular variant of APL typically show indented or bilobated "butterfly-shaped" nuclei and intense azurophilic granulation. Single or bundles of Auer rods may also be appreciated (Fig. 1.1). This contrasts with the microgranular variant of APL, in which the abnormal promyelocytes have very small and indistinct azurophilic granules and show

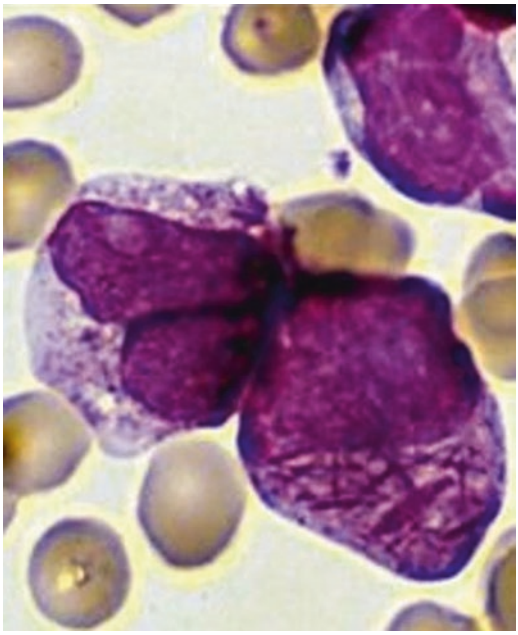

**Fig. 1.1** Acute promyelocytic leukemia, hypergranular variant showing prominent azurophilic granules bilobed nuclei and bundles of Auer rods

predominantly bilobed nuclei. The blast/blast equivalent count in the microgranular variant of APL also tends to be higher than in the hypergranular variant of APL. The azurophilic granules in both types are positive for myeloperoxidase (MPO) [1].

The flow immunophenotype of APL is also characteristic and often aids in the diagnosis, especially in cases in which the morphologic features are less developed. The hypergranular variant of APL is classically CD34 and HLA-DR negative, shows high side scatter (where granulocytes typically are seen) and bright MPO, and will often show dim CD64 expression, without CD16 expression. The microgranular variant of APL, on the other hand, frequently expresses CD34 and occasionally HLA-DR (usually subset of neoplastic cells and week), shows a lower side scatter than the hypergranular variant of APL (residing in the normal blast area on the CD45/SSC plots), and frequently shows dim CD2 expression.

## Cytogenetic Abnormality

At the genome level, APL with *PML-RARA* is characterized by a t(15;17)(q24.1;q21.2) translocation (Fig. 1.2) [1]. The breakpoint in the *RARA* gene is within intron 2. The breakpoints in the *PML* gene can occur at three different locations resulting in different sized *PML-RARA* transcripts. Breakpoint 1 (BCR1) at intron 6 will result in a long transcript; breakpoint 2 (BCR2) at exon 6 will result in variable transcripts, which can be of different sizes in different patients; and

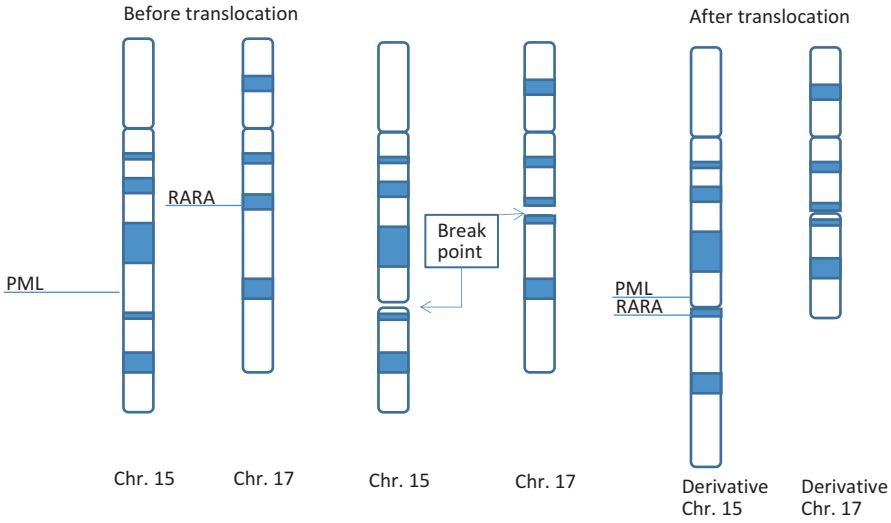


**Fig. 1.2** APL with PML-RARA is characterized by a t(15;17)(q24.1;q21.2) translocation

breakpoint 3 (BCR3) at intron 3 will result in short transcript [6, 7]. As a result, a nuclear regulatory gene (promyelocytic leukemia or *PML* gene) on 15q24.1 is translocated to the vicinity of the retinoic acid receptor alpha gene on 17q21.2, forming a *PML-RARA* fusion gene.

The translocation can be detected by karyotyping and FISH assay. Different FISH assay designs have been used. One assay is called dual-color dual-fusion fluorescence FISH assay [8]. In this assay, two probes are designed to hybridize to *PML* at 15q24 and *RARA* at 17q21, respectively, overlapping the breakpoints on each chromosome (Fig. 1.3). The two probes can be labeled with different fluorescent dyes. For example, the probe specific for *PML* at 15q24 can be labeled with red fluorescent dye and the probe for *RARA* at 17q21 green. A normal cell will show two red and two green dots (Fig. 1.3). A cell with t(15;17)(q24.1;q21.2) will show one red, one green, and two yellow dots (Fig. 1.3). This assay works very well in detecting t(15;17)(q24.1;q21.2) *PML-RARA* translocation. This translocation is the most common change found in APL with *PML-RARA*. However, the *RARA* gene has been found to have other translocation partner genes in some cases. Such translocations are called variant *RARA* translocations. These variants include t(5;17)(q35;q21) *NPM1-RAR*A [9], t(11;17)(q23;q21) *PLZF* (also known as *ZBTB16)-RARA* [10, 11], t(11;17)(q13;q21) *NUMA-RARA* [12], t(4;17)(q12;q21) *FIP1L1-RARA* [13, 14], t(2;17)(q32;q21) *OBFC2A-RARA* [15], t(7;17)(q11;q21) *GTF2I-RARA* [16], t(1;**17**)(q42;q21) *IRF2BP2-RARA* [17, 18], der(17) with duplication of 17q21.3-q23 *STAT5b-RARA* [19], and der(17) *PRKAR1A-RARA* with del(17)(q21) [20]. Generally speaking, the dual-color dual-fusion FISH assay does not work well for these variants. Therefore, a *RARA* dual-color break-apart FISH assay has been designed to address this issue. This assay uses dual-color probe, red and green. The red part of the probe hybridizes to *RARA* centromeric to the breaking point and the green part of the probe telemetric to the breaking point (Fig. 1.4). A normal interphase cell will

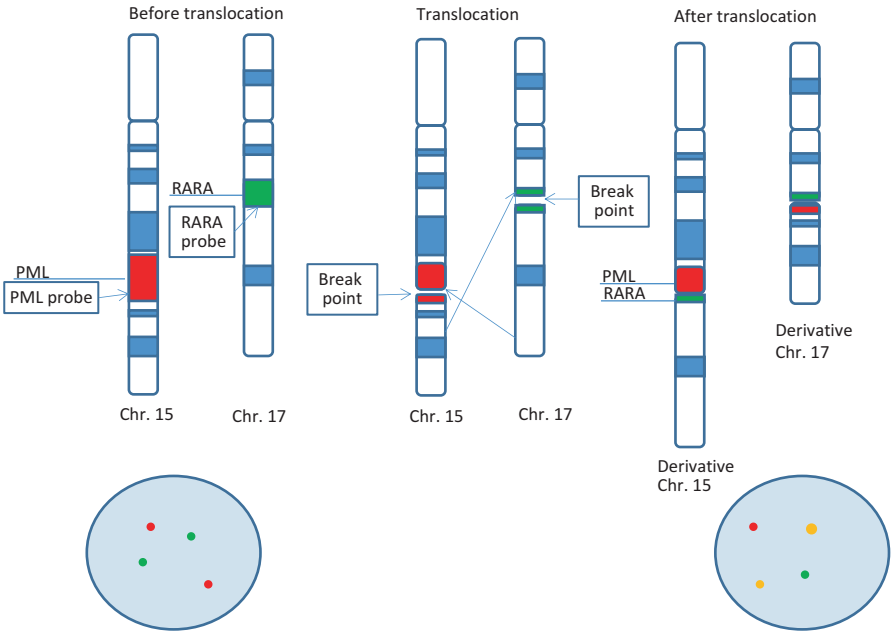


**Fig. 1.3** Two probes are designed to hybridize to PML at 15q24 and RARA at 17q21, respectively, overlapping the breakpoints on each chromosome. The two probes can be labeled with different fluorescent dyes. For example, the probe specific for PML at 15q24 can be labeled with red fluorescent dye and the probe for RARA at 17q21 green. A normal cell will show two red and two green dots. A cell with t(15;17)(q24.1;q21.2) will show one red, one green, and two yellow dots

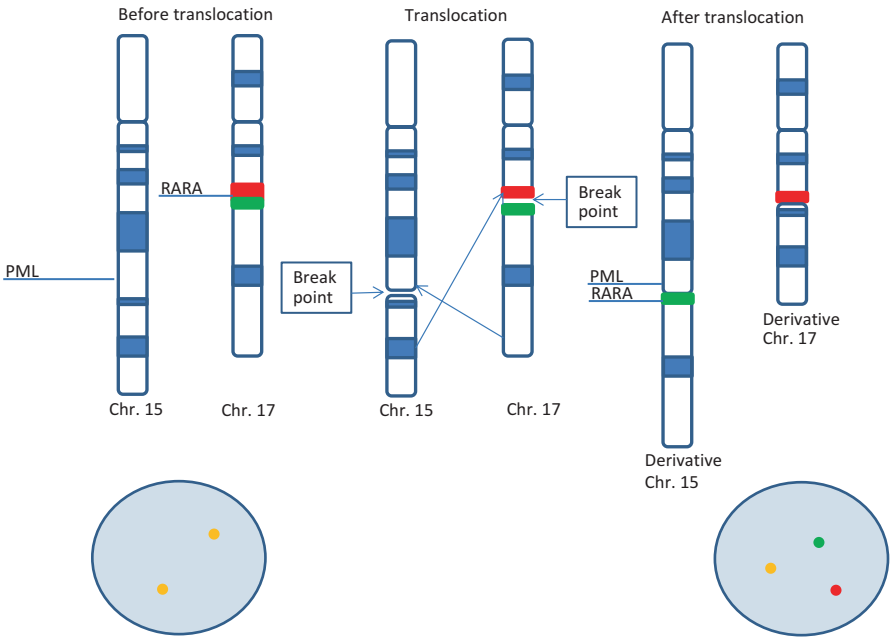


**Fig. 1.4** A normal interphase cell will show two yellow dots. A cell with translocation will show one green, one red and one yellow dots

show two yellow dots (Fig. 1.4). An interphase cell with *RARA* translocation, regardless of the partner genes, will show one yellow, one red, and one green dot (Fig. 1.4). This assay works better than dual-color dual-fusion assay for different *RARA* translocation variants. In rare cases, cryptic *PML-RARA* translocations cannot be detected by karyotyping or FISH assay. For such cases, RT-PCR assay can be used to detect translocations [21, 22]. RT-PCR assay can also be used to detect minimal residual disease (MRD) with *PML-RARA* [23]. Different RT-PCR designs have been used to improve the performance [7, 24]. In general, RNA is extracted from patients' blood or bone marrow samples. Random hexamer primers are used to make cDNA. The target in cDNA is amplified using different primer pairs flanking fusion site of *PML-RARA* [23].

## Somatic Gene Mutations

Somatic gene mutations in *FLT3* are relatively common (50% of cases) with the *FLT3*-ITD being most common (30–40%) and the *FLT3*-D835 mutation occurring in approximately 10% of cases. However, the prognostic significance of *FLT3* mutation remains unclear [25, 26].

Other gene mutations including alterations in *KIT* and *RAS* genes have been described relatively rare (generally reported frequency of 5% or less) [27].

## Clinical Significance

Clinically, patients with APL often develop disseminated intravascular coagulation (DIC), which may result in rapidly unfavorable consequences (e.g., intracranial hemorrhage). However, APL is generally sensitive to the treatment using all-trans-retinoic acid (ATRA). Therefore, early diagnosis of APL is critical for improving patient outcome. Morphologic and immunophenotypic evaluation, in combination with FISH and PCR assays, plays an important role in patient care. Resistance to ATRA can occur due to genetic mutations in the retinoic acid receptor alpha (RARA) ligand-binding domain or the PML-B2 domain of PML-RARA. Additionally, cases of APL with variant RARA translocations may exhibit ATRA resistance [28].

## Acute Myeloid Leukemia with t(8;21)(q22;q22);*RUNX1-RUNX1T1*

AML with t(8;21)(q22;q22);*RUNX1-RUNX1T1* is one of the AMLs with recurrent genetic abnormalities, first described in 1973 [29, 30], and comprises approximately 5% of all AML cases. The key cytogenetic abnormality is a *RUNX1-RUNX1T1* fusion gene [1]. *RUNX1* (runt-related transcription factor 1) is also known as acute myeloid

leukemia 1 gene (*AML1*), polyomavirus enhancer binding protein 2 subunit a (*PEBP2a*), and core-binding factor subunit A2 (*CBFA2*) [29]. *RUNX1T1* (runt-related transcription factor 1 translocated to 1) is also known as *ETO* (eight twenty one).

Morphologically, AML with t(8;21)(q22;q22) typically shows large myeloblasts with abundant basophilic cytoplasm, perinuclear hofs, and azurophilic granules, some of which coalesce into larger salmon-colored granules (pseudo-Chédiak-Higashi granules) (Fig. 1.5). In rare cases, the blasts may be less than 20%, which should not invalidate the diagnosis of AML with t(8;21)(q22;q22). Apart from myeloid blasts, the granulocytic lineage may show features of dysplasia, including hypogranulation or irregular lobation. Dysplasia in other lineages is not typically seen. Eosinophilic precursors are often increased, and occasionally basophils and mast cells are present in excess. [1, 30, 31].

By immunophenotypic evaluation, AML with t(8;21)(q22;q22)is positive for CD34, HLA-DR, MPO, and CD13, with dim CD33. Of note, these leukemias can often express lymphoid markers, including CD19, CD79a, PAX5, and on occasion dim Tdt. A diagnosis of biphenotypic leukemia should not be made in this setting.

## Cytogenetic Abnormality

At the genome level, AML with t(8;21)(q22;q22) is characterized by a chromosome 8 and 21 translocation (Fig. 1.6) resulting in a *RUNX1-RUNX1T1* fusion gene [1]. The *RUNX1* gene is on chromosome 21 and *RUNX1T1* gene on chromosome 8. The breakpoint in the *RUNX1T1* gene is within an intron at the 5′ end of *RUNX1T1*. The breakpoint in the *RUNX1* gene occurs between the exon 5 and 6. Therefore, the fusion gene contains the N-terminal 177 amino acids of *RUNX1*, which is fused in frame to nearly full-length *RUNX1T1* protein [6, 32]. The fusion gene resides on derivative chromosome 8 after the translocation [6] (Fig. 1.6).

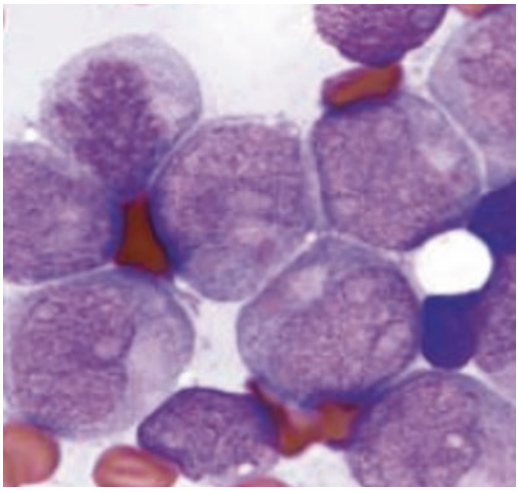

**Fig. 1.5** Acute myeloid leukemia with t(8;21) showing basophilic cytoplasm and paranuclear hofs

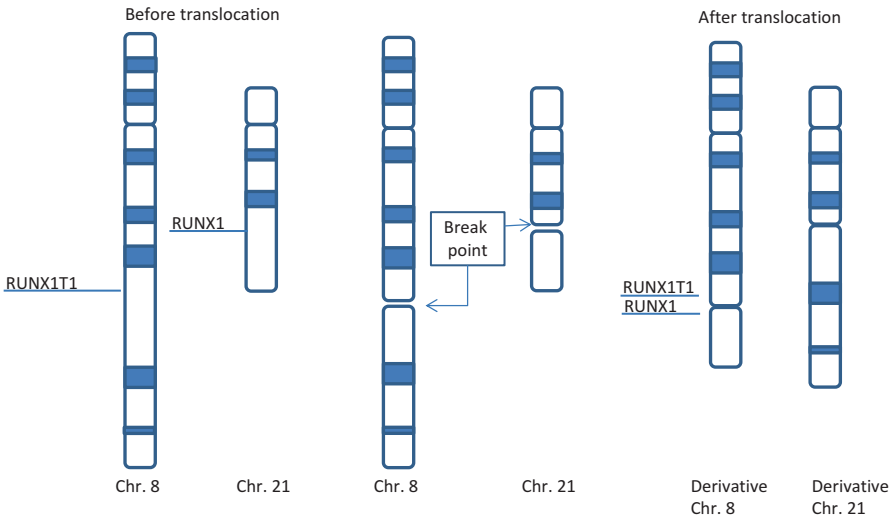


**Fig. 1.6** At the genome level, AML with t(8;21)(q22;q22) is characterized by a chromosome 8 and 21 translocation

*RUNX1-RUNX1T1* translocation can be detected using karyotyping and FISH. One of the FISH assay designs is a dual-color dual-fusion FISH assay. In this assay, two probes are designed to hybridize to *RUNX1T1* at 8q22 and *RUNX1* at 21q22, respectively, overlapping the breakpoints on each chromosome. The two probes can be labeled with different fluorescent dyes. For example, the probe specific for *RUNX1T1* can be labeled with red fluorescent dye and the probe for *RUNX1* green. A normal cell will show two red and two green dots (Fig. 1.7). A cell with t(8;21)(q22;q22) will show one red, one green, and two yellow dots (Fig. 1.7). This assay is typically more sensitive than karyotyping.

In rare cases, cryptic *RUNX1-RUNX1T1* translocations may not be detected by karyotyping or FISH assays. RT-PCR assays in these instances can be used to detect such cryptic translocations [30]. RT-PCR assays may also be used to detect minimal residual disease (MRD) of *RUNX1-RUNX1T1* with a sensitivity of $10^{-3}$ to $10^{-4}$. A nested PCR design can reach a sensitivity of $10^{-4}$ to $10^{-5}$ [30].

## Somatic Mutations

Several genes additionally deserve discussion here with regard to AML with t(8;21)(q22;q22): *KIT*, *FLT3*, and the *RAS* genes, *NRAS* and *KRAS*. *KIT* mutations are the most frequent and vary in incidence in these AMLs (5–30%); most mutations are within the tyrosine kinase domain [33–44].

These mutations in *KIT* are activating mutations which increase cell proliferation and survival.

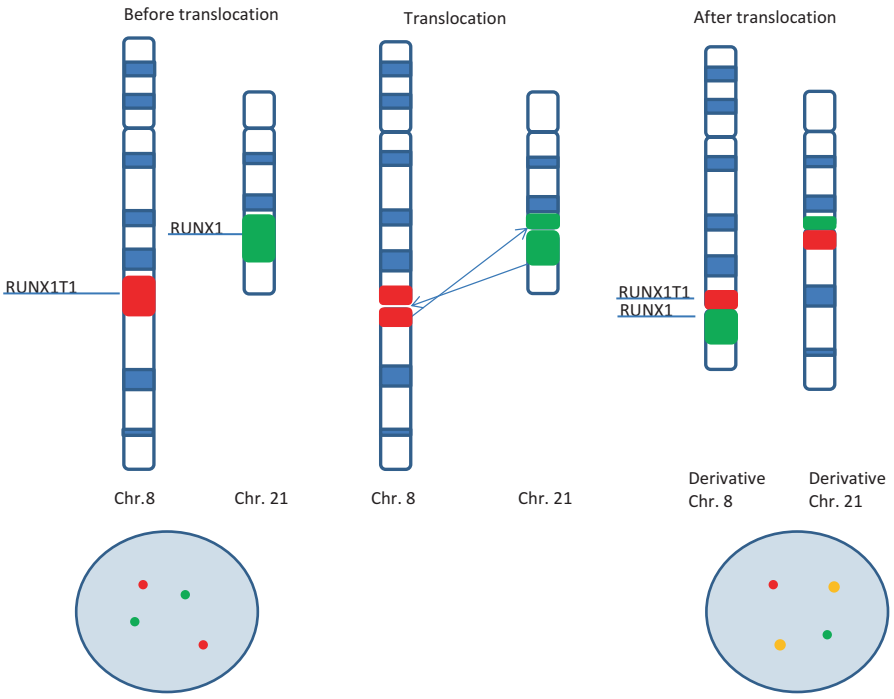


**Fig. 1.7** The probe specific for RUNX1T1 can be labeled with red fluorescent dye and the probe for RUNX1 green. A normal cell will show two red and two green dots. A cell with t(8;21)(q22;q22) will show one red, one green, and two yellow dots

Three other genes infrequently mutated in AML with t(8;21)(q22;q22) are *FLT3* and the *RAS* genes *KRAS* and *NRAS*. As in many other AML subtypes, *FLT3* internal tandem duplication (ITD) mutations may be seen, though at a low frequency (~5%) in AML with t(8;21)(q22;q22) [36].

In vivo mouse models have shown a cooperative effect in *FLT3*-ITD mutations in leukemogenesis [45].

*FLT3* D835 mutations are additionally rare though the significance of these mutations in AML with t(8;21)(q22;q22) is even less certain. Similarly, *NRAS* and *KRAS* mutations are relatively rare and like *FLT3-ITD* mutations may have a role in leukemogenesis without impacting chemosensitivity [36].

## Clinical Significance

AML with the *RUNX1-RUNX1T1* translocation is considered to have a favorable prognosis when compared with other AML subtypes [46, 47]. Somatic gene mutations in *KIT* and *FLT3-ITD* mutations may modify the prognostic outcomes of patients. Several studies have shown that patients with AML with the *RUNX1-RUNX1T1*

translocation and *KIT* mutations may have an adverse outcome though some studies have shown no prognostic impact [33–37, 39, 41, 42].

*FLT3-ITD* mutations are rare but are associated with poor outcomes in patients with t(8;21)(q22;q22) [38].

## Acute Myeloid Leukemia with inv(16)(p13.1q22);*CBFB-MYH11* or t(16;16)(p13.1;q22);*CBFB-MYH11*

AML with inv(16)(p13.1q22);*CBFB-MYH11* comprises 5–8% of AML cases and is predominantly seen in younger patients [1]. Morphologically, these leukemias demonstrate myelomonocytic differentiation, but more uniquely, the marrow shows increased eosinophils, which can show dysplastic features, including prominent granulation with aberrant purple-blue coloration and, on occasion, nuclear hyposegmentation (Fig. 1.8). While most cases show eosinophilia, occasionally, eosinophils are not increased, and myelomonocytic differentiation may be the only feature present. Like APL with *PML-RARA* and AML with t(8;21)(q22;q22), the translocation is diagnostic, and a blast count of greater than or equal to 20% is not required.

The immunophenotype of these leukemias may be heterogeneous, reflecting its myelomonocytic differentiation. It can include an immature blast population expression CD34 and CD117, as maturing granulocytes expressing CD13, CD33, CD65, and MPO and a monocyte population expressing CD4, CD14, and CD64.

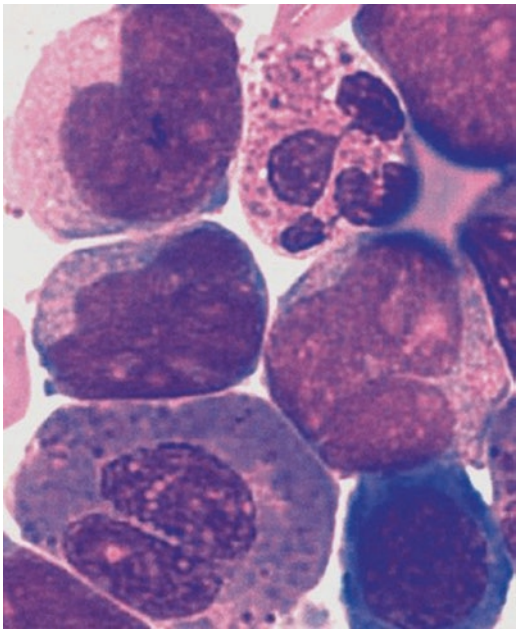

**Fig. 1.8** Acute myeloid leukemia with inv(16) showing abnormal eosinophils with violet-blue granules

## Cytogenetic Abnormality

At the genome level, AML with inv(16)(p13.1q22) is characterized by an inversion of a segment of chromosome 16 resulting in *CBFB-MYH11* fusion gene. *CBFB* is the core binding factor beta subunit located at 16q22. The *MYH11* gene encodes a smooth muscle myosin heavy chain, which is normally located at 16p13.1 [1, 48]. The breakpoints at *CBFB* are near the 3-prime end of the coding region of *CBFB* and are found to be the same in different cases, though the breakpoints at *MYH11* are more variable. The N-terminus and most of *CBFB* gene are fused to the C-terminus of *MYH11* gene with its multimerization domain. All rearrangements maintain the reading frame of the fusion transcript [49, 50]. This chromosome 16 inversion disrupts *CBFB* resulting in impaired differentiation [51]. A similar fusion gene can also result from t(16;16)(p13.1;q22) and bears a similar consequence. The common chromosome 16 inversion is depicted in Fig. 1.9. Such an inversion comprises about 95% of cases generating a *CBFB-MYH11* fusion gene. Karyotyping is a common method used and is capable of detecting most cases. A FISH assay has been used in detecting AML with inv(16)(p13.1q22) with higher sensitivity when compared to karyotyping. Generally speaking, RT-PCR assay has the highest sensitivity since it can detect subtle *CBFB-MYH11* fusion transcripts [52–55].

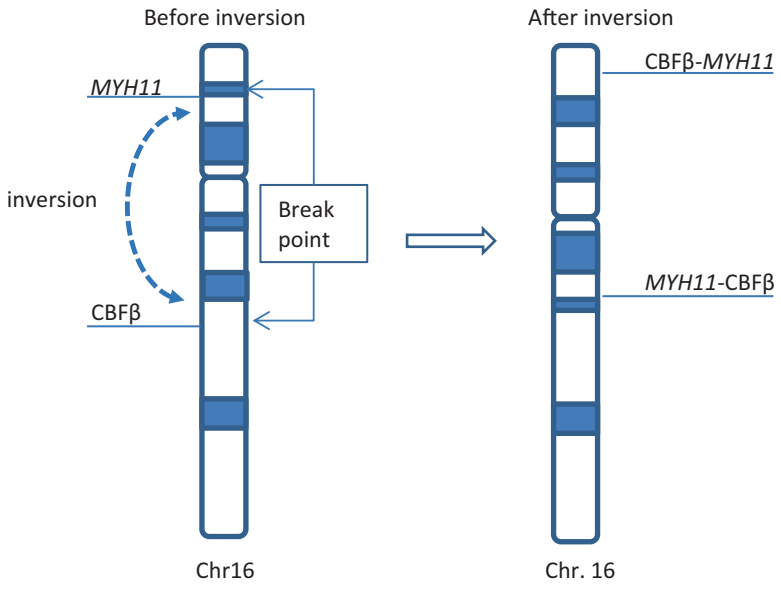


**Fig. 1.9** The common chromosome 16 inversion

## Somatic Gene Mutations

Similar to AML with t(8;21)(q22;q22), AML with inv(16)(p13.1q22) also has frequent mutations in the *KIT* gene (10–40%); additionally mutations in *FLT3* are seen with lesser frequency (10%) [34, 36, 42, 43, 56, 57].

*RAS* mutations are frequently seen in AML with inv(16)(p13.1q22) (35–50%); mutations in the RAS family of genes may function cooperatively with *RUNX1-RUNX1T1* to promote leukemogenesis [36, 56, 58].

## Clinical Significance

Like AML with *RUNX1-RUNX1T1*, AML with *CBFB-MYH11* has a favorable prognosis when compared with other AML subtypes [59]. The significance of *KIT* and *RAS* mutations in these AMLs is uncertain; however, *FLT3*-ITD mutations have been shown in some studies to be associated with poorer prognosis within this category [34, 36, 42, 43, 56, 57].

## Acute Myeloid Leukemia with t(9;11)(p22;q23);*KMT2A-MLLT3*

AML with t(9;11)(p22;q23) results in the fusion of KMT2A, also known as MLL, to MLLT3 and is more often seen in the pediatric setting, comprising 9–12% of pediatric AMLs and 2% of adult AMLs [1]. Morphologically, AML with t(9;11)(p22;q23) is characterized primarily by monocytic differentiation. AML with *KMT2A-MLLT3* is often associated with acute monocytic and myelomonocytic leukemias [1]. Monoblasts, as well as promonocytes (considered blast equivalents), predominate. Monoblasts demonstrate round/oval nuclei, delicate chromatin with prominent nucleoli, basophilic cytoplasm, and rare azurophilic granules. Promonocytes, on the hand, have more convoluted or indented nuclei and less basophilic cytoplasm but can also demonstrate delicate chromatin and visible nucleoli.

In terms of the immunophenotype, the most commonly expressed markers in AML with t(9;11)(p22;q23) include CD13, CD33, CD15, CD38, CD64, CD4, and HLA-DR, whereas CD14, CD117, and MPO are less frequent. Interestingly, pediatric patients will often lack CD14 and CD34 expression, while adult patients will more frequently show CD14 expression and variable CD34.

## Cytogenetic Abnormality

At the genome level, AML with t(9;11)(p22;q23) involves *MLLT3* (mixed-lineage leukemia translocated to 3) and *KMT2A* genes. *MLLT3* is located at 9p22 and *KMT2A* gene at 11q23. The breakpoints of *KMT2A* gene are clustered in a region

that spans the 3′-portion of exon 8, exons 9–13 and the 5′-portion of exon 14 [60, 61]. The breakpoints of *MLLT3* are clustered in introns 4, 7, and 8 [60, 62]. The translocation results in a *MLLT-KMT2A* fusion gene, which consists of the 5′ portion of *KMT2A* gene and the 3′ portion of *MLLT3* gene [60]. Although *MLLT3* is a major partner gene in the translocation associated with *KMT2A*, it is well known that *KMT2A* has many other translocation partner genes. About 80 of all partner genes have been identified [63–65]. In general, karyotyping can detect the majority of translocations. However, since *KMT2A* may have different partner genes and may have cryptic translocations, a significant portion of the translocations may be missed by karyotyping. FISH assays are a good compliment in detecting those cryptic or variant translocations. Considering the fact that *KMT2A* has many possible partner genes, FISH assays usually use dual-color, break-apart designs. The probes span over the *KMT2A* gene and cover the region on either side of *KMT2A* breakpoints. The probe on one side of the breakpoints will be labeled with red florescent dye, and the probe on the other side of the breakpoints will be labeled with green florescent dye (Fig. 1.10). Normal interphase cells will show two yellow dots. The cells with *KMT2A* translocation will show one yellow dot, one green dot, and one red dot. Next-generation sequencing (NGS) offers a new opportunity for *KMT2A* translocation testing as well.

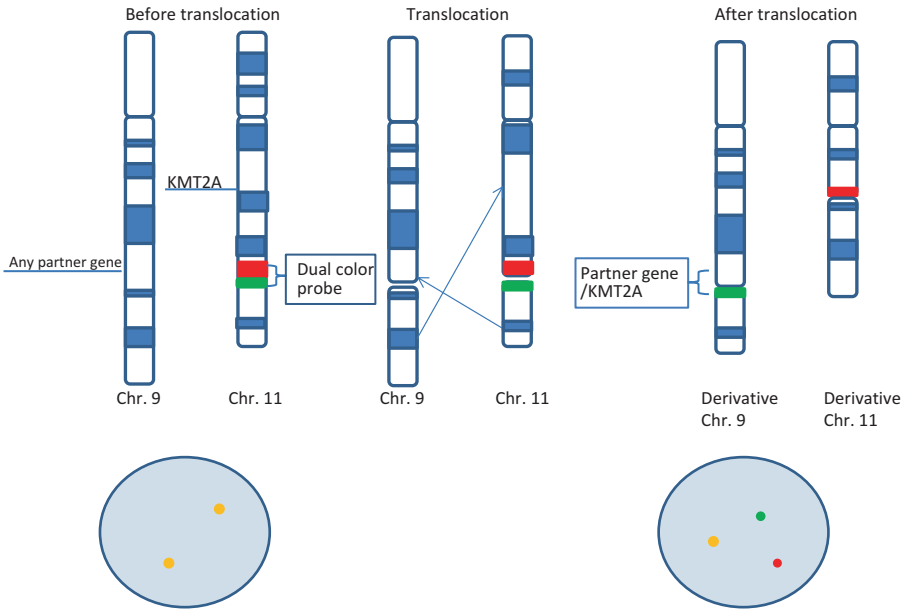


**Fig. 1.10** FISH assays usually use dual-color, break-apart designs. The probes span over the KMT2A gene and cover the region on either side of KMT2A breakpoints. The probe on one side of the breakpoints will be labeled with red florescent dye, and the probe on the other side of the breakpoints will be labeled with green florescent dye

## Clinical Significance

AML with *KMT2A* translocations are often associated with an unfavorable prognosis when compared to other AML subtypes; however, AML with 11q23 translocations can also be seen in therapy-related leukemias after topoisomerase II inhibitor therapy [66, 67]; but such cases should be diagnosed as therapy-related leukemias [1].

## Acute Myeloid Leukemia with t(6;9)(p23;q34);*DEK-NUP214*

AML with t(6;9)(p23;q34) can be seen in both children and adults, consisting of approximately 1% of total AML [1]. AML with t(6;9)(p23;q34) can be seen in any FAB subtype of AML. Patients usually present with anemia, pancytopenia, and thrombocytopenia. In addition to general AML morphology, this subtype is often associated with basophilia (often >2%) and multilineage dysplasia [1].

Morphologically, AML with t(6;9)(p23;q34) can be seen in any FAB subtype of AML, except APL and acute megakaryoblastic leukemia. In addition, this subtype is often associated with marrow and peripheral blood basophilia (defined as >2%). Multilineage dysplasia is often seen, with granulocytic and erythroid dysplasia being more common than megakaryocytic dysplasia. Ringed sideroblasts are present in a subset of cases [1].

The immunophenotype of AML with t(6;9)(p23;q34) is fairly nonspecific, showing typical myeloblast markers including CD34, CD117, MPO, CD13, CD33, and CD15. A subset of cases also express CD64, and Tdt expression is not uncommon.

## Cytogenetic Abnormality

At the genome level, AML with t(6;9)(p23;q34) translocation results in *DEK-NUP214* fusion gene on derivative chromosome 6 [6, 68, 69]. The *DEK* gene is located at 6p23 and the *NUP214* (nuclear pore complex protein 214 kDa) gene at 9q34. The *NUP214* gene is also known as the *CAN* gene. The breakpoints in the *DEK* and *NUP214* genes occur in the intron of each gene. The *DEK-NUP214* fusion gene consists of the 3′ portion of the *NUP214* gene from chromosome 9 and the 5′ portion of the *DEK* gene from chromosome 6. The fusion gene contains the open reading frames of both genes [69–71]. Since most translocation breakpoints occur in a single intron of the *NUP214* gene and in a single intron of the DEK gene, most translocations can be detected using FISH and PCR assays. PCR assays usually have higher sensitivity and real-time PCR assays can be used to follow patients for minimal residual disease [72–75].

## Somatic Gene Mutations

AML with t(6;9)(p23;q34) is frequently associated with a *FLT3* mutation, often *FLT3*-ITD (as high as 70% of cases); other mutations in myeloid mutation-associated genes are much rarer in this AML [76].

## Clinical Significance

Overall, AMLs with t(6;9)(p23;q34) usually have an unfavorable prognosis, and *FLT3*-ITD mutations confer even faster relapse than those patients without *FLT3*-ITD mutation [76].

## Acute Myeloid Leukemia with inv(3)(q21q26.2) or t(3;3)(q21;q26.2)

AMLs with inv(3)(q21q26.2) are rare (2% of AMLs) but usually seen adults [1]. Patients usually present with anemia, thrombocytopenia, or pancytopenia. Morphologically, AML with t(6;9)(p23;q34) can show variable features, but promyelocytic and megakaryoblastic differentiation are not typically seen. In addition, this subtype is often associated with marrow and peripheral blood basophilia (defined as >2%). Multilineage dysplasia is frequently present, with granulocytic and erythroid dysplasia being more common than megakaryocytic dysplasia. Ring sideroblasts can also be demonstrated in a subset of cases [1]. The immunophenotype of AML with t(6;9)(p23;q34) is fairly nonspecific, showing typical myeloblast markers including CD34, CD117, MPO, CD13, CD33, and CD15. A subset of cases also express CD64, and Tdt expression is not uncommon.

## Cytogenetic Abnormality

At the genome level, the result of inv(3)(q21q26.2) brings a *GATA2* distal hematopoietic enhancer (G2DHE) to the vicinity of the *EVI1* gene, causing aberrant *EVI1* expression and *GATA2* haploinsufficiency [2, 4, 5]. This is unlike other AML translocations that usually result in fusion genes. The breakpoints in 3q26 are distributed over several kilobases [77–81]. The breakpoints in 3q21 are distributed over 100 kb [81–83]. The inv(3)(q21q26.2) and t(3;3)(q21;q26.2) can be detected using karyotyping; however, karyotyping may miss some cryptic changes. A dual-color, double-fusion FISH assay

has better sensitivity than that of karyotyping [84]. As in other translocations, RT-PCR assays can be used to detect the inversion and translocation [83, 85, 86]. Due to the wide spread of breakpoints in the inversion and translocation, designing a multiplex RT-PCR to reach a good sensitivity is difficult. However, RNA NGS assays may overcome such challenges and offer practical solutions with good sensitivity and specificity.

## Somatic Gene Mutations

The somatic gene mutational landscape of AML with inv(3)(q21q26.2) has been preliminarily mapped, and mutations in *RAS* genes (*NRAS, KRAS*) and *RUNX1* are most common, with 40–50% and 20% of cases, respectively. *NF1* deletions, *FLT3*, and *CBL* and *IDH1* mutations are less frequent, with 10%, 10%, 10%, and 5% of cases, respectively [87, 88].

## Clinical Significance

The AML patients with inv(3)(q21q26.2) and t(3;3)(q21;q26.2) tend to have unfavorable prognosis with short overall survival and poor response to conventional chemotherapy [77, 78, 87]. The role of other somatic gene mutations in AML patients with inv(3)(q21q26.2) and t(3;3)(q21;q26.2) such as those in *NRAS, KRAS*, *RUNX1*, *NF1*, *FLT3*, *CBL*, and *IDH1* is less certain.

## Acute Myeloid Leukemia with t(1;22)(p13;q13);*RBM15-MKL1*

AML with t(1;22)(p13;q13) is usually seen in infants without Down syndrome, more often in female infants. It consists of less than 1% of all AML cases [1] and about 10% of pediatric AMLs [89–92]. Morphologically, the blasts in AML with t(1;22)(p13;q13) present as small or large megakaryoblasts resembling those of acute megakaryoblastic leukemia [1].

## Cytogenetic Abnormality

At the genome level, t(1;22)(p13;q13) results in a fusion gene, *RBM15-MKL1*. *RBM15* (RNA-binding motif protein 15) is located at 1p13 and is also known as *OTT* (one twenty-two). *MKL1* (megakaryocyte leukemia 1) is located at 22q13. The breakpoints

on chromosome 1p13 are located at a 6 kb genomic region. The breakpoints on chromosome 22q13 are located in a 28 kb intron [93]. Although both reciprocal fusion genes are expressed, *RBM15-MKL1* fusion gene has all the functional motifs encoded by each partner gene [93]. The translocation can be detected by karyotyping, FISH, and PCR [93], with PCR assays being used to follow minimal residual disease after treatment [94].

## Clinical Significance

The early studies suggested that AMLs with t(1;22)(p13;q13) carry an unfavorable prognosis [1, 95, 96]; however, more intensive therapy has improved the long-term survival of such patients [97, 98].

## De Novo Acute Myeloid Leukemia with t(9;22)(q34;q11.2)

AML with t(9;22)(q34;q11.2) is seen in 0.5–3.0% of all AML cases . In the cases of de novo AML, the patients should not have a history of *BCR-ABL1* positive chronic myeloid leukemia (CML), as such splenomegaly and peripheral basophilia are less common than in CML in blast crisis. The translocation t(9;22)(q34;q11.2) results in a *BCR-ABL1* fusion gene and is often the specific Philadelphia chromosome [99–105]. It is now considered a provisional category of AML with recurrent genetic abnormalities [2].

As it is a newly defined provisional entity, our experience with AML with t(9;22)(q34;q11.2) is somewhat limited. To date, no specific histologic or immunophenotypic features which distinguish this entity from typical AML have been identified [99–105].

## Cytogenetic Abnormality

At the genome level, the t(9;22)(q34;q11.2) in this AML is the same as that seen in CML. Karyotyping, FISH, and PCR assays can be used to detect the translocation. As one may expect, FISH has higher sensitivity than karyotyping. Quantitative PCR assay is the most sensitive among the three assays and can be used to monitor minimal residual disease. Differentiating between the blast phase of CML and AML with t(9;22)(q34;q11.2) has been difficult and heavily depends on the patient's history. However, genomic analysis of de novo cases in comparison to the blast phase of CML has demonstrated specific deletions in *IKZF1* and *CDNK1A/B* as well as loss of *IGH/VDJ* and *TARP* regions [104].

## Clinical Significance

These cases are so rare that tailored therapies are still in their infancy. It is thought that, like CML in blast phase crisis, AML with t(9;22)(q34;q11.2) may benefit from TKI (tyrosine kinase inhibitor) therapy [103–106].

## Conclusion

Understanding cytogenetic changes in acute myeloid leukemia is critical not only for diagnostic subtyping but also for understanding the pathogenesis and potential clinical progression of patients. While the majority of cytogenetically defined categories of AML have been elucidated, understanding how these genetic changes result in leukemia is still far from complete. Additionally, the significance and prevalence of other somatic gene mutations in these leukemias require further study.

## References

1. Steven H, Swerdlow EC, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW. WHO classification of tumours of haematopoietic and lymphoid tissues. In: Bosman FT, Jaffe ES, Lakhani SR, Ohgaki H, editors. IARC WHO Classification of Tumours. 4th ed. Lyon: International Agency for Research on Cancer; 2008. p. 439.
2. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–405.
3. Grimwade D, Hills RK, Moorman AV, Walker H, Chatters S, Goldstone AH, et al. Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. Blood. 2010;116(3):354–65.
4. Yamazaki H, Suzuki M, Otsuki A, Shimizu R, Bresnick EH, Engel JD, et al. A remote GATA2 hematopoietic enhancer drives leukemogenesis in inv(3)(q21;q26) by activating EVI1 expression. Cancer Cell. 2014;25(4):415–27.
5. Groschel S, Sanders MA, Hoogenboezem R, de Wit E, Bouwman BA, Erpelinck C, et al. A single oncogenic enhancer rearrangement causes concomitant EVI1 and GATA2 deregulation in leukemia. Cell. 2014;157(2):369–81.
6. William B, Coleman GJT. Molecular diagnostics for the clinical laboratorian. Totowa: Humana Press Inc.; 2006.
7. Tobal K, Liu Yin JA. RT-PCR method with increased sensitivity shows persistence of PML-RARA fusion transcripts in patients in long-term remission of APL. Leukemia. 1998;12(9):1349–54.
8. Brockman SR, Paternoster SF, Ketterling RP, Dewald GW. New highly sensitive fluorescence in situ hybridization method to detect PML/RARA fusion in acute promyelocytic leukemia. Cancer Genet Cytogenet. 2003;145(2):144–51.
9. Redner RL, Rush EA, Faas S, Rudert WA, Corey SJ. The t(5;17) variant of acute promyelocytic leukemia expresses a nucleophosmin-retinoic acid receptor fusion. Blood. 1996;87(3):882–6.
10. Chen SJ, Zelent A, Tong JH, Yu HQ, Wang ZY, Derre J, et al. Rearrangements of the retinoic acid receptor alpha and promyelocytic leukemia zinc finger genes resulting from t(11;17)(q23;q21) in a patient with acute promyelocytic leukemia. J Clin Invest. 1993;91(5):2260–7.

11. Chen Z, Brand NJ, Chen A, Chen SJ, Tong JH, Wang ZY, et al. Fusion between a novel Kruppel-like zinc finger gene and the retinoic acid receptor-alpha locus due to a variant t(11;17) translocation associated with acute promyelocytic leukaemia. EMBO J. 1993;12(3):1161–7.
12. Wells RA, Catzavelos C, Kamel-Reid S. Fusion of retinoic acid receptor alpha to NuMA, the nuclear mitotic apparatus protein, by a variant translocation in acute promyelocytic leukaemia. Nat Genet. 1997;17(1):109–13.
13. Buijs A, Bruin M. Fusion of FIP1L1 and RARA as a result of a novel t(4;17)(q12;q21) in a case of juvenile myelomonocytic leukemia. Leukemia. 2007;21(5):1104–8.
14. Kondo T, Mori A, Darmanin S, Hashino S, Tanaka J, Asaka M. The seventh pathogenic fusion gene FIP1L1-RARA was isolated from a t(4;17)-positive acute promyelocytic leukemia. Haematologica. 2008;93(9):1414–6.
15. Won D, Shin SY, Park CJ, Jang S, Chi HS, Lee KH, et al. OBFC2A/RARA: a novel fusion gene in variant acute promyelocytic leukemia. Blood. 2013;121(8):1432–5.
16. Li J, Zhong HY, Zhang Y, Xiao L, Bai LH, Liu SF, et al. GTF2I-RARA is a novel fusion transcript in a t(7;17) variant of acute promyelocytic leukaemia with clinical resistance to retinoic acid. Br J Haematol. 2015;168(6):904–8.
17. Shimomura Y, Mitsui H, Yamashita Y, Kamae T, Kanai A, Matsui H, et al. A new variant of acute promyelocytic leukemia with IRF2BP2-RARA fusion. Cancer Sci. 2016;
18. Yin CC, Jain N, Mehrotra M, Zhagn J, Protopopov A, Zuo Z, et al. Identification of a novel fusion gene, IRF2BP2-RARA, in acute promyelocytic leukemia. J Natl Compr Canc Netw. 2015;13(1):19–22.
19. Arnould C, Philippe C, Bourdon V, Gr goire MJ, Berger R, Jonveaux P. The signal transducer and activator of transcription STAT5b gene is a new partner of retinoic acid receptor alpha in acute promyelocytic-like leukaemia. Hum Mol Genet. 1999;8(9):1741–9.
20. Catalano A, Dawson MA, Somana K, Opat S, Schwarer A, Campbell LJ, et al. The PRKAR1A gene is fused to RARA in a new variant acute promyelocytic leukemia. Blood. 2007;110(12):4073–6.
21. Lewis C, Patel V, Abhyankar S, Zhang D, Ketterling RP, McClure RF, et al. Microgranular variant of acute promyelocytic leukemia with normal conventional cytogenetics, negative PML/RARA FISH and positive PML/RARA transcripts by RT-PCR. Cancer Genet. 2011;204(9):522–3.
22. Blanco EM, Curry CV, Lu XY, Sarabia SF, Redell MS, Lopez-Terrada DH, et al. Cytogenetically cryptic and FISH-negative PML/RARA rearrangement in acute promyelocytic leukemia detected only by PCR: an exceedingly rare phenomenon. Cancer Genet. 2014;207(1–2):48–9.
23. Huang W, Sun GL, Li XS, Cao Q, Lu Y, Jiang GS, et al. Acute promyelocytic leukemia: clinical relevance of two major PML-RAR alpha isoforms and detection of minimal residual disease by retrotranscriptase/polymerase chain reaction to predict relapse. Blood. 1993;82(4):1264–9.
24. Borrow J, Goddard AD, Gibbons B, Katz F, Swirsky D, Fioretos T, et al. Diagnosis of acute promyelocytic leukaemia by RT-PCR: detection of PML-RARA and RARA-PML fusion transcripts. Br J Haematol. 1992;82(3):529–40.
25. Callens C, Chevret S, Cayuela JM, Cassinat B, Raffoux E, de Botton S, Thomas X, Guerci A, Fegueux N, Pigneux A, Stoppa AM, Lamy T, Rigal-Huguet F, Vekhoff A, Meyer-Monard S, Ferrand A, Sanz M, Chomienne C, Fenaux P, Dombret H, European APL Group. Prognostic implication of FLT3 and Ras gene mutations in patients with acute promyelocytic leukemia (APL): a retrospective study from the European APL Group. Leukemia. 2005;19(7):1153–60. PubMed PMID: 15889156
26. Schnittger S, Bacher U, Haferlach C, Kern W, Alpermann T, Haferlach T. Clinical impact of FLT3 mutation load in acute promyelocytic leukemia with t(15;17)/PML-RARA. Haematologica. 2011;96(12):1799–807.
27. Shen Y, Zhu YM, Fan X, Shi JY, Wang QR, Yan XJ, Gu ZH, Wang YY, Chen B, Jiang CL, Yan H, Chen FF, Chen HM, Chen Z, Jin J, Chen SJ. Gene mutation patterns and their prognostic impact in a cohort of 1185 patients with acute myeloid leukemia. Blood. 2011;118(20):5593–603.
28. Tomita A, Kiyoi H, Naoe T. Mechanisms of action and resistance to all-trans retinoic acid (ATRA) and arsenic trioxide (As2O 3) in acute promyelocytic leukemia. Int J Hematol. 2013;97(6):717–25.
29. Rowley JD. Identificaton of a translocation with quinacrine fluorescence in a patient with acute leukemia. Ann Genet. 1973;16(2):109–12.

30. van Dongen JJ, Macintyre EA, Gabert JA, Delabesse E, Rossi V, Saglio G, et al. Standardized RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia for detection of minimal residual disease. Report of the BIOMED-1 concerted action: investigation of minimal residual disease in acute leukemia. Leukemia. 1999;13(12):1901–28.
31. Foucar K, Anastasi J. Acute myeloid leukemia with recurrent cytogenetic abnormalities. Am J Clin Pathol. 2015;144(1):6–18.
32. Downing JR. The AML1-ETO chimaeric transcription factor in acute myeloid leukaemia: biology and clinical significance. Br J Haematol. 1999;106(2):296–308.
33. Wang YY, Zhou GB, Yin T, et al. AML1-ETO and C-KIT mutation/overexpression in t(8;21) leukemia: implication in stepwise leukemogenesis and response to Gleevec. Proc Natl Acad Sci U S A. 2005;102(4):1104–9.
34. Paschka P, Marcucci G, Ruppert AS, et al. Adverse prognostic significance of KIT mutations in adult acute myeloid leukemia with inv(16) and t(8;21): a Cancer and Leukemia Group B study. J Clin Oncol. 2006;24(24):3904–11.
35. Shimada A, Taki T, Tabuchi K, et al. KIT mutations, and not FLT3 internal tandem duplication, are strongly associated with a poor prognosis in pediatric acute myeloid leukemia with t(8;21): a study of the Japanese Childhood AML Cooperative Study Group. Blood. 2006;107(5):1806–9.
36. Boissel N, Leroy H, Brethon B, et al. Incidence and prognostic impact of c-Kit, FLT3, and Ras gene mutations in core binding factor acute myeloid leukemia (CBF-AML). Leukemia. 2006;20(6):965–70.
37. Cairoli R, Beghini A, Grillo G, et al. Prognostic impact of c-KIT mutations in core binding factor leukemias: an Italian retrospective study. Blood. 2006;107(9):3463–8.
38. Paschka P, Du J, Schlenk RF, et al. Mutations in the Fms-related tyrosine kinase 3 (FLT3) gene independently predict poor outcome in acute myeloid leukemia (AML) with t(8;21): a study of the outcome in acute myeloid leukemia (AML) with t(8;21): a study of the German-Austrian AML Study Group (AMLSG) ASH Annual Meeting Abstracts. 2009;114(22) abstract no 825.
39. Schnittger S, Bacher U, Kern W, Haferlach T, Haferlach C. JAK2V617F as progression marker in CMPD and as cooperative mutation in AML with trisomy 8 and t(8;21): a comparative study on 1103 CMPD and 269 AML cases. Leukemia. 2007;21(8):1843–5.
40. Sritana N, Auewarakul CU. KIT and FLT3 receptor tyrosine kinase mutations in acute myeloid leukemia with favorable cytogenetics: two novel mutations and selective occurrence in leukemia subtypes and age groups. Exp Mol Pathol. 2008;85(3):227–31.
41. Goemans BF, Zwaan CHM, Miller M, et al. Mutations in KIT and RAS are frequent events in pediatric core-binding factor acute myeloid leukemia. Leukemia. 2005;19(9):1536–42.
42. Care RS, Valk PJM, Goodeve AC, et al. Incidence and prognosis of c-KIT and FLT3 mutations in core binding factor (CBF) acute myeloid leukaemias. Br J Haematol. 2003;121(5):775–7.
43. Pollard JA, Alonzo TA, Gerbing RB, et al. Prevalence and prognostic significance of KIT mutations in pediatric patients with core binding factor AML enrolled on serial pediatric cooperative trials for de novo AML. Blood. 2010;115(12):2372–9.
44. Schnittger S, Kohl TM, Haferlach T, et al. KIT-D816 mutations in AML1-ETO-positive AML are associated with impaired event-free and overall survival. Blood. 2006;107(5):1791–9.
45. Schessl C, Rawat VPS, Cusan M, et al. The AML1-ETO fusion gene and the FLT3 length mutation collaborate in inducing acute leukemia in mice. J Clin Investig. 2005;115(8):2159–68.
46. Dombret H, Preudhomme C, Boissel N. Core binding factor acute myeloid leukemia (CBF-AML): is high-dose Ara-C (HDAC) consolidation as effective as you think? Curr Opin Hematol. 2009;16(2):92–7.
47. Sangle NA, Perkins SL. Core-binding factor acute myeloid leukemia. Arch Pathol Lab Med. 2011;135(11):1504–9.
48. Dash A, Gilliland DG. Molecular genetics of acute myeloid leukaemia. Best Pract Res Clin Haematol. 2001;14(1):49–64.
49. Dauwerse JG, Wessels JW, Giles RH, Wiegant J, van der Reijden BA, Fugazza G, et al. Cloning the breakpoint cluster region of the inv(16) in acute nonlymphocytic leukemia M4 Eo. Hum Mol Genet. 1993;2(10):1527–34.

50. Liu P, Tarle SA, Hajra A, Claxton DF, Marlton P, Freedman M, et al. Fusion between transcription factor CBF beta/PEBP2 beta and a myosin heavy chain in acute myeloid leukemia. Science. 1993;261(5124):1041–4.
51. Speck NA, Gilliland DG. Core-binding factors in haematopoiesis and leukaemia. Nat Rev Cancer. 2002;2(7):502–13.
52. Mancini M, Cedrone M, Diverio D, Emanuel B, Stul M, Vranckx H, et al. Use of dual-color interphase FISH for the detection of inv(16) in acute myeloid leukemia at diagnosis, relapse and during follow-up: a study of 23 patients. Leukemia. 2000;14(3):364–8.
53. Dauwerse HG, Smit EM, Giles RH, Slater R, Breuning MH, Hagemeijer A, et al. Two-colour FISH detection of the inv(16) in interphase nuclei of patients with acute myeloid leukaemia. Br J Haematol. 1999;106(1):111–4.
54. Martinet D, Muhlematter D, Leeman M, Parlier V, Hess U, Gmur J, et al. Detection of 16 p deletions by FISH in patients with inv(16) or t(16;16) and acute myeloid leukemia (AML). Leukemia. 1997;11(7):964–70.
55. Hernandez JM, Gonzalez MB, Granada I, Gutierrez N, Chillon C, Ramos F, et al. Detection of inv(16) and t(16;16) by fluorescence in situ hybridization in acute myeloid leukemia M4Eo. Haematologica. 2000;85(5):481–5.
56. Jones D, Yao H, Romans A, et al. Modeling interactions between leukemia-specific chromosomal changes, somatic mutations, and gene expression patterns during progression of core-binding factor leukemias. Genes Chromosomes Cancer. 2010;49(2):182–91.
57. Park SH, Chi HS, Min SK, Park BG, Jang S, Park CJ. Prognostic impact of c-KIT mutations in core binding factor acute myeloid leukemia. Leuk Res. 2011;35(10):1376–83.
58. Paschka P, Du J, Schlenk RF, et al. Secondary genetic lesions in acute myeloid leukemia with inv(16) or t(16;16): a study of the German-Austrian AML Study Group (AMLSG). Blood. 2013;121(1):170–7.
59. Larson RA, Williams SF, Le Beau MM, Bitter MA, Vardiman JW, Rowley JD. Acute myelomonocytic leukemia with abnormal eosinophils and inv(16) or t(16;16) has a favorable prognosis. Blood. 1986;68(6):1242–9.
60. Alonso CN, Longo PL, Gallego MS, Medina A, Felice MS. A novel AF9 breakpoint in MLL-AF9-positive acute monoblastic leukemia. Pediatr Blood Cancer. 2008;50(4):869–71.
61. Nilson I, Lochner K, Siegler G, Greil J, Beck JD, Fey GH, et al. Exon/intron structure of the human ALL-1 (MLL) gene involved in translocations to chromosomal region 11q23 and acute leukaemias. Br J Haematol. 1996;93(4):966–72.
62. Strissel PL, Strick R, Tomek RJ, Roe BA, Rowley JD, Zeleznik-Le NJ. DNA structural properties of AF9 are similar to MLL and could act as recombination hot spots resulting in MLL/AF9 translocations and leukemogenesis. Hum Mol Genet. 2000;9(11):1671–9.
63. Meyer C, Hofmann J, Burmeister T, Groger D, Park TS, Emerenciano M, et al. The MLL recombinome of acute leukemias in 2013. Leukemia. 2013;27(11):2165–76.
64. Meyer C, Kowarz E, Hofmann J, Renneville A, Zuna J, Trka J, et al. New insights to the MLL recombinome of acute leukemias. Leukemia. 2009;23(8):1490–9.
65. Meyer C, Schneider B, Jakob S, Strehl S, Attarbaschi A, Schnittger S, et al. The MLL recombinome of acute leukemias. Leukemia. 2006;20(5):777–84.
66. Schoch C, Schnittger S, Klaus M, Kern W, Hiddemann W, Haferlach T. AML with 11q23/MLL abnormalities as defined by the WHO classification: incidence, partner chromosomes, FAB subtype, age distribution, and prognostic impact in an unselected series of 1897 cytogenetically analyzed AML cases. Blood. 2003;102(7):2395–402.
67. Langer T, Metzler M, Reinhardt D, Viehmann S, Borkhardt A, Reichel M, et al. Analysis of t(9;11) chromosomal breakpoint sequences in childhood acute leukemia: almost identical MLL breakpoints in therapy-related AML after treatment without etoposides. Genes Chromosomes Cancer. 2003;36(4):393–401.
68. Soekarman D, von Lindern M, Daenen S, de Jong B, Fonatsch C, Heinze B, et al. The translocation (6;9) (p23;q34) shows consistent rearrangement of two genes and defines a myeloproliferative disorder with specific clinical features. Blood. 1992;79(11):2990–7.

69. von Lindern M, Fornerod M, van Baal S, Jaegle M, de Wit T, Buijs A, et al. The translocation (6;9), associated with a specific subtype of acute myeloid leukemia, results in the fusion of two genes, dek and can, and the expression of a chimeric, leukemia-specific dek-can mRNA. Mol Cell Biol. 1992;12(4):1687–97.
70. von Lindern M, Fornerod M, Soekarman N, van Baal S, Jaegle M, Hagemeijer A, et al. Translocation t(6;9) in acute non-lymphocytic leukaemia results in the formation of a DEK-CAN fusion gene. Baillieres Clin Haematol. 1992;5(4):857–79.
71. Soekarman D, von Lindern M, van der Plas DC, Selleri L, Bartram CR, Martiat P, et al. Dek-can rearrangement in translocation (6;9)(p23;q34). Leukemia. 1992;6(6):489–94.
72. Shearer BM, Knudson RA, Flynn HC, Ketterling RP. Development of a D-FISH method to detect DEK/CAN fusion resulting from t(6;9)(p23;q34) in patients with acute myelogenous leukemia. Leukemia. 2005;19(1):126–31.
73. Tobal K, Frost L, Liu Yin JA. Quantification of DEK-CAN fusion transcript by real-time reverse transcription polymerase reaction in patients with t(6;9) acute myeloid leukemia. Haematologica. 2004;89(10):1267–9.
74. Ostergaard M, Stentoft J, Hokland P. A real-time quantitative RT-PCR assay for monitoring DEK-CAN fusion transcripts arising from translocation t(6;9) in acute myeloid leukemia. Leuk Res. 2004;28(11):1213–5.
75. Garcon L, Libura M, Delabesse E, Valensi F, Asnafi V, Berger C, et al. DEK-CAN molecular monitoring of myeloid malignancies could aid therapeutic stratification. Leukemia. 2005;19(8):1338–44.
76. Ommen HB, Touzart A, MacIntyre E, Kern W, Haferlach T, Haferlach C, et al. The kinetics of relapse in DEK-NUP214-positive acute myeloid leukemia patients. Eur J Haematol. 2015;95(5):436–41.
77. Fonatsch C, Gudat H, Lengfelder E, Wandt H, Silling-Engelhardt G, Ludwig WD, et al. Correlation of cytogenetic findings with clinical features in 18 patients with inv(3)(q21q26) or t(3;3)(q21;q26). Leukemia. 1994;8(8):1318–26.
78. Secker-Walker LM, Mehta A, Bain B. Abnormalities of 3q21 and 3q26 in myeloid malignancy: a United Kingdom Cancer Cytogenetic Group study. Br J Haematol. 1995;91(2):490–501.
79. Morishita K, Parganas E, William CL, Whittaker MH, Drabkin H, Oval J, et al. Activation of EVI1 gene expression in human acute myelogenous leukemias by translocations spanning 300-400 kilobases on chromosome band 3q26. Proc Natl Acad Sci U S A. 1992;89(9):3937–41.
80. Levy ER, Parganas E, Morishita K, Fichelson S, James L, Oscier D, et al. DNA rearrangements proximal to the EVI1 locus associated with the 3q21q26 syndrome. Blood. 1994;83(5):1348–54.
81. Suzukawa K, Parganas E, Gajjar A, Abe T, Takahashi S, Tani K, et al. Identification of a breakpoint cluster region 3′ of the ribophorin I gene at 3q21 associated with the transcriptional activation of the EVI1 gene in acute myelogenous leukemias with inv(3)(q21q26). Blood. 1994;84(8):2681–8.
82. Pekarsky Y, Zabarovsky E, Kashuba V, Drabkin H, Sandberg AA, Morgan R, et al. Cloning of breakpoints in 3q21 associated with hematologic malignancy. Cancer Genet Cytogenet. 1995;80(1):1–8.
83. Rynditch A, Pekarsky Y, Schnittger S, Gardiner K. Leukemia breakpoint region in 3q21 is gene rich. Gene. 1997;193(1):49–57.
84. Shearer BM, Sukov WR, Flynn HC, Knudson RA, Ketterling RP. Development of a dual-color, double fusion FISH assay to detect RPN1/EVI1 gene fusion associated with inv(3), t(3;3), and ins(3;3) in patients with myelodysplasia and acute myeloid leukemia. Am J Hematol. 2010;85(8):569–74.
85. Martinelli G, Ottaviani E, Buonamici S, Isidori A, Borsaru G, Visani G, et al. Association of 3q21q26 syndrome with different RPN1/EVI1 fusion transcripts. Haematologica. 2003;88(11):1221–8.
86. Lahortiga I, Vazquez I, Agirre X, Larrayoz MJ, Vizmanos JL, Gozzetti A, et al. Molecular heterogeneity in AML/MDS patients with 3q21q26 rearrangements. Genes Chromosomes Cancer. 2004;40(3):179–89.

87. Gröschel S, Sanders MA, Hoogenboezem R, Zeilemaker A, Havermans M, Erpelinck C, Bindels EM, Beverloo HB, Döhner H, Löwenberg B, Döhner K, Delwel R, Valk PJ. Mutational spectrum of myeloid malignancies with inv(3)/t(3;3) reveals a predominant involvement of RAS/RTK signaling pathways. Blood. 2015;125(1):133–9. doi:10.1182/blood-2014-07-591461. PubMed PMID: 25381062; PubMed Central PMCID: PMC4334729
88. Haferlach C, Bacher U, Haferlach T, Dicker F, Alpermann T, Kern W, Schnittger S. The inv(3)(q21q26)/t(3;3)(q21;q26) is frequently accompanied by alterations of the RUNX1, KRAS and NRAS and NF1 genes and mediates adverse prognosis both in MDS and in AML: a study in 39 cases of MDS or AML. Leukemia. 2011;25(5):874–7. doi:10.1038/leu.2011.5. PubMed PMID: 21283084
89. Reiter E, Greinix H, Rabitsch W, Keil F, Schwarzinger I, Jaeger U, et al. Low curative potential of bone marrow transplantation for highly aggressive acute myelogenous leukemia with inversioin inv (3)(q21q26) or homologous translocation t(3;3) (q21;q26). Ann Hematol. 2000;79(7):374–7.
90. Athale UH, Razzouk BI, Raimondi SC, Tong X, Behm FG, Head DR, et al. Biology and outcome of childhood acute megakaryoblastic leukemia: a single institution's experience. Blood. 2001;97(12):3727–32.
91. Hama A, Yagasaki H, Takahashi Y, Nishio N, Muramatsu H, Yoshida N, et al. Acute megakaryoblastic leukaemia (AMKL) in children: a comparison of AMKL with and without Down syndrome. Br J Haematol. 2008;140(5):552–61.
92. Reinhardt D, Diekamp S, Langebrake C, Ritter J, Stary J, Dworzak M, et al. Acute megakaryoblastic leukemia in children and adolescents, excluding Down's syndrome: improved outcome with intensified induction treatment. Leukemia. 2005;19(8):1495–6.
93. Ma Z, Morris SW, Valentine V, Li M, Herbrick JA, Cui X, et al. Fusion of two novel genes, RBM15 and MKL1, in the t(1;22)(p13;q13) of acute megakaryoblastic leukemia. Nat Genet. 2001;28(3):220–1.
94. Takeda A, Shimada A, Hamamoto K, Yoshino S, Nagai T, Fujii Y, et al. Detection of RBM15-MKL1 fusion was useful for diagnosis and monitoring of minimal residual disease in infant acute megakaryoblastic leukemia. Acta Med Okayama. 2014;68(2):119–23.
95. Bernstein J, Dastugue N, Haas OA, Harbott J, Heerema NA, Huret JL, et al. Nineteen cases of the t(1;22)(p13;q13) acute megakaryblastic leukaemia of infants/children and a review of 39 cases: report from a t(1;22) study group. Leukemia. 2000;14(1):216–8.
96. Carroll A, Civin C, Schneider N, Dahl G, Pappo A, Bowman P, et al. The t(1;22) (p13;q13) is nonrandom and restricted to infants with acute megakaryoblastic leukemia: a Pediatric Oncology Group Study. Blood. 1991;78(3):748–52.
97. Duchayne E, Fenneteau O, Pages MP, Sainty D, Arnoulet C, Dastugue N, et al. Acute megakaryoblastic leukaemia: a national clinical and biological study of 53 adult and childhood cases by the Groupe Francais d'Hematologie Cellulaire (GFHC). Leuk Lymphoma. 2003;44(1):49–58.
98. Inaba H, Zhou Y, Abla O, Adachi S, Auvrignon A, Beverloo HB, et al. Heterogeneous cytogenetic subgroups and outcomes in childhood acute megakaryoblastic leukemia: a retrospective international study. Blood. 2015;126(13):1575–84.
99. Cuneo A, Ferrant A, Michaux JL, Demuynck H, Boogaerts M, Louwagie A, et al. Philadelphia chromosome-positive acute myeloid leukemia: cytoimmunologic and cytogenetic features. Haematologica. 1996;81(5):423–7.
100. Keung YK, Beaty M, Powell BL, Molnar I, Buss D, Pettenati M. Philadelphia chromosome positive myelodysplastic syndrome and acute myeloid leukemia-retrospective study and review of literature. Leuk Res. 2004;28(6):579–86.
101. Khan MH. Heteromorphic pair of metacentric chromosomes with fused arms and the Philadelphia chromosome in a case of acute myeloid leukemia. Acta Haematol. 1972;48(5):312–9.
102. Price CM, Rassool F, Shivji MK, Gow J, Tew CJ, Haworth C, et al. Rearrangement of the breakpoint cluster region and expression of P210 BCR-ABL in a “masked” Philadelphia chromosome-positive acute myeloid leukemia. Blood. 1988;72(5):1829–32.
103. Soupir CP, Vergilio JA, Dal Cin P, Muzikansky A, Kantarjian H, Jones D, et al. Philadelphia chromosome-positive acute myeloid leukemia: a rare aggressive leukemia with clinicopathologic features distinct from chronic myeloid leukemia in myeloid blast crisis. Am J Clin Pathol. 2007;127(4):642–50.

104. Bloomfield CD, Lindquist LL, Brunning RD, Yunis JJ, Coccia PF. The Philadelphia chromosome in acute leukemia. Virchows Arch B Cell Pathol. 1978;29(1–2):81–91.
105. Konoplev S, Yin CC, Kornblau SM, Kantarjian HM, Konopleva M, Andreeff M, et al. Molecular characterization of de novo Philadelphia chromosome-positive acute myeloid leukemia. Leuk Lymphoma. 2013;54(1):138–44.
106. Nacheva EP, Grace CD, Brazma D, Gancheva K, Howard-Reeves J, Rai L, Gale RE, Linch DC, Hills RK, Russell N, Burnett AK, Kottaridis PD. Does BCR/ABL1 positive acute myeloid leukaemia exist? Br J Haematol. 2013;161(4):541–50. doi:10.1111/bjh.12301. PubMed PMID: 23521501

## Recommended Reading

Bejar R, Stevenson K, Abdel-Wahab O, Galili N, Nilsson B, Garcia-Manero G, et al. Clinical effect of point mutations in myelodysplastic syndromes. N Engl J Med. 2011;364(26):2496–506.
Birkenmeier EH, Gwynn B, Howard S, Jerry J, Gordon JI, Landschulz WH, et al. Tissue-specific expression, developmental regulation, and genetic mapping of the gene encoding CCAAT/enhancer binding protein. Genes Dev. 1989;3(8):1146–56.
Brown P, McIntyre E, Rau R, Meshinchi S, Lacayo N, Dahl G, et al. The incidence and clinical significance of nucleophosmin mutations in childhood AML. Blood. 2007;110(3):979–85.
Chen W, Rassidakis GZ, Medeiros LJ. Nucleophosmin gene mutations in acute myeloid leukemia. Arch Pathol Lab Med. 2006;130(11):1687–92.
Chiu A, Orazi A. Mastocytosis and related disorders. Semin Diagn Pathol. 2012;29(1):19–30.
Dicker F, Haferlach C, Sundermann J, Wendland N, Weiss T, Kern W, et al. Mutation analysis for RUNX1, MLL-PTD, FLT3-ITD, NPM1 and NRAS in 269 patients with MDS or secondary AML. Leukemia. 2010;24(8):1528–32.
Ettinger DS, Wood DE, Akerley W, Bazhenova LA, Borghaei H, Camidge DR, et al. NCCN guidelines insights: non-small cell lung cancer, version 4.2016. J Natl Compr Cancer Netw. 2016;14(3):255–64.
Fathi AT, Chen YB. The role of FLT3 inhibitors in the treatment of FLT3 mutated acute myeloid leukemia. Eur J Haematol. 2017;98(4):330–336
Gaidzik VI, Bullinger L, Schlenk RF, Zimmermann AS, Rock J, Paschka P, et al. RUNX1 mutations in acute myeloid leukemia: results from a comprehensive genetic and clinical analysis from the AML study group. J Clin Oncol. 2011;29(10):1364–72.
Heinrich MC, Corless CL, Blanke CD, Demetri GD, Joensuu H, Roberts PJ, et al. Molecular correlates of imatinib resistance in gastrointestinal stromal tumors. J Clin Oncol. 2006;24(29):4764–74.
Hsueh YS, Lin CL, Chiang NJ, Yen CC, Li CF, Shan YS, et al. Selecting tyrosine kinase inhibitors for gastrointestinal stromal tumor with secondary KIT activation-loop domain mutations. PLoS One. 2013;8(6):e65762.
Ichikawa K, Aritaka N, Sekiguchi Y, Sugimoto KJ, Imai H, Komatsu N, et al. C-kit-positive acute myelogenous leukemia effectively treated with imatinib: a case report and review of the literature. Geriatr Gerontol Int. 2012;12(4):762–4.
Kemmer K, Corless CL, Fletcher JA, McGreevey L, Haley A, Griffith D, et al. KIT mutations are common in testicular seminomas. Am J Pathol. 2004;164(1):305–13.
Koschmieder S, Halmos B, Levantini E, Tenen DG. Dysregulation of the C/EBPalpha differentiation pathway in human cancer. J Clin Oncol. 2009;27(4):619–28.
Levis M. FLT3 mutations in acute myeloid leukemia: what is the best approach in 2013? Hematology Am Soc Hematol Educ Program. 2013;2013:220–6.
Ma F, Liu P, Lei M, Liu J, Wang H, Zhao S, et al. Design, synthesis and biological evaluation of indolin-2-one-based derivatives as potent, selective and efficacious inhibitors of FMS-like tyrosine kinase3 (FLT3). Eur J Med Chem. 2016;127:72–86.

Mangan JK, Speck NA. RUNX1 mutations in clonal myeloid disorders: from conventional cytogenetics to next generation sequencing, a story 40 years in the making. Crit Rev Oncog. 2011;16(1–2):77–91.

Marcucci G, Haferlach T, Dohner H. Molecular genetics of adult acute myeloid leukemia: prognostic and therapeutic implications. J Clin Oncol. 2011;29(5):475–86.

Ng SW, Mitchell A, Kennedy JA, Chen WC, McLeod J, Ibrahimova N, et al. A 17-gene stemness score for rapid determination of risk in acute leukaemia. Nature. 2016;540(7633):433–7.

Oh HS, Smart RC. Expression of CCAAT/enhancer binding proteins (C/EBP) is associated with squamous differentiation in epidermis and isolated primary keratinocytes and is altered in skin neoplasms. J Invest Dermatol. 1998;110(6):939–45.

Oh C, Kim H, Kang JS, Yun J, Sim J, Kim HM, et al. Synthetic strategy for increasing solubility of potential FLT3 inhibitor thieno[2,3-d]pyrimidine derivatives through structural modifications at the C2 and C6 positions. Bioorg Med Chem Lett. 2016;

Ohgami RS, Arber DA. The diagnostic and clinical impact of genetics and epigenetics in acute myeloid leukemia. Int J Lab Hematol. 2015;37(Suppl 1):122–32.

Schnittger S, Bacher U, Kern W, Alpermann T, Haferlach C, Haferlach T. Prognostic impact of FLT3-ITD load in NPM1 mutated acute myeloid leukemia. Leukemia. 2011;25(8):1297–304.

Smith ML, Hills RK, Grimwade D. Independent prognostic variables in acute myeloid leukaemia. Blood Rev. 2011;25(1):39–51.

Ziai JM, Siddon AJ. Education Committee of the Academy of Clinical Laboratory P, Scientists. Pathology consultation on gene mutations in acute myeloid leukemia. Am J Clin Pathol. 2015;144(4):539–54.

# Chapter 2
# Acute Myeloid Leukemia with Recurrent Genetic Abnormalities, Part II: Mutations Involving *CEBPA*, *NPM1*, and *RUNX1*

**Ryan S. Robetorye**

## Introduction

Recurrent chromosomal structural abnormalities identified by conventional cytogenetic studies and fluorescence in situ hybridization (FISH) analysis have a well-established role in the classification and risk stratification of acute myelogenous leukemia (AML). For example, patients with cytogenetic alterations associated with favorable risk, such as inv(16)(p13.1q22) or t(16;16)(p13.1;q22);*CBFB-MYH11*, t(15;17)(q22;q12);*PML-RARA*, and t(8;21)(q22;q22);*RUNX1-RUN1T1*, have relatively good responses to chemotherapy-based regimens. Patients with unfavorable risk profiles, including monosomies of chromosomes 5, 7, and 17, deletions of chromosomes 5q and 7q, 11q23 abnormalities other than t(9;11), 17p abnormalities, complex karyotypes (>3 abnormalities), and other specific chromosomal rearrangements such as inv(3)/t(3;3) and t(6;9), often require allogeneic stem cell transplantation to improve their prognosis. However, approximately one-half of AML cases have an intermediate cytogenetic risk associated with a normal karyotype and cannot be further subclassified using cytogenetics [1–3]. During the past decade, numerous molecular genetic tests and gene sequencing approaches have been employed to identify specific gene mutations that could be used to further divide cytogenetically normal AML (CN-AML) cases into clinically relevant prognostic subsets. The most frequently mutated genes in CN-AML occur in the *NPM1* (~50–60% of cases), *FLT3* and *DNMT3A* (~30% of cases), *TET2* (23%), *IDH2* (19%), *IDH1* (14%), *CEBPA* (10–18%), *NRAS* (13%), *ASXL1* (~10%), *WT1* (~7–13%, with increased mutation frequency in younger patients), and *RUNX1* (~6–26%, with increased mutation frequency in elderly individuals) genes [4–19].

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The discovery of such gene mutations in CN-AML resulted in inclusion of the provisional diagnostic entities "AML with mutated *CEBPA*" and "AML with mutated *NPM1*" in the "Acute myeloid leukemia with recurrent genetic abnormalities" category in the 2008 WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues [1]. However, a recent revision of the 2008 WHO Classification changed these two provisional gene mutation entities to distinct diagnostic entities and also added "AML with mutated *RUNX1*" as a new provisional diagnostic entity [20, 21]. Further refinement of CN-AML cases within the AML classification will improve the identification of molecularly defined subsets of AML patients with different risk categories and will also provide the possibility of minimal residual disease monitoring using such mutations in specific quantitative molecular assays [22, 23]. Among the most frequent CN-AML-associated mutations, those affecting the *CEBPA*, *NPM1*, and *RUNX1* genes are associated with distinct biological and clinical features and gene expression profiles [5, 24–28]. Therefore, this chapter will focus on the characteristic clinicopathologic features of AML with mutations in the *CEBPA*, *NPM1*, and *RUNX1* genes.

## AML with Biallelic Mutations of CEBPA

The *CEBPA* gene on chromosome 19q13.1 has a GC-rich coding region (greater than 70%) contained within a single exon and encodes for the CCAAT/enhancer binding protein alpha, a basic region leucine zipper transcription factor important for regulation of cell proliferation and differentiation of myeloid precursors [29–31]. *CEBPA* is transcribed as a single mRNA that is translated into two isoforms due to an alternative start site, resulting in a full-length 42 kDa protein and shorter 30 kDa N-terminal truncated isoform. Both CEBPA isoforms share a transactivation domain that mediates antimitotic effects through interactions with the cyclin dependent kinase inhibitor p21 [32] and regulates proliferation through interaction with the chromatin remodeling complex SWI/SNF [33] and inhibition of the cyclin dependent kinases CDK2 and CDK4 [34]. In *CEBPA* knockout mice, there is a selective block in neutrophil differentiation at the myeloblast stage that resembles the arrest in maturation observed in human AML patients [35]. Given the phenotype of the *CEBPA*-mutant mice, Pabst et al. looked for *CEBPA* mutations in a large collection of human AML and were the first to report the presence of these mutations in a subset of AML cases [36]. *CEBPA* mutations occur in 5–10% of de novo AML, but are most commonly found in CN-AML (10–18%) or AML with 9q deletion [19, 37]. Although there are no specific morphological characteristics of *CEBPA* mutation-positive leukemias, AML patients with these mutations tend to have higher hemoglobin levels, higher peripheral blood WBC and blast counts, lower platelet counts, and are less likely to present with lymphadenopathy or extramedullary leukemia [38].

Two major types of *CEBPA* mutations have been identified in AML that predominantly affect the N-terminal and C-terminal regions of the protein [39].

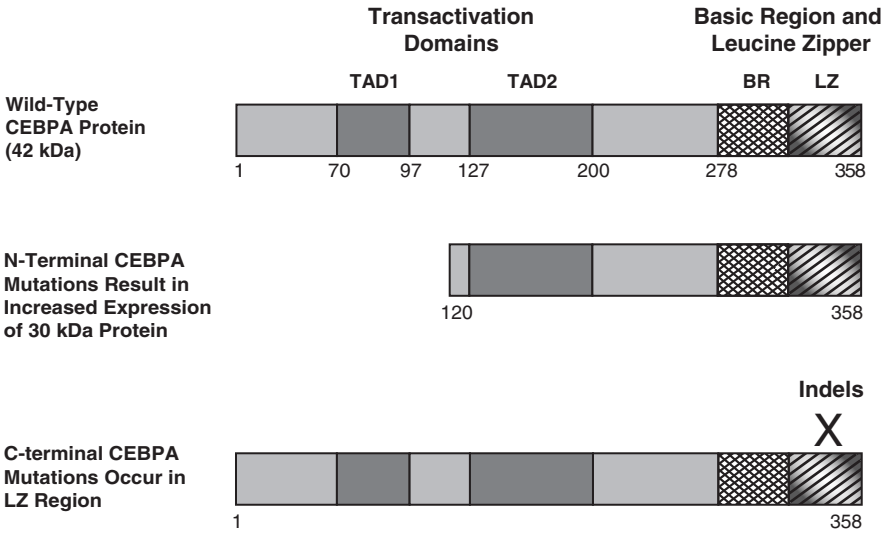


**Fig. 2.1** CEBPA protein structure. The wild-type CEBPA 42 kDa protein consists of two transactivation domains (*TAD1* and *TAD2*), a basic region (*BR*), and a leucine zipper region (*LZ*). The protein is translated as two isoforms due to an alternative start site, resulting in full-length 42 kDa and truncated 30 kDa isoforms (amino acid numbering is shown below each isoform). N-terminal mutations occur as frameshift mutations that cause premature termination of the 42 kDa protein and result in increased expression of the 30 kDa isoform that also acts as a dominant-negative inhibitor of wild-type *CEBPA*. C-terminal mutations occur as in-frame insertions or deletions in the *LZ* region and result in decreased DNA binding and/or dimerization activity

N-terminal mutations occur as frame-shift mutations that cause premature termination of the full-length 42 kDa protein and result in formation of a nonfunctional truncated 20 kDa protein, and increased expression of the 30 kDa isoform that also acts as a dominant-negative inhibitor of wild-type CEBPA (Fig. 2.1) [36]. C-terminal mutations occur as in-frame insertions or deletions in the leucine zipper region and result in decreased DNA binding and/or dimerization activity (Fig. 2.1). About two-thirds of AML with *CEBPA* mutations have two mutations (biallelic; also called double mutations), with the majority of cases (approximately 90%) being compound heterozygotes, harboring an N-terminal mutation on one allele and a C-terminal mutation on the second allele, and the rest of the cases being homozygous, with both mutations comprising either N-terminal or C-terminal mutations. Consequently, *CEBPA* double mutation cases lack detectable expression of wild-type 42 kDa CEBPA protein. The remaining one-third of AML with *CEBPA* mutations carry a single CEBPA mutation (single allele; also called single mutations) [40].

The role of *CEBPA* mutations in leukemogenesis was further clarified by Bereshchenko et al. using a mouse model for the spectrum of *CEBPA* mutations occurring in human AML [41]. They found that N-terminal and C-terminal *CEBPA*

mutations had different effects on hematopoietic stem cell expansion, homeostasis, and myeloid differentiation. The most efficient leukemogenesis occurred when pre-malignant hematopoietic stem cell expansion induced by the presence of a C-terminal *CEBPA* mutation was combined with maintenance of myeloid lineage commitment by the presence of a coexisting N-terminal *CEBPA* mutation. These findings in the mouse model are consistent with the prevalence of *CEBPA* double mutations in AML patients.

Germline *CEBPA* mutations have also been described in familial AML. These cases are typically heterozygous N-terminal frame-shift mutations that lead to preferential expression of the 30 kDa dominant-negative isoform of the CEBPA protein, suggesting that decreased CEBPA function might play a role in the subsequent development of AML [42–44]. Familial AML associated with mutated *CEBPA* is inherited in an autosomal dominant fashion and is highly penetrant, with most of the patients inheriting the mutated gene going on to develop AML. No genotype-phenotype correlations have been associated with germline *CEBPA* mutations, and most of these cases have a normal karyotype [45]. The majority of cases present at an early age and are associated with development of de novo disease without preceding cytopenias or myelodysplasia [46]. Most of the AML patients develop an additional C-terminal in-frame *CEBPA* mutation on a separate allele at diagnosis, with the C-terminal mutation subsequently disappearing on remission [47, 48]. Approximately 5–10% of *CEBPA*-mutated AML harbor germline mutations. Thus, germline N-terminal *CEBPA* mutations may predispose to the development of a subsequent somatic C-terminal mutation and progression to AML after a long latency period [47, 49]. Patients with familial *CEBPA*-mutated AML may also be at increased risk of developing additional leukemia clones after treatment of their initial leukemia and may acquire new *CEBPA* mutations that are different from those in the original leukemia [45]. Germline *CEBPA* mutations comprise a novel leukemia predisposition syndrome, with development of de novo AML without prodromal cytopenias. Genetic counseling is a critical component of management, and a thorough family history and germline DNA analysis of patients is recommended in patients over 50 years of age that present with double-mutated *CEBPA* in order to adequately identify families with germline *CEBPA* mutations requiring long-term surveillance [46].

Although more than 600 *CEBPA* mutations have been reported [37], several studies have also documented that the *CEBPA* gene can be inactivated (silenced) by methylation. Hypermethylation of the *CEBPA* core promoter (−141 to +103 from the transcription start site) was first reported in 2002 in two out of 70 (2.8%) AML cases, both of whom had AML-M2 morphology [50]. A correlation was also found by Wouters et al. between silenced *CEBPA* and core promoter hypermethylation in six out of 285 (1.4%) AML patients [51]. These cases also exhibited an expression profile that was similar to AML with mutated *CEBPA*, even though they lacked such mutations. However, Hackanson et al. observed that methylation of the distal *CEBPA* promoter region (1422 to −896 upstream from the transcription start site) was much more common than the core promoter, occurring in 20 out of 39 (51%) of AML patients, and that aberrant DNA methylation was strongly associated with

samples carrying the cytogenetically favorable inv(16) and t(15;17) abnormalities [52]. An evaluation of the *CEBPA* core, proximal (−1121 to −896), and distal promoter regions in 193 unselected patients with de novo AML by Lin et al. also found heterogeneous methylation in the distal promoter region, but not in the core or proximal promoter regions [53]. Hypermethylation of the distal *CEBPA* promoter was associated with decreased *CEBPA* expression, younger age, and the (15;17) translocation. Overall, AML patients with high *CEBPA* methylation also exhibited better response to induction chemotherapy than AML patients with low *CEBPA* methylation and showed longer disease-free and overall survival; however, the effects of *CEBPA* hypermethylation on patient prognosis have not been reproduced [54].

Initially, *CEBPA* single mutation and double mutation cases were considered to be a homogeneous group of AML with a favorable prognosis and precipitated the inclusion of these cases as a new provisional entity in the 2008 Classification of Tumours of Haematopoietic and Lymphoid Tissues [1]. However, numerous recent studies have clearly demonstrated that only AML harboring *CEBPA* double mutations represents a distinct entity with a favorable outcome, whereas AML with a *CEBPA* single mutation is associated with survival similar to that of AML with wild-type *CEBPA* [40, 49, 55–59]. Several clinical trials have demonstrated that the presence of a *CEBPA* double mutation is an independent prognostic factor for favorable outcome in AML cases [22], and this finding has also been biologically substantiated by the distinct gene expression signature associated with AML with *CEBPA* double mutation [40] and by the mouse experiments modeling the mutations by knock-in mutagenesis performed by Bereshchenko et al. [41]. Multilineage dysplasia can also occur in AML with mutated *CEBPA*, but this does not influence the favorable prognosis of these cases, further supporting the classification of these cases as a unique entity [60]. Interestingly, AML cases that harbor *CEBPA* double mutations usually exhibit a characteristic immunophenotype that includes aberrant expression of CD7 on the leukemic cells [61]. Therefore, with regard to disease classification and risk stratification, only AML cases with biallelic *CEBPA* mutations should be regarded and as a separate entity associated with a favorable prognosis. These findings are reflected in the decision to change the provisional category of "AML with mutated *CEBPA*" to the distinct diagnostic entity "AML with biallelic mutations of *CEBPA*" in the recent 2016 revision of the WHO classification of myeloid neoplasms and acute leukemia [21].

## AML with Mutated NPM1

The *NPM1* gene on chromosome 5q35 contains 12 exons and encodes for a 32 kDa nucleolar protein that shuttles continuously between the nucleus and the cytoplasm [37, 62–65]. Nucleophosmin (NPM) is a multifunctional protein that acts as a molecular chaperone to facilitate multiple protein-protein interactions and has both proliferative and growth suppressive roles [66, 67]. NPM is involved in critical cell functions, including ribosome biogenesis and export and regulation of centrosome

duplication [68–70], and also contributes to genomic stability by controlling DNA repair mechanisms [71, 72]. NPM is also involved in the control of cellular proliferation and apoptosis through interactions with the important regulatory proteins TP53, RB, $p19^{ARF}$, and HDM2 [73–77]. *NPM1* knockout mice show abnormal organogenesis and exhibit embryonic lethality due to severe anemia resulting from defects in primitive hematopoiesis. However, heterozygous *NPM1* knockout mice are viable and appear to thrive, but show unrestricted centrosome duplication and genetic instability, resulting in a hematologic syndrome with features similar to those observed in human patients with a myelodysplastic syndrome [78]. Heterozygous mice also show increased susceptibility to development of hematologic malignancies of both myeloid and lymphoid origin, with myeloid malignancies displaying the highest incidence [79]. The discovery of *NPM1* mutations in AML resulted from the observation that many cases of AML showed an aberrant expression of nucleophosmin protein in the cytoplasm of the leukemic cells [4]. Cytoplasmic NPM was detected in 208 out of 591 (35%) primary AML specimens, but not in 135 secondary AML or in 980 hematopoietic or extrahematopoietic neoplasms other than AML. This immunohistochemical finding led to sequencing of the *NPM1* gene and the identification of exon 12 mutations in nearly all of the AML cases with cytoplasmic NPM. There are no specific morphological characteristics exhibited by these leukemias; however, cytoplasmic/*NPM1*-mutated AML is associated with distinctive clinicopathological features, including a normal karyotype, female sex, strong expression of CD33, lack of CD34 expression in the leukemic cells (more than 95% are CD34-negative), hypercellular bone marrow with multilineage involvement, high white blood cell count at presentation, high blast percentage at diagnosis, high incidence of extramedullary dissemination (mostly confined to gingival hyperplasia and lymphadenopathy), and good response to induction chemotherapy [4, 80]. Cytoplasmic NPM is also readily detectable by immunohistochemistry in routinely-fixed paraffin-embedded tissue samples and can be used as a surrogate for molecular *NPM1* testing, making this technique potentially useful in the diagnosis of *NPM1*-mutated AML and myeloid sarcoma [81, 82].

*NPM1* mutations are the most common genetic abnormality in adult AML, comprising approximately 30% of all AML and 50–60% of CN-AML [19]. *NPM1* mutations are usually restricted to frameshift mutations in exon 12 and are characteristically heterozygous with a retained wild type allele [83]. Approximately 55 molecular variants of *NPM1* exon 12 mutations have been identified so far [84], and only rare cases with a mutation occurring outside exon 12 have been reported, including a splice-site donor mutation in exon 9 [85] and an 8 base pair insertion in exon 11 [86]. Greater than 95% of *NPM1* mutations occur as a 4 base pair insertion at nucleotide position 960. A tandem duplication of TCTG accounts for approximately 80% of cases (so-called “mutation A”), with CATG (“mutation B”) and CCTG (“mutation D”) tetranucleotide duplications accounting for an additional 10% and 5% of cases, respectively. Other mutations (so-called “mutation C” [CGTG], “mutation E” [CTCTTGCCC], and “mutation F” [CCCTGGAGA]) are extremely rare [4, 84]. All *NPM1* exon 12 mutation variants cause similar alterations in the C-terminus of the mutant proteins and result in the creation of a new

nuclear export motif. As a consequence, the NPM protein mutants aberrantly accumulate in the cytoplasm of leukemic cells [84]. *NPM1* mutations are highly stable and may be detected at AML relapse many years after initial diagnosis, in patients with more than one relapse, and even in relapses that occur at extramedullary sites [87–89]. Uncommon cases with loss of *NPM1* mutations at leukemia relapse may be associated with development of new cytogenetic abnormalities [90, 91]. *NPM1* mutations appear to be mutually exclusive of other recurrent cytogenetic abnormalities in AML [92]. A small number of *NPM1*-mutated AML also carry a *CEBPA* mutation, but analysis of *NPM1*/*CEBPA* mutated cases has shown that this rare association only occurs with *CEBPA* single mutation cases and is mutually exclusive of *CEBPA* double mutations [93].

The pathogenic role played by *NPM1* mutation in the development of AML is still not completely understood. *NPM1* can function as an oncogene as well as a tumor suppressor depending on gene dosage, expression level, interacting proteins, and cell localization, with many of the functions of NPM1 requiring continuous shuttling between the cytoplasm, nucleoplasm, and nucleoli [80, 93]. However, because the *NPM1* mutation always results in aberrant cytoplasmic localization of the mutant protein, this event appears to be critical for leukemogenesis. The *NPM1* mutation likely affects multiple cellular pathways by a combination of loss of function of the nucleophosmin nucleolar-interacting proteins that are delocalized by movement of the mutant protein into the leukemic cell cytoplasm, and gain of function resulting from the deregulated shuttling of the mutant protein between the nucleus and the cytoplasm [94]. Additional insights regarding the role of *NPM1* mutation in leukemogenesis have recently been provided by Vassiliou et al. [95]. These authors used a strategy of introducing a humanized *NPM1* mutation allele into the endogenous *NPM1* locus in a knock-in mouse model, thus keeping *NPM1* expression under the control of the endogenous promoter and mimicking the process of human AML development. Hematopoietic stem cells derived from these mice exhibited overexpression of homeodomain-containing transcription factor (*HOX*) genes (a characteristic also found in *NPM1*-mutated human AML [27, 28]), enhanced self-renewal, and expanded myelopoiesis. In addition, one-third of the mice also developed delayed-onset AML, demonstrating that the *NPM1* mutation can act as an AML-driving lesion [96].

The presence of *NPM1* mutation has emerged as an important favorable prognostic factor in AML patients. AML with mutated *NPM1* are highly responsive to induction chemotherapy [4, 80], and patients with CN-AML and mutated *NPM1* also exhibit higher rates of complete remission, disease-free survival, and overall survival compared to CN-AML with wild type *NPM1* [24]. However, the prognostic importance of the *NPM1* mutation in AML is also dependent upon the mutation status of the fms-like tyrosine kinase-3 (*FLT3*) gene [6]. Internal tandem duplication (ITD) mutations in the *FLT3* gene are found in approximately 40% of patients with AML and mutated *NPM1* compared with approximately 14% of the *NPM1* wild type cases [97]. The *FLT3*-ITD mutation in AML is usually associated with more aggressive disease, high white blood cell counts, early relapses, and poor survival [23]. Therefore, *NPM1* mutations confer a favorable prognosis in CN-AML

only in the absence of a concomitant *FLT3*-ITD mutation, and testing for both gene mutations should be performed together to provide the most accurate prognostic information [2, 80]. The favorable prognostic effects of *NPM1* mutations also appear to be dominant over other secondary AML features such as chromosomal abnormalities and multilineage dysplasia, which may be present in up to 15% and 23% of *NPM1*-mutated AML cases, respectively [98, 99].

The distinct biological properties of *NPM1*-mutated AML have also been confirmed by a number of gene expression profiling studies that showed upregulation of several *HOX* and transcription activator-like effector (*TALE*) gene family members [28, 100, 101]. These genes are known to be important for hematopoietic cell development and stem cell maintenance, supporting the possibility that *NPM1*-mutated AML is derived from a multipotent hematopoietic progenitor. However, the mechanism by which *NPM1* mutation leads to aberrant expression of *HOX* genes is unclear. Mutation of *NPM1* could directly influence the expression of *HOX* genes, or perhaps, *NPM1* mutation might cause arrested development of hematopoietic cell precursors at a primitive stage when the expression of *HOX* genes is elevated. It has also been suggested that microRNAs (miRNAs) could play a key role in the upregulation of *HOX* genes in *NPM1*-mutated AML. A unique miRNA signature was found in *NPM1*-mutated cases, with upregulation of *miR-10a*, *miR-10b*, *miR-196a*, *miR-196b*, and three families of tumor suppressor miRNAs, including *miR-15-a*/*miR-16-1*, *mir-29s* (*a/b/c*), and *let-7* family members (*7a/7b/7f*) [27, 102]. Several other miRNAs were found to be downregulated, including *miR-204* and *miR-128a*. Interestingly, miRNAs *10a*, *10b*, *196a*, and *196b* are all located within the genomic cluster of *HOX* genes, and *miR-204* has been shown to target the expression of the *HOXA10* and *MEIS1* genes, suggesting that *HOX* gene dysregulation in *NPM1*-mutated AML could be the result of altered miRNA expression. These findings are consistent with the possibility of an aberrant regulatory network including *NPM1*, *HOX* genes, and miRNAs that might be engaged in the arrest of cellular differentiation of hematopoietic precursors and development of AML with mutated *NPM1* [84].

Patients with AML that are found to have minimal residual disease after induction chemotherapy or before stem cell transplantation are more prone to relapse disease and show decreased overall survival [103–105]. The high incidence of the *NPM1* mutation in AML (present in approximately 30% of all AML and 50–60% in CN-AML), and the stability of the *NPM1* mutation in AML during disease evolution, makes this mutation a useful marker for the detection of submicroscopic levels of leukemia (minimal residual disease detection) after therapy. Numerous studies have now shown that assessment of *NPM1* mutation status in AML can serve as an important tool for prognosis prediction and therapy guidance [106–111]. A recent publication by Ivey et al. also clearly indicates that assessment of minimal residual disease in AML patients (based on the detection and quantitation of *NPM1*-mutated transcripts) provides prognostic information that is independent of other risk factors [112]. Patients who developed morphologic remission after chemotherapy and showed evidence of minimal residual disease in peripheral blood, as compared to patients with no evidence of minimal residual disease, had a significantly greater

risk of relapse (82% versus 30%) and a lower rate of survival (24% versus 75%). Multivariate analysis showed that the presence of minimal residual disease, as evidenced by persistence of *NPM1*-mutated transcripts, was the only significant prognostic factor for relapse and survival [112]. The ability to reclassify standard-risk or low-risk patients as high risk based on persistence of *NPM1*-mutated transcripts may help to appropriately stratify patients who would benefit from stem cell transplant rather than chemotherapy alone, and bone marrow transplant might be also be appropriately avoided in high-risk patients that have no evidence of minimal residual disease after therapy [113].

## AML with Mutated RUNX1

In 1973, Dr. Janet Rowley identified a reciprocal translocation between chromosomes 8 and 21 in two patients with AML [114]. Eighteen years later, the Acute Myeloid Leukemia 1 (*AML1*) gene located at the breakpoint of the (8;21)(q22;q22) translocation on chromosome 21q22.12 was cloned and later renamed as the Runt-related Transcription Factor 1 (*RUNX1*) gene (named after the Drosophila RUNT protein, the first member of this gene family to be cloned) [115, 116]. The *RUNX1* gene contains 10 exons and encodes a sequence-specific DNA binding protein characterized by an N-terminal RUNT homology domain (RHD; exons 3–5) that mediates DNA binding and interaction with core-binding factor beta (CBFB), and a C-terminal transactivation domain (TAD; exons 6–8) [117]. *RUNX1* is widely expressed in hematopoietic cells and is required for the establishment of definitive hematopoiesis. In mouse models, *RUNX1* is absolutely required for embryogenesis and hematopoiesis, and lack of the *RUNX1* gene in homozygous mutants results in midgestation embryonic death due to necrosis and extensive hemorrhage in the central nervous system [118]. Because germline deletions are lethal, conditional *RUNX1* deletion strategies have been necessary to determine the role of this gene in adult hematopoiesis. Disruption of the *RUNX1* gene in adult hematopoietic stem cells using an inducible gene-targeting method causes inhibition of megakaryocytic maturation, increased hematopoietic progenitor cells, and defective B-lymphocyte and T-lymphocyte development [119]. Interestingly, loss of *RUNX1* function in hematopoietic stem cells does not result in AML, but rather establishes a preleukemic state that predisposes to AML following acquisition of additional mutations [117].

In human acute leukemia, the *RUNX1* gene is involved in a number of recurrent chromosomal translocations, including t(8;21)(q22;q22); *RUNX1-RUNX1T1* and t(3;21)(q26.2;q22); *MECOM(EVI1)-RUNX1* in AML, and t(12;21)(p13;q22); *ETV6-RUNX1* in B-lymphoblastic leukemia. Recurrent intragenic mutations have also been identified in AML, myelodysplastic syndromes (MDS), AML after MDS, therapy-related MDS and AML, radiation-exposed patients with MDS/AML, chronic myelomonocytic leukemia, and T-lymphoblastic leukemia [8, 120]. In AML, *RUNX1* mutations are found in both of the functional domains of the protein

(N-terminal RHD and C-terminal TAD), with the majority of mutations occurring in the RHD [5, 8, 14, 15, 26, 120]. Most *RUNX1* mutations are mono-allelic, and the mutational spectrum includes N-terminal missense mutations and C-terminal truncating mutations that result in deletion of the TAD of the protein. Both missense and truncating mutations cause loss of normal *RUNX1* function and also appear to have a dominant negative effect on the transactivation activity of wild type *RUNX1* in a dose-dependent fashion [121].

Rare germline mutations in the *RUNX1* gene have also been reported that are associated with an autosomal dominant disorder known as familial platelet disorder with predisposition to acute myeloid leukemia (FPD/AML). In FPD/AML families, there is thrombocytopenia with normal platelet size and platelet dysfunction with abnormal aggregation and secretion responses. Most FPD/AML patients exhibit mucocutaneous bleeding symptoms that include easy bruising, epistaxis, and bleeding after minor surgical or dental procedures. Up to 35% of these individuals eventually develop AML [122]. Among the more than 30 families described with FPD/AML, most carriers have a hemizygous *RUNX1* gene mutation. However, within individual FPD/AML families there is variable penetrance, with carriers of the same *RUNX1* mutations displaying heterogeneity in the degree of thrombocytopenia, platelet dysfunction, and bleeding. In addition, some family members develop myeloid malignancies, though not always of the same type, while other family members may remain unaffected. Different families also exhibit varying risks of progression to myeloid malignancy, likely due to the unique *RUNX1* mutations in each family group. The specific mechanisms underlying progression to acute leukemia in *RUNX1* mutation carriers are unknown, but could include haplosufficiency, dominant negative effects on normal *RUNX1* function, acquisition of another *RUNX1* mutation in the nonmutated germline allele, or acquisition of additional cooperating mutations [45]. Familial clustering of myelodysplastic syndromes and acute myeloid leukemia (MDS/AML) is also seen in patients with inherited disorders such as Fanconi anemia and congenital neutropenia, and these patients have also frequently been shown to carry *RUNX1* mutations [120, 123]. Since not all family members with familial *RUNX1* mutations exhibit clinical findings, testing for germline *RUNX1* mutations is recommended when more than two individual family members have been diagnosed with a myeloid malignancy [124].

*RUNX1* mutations in AML are almost entirely mutually exclusive of the recurrent genetic abnormalities recognized in the WHO classification and complex abnormal karyotypes, but are frequently associated with trisomy 13 and monosomy 7/del(7q) [8, 120, 125]. Somatic alterations of the second *RUNX1* allele can also be found in a small percentage of AML cases, suggesting a role as a classical tumor suppressor gene [125, 126]. AML with *RUNX1* mutation has been associated with specific clinical and pathological features, including male gender, older age, more immature morphology (M0 French-American-British subtype), and secondary AML evolving from MDS [15, 121, 127]. These parameters also have well-recognized adverse effects on clinical outcome. In numerous studies, the *RUNX1* mutation has been shown to be a significant predictor of resistance to standard induction therapy and for inferior survival rate [5, 8, 14, 15, 25]. In univariate analy-

ses, *RUNX1* mutations are associated with refractory disease and inferior rates of event free survival, relapse free survival, and overall survival [120]. Gene expression profiling analysis has also provided evidence that *RUNX1*-mutated AML shares distinct biological features with other high-risk AML, with enrichment of *RUNX1*-mutated cases in complex karyotype AML and cytogenetic adverse-risk cases with monosomy 7, del(7q), inv(3), and t(3;3) [5]. *RUNX1*-mutated AML also exhibits upregulation of genes normally expressed in primitive hematopoietic cells and B-cell progenitors, and downregulation of promoters of myelopoiesis [26]. These cases also exhibit characteristic microRNA expression signatures, with downregulation microRNAs normally expressed in definitive myeloid progenitors (*miR-223*) and distinctly myeloid AML blasts (*miR-99a* and *miR-100*) [26]. The poor outcome of patients with the *RUNX1*-mutated AML suggests that novel therapeutic approaches should be used, including allogeneic stem cell transplant in younger patients, and investigational therapies in older patients [15, 120].

## Future Perspectives and Conclusions

Emerging data from newer molecular techniques such as next generation sequencing (NGS) have revealed that CN-AML harbor numerous gene mutations, with most of them representing background mutations, and only a limited number having important prognostic and therapeutic implications [22]. The evaluation of these mutations as prognostic and predictive markers is a highly active research area, and some of these findings have been incorporated in a recent revision of the 2008 WHO Classification that changed two provisional gene mutation entities "AML with mutated *CEBPA*" and "AML with mutated *NPM1*" to clinically distinct diagnostic entities and also added "AML with mutated *RUNX1*" as a new provisional diagnostic entity [21]. Currently, there are three main genes (*NPM1*, *CEBPA*, and *FLT3*) routinely analyzed for molecular mutations that are used in clinical practice for the diagnosis and management of AML [128]. However, the addition of "AML with mutated *RUNX1*" as a provisional diagnostic entity will also require adding mutational analysis of this gene to current practice. The characteristic clinical and biological features of AML with *CEBPA*, *NPM1*, *RUNX1* gene mutations are summarized in Table 2.1. Evaluation for additional molecular mutations in other genes consistently associated with inferior outcomes, such as *ASXL1* and *TP53*, are also likely to be included in updated recommendations [20, 129]. The prognostic importance of other genes commonly mutated in AML such as *DNMT3A*, *TET2*, *IDH1*, *IDH2*, *NRAS*, *WT1* is less clear, but mutation testing for some of these genes may also be indicated due to the existence of therapies targeting specific gene mutations or combinations of mutations.

Within the past decade, nearly a dozen adult-onset inherited myelodysplastic syndrome and leukemia predisposition syndromes have also been identified. Individuals with inherited forms of hematologic malignancies are currently underdiagnosed due to the low frequency of cases and low level of clinician awareness of

**Table 2.1** Characteristic clinical and biological features of acute myeloid leukemia with gene mutations

| | AML with biallelic *CEBPA* mutations | AML with mutated *NPM1* | AML with mutated *RUNX1* |
|---|---|---|---|
| Frequency in cytogenetically normal AML | 10–18% | 50–60% | 6–26%; increased frequency in elderly individuals |
| Clinical characteristics | High hemoglobin levels<br>High WBC and blast counts<br>Low platelet counts<br>Low extramedullary involvement | Female sex<br>High WBC and blast counts<br>High extramedullary involvement | Male sex<br>Older age<br>Less blast differentiation (FAB M0) |
| Surface ntigen expression characteristics | Aberrant expression of CD7 | Strong CD33 expression<br>Lack of CD34 expression (>95% CD34-negative) | None |
| Germline mutations | 10–15% | No | Rare |
| Response to chemotherapy | Good | Good | Poor |
| Distinct gene expression profile | Yes | Yes | Yes |
| Distinct microRNA expression profile | Yes | Yes | Yes |
| Gene mutation results in abnormal protein localization | No | Yes (aberrant cytoplasmic expression) | No |
| Prognosis | Good | Good | Poor |

these syndromes. However, these individuals are increasingly likely to be encountered in clinical practice with wider adoption of NGS-based testing for the detection of prognostically significant or targetable genomic alterations in hematologic malignancies [130]. FDP/AML due to inherited *RUNX1* mutations was the first hereditary myeloid malignancy syndrome (HMMS) to be defined in 1999, followed by familial AML with *CEBPA* mutation in 2004 (both discussed earlier in this chapter). Expanding use of NGS has also contributed to the rapid identification of several additional HMMS, including familial MDS/AML with *GATA2* mutation, thrombocytopenia 2 (*ANKRD26*), myeloid neoplasms with germline predisposition (*ATG2B/GSKIP*), familial MDS/AML with mutated *DDX41*, thrombocytopenia 5

(*ETV6*), familial aplastic anemia/MDS with *SRP72* mutation, and an adult-onset inherited bone marrow failure/telomere syndrome with familial MDS/AML (*TERC/TERT*) [130, 131]. Clinicians must increasingly recognize the possibility that mutations identified in some genes, like *CEBPA* and *RUNX1* and others listed above, may represent pathogenic germline mutations and initiate appropriate follow-up germline genetic testing. The increasing importance of recognition of germline mutations is evidenced by the inclusion of a new category in the 2016 revision of the WHO classification of myeloid neoplasms and acute leukemia designated "Classification of myeloid neoplasm with germline predisposition" [21].

Given the growing number of gene mutations that are considered prognostically and therapeutically important in MDS/AML, there is no doubt that NGS testing will be showing increased use in conjunction with standard methods such as morphology and flow cytometry for diagnostic purposes. A major advantage of NGS-based assays is that they can detect and monitor multiple mutations simultaneously in a single cost-effective assay. This capability will also allow the possibility of NGS-based minimal residual disease monitoring assays in the future for detection of small clonal populations and low-frequency somatic mutations associated with MDS/AML [132].

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## References

1. Swerdlow SH, Campo E, Harris NL, Jaffee ES, Pileri SA, Stein H, Thiele J, Vardiman JW. WHO classification of tumours of haematopoietic and lymphoid tissues. 4th ed. Lyon: International Agency for Research on Cancer; 2008.
2. Wang ML, Bailey NG. Acute myeloid leukemia genetics: risk stratification and implications for therapy. Arch Pathol Lab Med. 2015;139(10):1215–23.
3. Estey EH. Acute myeloid leukemia: 2014 update on risk-stratification and management. Am J Hematol. 2014;89(11):1063–81.
4. Falini B, Mecucci C, Tiacci E, Alcalay M, Rosati R, Pasqualucci L, et al. Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. N Engl J Med. 2005;352(3):254–66.
5. Gaidzik VI, Bullinger L, Schlenk RF, Zimmermann AS, Rock J, Paschka P, et al. RUNX1 mutations in acute myeloid leukemia: results from a comprehensive genetic and clinical analysis from the AML study group. J Clin Oncol. 2011;29(10):1364–72.
6. Schlenk RF, Dohner K, Krauter J, Frohling S, Corbacioglu A, Bullinger L, et al. Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. N Engl J Med. 2008;358(18):1909–18.
7. Schnittger S, Schoch C, Kern W, Mecucci C, Tschulik C, Martelli MF, et al. Nucleophosmin gene mutations are predictors of favorable prognosis in acute myelogenous leukemia with a normal karyotype. Blood. 2005;106(12):3733–9.
8. Schnittger S, Dicker F, Kern W, Wendland N, Sundermann J, Alpermann T, et al. RUNX1 mutations are frequent in de novo AML with noncomplex karyotype and confer an unfavorable prognosis. Blood. 2011;117(8):2348–57.
9. Metzeler KH, Becker H, Maharry K, Radmacher MD, Kohlschmidt J, Mrozek K, et al. ASXL1 mutations identify a high-risk subgroup of older patients with primary cytogenetically normal AML within the ELN Favorable genetic category. Blood. 2011;118(26):6920–9.

10. Marcucci G, Maharry K, YZ W, Radmacher MD, Mrozek K, Margeson D, et al. IDH1 and IDH2 gene mutations identify novel molecular subsets within de novo cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B study. J Clin Oncol. 2010;28(14):2348–55.
11. Hou HA, Kuo YY, Liu CY, Chou WC, Lee MC, Chen CY, et al. DNMT3A mutations in acute myeloid leukemia: stability during disease evolution and clinical implications. Blood. 2012;119(2):559–68.
12. Metzeler KH, Maharry K, Radmacher MD, Mrozek K, Margeson D, Becker H, et al. TET2 mutations improve the new European LeukemiaNet risk classification of acute myeloid leukemia: a Cancer and Leukemia Group B study. J Clin Oncol. 2011;29(10):1373–81.
13. Schnittger S, Eder C, Jeromin S, Alpermann T, Fasan A, Grossmann V, et al. ASXL1 exon 12 mutations are frequent in AML with intermediate risk karyotype and are independently associated with an adverse outcome. Leukemia. 2013;27(1):82–91.
14. Greif PA, Konstandin NP, Metzeler KH, Herold T, Pasalic Z, Ksienzyk B, et al. RUNX1 mutations in cytogenetically normal acute myeloid leukemia are associated with a poor prognosis and up-regulation of lymphoid genes. Haematologica. 2012;97(12):1909–15.
15. Tang JL, Hou HA, Chen CY, Liu CY, Chou WC, Tseng MH, et al. AML1/RUNX1 mutations in 470 adult patients with de novo acute myeloid leukemia: prognostic implication and interaction with other gene alterations. Blood. 2009;114(26):5352–61.
16. Becker H, Marcucci G, Maharry K, Radmacher MD, Mrozek K, Margeson D, et al. Mutations of the Wilms tumor 1 gene (WT1) in older patients with primary cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B study. Blood. 2010;116(5):788–92.
17. Virappane P, Gale R, Hills R, Kakkas I, Summers K, Stevens J, et al. Mutation of the Wilms' tumor 1 gene is a poor prognostic factor associated with chemotherapy resistance in normal karyotype acute myeloid leukemia: the United Kingdom Medical Research Council Adult Leukaemia Working Party. J Clin Oncol. 2008;26(33):5429–35.
18. Gaidzik VI, Schlenk RF, Moschny S, Becker A, Bullinger L, Corbacioglu A, et al. Prognostic impact of WT1 mutations in cytogenetically normal acute myeloid leukemia: a study of the German-Austrian AML Study Group. Blood. 2009;113(19):4505–11.
19. Marcucci G, Haferlach T, Dohner H. Molecular genetics of adult acute myeloid leukemia: prognostic and therapeutic implications. J Clin Oncol. 2011;29(5):475–86.
20. Dohner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia. N Engl J Med. 2015;373(12):1136–52.
21. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–405.
22. Martelli MP, Sportoletti P, Tiacci E, Martelli MF, Falini B. Mutational landscape of AML with normal cytogenetics: biological and clinical implications. Blood Rev. 2013;27(1):13–22.
23. Falini B, Martelli MP. Impact of genomics in the clinical management of patients with cytogenetically normal acute myeloid leukemia. Best Pract Res Clin Haematol. 2015;28(2–3):90–7.
24. Marcucci G, Maharry K, Radmacher MD, Mrózek K, Vukosavljevic T, et al. Prognostic significance of, and gene and microRNA expression signatures associated with, CEBPA mutations in cytogenetically normal acute myeloid leukemia with high-risk molecular features: a Cancer and Leukemia Group B Study. J Clin Oncol. 2008;26(31):5078–87.
25. Becker H, Marcucci G, Maharry K, Radmacher MD, Mrozek K, Margeson D, et al. Favorable prognostic impact of NPM1 mutations in older patients with cytogenetically normal de novo acute myeloid leukemia and associated gene- and microRNA-expression signatures: a Cancer and Leukemia Group B study. J Clin Oncol. 2010;28(4):596–604.
26. Mendler JH, Maharry K, Radmacher MD, Mrozek K, Becker H, Metzeler KH, et al. RUNX1 mutations are associated with poor outcome in younger and older patients with cytogenetically normal acute myeloid leukemia and with distinct gene and MicroRNA expression signatures. J Clin Oncol. 2012;30(25):3109–18.
27. Garzon R, Garofalo M, Martelli MP, Briesewitz R, Wang L, Fernandez-Cymering C, et al. Distinctive microRNA signature of acute myeloid leukemia bearing cytoplasmic mutated nucleophosmin. Proc Natl Acad Sci U S A. 2008;105(10):3945–50.

28. Alcalay M, Tiacci E, Bergomas R, Bigerna B, Venturini E, Minardi SP, et al. Acute myeloid leukemia bearing cytoplasmic nucleophosmin (NPMc+ AML) shows a distinct gene expression profile characterized by up-regulation of genes involved in stem-cell maintenance. Blood. 2005;106(3):899–902.
29. Antonson P, Xanthopoulos KG. Molecular cloning, sequence, and expression patterns of the human gene encoding CCAAT/enhancer binding protein alpha (C/EBP alpha). Biochem Biophys Res Commun. 1995;215(1):106–13.
30. Tenen DG. Disruption of differentiation in human cancer: AML shows the way. Nat Rev Cancer. 2003;3(2):89–101.
31. Ohlsson E, Schuster MB, Hasemann M, Porse BT. The multifaceted functions of C/EBPalpha in normal and malignant haematopoiesis. Leukemia. 2016;30(4):767–75.
32. Timchenko NA, Harris TE, Wilde M, Bilyeu TA, Burgess-Beusse BL, Finegold MJ, et al. CCAAT/enhancer binding protein alpha regulates p21 protein and hepatocyte proliferation in newborn mice. Mol Cell Biol. 1997;17(12):7353–61.
33. Muller C, Calkhoven CF, Sha X, Leutz A. The CCAAT enhancer-binding protein alpha (C/EBPalpha) requires a SWI/SNF complex for proliferation arrest. J Biol Chem. 2004;279(8):7353–8.
34. Wang H, Iakova P, Wilde M, Welm A, Goode T, Roesler WJ, et al. C/EBPalpha arrests cell proliferation through direct inhibition of Cdk2 and Cdk4. Mol Cell. 2001;8(4):817–28.
35. Zhang DE, Zhang P, Wang ND, Hetherington CJ, Darlington GJ, Tenen DG. Absence of granulocyte colony-stimulating factor signaling and neutrophil development in CCAAT enhancer binding protein alpha-deficient mice. Proc Natl Acad Sci U S A. 1997;94(2):569–74.
36. Pabst T, Mueller BU, Zhang P, Radomska HS, Narravula S, Schnittger S, et al. Dominant-negative mutations of CEBPA, encoding CCAAT/enhancer binding protein-alpha (C/EBPalpha), in acute myeloid leukemia. Nat Genet. 2001;27(3):263–70.
37. Ziai JM, Siddon AJ. Pathology consultation on gene mutations in acute myeloid leukemia. Am J Clin Pathol. 2015;144(4):539–54.
38. Fröhling S, Schlenk RF, Stolze I, Bihlmayr J, Benner A, Kreitmeier S, et al. CEBPA mutations in younger adults with acute myeloid leukemia and normal cytogenetics: prognostic relevance and analysis of cooperating mutations. J Clin Oncol. 2004;22(4):624–33.
39. Koschmieder S, Halmos B, Levantini E, Tenen DG. Dysregulation of the C/EBPalpha differentiation pathway in human cancer. J Clin Oncol. 2009;27(4):619–28.
40. Wouters BJ, Löwenberg B, Erpelinck-Verschueren CA, van Putten WL, Valk PJ, Delwel R. Double CEBPA mutations, but not single CEBPA mutations, define a subgroup of acute myeloid leukemia with a distinctive gene expression profile that is uniquely associated with a favorable outcome. Blood. 2009;113(13):3088–91.
41. Bereshchenko O, Mancini E, Moore S, Bilbao D, Mansson R, Luc S, et al. Hematopoietic stem cell expansion precedes the generation of committed myeloid leukemia-initiating cells in C/EBPalpha mutant AML. Cancer Cell. 2009;16(5):390–400.
42. Smith ML, Cavenagh JD, Lister TA, Fitzgibbon J. Mutation of CEBPA in familial acute myeloid leukemia. N Engl J Med. 2004;351(23):2403–7.
43. Sellick GS, Spendlove HE, Catovsky D, Pritchard-Jones K, Houlston RS. Further evidence that germline CEBPA mutations cause dominant inheritance of acute myeloid leukaemia. Leukemia. 2005;19(7):1276–8.
44. Pabst T, Mueller BU. Complexity of CEBPA dysregulation in human acute myeloid leukemia. Clin Cancer Res. 2009;15(17):5303–7.
45. Nickels EM, Soodalter J, Churpek JE, Godley LA. Recognizing familial myeloid leukemia in adults. Ther Adv Hematol. 2013;4(4):254–69.
46. Tawana K, Wang J, Renneville A, Bödör C, Hills R, Loveday C, et al. Disease evolution and outcomes in familial AML with germline CEBPA mutations. Blood. 2015;126(10):1214–23.
47. Pabst T, Eyholzer M, Haefliger S, Schardt J, Mueller BU. Somatic CEBPA mutations are a frequent second event in families with germline CEBPA mutations and familial acute myeloid leukemia. J Clin Oncol. 2008;26(31):5088–93.

48. Nanri T, Uike N, Kawakita T, Iwanaga E, Mitsuya H, Asou N. A family harboring a germline N-terminal C/EBPalpha mutation and development of acute myeloid leukemia with an additional somatic C-terminal C/EBPalpha mutation. Genes Chromosomes Cancer. 2010;49(3):237–41.
49. Taskesen E, Bullinger L, Corbacioglu A, Sanders MA, Erpelinck CA, Wouters BJ, et al. Prognostic impact, concurrent genetic mutations, and gene expression features of AML with CEBPA mutations in a cohort of 1182 cytogenetically normal AML patients: further evidence for CEBPA double mutant AML as a distinctive disease entity. Blood. 2011;117(8):2469–75.
50. Chim CS, Wong AS, Kwong YL. Infrequent hypermethylation of CEBPA promotor in acute myeloid leukaemia. Br J Haematol. 2002;119(4):988–90.
51. Wouters BJ, Jordà MA, Keeshan K, Louwers I, Erpelinck-Verschueren CA, Tielemans D, et al. Distinct gene expression profiles of acute myeloid/T-lymphoid leukemia with silenced CEBPA and mutations in NOTCH1. Blood. 2007;110(10):3706–14.
52. Hackanson B, Bennett KL, Brena RM, Jiang J, Claus R, Chen SS, et al. Epigenetic modification of CCAAT/enhancer binding protein alpha expression in acute myeloid leukemia. Cancer Res. 2008;68(9):3142–51.
53. Lin TC, Hou HA, Chou WC, DL O, SL Y, Tien HF, et al. CEBPA methylation as a prognostic biomarker in patients with de novo acute myeloid leukemia. Leukemia. 2011;25(1):32–40.
54. Fasan A, Alpermann T, Haferlach C, Grossmann V, Roller A, Kohlmann A, et al. Frequency and prognostic impact of CEBPA proximal, distal and core promoter methylation in normal karyotype AML: a study on 623 cases. PLoS One. 2013;8(2):e54365.
55. Pabst T, Eyholzer M, Fos J, Mueller BU. Heterogeneity within AML with CEBPA mutations; only CEBPA double mutations, but not single CEBPA mutations are associated with favourable prognosis. Br J Cancer. 2009;100(8):1343–6.
56. Dufour A, Schneider F, Metzeler KH, Hoster E, Schneider S, Zellmeier E, et al. Acute myeloid leukemia with biallelic CEBPA gene mutations and normal karyotype represents a distinct genetic entity associated with a favorable clinical outcome. J Clin Oncol. 2010;28(4):570–7.
57. Green CL, Koo KK, Hills RK, Burnett AK, Linch DC, Gale RE. Prognostic significance of CEBPA mutations in a large cohort of younger adult patients with acute myeloid leukemia: impact of double CEBPA mutations and the interaction with FLT3 and NPM1 mutations. J Clin Oncol. 2010;28(16):2739–47.
58. Shen Y, Zhu YM, Fan X, Shi JY, Wang QR, Yan XJ, et al. Gene mutation patterns and their prognostic impact in a cohort of 1185 patients with acute myeloid leukemia. Blood. 2011;118(20):5593–603.
59. Fasan A, Haferlach C, Alpermann T, Jeromin S, Grossmann V, Eder C, et al. The role of different genetic subtypes of CEBPA mutated AML. Leukemia. 2014;28(4):794–803.
60. Bacher U, Schnittger S, Macijewski K, Grossmann V, Kohlmann A, Alpermann T, et al. Multilineage dysplasia does not influence prognosis in CEBPA-mutated AML, supporting the WHO proposal to classify these patients as a unique entity. Blood. 2012;119(20):4719–22.
61. Lin LI, Chen CY, Lin DT, Tsay W, Tang JL, Yeh YC, et al. Characterization of CEBPA mutations in acute myeloid leukemia: most patients with CEBPA mutations have biallelic mutations and show a distinct immunophenotype of the leukemic cells. Clin Cancer Res. 2005;11(4):1372–9.
62. Chan WY, Liu QR, Borjigin J, Busch H, Rennert OM, Tease LA, et al. Characterization of the cDNA encoding human nucleophosmin and studies of its role in normal and abnormal growth. Biochemistry. 1989;28(3):1033–9.
63. Li XZ, McNeilage LJ, Whittingham S. The nucleotide sequence of a human cDNA encoding the highly conserved nucleolar phosphoprotein B23. Biochem Biophys Res Commun. 1989;163(1):72–8.
64. Chang JH, Olson MO. Structure of the gene for rat nucleolar protein B23. J Biol Chem. 1990;265(30):18227–33.
65. Borer RA, Lehner CF, Eppenberger HM, Nigg EA. Major nucleolar proteins shuttle between nucleus and cytoplasm. Cell. 1989;56(3):379–90.

66. Dumbar TS, Gentry GA, Olson MO. Interaction of nucleolar phosphoprotein B23 with nucleic acids. Biochemistry. 1989;28(24):9495–501.
67. Grisendi S, Mecucci C, Falini B, Pandolfi PP. Nucleophosmin and cancer. Nat Rev Cancer. 2006;6(7):493–505.
68. Tarapore P, Okuda M, Fukasawa K. A mammalian in vitro centriole duplication system: evidence for involvement of CDK2/cyclin E and nucleophosmin/B23 in centrosome duplication. Cell Cycle. 2002;1(1):75–81.
69. Maggi LB Jr, Kuchenruether M, Dadey DY, Schwope RM, Grisendi S, Townsend RR, et al. Nucleophosmin serves as a rate-limiting nuclear export chaperone for the Mammalian ribosome. Mol Cell Biol. 2008;28(23):7050–65.
70. Yu Y, Maggi LB Jr, Brady SN, Apicelli AJ, Dai MS, Lu H, et al. Nucleophosmin is essential for ribosomal protein L5 nuclear export. Mol Cell Biol. 2006;26(10):3798–809.
71. Lee SY, Park JH, Kim S, Park EJ, Yun Y, Kwon J. A proteomics approach for the identification of nucleophosmin and heterogeneous nuclear ribonucleoprotein C1/C2 as chromatin-binding proteins in response to DNA double-strand breaks. Biochem J. 2005;388(Pt 1):7–15.
72. MH W, Chang JH, Yung BY. Resistance to UV-induced cell-killing in nucleophosmin/B23 over-expressed NIH 3T3 fibroblasts: enhancement of DNA repair and up-regulation of PCNA in association with nucleophosmin/B23 over-expression. Carcinogenesis. 2002;23(1):93–100.
73. Li J, Zhang X, Sejas DP, Bagby GC, Pang Q. Hypoxia-induced nucleophosmin protects cell death through inhibition of p53. J Biol Chem. 2004;279(40):41275–9.
74. Takemura M, Sato K, Nishio M, Akiyama T, Umekawa H, Yoshida S. Nucleolar protein B23.1 binds to retinoblastoma protein and synergistically stimulates DNA polymerase alpha activity. J Biochem. 1999;125(5):904–9.
75. Bertwistle D, Sugimoto M, Sherr CJ. Physical and functional interactions of the Arf tumor suppressor protein with nucleophosmin/B23. Mol Cell Biol. 2004;24(3):985–96.
76. Colombo E, Bonetti P, Lazzerini Denchi E, Martinelli P, Zamponi R, Marine JC, et al. Nucleophosmin is required for DNA integrity and p19Arf protein stability. Mol Cell Biol. 2005;25(20):8874–86.
77. Kurki S, Peltonen K, Latonen L, Kiviharju TM, Ojala PM, Meek D, et al. Nucleolar protein NPM interacts with HDM2 and protects tumor suppressor protein p53 from HDM2-mediated degradation. Cancer Cell. 2004;5(5):465–75.
78. Grisendi S, Bernardi R, Rossi M, Cheng K, Khandker L, Manova K, et al. Role of nucleophosmin in embryonic development and tumorigenesis. Nature. 2005;437(7055):147–53.
79. Sportoletti P, Grisendi S, Majid SM, Cheng K, Clohessy JG, Viale A, et al. Npm1 is a haploinsufficient suppressor of myeloid and lymphoid malignancies in the mouse. Blood. 2008;111(7):3859–62.
80. Falini B, Nicoletti I, Martelli MF, Mecucci C. Acute myeloid leukemia carrying cytoplasmic/mutated nucleophosmin (NPMc+ AML): biologic and clinical features. Blood. 2007;109(3):874–85.
81. Falini B, Martelli MP, Bolli N, Bonasso R, Ghia E, Pallotta MT, et al. Immunohistochemistry predicts nucleophosmin (NPM) mutations in acute myeloid leukemia. Blood. 2006;108(6):1999–2005.
82. Falini B, Lenze D, Hasserjian R, Coupland S, Jaehne D, Soupir C, et al. Cytoplasmic mutated nucleophosmin (NPM) defines the molecular status of a significant fraction of myeloid sarcomas. Leukemia. 2007;21(7):1566–70.
83. Falini B, Nicoletti I, Bolli N, Martelli MP, Liso A, Gorello P, et al. Translocations and mutations involving the nucleophosmin (NPM1) gene in lymphomas and leukemias. Haematologica. 2007;92(4):519–32.
84. Rau R, Brown P. Nucleophosmin (NPM1) mutations in adult and childhood acute myeloid leukaemia: towards definition of a new leukaemia entity. Hematol Oncol. 2009;27(4):171–81.
85. Mariano AR, Colombo E, Luzi L, Martinelli P, Volorio S, Bernard L, et al. Cytoplasmic localization of NPM in myeloid leukemias is dictated by gain-of-function mutations that create a functional nuclear export signal. Oncogene. 2006;25(31):4376–80.

86. Albiero E, Madeo D, Bolli N, Giaretta I, Bona ED, Martelli MF, et al. Identification and functional characterization of a cytoplasmic nucleophosmin leukaemic mutant generated by a novel exon-11 NPM1 mutation. Leukemia. 2007;21(5):1099–103.
87. Falini B, Martelli MP, Mecucci C, Liso A, Bolli N, Bigerna B, et al. Cytoplasmic mutated nucleophosmin is stable in primary leukemic cells and in a xenotransplant model of NPMc+ acute myeloid leukemia in SCID mice. Haematologica. 2008;93(5):775–9.
88. Meloni G, Mancini M, Gianfelici V, Martelli MP, Foa R, Falini B. Late relapse of acute myeloid leukemia with mutated NPM1 after eight years: evidence of NPM1 mutation stability. Haematologica. 2009;94(2):298–300.
89. Bolli N, Galimberti S, Martelli MP, Tabarrini A, Roti G, Mecucci C, et al. Cytoplasmic nucleophosmin in myeloid sarcoma occurring 20 years after diagnosis of acute myeloid leukaemia. Lancet Oncol. 2006;7(4):350–2.
90. Suzuki T, Kiyoi H, Ozeki K, Tomita A, Yamaji S, Suzuki R, et al. Clinical characteristics and prognostic implications of NPM1 mutations in acute myeloid leukemia. Blood. 2005;106(8):2854–61.
91. Chou WC, Tang JL, Lin LI, Yao M, Tsay W, Chen CY, et al. Nucleophosmin mutations in de novo acute myeloid leukemia: the age-dependent incidences and the stability during disease evolution. Cancer Res. 2006;66(6):3310–6.
92. Falini B, Mecucci C, Saglio G, Lo Coco F, Diverio D, Brown P, et al. NPM1 mutations and cytoplasmic nucleophosmin are mutually exclusive of recurrent genetic abnormalities: a comparative analysis of 2562 patients with acute myeloid leukemia. Haematologica. 2008;93(3):439–42.
93. Federici L, Falini B. Nucleophosmin mutations in acute myeloid leukemia: a tale of protein unfolding and mislocalization. Protein Sci. 2013;22(5):545–56.
94. Falini B, Martelli MP, Bolli N, Sportoletti P, Liso A, Tiacci E, et al. Acute myeloid leukemia with mutated nucleophosmin (NPM1): is it a distinct entity? Blood. 2011;117(4):1109–20.
95. Vassiliou GS, Cooper JL, Rad R, Li J, Rice S, Uren A, et al. Mutant nucleophosmin and cooperating pathways drive leukemia initiation and progression in mice. Nat Genet. 2011;43(5):470–5.
96. Sportoletti P, Varasano E, Rossi R, Mupo A, Tiacci E, Vassiliou G, et al. Mouse models of NPM1-mutated acute myeloid leukemia: biological and clinical implications. Leukemia. 2015;29(2):269–78.
97. Thiede C, Koch S, Creutzig E, Steudel C, Illmer T, Schaich M, et al. Prevalence and prognostic impact of NPM1 mutations in 1485 adult patients with acute myeloid leukemia (AML). Blood. 2006;107(10):4011–20.
98. Haferlach C, Mecucci C, Schnittger S, Kohlmann A, Mancini M, Cuneo A, et al. AML with mutated NPM1 carrying a normal or aberrant karyotype show overlapping biologic, pathologic, immunophenotypic, and prognostic features. Blood. 2009;114(14):3024–32.
99. Falini B, Macijewski K, Weiss T, Bacher U, Schnittger S, Kern W, et al. Multilineage dysplasia has no impact on biologic, clinicopathologic, and prognostic features of AML with mutated nucleophosmin (NPM1). Blood. 2010;115(18):3776–86.
100. Mullighan CG, Kennedy A, Zhou X, Radtke I, Phillips LA, Shurtleff SA, et al. Pediatric acute myeloid leukemia with NPM1 mutations is characterized by a gene expression profile with dysregulated HOX gene expression distinct from MLL-rearranged leukemias. Leukemia. 2007;21(9):2000–9.
101. Andreeff M, Ruvolo V, Gadgil S, Zeng C, Coombes K, Chen W, et al. HOX expression patterns identify a common signature for favorable AML. Leukemia. 2008;22(11):2041–7.
102. Jongen-Lavrencic M, Sun SM, Dijkstra MK, Valk PJ, Löwenberg B. MicroRNA expression profiling in relation to the genetic heterogeneity of acute myeloid leukemia. Blood. 2008;111(10):5078–85.
103. Loken MR, Alonzo TA, Pardo L, Gerbing RB, Raimondi SC, Hirsch BA, et al. Residual disease detected by multidimensional flow cytometry signifies high relapse risk in patients with de novo acute myeloid leukemia: a report from Children's Oncology Group. Blood. 2012;120(8):1581–8.

104. Walter RB, Buckley SA, Pagel JM, Wood BL, Storer BE, Sandmaier BM, et al. Significance of minimal residual disease before myeloablative allogeneic hematopoietic cell transplantation for AML in first and second complete remission. Blood. 2013;122(10):1813–21.
105. Othus M, Wood BL, Stirewalt DL, Estey EH, Petersdorf SH, Appelbaum FR, et al. Effect of measurable ('minimal') residual disease (MRD) information on prediction of relapse and survival in adult acute myeloid leukemia. Leukemia. 2016;30(10):2080–3.
106. Gorello P, Cazzaniga G, Alberti F, Dell'Oro MG, Gottardi E, Specchia G, et al. Quantitative assessment of minimal residual disease in acute myeloid leukemia carrying nucleophosmin (NPM1) gene mutations. Leukemia. 2006;20(6):1103–8.
107. Chou WC, Tang JL, SJ W, Tsay W, Yao M, Huang SY, et al. Clinical implications of minimal residual disease monitoring by quantitative polymerase chain reaction in acute myeloid leukemia patients bearing nucleophosmin (NPM1) mutations. Leukemia. 2007;21(5):998–1004.
108. Schnittger S, Kern W, Tschulik C, Weiss T, Dicker F, Falini B, et al. Minimal residual disease levels assessed by NPM1 mutation-specific RQ-PCR provide important prognostic information in AML. Blood. 2009;114(11):2220–31.
109. Krönke J, Schlenk RF, Jensen KO, Tschürtz F, Corbacioglu A, Gaidzik VI, et al. Monitoring of minimal residual disease in NPM1-mutated acute myeloid leukemia: a study from the German-Austrian acute myeloid leukemia study group. J Clin Oncol. 2011;29(19):2709–16.
110. Shayegi N, Kramer M, Bornhäuser M, Schaich M, Schetelig J, Platzbecker U, et al. The level of residual disease based on mutant NPM1 is an independent prognostic factor for relapse and survival in AML. Blood. 2013;122(1):83–92.
111. Hubmann M, Köhnke T, Hoster E, Schneider S, Dufour A, Zellmeier E, et al. Molecular response assessment by quantitative real-time polymerase chain reaction after induction therapy in NPM1-mutated patients identifies those at high risk of relapse. Haematologica. 2014;99(8):1317–25.
112. Ivey A, Hills RK, Simpson MA, Jovanovic JV, Gilkes A, Grech A, et al. Assessment of minimal residual disease in standard-risk AML. N Engl J Med. 2016;374(5):422–33.
113. Burke MJ. Minimal residual disease in NPM1-mutated AML. N Engl J Med. 2016;374(5):481–2.
114. Rowley JD. Identificaton of a translocation with quinacrine fluorescence in a patient with acute leukemia. Ann Genet. 1973;16(2):109–12.
115. Miyoshi H, Shimizu K, Kozu T, Maseki N, Kaneko Y, Ohki M. t(8;21) breakpoints on chromosome 21 in acute myeloid leukemia are clustered within a limited region of a single gene, AML1. Proc Natl Acad Sci U S A. 1991;88(23):10431–4.
116. van Wijnen AJ, Stein GS, Gergen JP, Groner Y, Hiebert SW, Ito Y, et al. Nomenclature for Runt-related (RUNX) proteins. Oncogene. 2004;23(24):4209–10.
117. Mangan JK, Speck NA. RUNX1 mutations in clonal myeloid disorders: from conventional cytogenetics to next generation sequencing, a story 40 years in the making. Crit Rev Oncog. 2011;16(1–2):77–91.
118. Wang Q, Stacy T, Binder M, Marin-Padilla M, Sharpe AH, Speck NA. Disruption of the Cbfa2 (AML1) gene causes necrosis and hemorrhaging in the central nervous system and blocks definitive hematopoiesis. Proc Natl Acad Sci U S A. 1996;93(8):3444–9.
119. Ichikawa M, Asai T, Saito T, Seo S, Yamazaki I, Yamagata T, et al. AML-1 is required for megakaryocytic maturation and lymphocytic differentiation, but not for maintenance of hematopoietic stem cells in adult hematopoiesis. Nat Med. 2004;10(3):299–304.
120. Gaidzik VI, Teleanu V, Papaemmanuil E, Weber D, Paschka P, Hahn J, et al. RUNX1 mutations in acute myeloid leukemia are associated with distinct clinico-pathologic and genetic features. Leukemia. 2016;30(11):2160–8.
121. Harada Y, Harada H. Molecular pathways mediating MDS/AML with focus on AML1/RUNX1 point mutations. J Cell Physiol. 2009;220(1):16–20.
122. Lambert MP. Update on the inherited platelet disorders. Curr Opin Hematol. 2015;22(5):460–6.
123. Churpek JE, Pyrtel K, Kanchi KL, Shao J, Koboldt D, Miller CA, et al. Genomic analysis of germ line and somatic variants in familial myelodysplasia/acute myeloid leukemia. Blood. 2015;126(22):2484–90.

124. Churpek JE, Lorenz R, Nedumgottil S, Onel K, Olopade OI, Sorrell A, et al. Proposal for the clinical detection and management of patients and their family members with familial myelodysplastic syndrome/acute leukemia predisposition syndromes. Leuk Lymphoma. 2013;54(1):28–35.
125. Cancer Genome Atlas Research Network. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J Med. 2013;368(22):2059–74.
126. Osato M, Asou N, Abdalla E, Hoshino K, Yamasaki H, Okubo T, et al. Biallelic and heterozygous point mutations in the runt domain of the AML1/PEBP2alphaB gene associated with myeloblastic leukemias. Blood. 1999;93(6):1817–24.
127. Silva FP, Swagemakers SM, Erpelinck-Verschueren C, Wouters BJ, Delwel R, Vrieling H, et al. Gene expression profiling of minimally differentiated acute myeloid leukemia: M0 is a distinct entity subdivided by RUNX1 mutation status. Blood. 2009;114(14):3001–7.
128. Döhner H, Estey EH, Amadori S, Appelbaum FR, Büchner T, Burnett AK, et al. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. Blood. 2010;115(3):453–74.
129. Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, Roberts ND, et al. Genomic classification and prognosis in acute myeloid leukemia. N Engl J Med. 2016;374(23):2209–21.
130. DiNardo CD, Bannon SA, Routbort M, Franklin A, Mork M, Armanios M, et al. Evaluation of patients and families with concern for predispositions to hematologic malignancies within the hereditary hematologic malignancy clinic (HHMC). Clin Lymphoma Myeloma Leuk. 2016;16(7):417–28.
131. Churpek JE, Godley LA. How I diagnose and manage individuals at risk for inherited myeloid malignancies. Blood. 2016;pii:blood-2016-05-670240. [Epub ahead of print].
132. Duncavage EJ, Tandon B. The utility of next-generation sequencing in diagnosis and monitoring of acute myeloid leukemia and myelodysplastic syndromes. Int J Lab Hematol. 2015;37(Suppl 1):115–21.

# Chapter 3
# Acute Myeloid Leukemia with Myelodysplasia-Related Changes, Therapy-Related Myeloid Neoplasms, and Acute Myeloid Leukemia, Not Otherwise Specified

**Peng Li and Robert S. Ohgami**

## Introduction

### *Acute Myeloid Leukemia with Myelodysplasia-Related Changes*

In 2016, the updated World Health Organization (WHO) classification of hematopoietic neoplasms revised this entity minimally, and it remains a subcategory of acute myeloid leukemias (AMLs) entitled "acute myeloid leukemia with myelodysplasia-related changes (AML-MRC)." AML-MRC (Table 3.1) is defined as AML (≥20% blasts in peripheral blood or bone marrow) with a history of myelodysplastic syndrome (MDS) or myelodysplastic/myeloproliferative neoplasm (MDS/MPN), or specific MDS-related cytogenetic abnormalities (Table 3.2), or morphologic features of multilineage myelodysplasia (≥50% dysplastic cells in at least two cell lineages), in the absence of *NPM1* or biallelic *CEBPA* mutations, because the morphologic dysplasia in AML cases with *NPM1* or biallelic *CEBPA* mutations appears not to impact patient outcome [1–4]. AMLs with *NPM1* or biallelic *CEBPA* mutations have been upgraded from provisional entities in the 2008 WHO classification to full entities in the 2016 WHO classification and are now included amongst AMLs with recurrent genetic abnormalities.

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**Table 3.1** Diagnostic criteria for AML with myelodysplasia-related changes

| 20% blasts or more in peripheral blood or bone marrow | |
|---|---|
| AND | Inclusion criteria: any of the following |
| | 1. Previous history of myelodysplastic syndrome |
| | 2. Myelodysplastic syndrome-related recurrent cytogenetic abnormality |
| | 3. Multilineage dysplasia (presence of 50% or more dysplastic cells in at least two cell lines) lacking a mutation of *NPM1* and biallelic mutation of *CEBPA* in absence of 1 and 2 |
| AND | Exclusion criteria: absence of both of the following |
| | 1. History of cytotoxic therapy for an unrelated disease |
| | 2. AML with recurrent genetic abnormalities |

Modified from the WHO classification

**Table 3.2** Cytogenetic abnormalities for AML with myelodysplasia-related changes

| Complex karyotype | >3 unrelated abnormalities, none of which are included in the AML with recurrent genetic abnormalities subgroup |
|---|---|
| *Unbalanced abnormalities* | |
| | −7/del(7q) |
| | del(5q)/t(5q) |
| | i(17q)/t(17p) |
| | −13/del(13q) |
| | del(11q) |
| | del(12p)/t(12p) |
| | idic(X)(q13) |
| *Balanced abnormalities* | |
| | t(11;16)(q23;p13.3)[a] |
| | t(3;21)(q26.2;q22.1)[a] |
| | t(2;11)(p21;q23.3)[a] |
| | t(1;3)(p36.3;q21) |
| | t(5;12)(q32;p13.2) |
| | t(5;7)(q32;q11.2) |
| | t(5;17)(q32;p13.2) |
| | t(5;10)(q32;q21.2) |
| | t(3;5)(q25.3;q35.1) |

Modified from the WHO classification
[a]Absence of previous history of cytotoxic therapy for an unrelated disease

## *Therapy-Related Myeloid Neoplasm*

Therapy-related myeloid neoplasms (t-MNs), including AML, MDS, and MDS/MPN, occur after cytotoxic insults by chemotherapy or radiation therapy (Table 3.3) for a neoplastic or nonneoplastic disorder [5–7]. Although t-MNs can be further subclassified to t-MDS or t-AML based on blast counts, all therapy-related neoplasms are best considered as one clinical syndrome [4]. Associated cytogenetic and molecular abnormalities, independent of blast count, are better predictors of disease

**Table 3.3** Cytotoxic agents inducing therapy-related myeloid neoplasms

| Cytotoxic agents | Specific medication and therapy |
|---|---|
| Alkylating agents | Melphalan, cyclophosphamide, nitrogen mustard, chlorambucil, busulfan, carboplatin, cisplatin, dacarbazine, procarbazine, carmustine, mitomycin C, thiotepa, lomustine, etc. |
| Ionizing radiation therapy | Large fields including active bone marrow and for autologous bone marrow transplant modification |
| Topoisomerase II inhibitors | Etoposide, teniposide, doxorubicin, daunorubicin, mitoxantrone, amsacrine, actinomycin |
| Others | Antimetabolites: thiopurines, mycophenolate, fludarabine<br>Antitubulin agents (usually in combination with other agents): vincristine, vinblastine, vindesine, paclitaxel, docetaxel |

Modified from the WHO classification

prognosis and therapy response, and these abnormalities should be incorporated into the final diagnosis [4].

## *Acute Myeloid Leukemia, Not Otherwise Specified (AML, NOS)*

All AML cases that do not fulfill the diagnostic criteria for AML with recurrent genetic abnormalities including *NPM1* and biallelic *CEBPA* mutations, AML-MRC, therapy-related myeloid neoplasm, or myeloid neoplasm with germline predisposition are classified as AML, not otherwise specified (NOS). The basis for subclassification within this category is primarily the morphologic features of blasts, although these morphologic subtypes lack clinical or biologic significance [8, 9]. In the updated 2016 WHO classification, the blast percentage of all AMLs including acute erythroid leukemia is based on total marrow nucleated cells, not nonerythroid cells. The attempt to achieve consistency in calculating blast percentage across all myeloid neoplasms can, at least in part, avoid overdiagnosis of AML primarily due to the low reproducibility of nonerythroid blasts counts. Further, cases previously subcategorized as acute erythroid leukemia, erythroid/myeloid type, share common clinical manifestations, morphologic, and cytogenetic features with cases of MDS [10–13]. Therefore, this subcategory of acute erythroid leukemia (defined as ≥50% BM erythroid precursors and ≥20% myeloblasts among nonerythroid cells) is removed from acute erythroid leukemia. The majority of such cases are now classified as MDS, especially MDS with excess blasts [4].

## *Epidemiology*

AML-MRC is more common in older patients, with a median age of 68 years, and shows a slight male predominance [14]. The diagnosis of AML-MRC accounts for approximately 25–35% of adult AMLs [8, 15–17]. AML-MRC more commonly

**Table 3.4** Cytotoxic agents inducing therapy-related myeloid neoplasms and their clinical manifestations

| Cytotoxic agents | Latency | Cytogenetics | Dysplastic phase | Prognosis |
|---|---|---|---|---|
| Alkylating agents | Long, 5–7 years [7] | Deletions of chromosomes 5 and 7, complex karyotypes | Yes | Poor |
| Ionizing radiation | Long, 5–7 years [7] | Deletions of chromosomes 5 and 7, complex karyotypes | Yes | Poor |
| Topoisomerase II inhibitors | Short, 1–3 years | Abnormalities of *KMT2A* or *RUNX1* | Often no | Poor |

occurs de novo [14] and may also arise from preexisting myelodysplasia (MDS or MDS/MPN). AML-MRC is generally associated with a worse prognosis and a lower rate of achieving complete remission in response to current chemotherapies compared to other types of AML. The prognosis for AML-MRC is also dependent on other individual factors, such as the patient's age, blast count [18], cytogenetic abnormalities [19, 20], specific molecular mutations [19, 20], and different therapies [5, 20, 21]. Patients with lower blast counts (20–29%) in the absence of MDS-related cytogenetic abnormalities, specifically in children, have relatively better clinical outcomes [21–23]. Recent studies also suggest that allogeneic hematopoietic stem cell transplantation may overcome the poor prognosis for AML-MRC, especially in patients who are 50 years old or younger [18].

Therapy-related myeloid neoplasms account for approximately 10% of AML and 20% of MDS, and are associated with a uniformly poor prognosis with a median survival of 6 months [5–7]. Overall, the median time to develop t-MDS/AML following cytotoxic therapy is approximately 3–5 years, with a marked risk reduction after the first decade [6, 7]. There are two types of t-MNs based on their causative therapeutic exposures (Table 3.4): a topoisomerase II inhibitor-related type and an alkylating agent/radiation-related type. Therapy-related myeloid neoplasms associated with chemotherapeutic administration of topoisomerase II inhibitors develop 1–3 years after exposure, and often show no morphologic dysplasia. Alkylating agent/radiation–related t-MNs occur 5–7 years after exposure to cytotoxic agents [6, 7]. Approximately two-thirds of these patients develop a myelodysplastic phase lasting months to years before overt AML. The remainder often presents with AML with myelodysplastic features [5, 6] in addition to myelodysplasia-related cytogenetic abnormalities involving chromosomes 5 (−5/del[5q]) and 7 (−7/del[7q]).

AML, NOS, encompasses a large heterogeneous group, representing 25–30% of adult AML. It occurs at a younger age than AML-MRC, and has an intermediate prognosis [14]. Generally, there are no common clinical features or consistent chromosomal abnormalities identified with this category, and this diagnosis is made by exclusion of other subcategories of AML.

### *Clinical Features*

AML-MRC displays substantial heterogeneity in presentation and clinical course. Adults with AML-MRC often present with severe pancytopenia with a rapid increase in blasts. In contrast, children with a blast percentage between 20% and 29% and preexisting MDS (especially RAEB) experience relatively slow progression and stable blast counts for weeks to months [23]. Therapy-related myeloid neoplasms occurring with a short latency often show abnormalities of either *KMT2A* at 11q23 or *RUNX1* at 21q22, and are often associated with use of topoisomerase II inhibitors (Table 3.4). Longer latency t-MNs often have a prolonged MDS phase, multilineage dysplasia, and MDS-associated karyotypic abnormalities, and these features are more likely to be related to alkylating agents or ionizing radiation (Table 3.4). Patients with AML, NOS usually present with evidence of bone marrow failure and pancytopenias. Beyond medullary symptoms, extramedullary manifestations including soft tissue masses, cutaneous and gingival infiltration, and central nervous system (CNS) involvement are more common in acute monoblastic and monocytic leukemia. Patients with acute basophilic leukemia often have cutaneous involvement, organomegaly, lytic bone lesions associated with hyperhistaminemia, and bone marrow failure.

## Morphology and Immunophenotype

Most cases of AML-MRC and t-MNs have morphologic evidence of multilineage dysplasia best evaluated with peripheral blood smears and bone marrow aspirates. Significant dysplastic features are defined as involving at least 50% of the cells of each particular lineage. Blood smears may be particularly useful in identifying myeloid dysplasia, while erythroid and megakaryocytic dysplasia are best evaluated with bone marrow aspirates and biopsies. Many of these dysplastic morphologic features in blood smears and bone marrow are nonspecific for AML-MRC/t-MNs and may be seen in a variety of other neoplastic and nonneoplastic hematologic disorders. Myeloid dysplasia is evidenced, for instance, by hypogranulation and abnormal nuclear segmentation. Dyserythropoiesis is the most common change seen in the bone marrow and includes bizarre erythroid precursors and ring sideroblasts. Dyserythropoiesis is also manifested by karyorrhexis, profound nuclear budding and irregularity, multinucleation, marked megaloblastoid maturation, and cytoplasmic vacuolization. Dysmegakaryopoiesis includes hypolobulated micromegakaryocytes, nonlobulated nuclei or odd numbers of nuclei, and widely spaced nuclei.

t-MN cases following alkylating agents/ionizing radiation often have increased blasts with associated multilineage dysplasia. Approximately 20–30% of t-MN cases, associated with topoisomerase II inhibitors and balanced recurrent chromosomal translocations involving 11q23 (*KMT2A*/*MLL*) or 21q22 (*RUNX1*), show

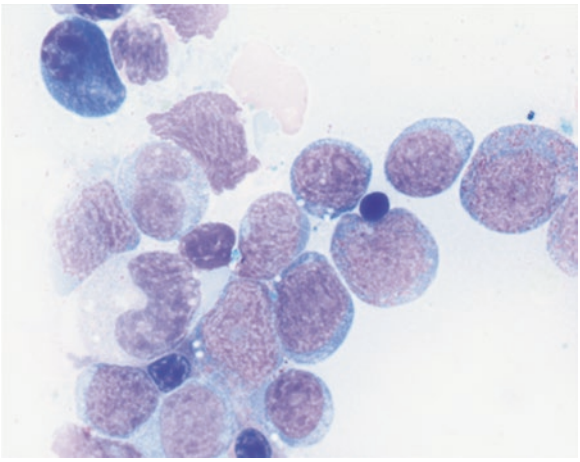

**Fig. 3.1** Bone marrow aspirates in a patient with acute myelomonocytic leukemia. Bone marrow aspirates in a patient who is diagnosed with t-MN with *KMT2A* translocation

morphologic features similar to those identified in de novo acute monoblastic leukemia or myelomonocytic leukemia (Fig. 3.1).

No specific immunophenotypic feature typifies AML-MRC and t-MNs due to the heterogeneity of underlying causes and cytogenetic abnormalities [14]. Nonetheless, there is a general immunophenotypic overlap with AML-MRC and t-MNs, and blasts are CD34+ and CD117+ and express the pan-myeloid markers such as CD13 and CD33 [14, 24]. Aberrant expression of CD7, CD10, and CD56 in blasts is not uncommon. In secondary AMLs with monocytic differentiation, blasts often express CD4 and CD14 and lack CD34. Cases of AML-MRC and t-MNs with abnormalities of chromosomes 5 and 7 may show aberrant expression of TDT and CD7.

Most morphologic subtypes of AML, NOS, are defined by previous FAB criteria, and a 20% marrow blast cell count is sufficient for a diagnosis of acute leukemia [24]. This category includes many cases of AML with a normal karyotype, and mutation analysis, rather than morphologic or immunophenotypic features, and is probably the most predictive prognostic marker in this group.

## Cytogenetic Abnormalities

Cytogenetic studies including conventional karyotyping remain a mainstream technology and are required in the evaluation of acute myeloid leukemia and other hematologic and nonhematologic disorders. The value of cytogenetic studies in AML can be divided into four main aspects: diagnosis, prognosis, prediction of therapeutic response, and monitoring minimal residual disease or early recurrence. Conventional karyotyping, although considered somewhat outdated compared to more specific and sensitive methods such as fluorescence in situ hybridization (FISH), provides a comprehensive view of the genome that cannot be obtained with

FISH or other more specific molecular studies, particularly in AMLs with a complex karyotype. Consequently, both conventional karyotyping and FISH remain the routine studies performed for AML diagnoses in clinical laboratories.

## *Cytogenetic Abnormalities in AML-MRC*

The presence of MDS-related cytogenetic abnormalities is sufficient to make a diagnosis of AML with MRC (Table 3.1). A complex karyotype, seven unbalanced chromosomal abnormalities, and nine balanced chromosomal abnormalities are considered diagnostic of AML-MRC (Table 3.2).

### Complex Karyotype

A complex karyotype (Fig. 3.2) is defined as three or more unrelated chromosomal abnormalities often leading to loss of genetic material at 5q, 14q33, 7q32q35, and 17p13, thus translating into decreased expression of genes at these chromosomal

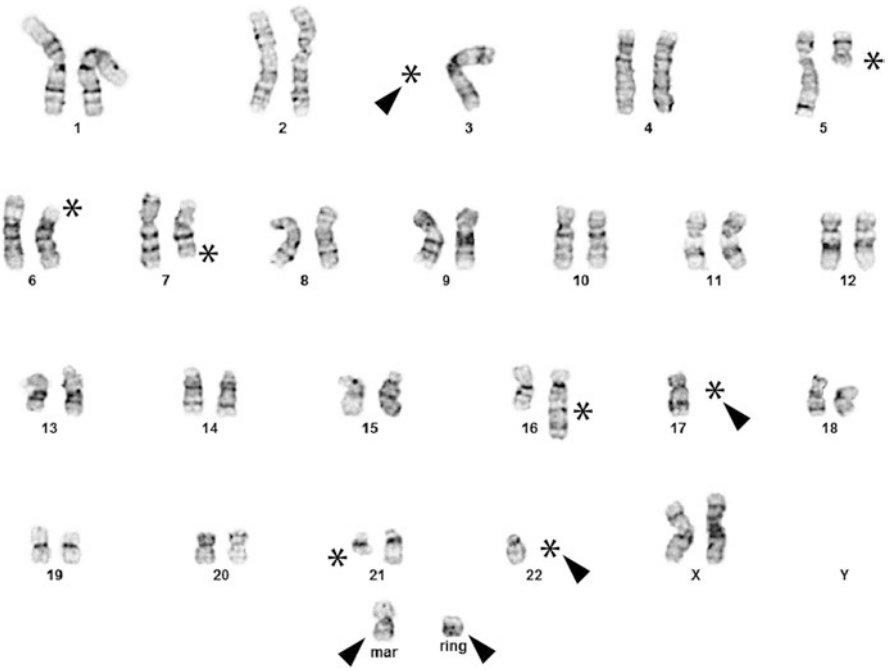


**Fig. 3.2** Bone marrow karyotype from a patient with AML-MRC. Bone marrow from a patient shows a complex aberrant karyotype. A diagnosis of AML-MRC was made based on the cytogenetic findings in addition to morphologic features. *Indicates translocation; * with *arrow head* indicates absence of the chromosome; *arrowhead* indicates additional aberrant chromosomal materials

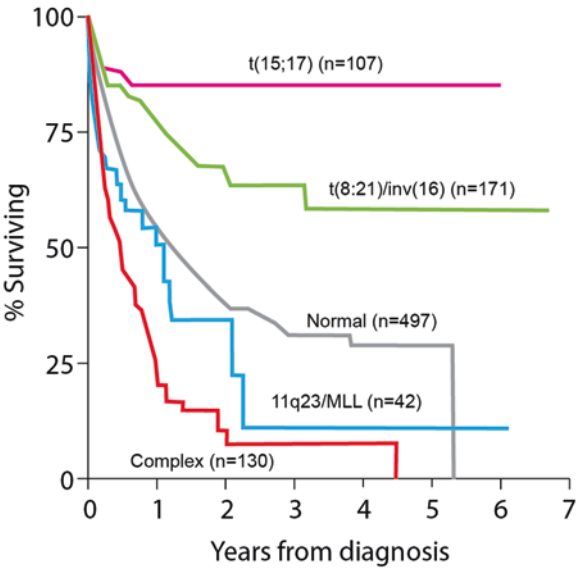


**Fig. 3.3** Overall survival of patients with de novo AMLs. Patients with a complex karyotype (*red*) show a dismal clinical outcome indicated by overall survival in comparison to those with AML with balanced abnormalities or with a normal karyotype (Graph modified from [25])

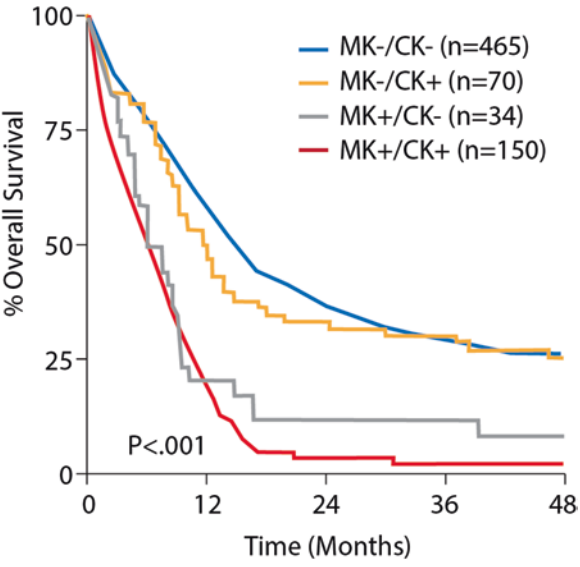


**Fig. 3.4** Overall survival of patients with AMLs and noncore binding factor chromosomal abnormalities. Patients with a monosomal karyotype (MK+, −7, *gray* and *red*, 4 year OS 27% vs MK-, *yellow* and *blue*, 4-year OS 0–6%, $p < 0.001$) show a reduced overall survival regardless complex karyotypes (CK). Therefore, a single autosomal monosomy is a better predictor for very poor prognosis than a complex karyotype (Graph modified from [26])

loci. It is invariably associated with a poor prognosis [25] (Fig. 3.3). Recent studies demonstrated that autosomal monosomy, defined as the presence of two or more distinct autosomal chromosome monosomies or a single autosomal monosomy with one or more other chromosomal abnormalities, is a better predictor for poor prognosis than a complex karyotype in AML [26] (Fig. 3.4). A monosomal karyotype (Fig. 3.5), occurring in 5–10% of AMLs is associated with a dismal prognosis, with

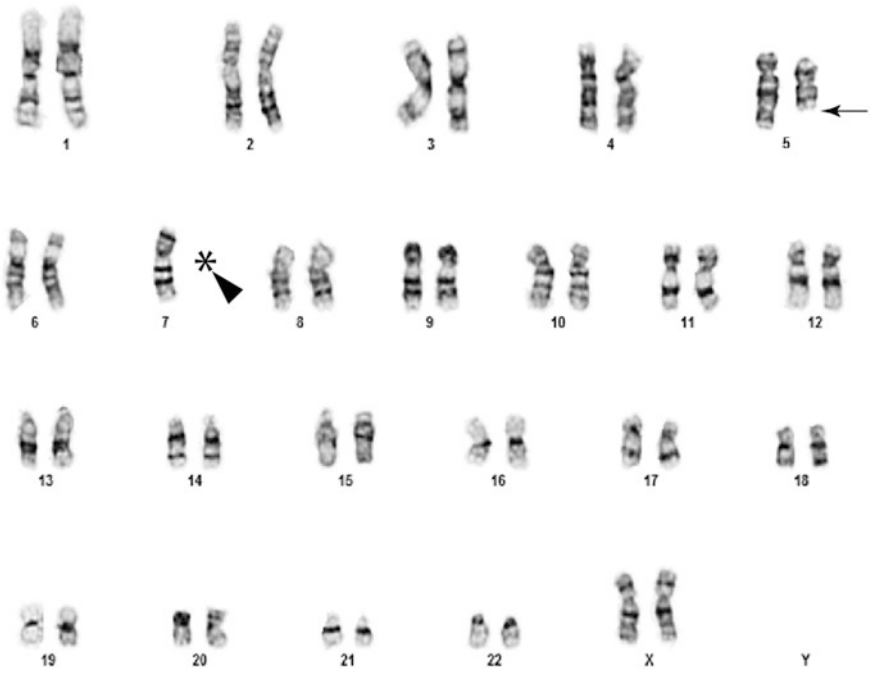


**Fig. 3.5** Bone marrow karyotype from a patient with AML-MRC. Bone marrow karyotype from a patient shows a monosomy 7 (* and *arrowhead*) in addition to deletion 5q (*arrow*)

a 5-year overall survival of less than 5%. Monosomies 5 and 7, included by MDS-related cytogenetic abnormality and among the most frequent autosomal monosomies in AML, are associated with poor clinical outcomes, which appear to be independent for each specific monosomic chromosome [26].

### Unbalanced Chromosomal Abnormalities

Unbalanced chromosomal abnormalities include, in order of decreasing frequency: deletion of 5q, loss of 7q, loss of 17p, loss of 13q, loss of 11q, and loss of 12p. They result in the loss of genomic integrity (Fig. 3.6). These lesions are the most common genetic aberrance in AML with MRC associated with poor clinical outcomes. In the updated WHO classification, del(9q) is no longer considered a defining MDS-related cytogenetic abnormality for AML-MRC [4]. Del(9q) is among the most frequent abnormalities associated with AML carrying *NPM1* or biallelic *CEBPA* mutations, and the presence of del(9q) is not an independent prognostic factor [2–4, 27]. Further, common recurring chromosomal abnormalities in MDS, such as trisomy 8, del(20q), and loss of chromosome Y, are not considered as defining abnormalities in AML-MRC, since they are also associated with AML carrying *NPM1* or biallelic *CEBPA* mutations and they lack prognostic significance in those settings [2–4, 27].

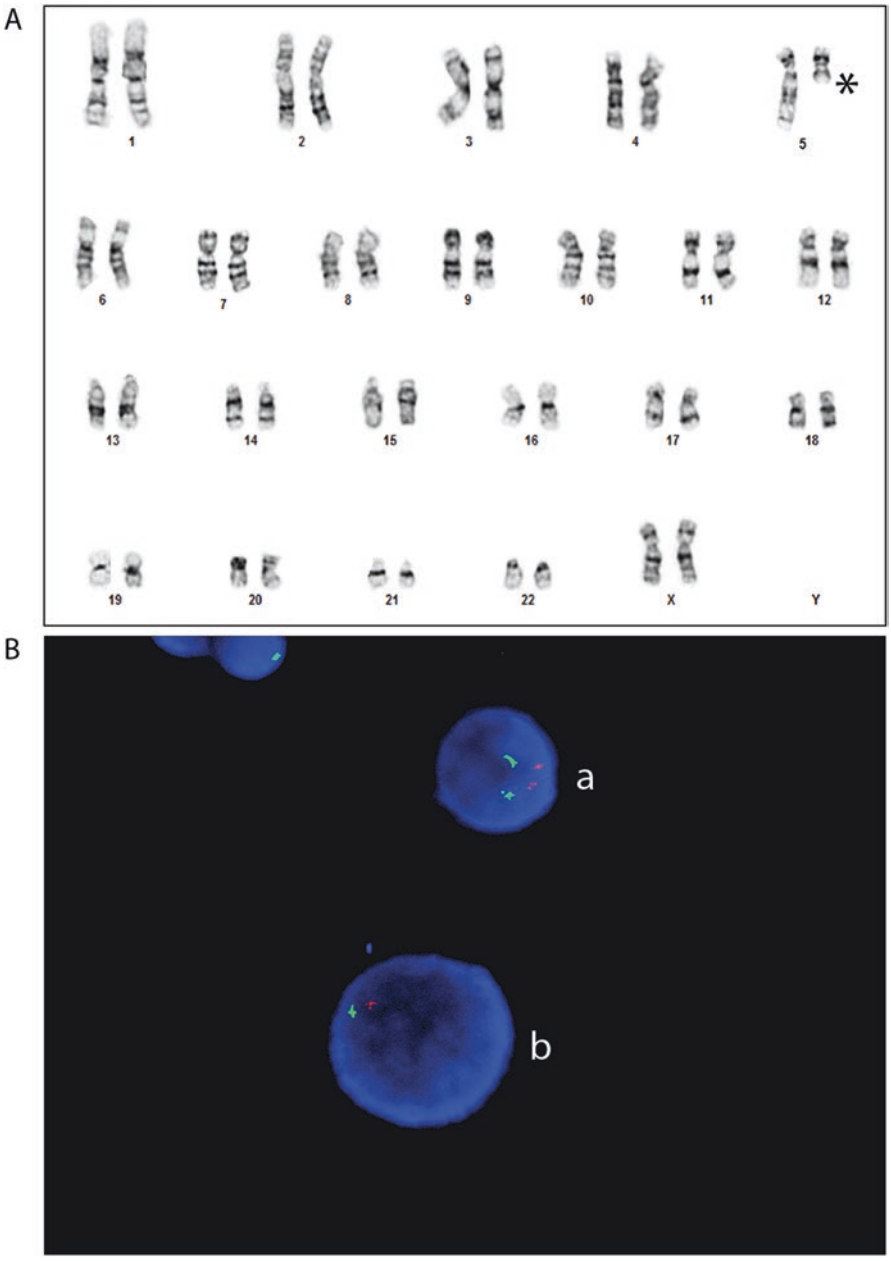


**Fig. 3.6** Bone marrow karyotype and fluorescence in situ hybridization from a patient with AML-MRC. (**a**, **b**) Bone marrow karyotype (**a**) from a patient shows a deletion 5q (*) and this karyotypic finding is confirmed by FISH employing specific probes (*red* and *green*) targeting 5q (**b**, *a* a cell with normal karyotype; *b* a cell with del 5q)

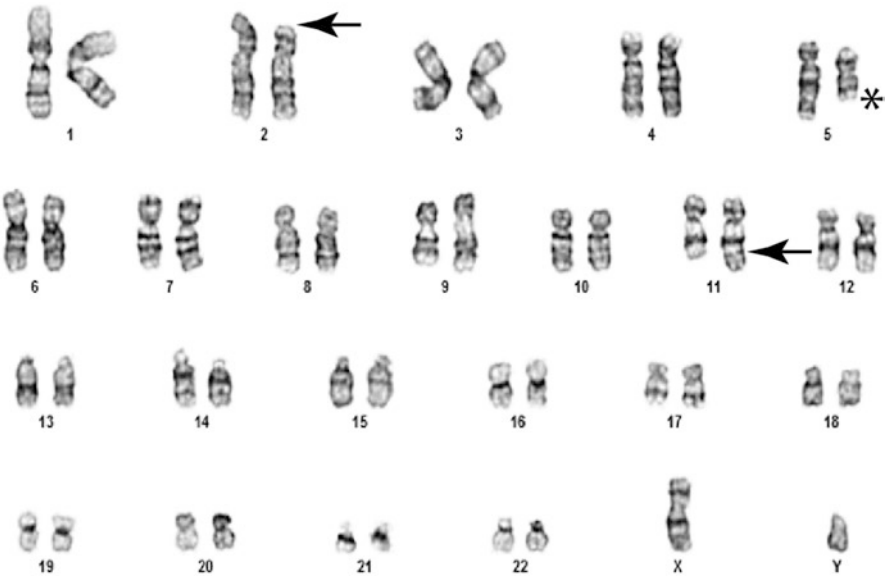


**Fig. 3.7** Bone marrow karyotype from a patient with AML-MRC. Bone marrow karyotype from a patient shows t(2;11)(p21;q23), indicated by *arrows*, in addition to del(5q)*

### Balanced Chromosomal Abnormalities

Nine balanced chromosomal abnormalities diagnostic of AML-MRC include, in order of decreasing frequency: t(11;16), t(3;21), t(1;3), t(2;11), t(5;12), t(5;7), t(5;17), t(5;10), and t(3;5). Among these balanced gene rearrangements, t(11;16), t(3;21), and t(2;11) are also commonly seen in t-MNs (Fig. 3.7). A previous history of cytotoxic therapy including chemotherapy or radiation warrants a diagnosis of therapy-related myeloid neoplasm instead of AML-MRC. 5q33 is involved in four of the nine gene rearrangements and rearrangement of this locus leads to activation of the platelet-derived growth factor receptor-β (PDGFRB), a member of class III tyrosine kinase receptors (RTKIII) [28]. Two other rearrangements involve the *EVI1* locus at 3q26 and the *GATA2* and *RPN1* loci at 3q21 [26, 29–31]. Moreover, two other rearrangements involving a myeloid lymphoid leukemia (*MLL* or *KMT2A*) locus at 11q23 (Fig. 3.8), rather than AML with t(9;11), are also diagnostic of AML-MRC [4]. There are over 80 different chromosomal translocations involving *KMT2A*, and over 50 translocation partner genes have been characterized in adult and pediatric acute leukemias [32–36]. Translocations involving *MLLT3* (AF9), resulting predominantly in AML, are the most common. Other *KMT2A* translocations resulting in AML include *MLLT1* (*ENL*), *MLLT10* (*AF10*), *MLLT4* (*AF6*), and *ELL* as partner genes. Up to one-third of *KMT2A* translocations in AMLs are detectable by conventional karyotyping, and FISH or other molecular studies may be necessary to identify these variant translocations [32, 33]. The remaining t(3;5)(q25;q35) rearrangement is relatively unique in AML-MRC, and the overall

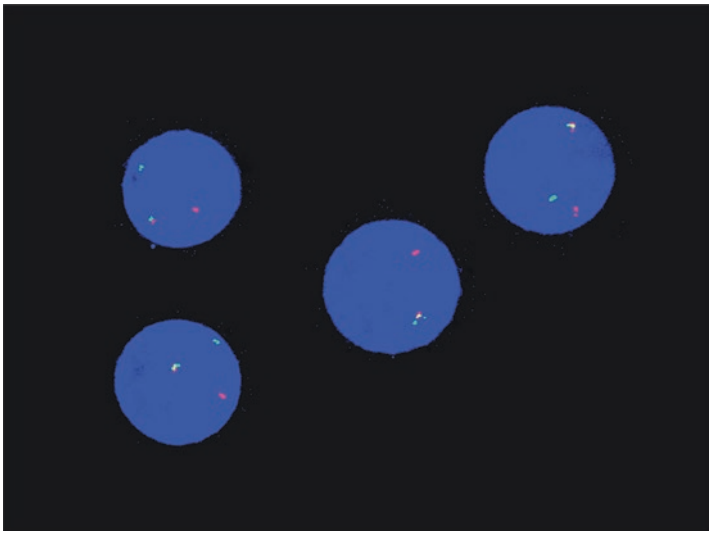

**Fig. 3.8** Bone marrow fluorescence in situ hybridization from a patient with therapy related myeloid neoplasm (t-MNs). FISH study shows 11q23 rearrangement involving *KMT2A* gene with different translocations employing break-apart rearrangement probes. 11q23 rearrangement is revealed by segregation of *red* and *green* fluorescence labeled probes

incidence is less than 0.5% of AMLs [19, 20, 37]. This gene rearrangement leads to the fusion transcript *NPM1-MLF1* [38] and tends to occur in young adults. These patients may respond well to stem cell transplants [8, 39].

## *Cytogenetic Abnormalities in t-MNs*

Therapy-induced AML secondary to alkylating agents is a prototypic AML, in which successive genetic hits occur and result in numerous genetic aberrations. t-MNs associated with alkylating cytotoxic agents/radiation and relatively long latency have abnormal cytogenetics most similar to those identified in AML-MRC [40]. As discussed earlier, these cytogenetic abnormalities are often associated with loss of chromosomes, particularly -5q or -7q, often in the setting of a complex karyotype [6, 7, 40].

In contrast, t-MNs associated with topoisomerase II inhibitors and short latency usually have balanced chromosomal abnormalities involving the *KMT2A* locus at 11q23 and the *RUNX1* locus at 21q22. Translocations involving the *KMT2A* locus at chromosome 11q23 are found in approximately 6% of t-MN cases.

Some patients with therapy-related AML have karyotypic changes identical to those of de novo AML, including types similar to those of the core binding factor leukemias, inv(16) or APL [41, 42]. In contrast to the dismal prognosis of most

**Table 3.5** Cytogenetic abnormalities in patients with t-MNs

| Cytogenetics | | Frequency (%) |
|---|---|---|
| Karyotype | Normal | 9.6 |
| | Abnormal | 90.4 |
| Abnormal chromosome 5, 7, or both | Abnormal chromosome 5 | 20 |
| | Abnormal chromosome 7 | 25 |
| | Abnormal chromosome 5 and 7 | 22 |
| Recurring balanced rearrangements | t(11q23) | 3 |
| | t(3;21) or t(8;21) or t(21q22) | 3 |
| | t(15;17) | 2 |
| | inv(16) | 2 |
| Others | +8, −13/del(13q), −Y, +11, del(11q), del(20q), +21, and others | 18.5 |

Modified from [43]

therapy-related AMLs, some studies suggest that cases with t(15;17) or inv(16) may have good prognoses similar to their de novo counterparts [41]. A summary of cytogenetic abnormalities in patients with t-MNs is listed in Table 3.5 [43].

## Somatic Mutations and Prognosis

The molecular dissection of many nonrandom recurrent gene rearrangements originally detected by conventional karyotyping and FISH has paved the way for the development of more specific and sensitive molecular tools in the diagnosis of AML. Compared to cytogenetic studies, these new approaches, particularly polymerase chain reaction (PCR) and sequencing-based methods, are more timely, sensitive, accurate, and quantitative, and overcome some of the limitations of karyotyping, such as the requirement of fresh material and viable dividing cells. Increasingly, next-generation sequencing (NGS) technologies, also known as high-throughput sequencing, have superseded traditional sequencing methods such as Sanger sequencing and pyrosequencing in clinical laboratories. NGS-based assays have numerous technical advantages over traditional methods, with the capacity to fully and rapidly sequence many genes in a single reaction that requires less DNA input. NGS can detect a large variety of genetic lesions in hematologic malignancies, including point mutations, small and intermediate-size deletions and insertions, copy number variants, and even translocations. The use of NGS-based assays in clinical and research laboratories has led to the discovery of a vast number of new mutations in myeloid neoplasms.

## *Common Mutations in Cytogenetically Normal Acute Myeloid Leukemia (CN-AML)*

A large number of somatic, point, and insertion/deletion mutations have been identified, in addition to those cytogenetically detectable lesions described previously, and these mutations require distinct molecular genetic analyses beyond cytogenetic studies for sensitive and accurate detection. The genes likely to have clinical and prognostic significance in CN-AMLs include *FLT3* abnormalities; *KMT2A* partial tandem duplications (*KMT2A* PTD); *NPM1*, *CEBPA*, *RUNX1*, *ASXL1/2*, *IDH1/2*, *KIT*, *TET2*, *RAS, CBL, ND4*, and *DNMT3A* mutations; and overexpression of a large number of different genes (Table 3.6). Most, but not all, of these mutations are enriched in CN-AMLs and, to a lesser degree, in AML-MRC and t-MNs (Table 3.6). Indeed, almost all AMLs have mutations in one of the eight categories of genes that are highly relevant for myeloid tumorigenesis, including (1) myeloid transcription-factor fusions, (2) the gene encoding nucleophosmin (*NPM1*), (3) multifunctional tumor-suppressor genes, (4) DNA methylation-related genes, (5) signal transduction genes, (6) chromatin-modifying genes, (7) spliceosome-complex genes, and (8) cohesin-complex genes. Among these genes, some mutations are either cooperative or mutually exclusive, and these specific patterns suggest biologic relationships among many of the genes and categories [44–46]. For instance, mutations of *FLT3*, *DNMT3A*, and *NPM1* are often present simultaneously, while other mutations of *NPM1*, *RUNX1*, *CEBPA*, and *TP53* are almost always mutually exclusive both at diagnosis and at the time of disease transformation (Fig. 3.9) [47–50]. Further, the AML morphologic phenotype might be driven by a more complex pattern of concurrent mutations rather than individual genes.

A major new area of emphasis in the revised 2016 WHO classification is on molecular genetics in cytogenetically normal AML. The three genes with the mutations most commonly detected and clinically relevant in CN-AMLs are *FLT3*, *NPM1*, and *CEBPA* (Table 3.6). Unlike *FLT3* mutations, which can also be identified in AML with recurrent genetic abnormalities and many other AML subtypes, AML with mutations in either *NPM1* or biallelic *CEBPA* are defined as distinct biologic subtypes of AML, and they are considered full AML entities in the most updated WHO classification: AML with mutated *NPM1*, and AML with biallelic mutations of *CEBPA*. Mutations in *NPM1* and biallelic *CEBPA*, diagnostic for entities are discussed elsewhere.

### Fms-Like Tyrosine Kinase 3 (FLT3)

*FLT3* mutations are identified in many AML subtypes (including cases with karyotypic abnormalities), thus *FLT3* mutations alone do not define a distinct biologic subtype [46]. Interestingly, mutations in *FLT3* and in genes encoding other kinases including tyrosine kinases, serine–threonine kinases, protein tyrosine phosphatases, and RAS family proteins are mutually exclusive in AMLs [46]. FLT3 is a class III

**Table 3.6** Examples of recurrent mutations in AML, NOS, and AML with MRC and t-MNs [19, 20, 46, 54, 55, 85, 87, 126, 143–146]

| Genes | Locus | Mutations/effects | % in AML, NOS | % in AML-MRC | % in t-MNs | Prognosis | Putative biologic mechanism | Biology and genetic correlation |
|---|---|---|---|---|---|---|---|---|
| *NPM1* | 5q35.1 | Indel | 25–35 | – | 3–16 | Good | Cytoplasmic mislocalization; dysregulated P53 | Cup-like nuclei |
| *CEBPA*<br>*Bi-CEBPA* | 19q13.1 | Indel/nonsense; NF | 7–15<br>4–6 | – | – | –<br>Good | ↓Transcription | Coexpression of T-cell antigens |
| *FLT3-ITD* | 13q12 | Insertion; activation | 19–28 | 8 | 7 | Bad | ↑Signal transduction | Cup-like nuclei |
| *FLT3-TKD* | 13q12 | Missense activation | 5–10 | – | 15 | Bad | ↑Signal transduction | Cup-like nuclei |
| *RUNX1* | 21q22 | Missense DN<br>Nonsense/indel/splice site; NF | 5–21 | 31 | 10 | Bad | ↓Transcription | Minimal differentiation |
| *NRAS* | 1p13 | Missense; activation | 8–13 | 8 | 6 | None | ↑Signal transduction | CBF AML |
| *KIT* | 4q11-q12 | Missense; activation | 2–8 | – | 3 | Bad | ↑Signal transduction | CBF AML |
| *KMT2A*<br>*PTD* | 11q23 | Activation | 5–10 | – | – | Bad | Chromatin modification | Trisomy 11 |
| *IDH1* | 2q33 | Missense; altered function | 8 | 11 | 7 | Unclear/Bad | Metabolite | Monosomy 8<br>Cup-like nuclei |
| *IDH2* | 15q26 | Missense; altered function | 8–15 | 9–14 | 9.5 | Unclear/<br>Good | Metabolite | Cup-like nuclei |
| *ASXL1* | 20q11 | Nonsense/indel; DN or activation | 17–25 | 32 | 16 | Bad | Chromatin modification | – |
| *TET2* | 4q24 | Nonsense/indel; NF | 8–30 | 20–24 | 13 | Bad | Epigenetic regulation | – |
| *CBL* | 11q23 | Missense; Inactivation, DN | 1 | 5 | 4 | Unclear | ↑ Signal transduction | CBF AML |
| *ND4* | MT | Missense; inactivation | 6 | – | – | Good | Electron transport | – |
| *DNMT3A* | 2p23 | Missense; DN | 18–36 | 19–35 | 13–17 | Bad | Epigenetic regulation | – |
| *KRAS* | 12p12 | Missense; activation | 2–4 | 7 | 14 | None | ↑Signal transduction | CBF AML |

*DN* dominant negative, *MT* mitochondria, *NF* nonfunctional

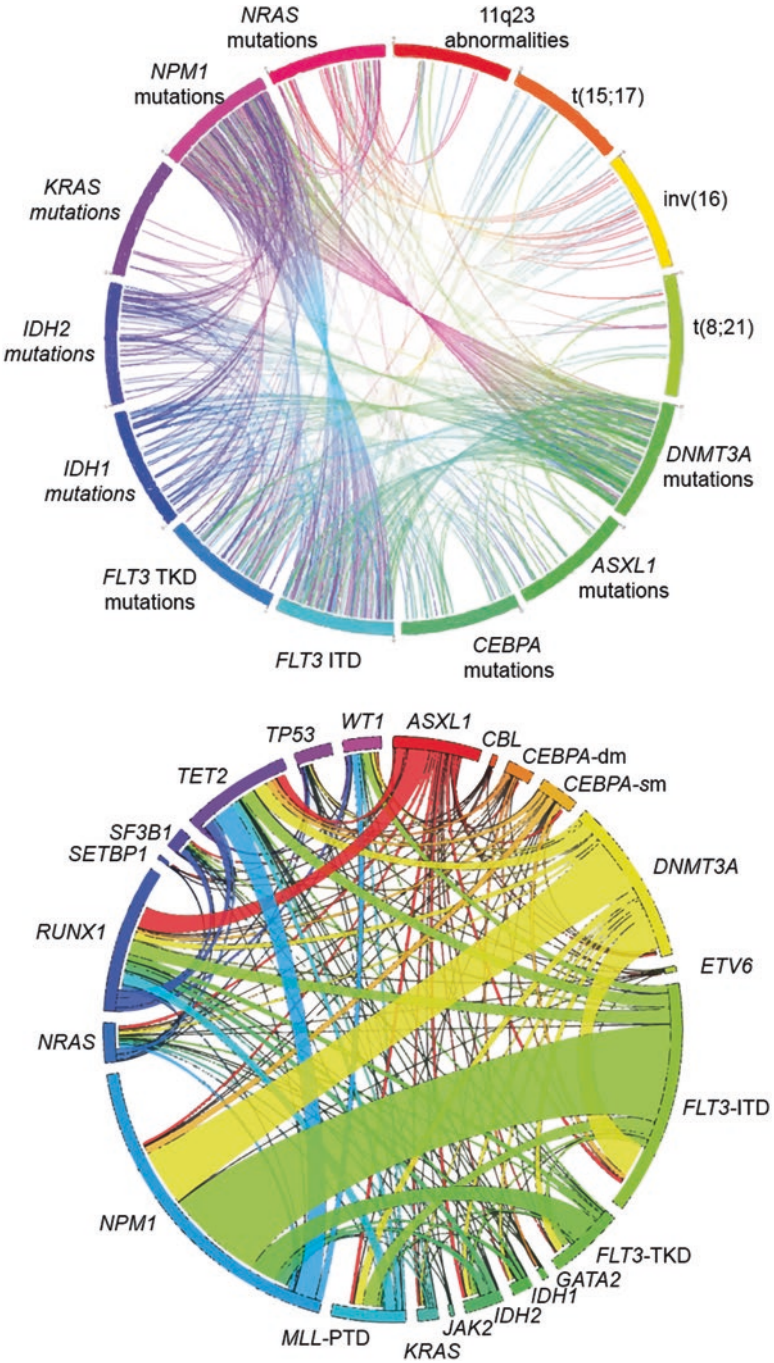


**Fig. 3.9** Molecular heterogeneity and complexity of AML. (**a**) Circos plots demonstrate the molecular heterogeneity of de novo AMLs and the specific patterns of cooperation and mutual exclusivity. *Colored lines* indicate concurrent genetic abnormalities. (**b**) The *outer circular segments* indicate the particular molecular maturations and the length of each segment indicates their relative frequencies in AML. *Outer segments* indicate a particular subcohort being positive for the given marker. *Ribbon widths* indicate relative frequencies of co-occurrences (Graph modified from [50, 143])

receptor tyrosine kinase and Ig receptor superfamily member that is expressed by hematopoietic progenitor cells and downregulated during differentiation. Independent of ligand binding, phosphorylation of regions in the juxtamembranous (JM) domain of FLT3, resulting in a persistent "on" signal, leads to uncontrolled proliferation and inhibition of differentiation and apoptosis via STAT5 and mitogen-activated protein kinase (MAPK) signaling [51–53] in transformed leukemic cells. There are two fundamental types of *FLT3* abnormalities: internal tandem duplication (ITD) of the JM domain, and a missense mutation at Asp835 (TKD) [54, 55]. *FLT3*-ITD is relatively more common [54], and occurs in 12–28% of AML, NOS, approximately 8% of AML-MRC, and about 7% of t-MNs (Table 3.6). Point mutations of *FLT3*-TKD are reported in about 7% of all AML, NOS [54]. Overall, *FLT3* mutations, among the most commonly identified somatic abnormalities in AML, are seen in approximately 35% of all AMLs, and are enriched in up to 50% of AMLs with normal cytogenetics [54, 55]. Overactivation of the FLT3 signaling pathway appears to be the single most important prognostic factor for overall survival in AML patients younger than 60 years [53]. It is correlated with a poor prognosis, and this correlation appears to be independent of the karyotypically aberrant AML groups especially in AMLs with mutant *NPM1* and *DNMT3A* [53, 54, 56]. In addition, *FLT3*-TKD mutations with high allele fraction (>10%), similar to *KIT* mutations, are associated with a higher cumulative incidence of relapse in core binding factor AML patients [57].

**KIT (CD117)**

KIT is a member of a type III tyrosine kinase receptor (RTK) family. Mutations in RTKs and their associated pathways in AML result in constitutive activation of downstream signaling cascades. KIT activation is triggered by binding of its ligands, stem cell factors, and receptor dimerization, which in turn facilitates receptor autophosphorylation. Intracellular signal transduction is mediated through multiple signaling pathways, including RAS–RAF–MAPK, JAK-STAT, and PI3K-AKT pathways. Mutations in *KIT* result in either KIT overexpression or constitutively active tyrosine kinase receptors. This gain of function confers a proliferative and survival advantage to hematopoietic progenitors thus promoting malignant transformation in myeloid cells. Similar to KRAS and NRAS, the critical components of its downstream signaling pathways, *KIT* mutations can be seen in AMLs with normal cytogenetics (Table 3.6) but are particularly common in core binding factor AMLs, occurring in 7–46% of AMLs with t(8;21) or inv(16) [58]. These mutations tend to be associated with a poor prognosis in these cytogenetically favorable AMLs. In AML, *KIT* mutations are found in the extracellular domain (exon 8), kinase domain (exon 17, especially KIT D816V mutations), and rarely in transmembrane (exon 10) and juxtamembrane domains (exon 11) [59].

### RAS

The *RAS* (rat sarcoma) gene family encodes approximately 50 structurally homologous proteins which contain a consensus guanosine triphosphate-binding motif, hence the name, small G-protein. RAS proteins, located on the cell surface, function as conduits for RTK signaling through downstream cascades to nuclear transcription factors regulating cell growth and cell-cycling proteins. Mutations in both *NRAS* (neuroblastoma RAS) and *KRAS* (Kirsten RAS) have been reported in CN-AML and AML with MRC (Table 3.6). Single-point mutations in RAS lead to constitutive activation by locking RAS into its active conformation or reducing its sensitivity to the GTPase-activating proteins (GAPs), thus resulting in overall gain-of-function effects [60, 61], and almost exclusively occur in "hot spot" codons 12, 13, and 61 [61]. *RAS* mutations are detected by PCR followed by pyrosequencing and NGS in most clinical laboratories.

### Histone-Lysine N-Methyltransferase 2A (KMT2A or MLL)

Partial tandem duplication (PTD) of the *KMT2A* gene, also known as acute lymphoblastic leukemia 1 (*ALL-1*) or myeloid/lymphoid or mixed-lineage leukemia (*MLL*), is another example of a biologically relevant genetic abnormality in AML with normal karyotype. KMT2A is a histone methyltransferase mediating chromatin modification and, as a positive regulator of gene expression for known targets such as *HOX* genes, plays an essential role in regulating gene expression during embryonic development and hematopoiesis. *KMT2A* PTD, containing a varied number of exons 5–12 duplicated and inserted before exon 12 giving rise to an in-frame repetition and an elongated protein [62], is associated with an unfavorable outcome [63] in AMLs. Although ~90% of cases with trisomy 11 are associated with *KMT2A* PTD, it is also present in ~10% of AMLs with normal cytogenetics [62, 64]. This mutation is readily detected by RT-PCR amplifying exons 2–12 in most clinical laboratories [62].

### Isocitrate Dehydrogenase 1 and 2 (IDH1/2)

IDH1 and IDH2 are key metabolic enzymes involved in the biosynthesis of central metabolites in the TCA cycle, the major pathway for cellular NADPH generation, and the pentose phosphate pathway. Loss of IDH1/2 function in malignancies impairs oxidative detoxification mechanisms, leading to DNA damage and genomic instability, and thus promoting tumorigenesis, especially in hypoxic setting [65, 66]. IDH converts αKG to (D)-2-hydroxyglutarate (2-HG) using NADPH as cofactors, and 2-HG in turns inhibits histone demethylation by suppressing αKG-dependent dioxygenases. Mutations in *IDH* are uniformly associated with elevated 2-HG in a vast number of cancers. Inhibition of histone demethylation (hypermethylated histone) and altered DNA methylation patterns can block the differentiation of non-transformed cells and facilitate myeloid neoplastic transformation [67]. The

prognostic value of *IDH* mutations in AML remains unknown since results vary across studies and a recent retrospective analysis found no impact of *IDH* mutation status on overall survival in patients with AMLs [68].

### Additional Sex Combs Like 1 (ASXL1)

The *ASXL1* gene encodes a human homologue of the additional sex combs (Asx) gene of *Drosophila*, located on chromosome 20q11. ASXL1 protein function in human remains largely unknown, although recent studies suggest that ASXL1 may be involved in the regulation of histone methylation and that *ASXL1* mutations are an early event contributing to leukemogenesis [69, 70]. *ASXL1* mutations are found in 5–12% of CN-AML and are enriched in secondary AMLs (Table 3.6), especially in older patients [71, 72]. *ASXL1* mutations, including nonsense or frameshift mutations, are almost exclusively in the "hot spot," exon 12, leading to disruption of the carboxy-terminal plant homeodomain (PHD) finger domain. Indeed, the mutation c.1934dupG (G646WfsX12) represents half of all *ASXL1* mutations and can be easily detected by sequencing. *ASXL1* mutations are also closely associated with other cytogenetic and molecular lesions, such as isolated trisomy 8 and *RUNX1*, and appear to be an independent adverse prognostic factor [73, 74].

### DNA Methyltransferases 3A (DNMT3A)

DNA methyltransferases (DNMTs) transfer a methyl group from the universal methyl donor, S-adenosyl-methionine (SAM), to carbon-5 (C5) of cytosine and are predominately responsible for the maintenance of genomic DNA methylation patterns. DNMT3A plays an important role in the maintenance of methylation patterns during primordial germ cell and early embryonic development [75]. Although *DNMT3A* was originally considered an oncogene in human tumorigenesis, recent studies identified several inactivating *DNMT3A* mutations and loss of DNMT3A activity in myeloid neoplasms, suggesting that DNMT3A may also behave as a tumor suppressor [76, 77]. Mutations include missense, nonsense, frameshift, and in-frame alterations throughout the gene across all functional domains, although mutations at arginine 882 (R882) in the catalytic domain resulting in impaired enzyme activity are the most common. However, most of the specific mutations identified in AMLs, presumably preleukemic lesions, have not been functionally characterized. *DNMT3A* mutations rarely occur alone in AMLs, and concurrent mutations in *FLT3*/ITD, *NPM1*, and *IDH* are common. These *DNMT3A* mutations show a negative correlation with AMLs with t(8;21), t(15;17), or inv(16). In addition, *DNMT3A* R882 mutations are associated with advanced age, high leukocyte and blast counts, and morphologic features of monocytic differentiation (FAB M4/M5) [78]. *DNMT3A* mutations also predict an adverse risk and poor outcome in AML patients [79] and can be detected by sequencing. Further, *DNMT3A* mutations at R882 can also be potential markers for minimal residual disease studies.

### Tet Methylcytosine Dioxygenase 2 (TET2)

As a member of the TET family, TET2 proteins convert 5-methylcytosine to 5-hydroxymethylcytosine (5-hmC) in DNA, with ferrous iron and α-KG as cofactors. By regulating DNA methylation and gene expression of downstream targets, TET2 inhibits cell proliferation, self-renewal, and differentiation. *TET2* mutations were first identified in myeloid malignancies via single-nucleotide polymorphism array and comparative genomic-hybridization arrays and confirmed by NGS. Deletion of *TET2* on chromosome 4q24 is common in AMLs [80, 81]. *TET2* mutations, including frameshift, nonsense, and deletion mutations, are detected in 8–30% of patients with CN-AML, and lead to loss of function, resulting in uncontrolled myeloid proliferation and abnormal differentiation [80, 81] (Table 3.6). The presence of single or double allelic *TET2* mutations is positively correlated with mutations of *NPM1*, *DNMT3A*, *ASXL1*, and *RUNX1*, but virtually mutually exclusive with *IDH* mutations. *TET2* mutations are also closely associated with older age, higher leukocyte count, normal karyotype, intermediate-risk cytogenetics, and isolated trisomy 8. Although studies regarding the prognostic impact of *TET2* mutations in different AML subgroups showed inconsistent results, a meta-analysis of over 2500 patients with de novo, secondary, or therapy-related AML revealed that *TET2* mutations appear to be an adverse prognostic factor and independently predict the risk of relapse [82, 83].

## *Gene Mutations Enriched in AML-MRC and t-MNs*

As previously mentioned, secondary AMLs, either AML secondary to a preexisting MDS or to cytogenetic toxic therapy, contain successive genetic hits and are characterized by numerous genetic aberrations. A subset of molecular mutations including *SRSF2*, *SF3B1*, *U2AF1*, *ZRSR2*, *ASXL1*, *EZH2*, *BCOR*, *STAG2*, and *TP53* are relatively specific for this group of secondary AMLs (Table 3.7) compared to those genes described above [19, 20, 40, 84–92]. These secondary AMLs have mutations in one of five categories of genes that are relevant for tumorigenesis and possibly for clinical prognosis, including (1) spliceosomes regulating mRNA splicing; (2) chromatin modifying genes; (3) transcription factors; (4) cohesin complex; and (5) *TP53*, which is a multifunctional tumor suppressor and particularly associated with t-MNs. Almost all of these mutations are detectable by sequencing. Among these genes, mutations in *ASXL1* are most common and their clinical significance has been discussed.

### Mutations in Splicing Factors

Mutations in spliceosome factor genes are most common in myeloid neoplasms including MDS, AMLs, and chronic lymphocytic leukemia. They are also present in several solid tumors, although at lower frequencies. Dysregulation in alternative

**Table 3.7** Gene mutations with clinical relevance in AML with myelodysplasia-related changes and therapy related myeloid neoplasm [19, 20, 40, 84–91]

| Genes | Full name | Locus | Putative biologic mechanism | Mutations/effects | % in AML-MRC | % in t-MNs | Prognosis |
|---|---|---|---|---|---|---|---|
| *SRSF2* | Serine/arginine-rich splicing factor 2 | 17q25 | mRNA splicing | Missense; DN or gain of function | 20–30 | 0–7 | Bad |
| *SF3B1* | Splicing factor 3b, subunit 1 | 2q33 | mRNA splicing | Missense; DN or gain of function | 11–20 | 0–3 | Good |
| *U2AF1* | U2 small nuclear RNA splicing factor 1 | 21q22 | mRNA splicing | Missense; DN or gain of function | 16–30 | 3–5 | Bad |
| *ZRSR2* | U2 small nuclear RNA splicing factor, subunit-related protein 2 | Xp22 | mRNA splicing | Nonsense/indel/splice sites; NF | 8 | 1 | Unclear |
| *ASXL1* | Additional sex combs like 1 | 20q11 | Chromatin modification | Nonsense/indel; DN oractivation | 32 | 3 | Bad |
| *EZH2* | Enhancer of zeste homolog 2 | 7q35-q36 | Chromatin modification | Missense/nonsense/indel; NF | 7–9 | 3 | Unclear |
| *BCOR* | BCL6 Co-repressor | Xp11 | Transcription regulation | Missense/nonsense/frame shift; indel; splicing-site/NF | 8–17 | 1 | Bad |
| *STAG2* | Cohesin subunit SA-2 | Xq25 | Cohesin complex | Missense/Nonsense/frame shift; NF | 14 | 5 | Neutral |
| *TP53* | Tumor protein 53 | 17p13 | DNA repair and tumor suppressor | Missense/indel; NF | 15 | 35–40 | Bad |

*DN* dominant negative, *NF* nonfunctional

mRNA splicing, potentially impacting more than 20,000 human genes, results in malignant transformation and tumorigenesis in various cell types. Prototypic examples of genes with alternative splicing impaired by spliceosome mutations include *BCL2* and *BCL-X*, *CD44*, and *GSK3B* [93]. These mutations promote cell proliferation and inhibit apoptosis, thus promoting tumorigenesis. Recent studies demonstrated that somatic mutations in not only downstream targets but also the splicing machinery itself are common mutations driving myeloid neoplasms including AMLs. *SRSF2*, *SF3B1*, and *U2AF1* frequently acquire heterozygous missense mutations in specific codons leading to gain of function. In contrast, *ZRSR2* mutations largely include loss-of-function variants. Commonly identified in MDS and AML with MRC, mutations in one of four genes, which are mutually exclusive accounting for 5–10% of all AMLs [56]. The clinical or prognostic significance of mutations in each individual gene remains unknown. However, lesions in these genes as a group, particularly in combination with accumulated somatic mutations in chromatin regulators, are frequently associated with a low overall survival rate in AMLs. Further, AMLs with mutations in regulators of chromatin, splicing, and transcription show a distinct clinical presentation including dysplastic features in older age and dismal clinical outcome. Therefore, these common and clinically significant mutations should be considered for incorporation into diagnostic and prognostic classification guidelines [56] (Fig. 3.10).

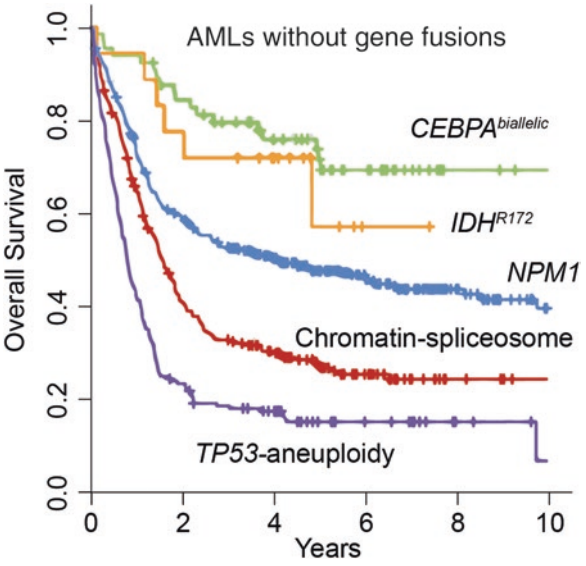


**Fig. 3.10** Overall survival of patients with cytogenetic normal AMLs and following five driver mutations. Patients with AML and *NPM1* (*blue*) and biallelic *CEBPA* mutations (*green*) show optimal overall survival. A subgroup of AMLs with *TP53*-aneuploidy has a distinct dismal long term outcome (*purple*). As compared with other subgroups, patients in the subgroup with chromatin-spliceosome mutations show a poor overall survival (*red*) and the long term outlook in the *IDH2R172* subgroup is similar to that in patients with *NPM1*-mutated AML (Graph modified from [56])

### Serine/Arginine-Rich Splicing Factor 2 Gene (SRSF2)

SRSF2 is an important component in the spliceosome, regulating many steps in RNA-related processes, including spliceosome assembly of the U1 snRNP, U2 snRNP binding at the branch point, and mRNA stabilization [94]. SRSF2 contains an RNA recognition motif and an RS domain rich in arginine and serine residues. Somatic mutations of *SRSF2* are predominantly missense mutations at a specific hotspot, codon Pro95. Proline at this codon is replaced by histidine, leucine, or arginine through missense mutations, or disrupted by in-frame insertions and deletions. Commonly identified in MDS (15%) and in chronic myelomonocytic leukemia (CMML, approximately 50%) [95], *SRSF2* mutations also preferentially occur in acute myeloid leukemia with preceding MDS or myelodysplastic morphology [90]. All patients with an initial mutation retain the same mutation after AML transformation, and approximately 15% of patients who are initially mutation-negative acquire this type mutation after AML progression. There appears to be no significant difference in the time to leukemic transformation between mutation-positive and -negative patients at the initial diagnosis of MDS [90].

### Splicing Factor 3B1 (SF3B1)

This gene encodes subunit 1 of the splicing factor 3b protein complex, which is an essential member of the U2 small ribonucleoprotein complex responsible for branch site recognition near the 3′ end of premessenger RNA. *SF3B1* mutations are frequently identified in a specific MDS subtype with refractory anemia and ring sideroblasts (RARS), and in other subtypes of MDS or MDS/MPN overlap syndromes, such as MDS/MPN with ring sideroblasts and thrombocytosis, a new full entity in the updated WHO classification. Ring sideroblasts are a morphological feature especially associated with *SF3B1* mutations. Missense substitutions are concentrated at codon 700, in the middle of four contiguous HEAT domains, and result in a gain of function or dominant negative activity, in which the mutant protein suppresses its wild-type counterpart. *SF3B1* mutations are associated with a lower rate of transformation to AML and appear to predict an indolent disease in MDS, while the same mutations alone have no detectable prognostic impact in secondary AMLs [91, 96]. In contrast, mutations in splicing factors in combination with *SRSF2*, *ZRSR2*, and *U2AF1* mutations appear to predict unfavorable prognosis in patients with de novo acute myeloid leukemia [97].

### U2-Complex Auxiliary Factor 1 (U2AF1)

*U2AF1* encodes a subunit of the U2 spliceosome required for the binding of U2 snRNP to the pre-mRNA branch site. The encoded protein contains four major functional domains: two zinc-finger regions, an arginine-serine domain, and a U2AF homology domain. U2AF1 binds directly to several other splicing factors important for myeloid neoplastic transformation, including *U2AF2*, *SRSF2*, and

*SF1*. There are two hotspots at codons S34 and Q157 where several missense substitutions occur [95]. Small in-frame insertions and deletions around codon 157 have also been identified but at a much lower frequency. These mutations lead to oncogenic gain-of-function phenotypes and directly interfere with RNA binding, resulting in splicing changes [98]. As with other spliceosome components, *U2AF1* mutations are associated with AML-MRC and morphologic trilineage dysplasia. Further, *U2AF1* mutations alone are an independent prognostic factor and associated with poor clinical outcomes in patients with AMLs [99].

### Zinc Finger, RNA-Binding Motif and Serine/Arginine Rich 2 Gene (ZRSR2)

*ZRSR2*, frequently mutated in myeloid malignancies, is located on the X chromosome and encodes a serine/arginine-rich splicing factor regulating the recognition of the 3′ splice acceptor site as a component of the U2 auxiliary factor heterodimer. In contrast to other splicing factors described above, *ZRSR2* mutations include out-of-frame insertions and deletions, splice-site mutations, and nonsense mutations, resulting in prematurely truncated proteins. Missense mutations across all exons also occur. These mutations result in loss of function, and occur in about 5% of patients with MDS, T-cell acute lymphoblastic leukemia, and plasmacytoid dendritic cell neoplasms [100–102]. Recent studies demonstrated no direct impact of *ZRSR2* mutations on clinical outcomes in patients with MDS, and the clinical relevance of *ZRSR2* mutations alone in patients with AMLs remains unknown.

### Enhancer of Zeste Homolog 2 (EZH2)

The polycomb repressive complex 2 (PRC2) maintains transcriptional silencing through posttranslational histone modification and regulation of homeotic gene expression [103], and is essential for hematopoiesis and lymphopoiesis in human. Comprising the catalytic subunit of PRC2 is EZH2 or EZH1, which serves as a H3K27 methyltransferase in the complex [103]. Overexpression of EZH2 is frequently identified in solid tumors such as prostate, breast, and endometrial cancers. Gain-of-function mutations in the catalytic domain are also commonly detected in patients with follicular lymphoma and diffuse large B-cell lymphoma [104]. In contrast, loss-of-function mutations across all 20 exons of *EZH2* are commonly detected in myeloid malignancies, especially MDS and acute myeloid leukemia. Inactivation of EZH2 leading to loss of H3K27 trimethylation contributes to myeloid neoplasms including MDS, MPN, and MDS/MPN overlap syndrome, but appears to attenuate its predisposition to leukemic transformation secondary to MDS [105–107] in animal models. Although frequently found in other myeloid neoplasms associated with a poor prognosis and clinical outcome, the prognostic relevance of *EZH2* mutations remains largely unknown in AMLs due to the low detection rate [78, 108].

## *BCL6 Corepressor (BCOR)*

The *BCOR* gene encodes a POZ/zinc finger transcriptional repressor, also known as an interacting corepressor of BCL6, which is required for germinal center formation and may influence apoptosis in lymphoid tissue. BCOR is a key component in the polycomb repressive complex 1 (PRC1) variant, which inhibits transcriptional activity and regulates early embryonic development, mesenchymal stem cell function, and hematopoiesis [89]. Recent studies demonstrated that *BCOR* mutations occur in 3.8% of unselected CN-AML patients and are enriched in a substantial fraction (17.1%) of CN-AML patients showing the same genotype as the AML index subjected to whole-exome sequencing. Disruptive somatic *BCOR* mutations include out-of-frame mutations, small insertions and deletions, nonsense mutations, and splice-site mutations along the *BCOR* coding exons and exon/intron junctions. *BCOR* mutations are associated with decreased *BCOR* mRNA levels, absence of full-length BCOR proteins, and low expression of a truncated BCOR protein. Further mutations in *BCOR* are closely associated with mutations in other histone modifiers such as *DNMT3A*, suggesting cooperativity among these genetic alterations, while *BCOR* mutations are virtually mutually exclusive with *NPM1* mutations. *BCOR* mutations are associated with an unfavorable outcome in patients with CN-AML [89].

## *Cohesin Complex*

The cohesin complex forms a ring structure regulating appropriate chromosomal segregation during mitosis and cell division, double-stranded DNA repair, and transcription. Genes encoding the cohesin complex in somatic vertebrate cells are *SMC1A*, *SMC3*, *RAD21* (*SCC1*), *STAG2* (*SA-2*), and *STAG1* (*SA-1*) [109]. Mutations in *STAGs* or other components in this complex result in chromosomal instability and contribute to myeloid malignant transformation [88]. Indeed, disruptive mutations of the core cohesin subunit *STAG2* occurs in a variety of human tumors, including glioblastoma, Ewing sarcoma, melanoma, cervical carcinoma, and hematologic cancers, as *STAG2* mutations lead to aneuploidy in a variety of human cancer cells [110]. Mutations throughout all coding exons of *STAG2* are common in antecedent MDS, secondary AML with predominantly normal cytogenetics, and de novo AMLs. Concurrent mutations in *NPM1*, *FLT3*, *DNMT3A*, and *PTPN11* correlate with *STAG2* mutation status. Cohesin complex or *STAG2* mutations alone are not independent predictive factors for overall survival or remission rates in AMLs [88, 111].

### *Tumor Protein 53 (TP53)*

The tumor protein 53 (TP53) is a transcription factor and prototypical tumor suppressor that arrests the cell cycle, promotes apoptosis, and coordinates DNA damage repair in response to various cellular stresses and cytotoxic insults. TP53 mutations result in resistance to protein degradation through MDM2, and in abnormal nuclear accumulation detectable by immunohistochemistry. TP53 mutations are found in up to 40% of therapy-related AML [40] and in over 50% of AML with complex cytogenetics. Somatic TP53 mutations are also detected in 10–15% of de novo AML and MDS [91]. A large variety of TP53 mutations have been identified to date, including the point mutations and loss of the TP53 locus at chromosome 17p commonly seen in AMLs. These TP53 mutations can be identified by cytogenetic studies and sequencing. It has been suggested that TP53 mutations are not directly induced by cytotoxic chemotherapy. Rather, they are likely to reflect rare age-related mutations that are resistant to chemotherapy and which expand preferentially following treatment. TP53 mutations are directly correlated with a poor prognosis in patients with MDS and AML, with resistance to chemotherapy and reductions in overall and disease-free survival, even in patients undergoing stem cell transplantation [91]. Moreover, TP53 mutations impart similarly poor prognoses in other malignancies as well. Recent studies suggest that a mutation in TP53 with aneuploidy may represent an acquired causative driver mutation involving discrete evolution pathways in the tumorigenesis of acute myeloid leukemia. AML patients with TP53 mutations and aneuploidy had dismal clinical outcomes, and beyond the current WHO classification, AML with TP53-aneuploidy may be defined as a distinct subtype [56, 87, 99].

## Standard and Targeted Therapy

As the molecular landscape of AML becomes more detailed, and we understand how various cytogenetic abnormalities and somatic mutations affect the development of AML, the role of targeted therapies will become more critical. While standard therapies for AML include induction with cytarabine and an anthracycline drug such as daunorubicin followed by consolidation therapy with cytarabine and/or stem cell transplant, here we discuss mainly targeted therapies, again which are gaining momentum in use.

In the case of DNMT3A, which is commonly mutated in AML, nonspecific inhibitors of DNMT, are already commonly used, such as azacitidine and decitabine, and, in preliminary studies, provide better responses in older patients harboring mutations in epigenetic modifiers including *DNMT3A* [112, 113]. DNMT inhibitors may be a superior treatment option for older patients ineligible for intensive chemotherapy. Additional large-scale studies are warranted.

Many targeted therapies focus on mutations in kinase proteins (FLT3, KIT). For instance, FLT3 is considered a potentially important target for leukemia therapy [54]. Although no FLT3-specific inhibitor has been developed to date, many tyrosine

kinase inhibitors (TKIs) appear to inhibit FLT3, and several potential FLT3 inhibitors are being evaluated in clinical trials [54, 114–117]. Midostaurin (PKC412, Novartis Pharmaceuticals, East Hanover, NJ, USA), a benzoylstaurosporine, is a first-generation FLT3 inhibitor, and inhibits the kinase activities of both FLT3/ITDs and FLT3/KDMs, as well as wild-type FLT3 [118]. Phase 2 studies of PKC412, alone or in combination with conventional chemotherapeutic agents in patients with AML or MDS, have shown that patients with *FLT3* mutations but not previously exposed to other FLT3 inhibitors realize the greatest benefit, although the duration of remission was short in some patients [119–121]. United States Food and Drug Administration (FDA) granted Breakthrough Therapy designation to PKC412 (midostaurin) recently primarily based upon the positive results from the Phase III RATIFY (CALGB 10603) clinical trial. Patients who received PKC412 (midostaurin) and standard induction and consolidation chemotherapy experienced a significant improvement in overall survival (OS) (hazard ratio = 0.77, $P = 0.0074$) compared with those who received standard induction and consolidation chemotherapy alone. The median OS for patients in the PKC412 (midostaurin) treatment group was 74.7 months (95% confidence interval [CI]: 31.7, not attained), versus 25.6 months (95% CI: 18.6, 42.9) for patients in the placebo group. Unfortunately, PKC412 alone has insufficient activity in AML patients, and off-target effects such as pulmonary edema are concerning. CEP-701 (Cephalon, Frazer, PA, USA), another relatively-specific first-generation inhibitor targeting FLT3 kinases, showed a transient decrease in circulating blasts [122] in patients with relapsed or refractory acute myeloid leukemia without achieving a complete remission [122, 123]. In recent years, a new generation of more potent and specific FLT3 inhibitors has been developed, with AC220 [124] and G-749 [125] as leading examples. These newer agents have shown significant promise in early phases of clinical investigation including animal models and are currently in more advanced clinical trials [123–125].

TKIs have been used in combination with conventional chemotherapy in *KIT*-positive relapsed/refractory AML and are in clinical trials [126]. Imatinib combined with traditional chemotherapy reagents including mitoxantrone, etoposide, and cytarabine in patients with *KIT*-positive relapsed/refractory AML achieved a complete response in approximately 60% of patients [127, 128]. A multicenter phase 2 trial for older patients with *KIT*-positive AML, who are not optimal candidates for intensive induction therapy, showed that imatinib combined with low-dose cytarabine achieved an 11% hematologic response [129]. However, in most of these studies, the *KIT* mutation status was evaluated by flow cytometry rather than molecular studies. In contrast, dasatinib alone as a maintenance therapy in patients with high-risk AMLs who achieved complete remission 1 (CR1) showed a poor 2-year disease-free survival rate of 25% [130].

Other targeted therapies may be exquisitely protein specific such as IDH inhibitors [65, 66], which are currently being assessed in clinical trials. Other therapies may be somewhat indirect and target a step in the pathway involving the gene of interest. For instance, *RAS* is another gene mutated highly in AML and since RAS activity is dependent on posttranslational farnesylation, specific inhibitors targeting farnesyltransferase have been examined in clinical trials involving patients with

AML [131]. Overall, responses to farnesyltransferase inhibitors in AMLs have not been encouraging. In a trial of 348 elderly patients with AML (aged ≥70 years) who received tipifarnib, less than 20% achieved a complete remission [132–134]. RAS signaling also activates downstream targets, most notably mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K)–AKT through a variety of pathways to promote cell proliferation. Combination therapy with inhibitors targeting the downstream RAS effectors, MEK and AKT, is currently being investigated in clinical trials in AML patients with RAS mutations [135, 136].

In the case of *MLL/KMT2A*, it is known that DOT1L, a histone methyltransferase, is required for the development and maintenance of *KMT2A*-rearranged leukemias [137]. Preclinical studies have shown potential clinical utility of DOT1L inhibition in AMLs [137–139], and small-molecule and competitive inhibitors of DOT1L have been developed (EPZ-5676; Epizyme Inc., Cambridge, MA, USA) which preliminarily show some promising responses, including complete morphologic and cytogenetic remissions, and resolution of leukemia cutis and treatment-related increases in neutrophils and/or monocytes [140].

Finally, modulation of the spliceosome complex may provide a new therapeutic approach in patients with MDS or AML containing spliceosome gene mutations; but studies on specific inhibitors targeting splicing factors in AMLs remain in the preclinical stage. E7107, a structurally distinct splicing inhibitor, is the only compound that specifically inhibits SF3B1, and has been tested in clinical trials. Preliminary data suggest that SF3B1 inhibition has therapeutic potential for the treatment of solid tumors with *SF3B1* mutations. Additional clinical studies with new inhibitors targeting splicing factors for patients with myeloid neoplasms are needed [141, 142].

## Conclusions

As our understanding of the molecular landscape of AML continues to grow, so too will our subclassification of this disease. Additionally, while such detailed understanding drives our diagnostic categorization of this disease, perhaps more importantly it also informs us of the mechanisms driving AML. Importantly, with an understanding of the mechanisms of this disease, continual development of specific targeted therapies is possible which will continue to positively change the course of this disease.

## References

1. Schlenk RF, Taskesen E, van Norden Y, Krauter J, Ganser A, Bullinger L, et al. The value of allogeneic and autologous hematopoietic stem cell transplantation in prognostically favorable acute myeloid leukemia with double mutant CEBPA. Blood. 2013;122(9):1576–82.
2. Haferlach C, Mecucci C, Schnittger S, Kohlmann A, Mancini M, Cuneo A, et al. AML with mutated NPM1 carrying a normal or aberrant karyotype show overlapping biologic, pathologic, immunophenotypic, and prognostic features. Blood. 2009;114(14):3024–32.

3. Falini B, Macijewski K, Weiss T, Bacher U, Schnittger S, Kern W, et al. Multilineage dysplasia has no impact on biologic, clinicopathologic, and prognostic features of AML with mutated nucleophosmin (NPM1). Blood. 2010;115(18):3776–86.
4. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–405.
5. Pedersen-Bjergaard J, Andersen MK, Christiansen DH. Therapy-related acute myeloid leukemia and myelodysplasia after high-dose chemotherapy and autologous stem cell transplantation. Blood. 2000;95(11):3273–9.
6. Sill H, Olipitz W, Zebisch A, Schulz E, Wölfler A. Therapy-related myeloid neoplasms: pathobiology and clinical characteristics. Br J Pharmacol. 2011;162(4):792–805.
7. Bhatia S. Therapy-related myelodysplasia and acute myeloid leukemia. Semin Oncol. 2013;40(6):666–75.
8. Arber DA, Stein AS, Carter NH, Ikle D, Forman SJ, Slovak ML. Prognostic impact of acute myeloid leukemia classification. Importance of detection of recurring cytogenetic abnormalities and multilineage dysplasia on survival. Am J Clin Pathol. 2003;119(5):672–80.
9. Tallman MS, Kim HT, Paietta E, Bennett JM, Dewald G, Cassileth PA, et al. Acute monocytic leukemia (French-American-British classification M5) does not have a worse prognosis than other subtypes of acute myeloid leukemia: a report from the eastern cooperative oncology group. J Clin Oncol. 2004;22(7):1276–86.
10. Hasserjian RP, Zuo Z, Garcia C, Tang G, Kasyan A, Luthra R, et al. Acute erythroid leukemia: a reassessment using criteria refined in the 2008 WHO classification. Blood. 2010;115(10):1985–92.
11. Liu W, Hasserjian RP, Hu Y, Zhang L, Miranda RN, Medeiros LJ, Wang SA, et al. Pure erythroid leukemia: a reassessment of the entity using the 2008 World Health Organization classification. Mod Pathol. 2011;24(3):375–83.
12. Porwit A, Vardiman JW. Acute myeloid leukemia with expanded erythropoiesis. Haematologica. 2011;96(9):1241–3.
13. Grossmann V, Bacher U, Haferlach C, Schnittger S, Pötzinger F, Weissmann S, et al. Acute erythroid leukemia (AEL) can be separated into distinct prognostic subsets based on cytogenetic and molecular genetic characteristics. Leukemia. 2013;27(9):1940–3.
14. Weinberg OK, Seetharam M, Ren L, Seo K, Ma L, Merker JD, et al. Clinical characterization of acute myeloid leukemia with myelodysplasia-related changes as defined by the 2008 WHO classification system. Blood. 2009;113(9):1906–8.
15. Haferlach T, Schoch C, Löffler H, Gassmann W, Kern W, Schnittger S, et al. Morphologic dysplasia in de novo acute myeloid leukemia (AML) is related to unfavorable cytogenetics but has no independent prognostic relevance under the conditions of intensive induction therapy: results of a multiparameter analysis from the German AML cooperative group studies. J Clin Oncol. 2003;21(2):256–65.
16. Miyazaki Y, Kuriyama K, Miyawaki S, Ohtake S, Sakamaki H, Matsuo T, et al. Cytogenetic heterogeneity of acute myeloid leukaemia (AML) with trilineage dysplasia: Japan adult Leukaemia study group-AML 92 study. Br J Haematol. 2003;120(1):56–62.
17. Yanada M, Suzuki M, Kawashima K, Kiyoi H, Kinoshita T, Emi N, et al. Long-term outcomes for unselected patients with acute myeloid leukemia categorized according to the World Health Organization classification: a single-center experience. Eur J Haematol. 2005;74(5):418–23.
18. Ikegawa S, Doki N, Kurosawa S, Yamaguchi T, Sakaguchi M, Harada K, et al. Allogeneic hematopoietic stem cell transplant overcomes poor prognosis of acute myeloid leukemia with myelodysplasia-related changes. Leuk Lymphoma. 2016;57(1):76–80.
19. Cazzola M, Della Porta MG, Malcovati L. The genetic basis of myelodysplasia and its clinical relevance. Blood. 2013;122(25):4021–34.
20. Malcovati L, Ambaglio I, Elena C. The genomic landscape of myeloid neoplasms with myelodysplasia and its clinical implications. Curr Opin Oncol. 2015;27(6):551–9.

21. Boogaerts MA. Stem cell transplantation and intensified cytotoxic treatment for myelodysplasia. Curr Opin Hematol. 1998;5(6):465–71.
22. Emanuel PD. Myelodysplasia and myeloproliferative disorders in childhood: an update. Br J Haematol. 1999;105(4):852–63.
23. Davis KL, Marina N, Arber DA, Ma L, Cherry A, Dahl GV, et al. Pediatric acute myeloid leukemia as classified using 2008 WHO criteria: a single-center experience. Am J Clin Pathol. 2013;139(6):818–25.
24. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood. 2009;114(5):937–51.
25. Schoch C, Kern W, Kohlmann A, Hiddemann W, Schnittger S, Haferlach T. Acute myeloid leukemia with a complex aberrant karyotype is a distinct biological entity characterized by genomic imbalances and a specific gene expression profile. Genes Chromosomes Cancer. 2005;43(3):227–38.
26. Breems DA, Van Putten WL, De Greef GE, Van Zelderen-Bhola SL, Gerssen-Schoorl KB, Mellink CH, et al. Monosomal karyotype in acute myeloid leukemia: a better indicator of poor prognosis than a complex karyotype. J Clin Oncol. 2008;26(29):4791–7.
27. Pabst T, Eyholzer M, Fos J, Mueller BU. Heterogeneity within AML with CEBPA mutations; only CEBPA double mutations, but not single CEBPA mutations are associated with favourable prognosis. Br J Cancer. 2009;100(8):1343–6.
28. Heldin CH, Lennartsson J. Structural and functional properties of platelet-derived growth factor and stem cell factor receptors. Cold Spring Harb Perspect Biol. 2013;5(8):a009100.
29. Lugthart S, van Drunen E, van Norden Y, van Hoven A, Erpelinck CA, et al. High EVI1 levels predict adverse outcome in acute myeloid leukemia: prevalence of EVI1 overexpression and chromosome 3q26 abnormalities underestimated. Blood. 2008;111(8):4329–37.
30. Groschel S, Sanders MA, Hoogenboezem R, de Wit E, Bouwman BA, Erpelinck C, et al. A single oncogenic enhancer rearrangement causes concomitant EVI1 and GATA2 deregulation in leukemia. Cell. 2014;157(2):369–81.
31. Yamazaki H, Suzuki M, Otsuki A, Shimizu R, Bresnick EH, Engel JD, Yamamoto M. A remote GATA2 hematopoietic enhancer drives leukemogenesis in inv(3)(q21;q26) by activating EVI1 expression. Cancer Cell. 2014;25(4):415–27.
32. Meyer C, Schneider B, Jakob S, Strehl S, Attarbaschi A, Schnittger S, et al. The MLL recombinome of acute leukemias. Leukemia. 2006;20(5):777–84.
33. Shih LY, Liang DC, Fu JF, Wu JH, Wang PN, Lin TL, et al. Characterization of fusion partner genes in 114 patients with de novo acute myeloid leukemia and MLL rearrangement. Leukemia. 2006;20(2):218–23.
34. Byrd JC, Mrózek K, Dodge RK, Carroll AJ, Edwards CG, Arthur DC, et al. Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: results from cancer and leukemia group B (CALGB 8461). Blood. 2002;100(13):4325–36.
35. Forestier E, Heim S, Blennow E, Borgström G, Holmgren G, Heinonen K, et al. Cytogenetic abnormalities in childhood acute myeloid leukaemia: a Nordic series comprising all children enrolled in the NOPHO-93-AML trial between 1993 and 2001. Br J Haematol. 2003;121(4):566–77.
36. Meyer C, Kowarz E, Hofmann J, Renneville A, Zuna J, Trka J, et al. New insights to the MLL recombinome of acute leukemias. Leukemia. 2009;23(8):1490–9.
37. Miesner M, Haferlach C, Bacher U, Weiss T, Macijewski K, Kohlmann A, et al. Multilineage dysplasia (MLD) in acute myeloid leukemia (AML) correlates with MDS-related cytogenetic abnormalities and a prior history of MDS or MDS/MPN but has no independent prognostic relevance: a comparison of 408 cases classified as “AML not otherwise specified” (AML-NOS) or “AML with myelodysplasia-related changes” (AML-MRC). Blood. 2010;116(15):2742–51.
38. Lim G, Choi JR, Kim MJ, Kim SY, Lee HJ, Suh JT, et al. Detection of t(3;5) and NPM1/MLF1 rearrangement in an elderly patient with acute myeloid leukemia: clinical and laboratory study with review of the literature. Cancer Genet Cytogenet. 2010;199(2):101–9.

39. Arber DA, Chang KL, Lyda MH, Bedell V, Spielberger R, Slovak ML, et al. Detection of NPM/MLF1 fusion in t(3;5)-positive acute myeloid leukemia and myelodysplasia. Hum Pathol. 2003;34(8):809–13.
40. Shih AH, Chung SS, Dolezal EK, Zhang SJ, Abdel-Wahab OI, Park CY, et al. Mutational analysis of therapy-related myelodysplastic syndromes and acute myelogenous leukemia. Haematologica. 2013;98(6):908–12.
41. Andersen MK, Larson RA, Mauritzson N, Schnittger S, Jhanwar SC, Pedersen-Bjergaard J. Balanced chromosome abnormalities inv(16) and t(15;17) in therapy-related myelodysplastic syndromes and acute leukemia: report from an international workshop. Genes Chromosomes Cancer. 2002;33(4):395–400.
42. Borthakur G, Lin E, Jain N, Estey EE, Cortes JE, O'Brien S, et al. Survival is poorer in patients with secondary core-binding factor acute myelogenous leukemia compared with de novo core-binding factor leukemia. Cancer. 2009;115(14):3217–21.
43. Qian Z, Joslin JM, Tennant TR, Reshmi SC, Young DJ, Stoddart A, et al. Cytogenetic and genetic pathways in therapy-related acute myeloid leukemia. Chem Biol Interact. 2010;184(1–2):50–7.
44. Takahashi S. Current findings for recurring mutations in acute myeloid leukemia. J Hematol Oncol. 2011;4:36.
45. Wouters BJ, Delwel R. Epigenetics and approaches to targeted epigenetic therapy in acute myeloid leukemia. Blood. 2016;127(1):42–52.
46. Cancer Genome Atlas Research Network, Ley TJ, Miller C, Ding L, Raphael BJ, Mungall AJ, et al. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J Med. 2013;368(22):2059–74.
47. Larsson CA, Cote G, Quintas-Cardama A. The changing mutational landscape of acute myeloid leukemia and myelodysplastic syndrome. Mol Cancer Res. 2013;11(8):815–27.
48. Hou HA, Chou WC, Kuo YY, Liu CY, Lin LI, Tseng MH, et al. TP53 mutations in de novo acute myeloid leukemia patients: longitudinal follow-ups show the mutation is stable during disease evolution. Blood Cancer J. 2015;5:e331.
49. Miller CA, Wilson RK, Ley TJ. Genomic landscapes and clonality of de novo AML. N Engl J Med. 2013;369(15):1473.
50. Sanders MA, Valk PJ. The evolving molecular genetic landscape in acute myeloid leukaemia. Curr Opin Hematol. 2013;20(2):79–85.
51. Drexler HG, Meyer C, Quentmeier H. Effects of FLT3 ligand on proliferation and survival of myeloid leukemia cells. Leuk Lymphoma. 1999;33(1–2):83–91.
52. Meyer C, Drexler HG. FLT3 ligand inhibits apoptosis and promotes survival of myeloid leukemia cell lines. Leuk Lymphoma. 1999;32(5–6):577–81.
53. Voutsadakis IA. Flt3 in acute myelogenous leukemia: biology, prognosis, and therapeutic implications. Med Oncol. 2003;20(4):311–24.
54. Kiyoi H, Naoe T. Biology, clinical relevance, and molecularly targeted therapy in acute leukemia with FLT3 mutation. Int J Hematol. 2006;83(4):301–8.
55. Kiyoi H, Naoe T. FLT3 mutations in acute myeloid leukemia. Methods Mol Med. 2006;125:189–97.
56. Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, Roberts ND, et al. Genomic classification and prognosis in acute myeloid leukemia. N Engl J Med. 2016;374(23):2209–21.
57. Duployez N, , Marceau-Renaut A, Boissel N, Petit A, Bucci M, Geffroy S, et al. Comprehensive mutational profiling of core binding factor acute myeloid leukemia. Blood 2016;127(20):2451–2459.
58. Yohe S. Molecular genetic markers in acute myeloid leukemia. J Clin Forensic Med. 2015;4(3):460–78.
59. Becker H, Pfeifer D, Afonso JD, Nimer SD, Veelken H, Schwabe M, Lübbert M. Two cell lines of t(8;21) acute myeloid leukemia with activating KIT exon 17 mutation: models for the 'second hit' hypothesis. Leukemia. 2008;22(9):1792–4.
60. Colicelli J. Human RAS superfamily proteins and related GTPases. Sci STKE. 2004;2004(250):RE13.

61. Johnson DB, Smalley KS, Sosman JA. Molecular pathways: targeting NRAS in melanoma and acute myelogenous leukemia. Clin Cancer Res. 2014;20(16):4186–92.
62. Krivtsov AV, Armstrong SA. MLL translocations, histone modifications and leukaemia stem-cell development. Nat Rev Cancer. 2007;7(11):823–33.
63. Kao HW, Liang DC, Kuo MC, Wu JH, Dunn P, Wang PN, et al. High frequency of additional gene mutations in acute myeloid leukemia with MLL partial tandem duplication: DNMT3A mutation is associated with poor prognosis. Oncotarget. 2015;6(32):33217–25.
64. Bacher U, Schnittger S, Haferlach T. Molecular genetics in acute myeloid leukemia. Curr Opin Oncol. 2010;22(6):646–55.
65. Dang L, Jin S, Su SM. IDH mutations in glioma and acute myeloid leukemia. Trends Mol Med. 2010;16(9):387–97.
66. Dang L, Yen K, Attar EC. IDH mutations in cancer and progress toward development of targeted therapeutics. Ann Oncol. 2016;27(4):599–608.
67. Lu C, Ward PS, Kapoor GS, Rohle D, Turcan S, Abdel-Wahab O, et al. IDH mutation impairs histone demethylation and results in a block to cell differentiation. Nature. 2012;483(7390):474–8.
68. DiNardo CD, Ravandi F, Agresta S, Konopleva M, Takahashi K, Kadia T, et al. Characteristics, clinical outcome, and prognostic significance of IDH mutations in AML. Am J Hematol. 2015;90(8):732–6.
69. Abdel-Wahab O, Adli M, LaFave LM, Gao J, Hricik T, Shih AH, et al. ASXL1 mutations promote myeloid transformation through loss of PRC2-mediated gene repression. Cancer Cell. 2012;22(2):180–93.
70. Inoue D, Kitaura J, Togami K, Nishimura K, Enomoto Y, Uchida T, et al. Myelodysplastic syndromes are induced by histone methylation-altering ASXL1 mutations. J Clin Invest. 2013;123(11):4627–40.
71. Chou WC, Huang HH, Hou HA, Chen CY, Tang JL, Yao M, et al. Distinct clinical and biological features of de novo acute myeloid leukemia with additional sex comb-like 1 (ASXL1) mutations. Blood. 2010;116(20):4086–94.
72. Paschka P, Schlenk RF, Gaidzik VI, Herzig JK, Aulitzky T, Bullinger L, et al. ASXL1 mutations in younger adult patients with acute myeloid leukemia: a study by the German-Austrian acute myeloid leukemia study group. Haematologica. 2015;100(3):324–30.
73. Pratcorona M, Abbas S, Sanders MA, Koenders JE, Kavelaars FG, Erpelinck-Verschueren CA, et al. Acquired mutations in ASXL1 in acute myeloid leukemia: prevalence and prognostic value. Haematologica. 2012;97(3):388–92.
74. Schnittger S, Eder C, Jeromin S, Alpermann T, Fasan A, Grossmann V, et al. ASXL1 exon 12 mutations are frequent in AML with intermediate risk karyotype and are independently associated with an adverse outcome. Leukemia. 2013;27(1):82–91.
75. Okano M, Bell DW, Haber DA, Li E. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell. 1999;99(3):247–57.
76. Gao Q, Steine EJ, Barrasa MI, Hockemeyer D, Pawlak M, Fu D, et al. Deletion of the de novo DNA methyltransferase Dnmt3a promotes lung tumor progression. Proc Natl Acad Sci U S A. 2011;108(44):18061–6.
77. Walter MJ, Ding L, Shen D, Shao J, Grillot M, McLellan M, et al. Recurrent DNMT3A mutations in patients with myelodysplastic syndromes. Leukemia. 2011;25(7):1153–8.
78. Hou HA, Tien HF. Mutations in epigenetic modifiers in acute myeloid leukemia and their clinical utility. Expert Rev Hematol. 2016;9(5):447–69.
79. Ley TJ, Ding L, Walter MJ, McLellan MD, Lamprecht T, Larson DE, et al. DNMT3A mutations in acute myeloid leukemia. N Engl J Med. 2010;363(25):2424–33.
80. Delhommeau F, Dupont S, Della Valle V, James C, Trannoy S, Massé A, et al. Mutation in TET2 in myeloid cancers. N Engl J Med. 2009;360(22):2289–301.
81. Langemeijer SM, Kuiper RP, Berends M, Knops R, Aslanyan MG, Massop M, et al. Acquired mutations in TET2 are common in myelodysplastic syndromes. Nat Genet. 2009;41(7):838–42.

82. Liu WJ, et al. Prognostic significance of TET methylcytosine dioxygenase 2 (TET2) gene mutations in adult patients with acute myeloid leukemia: a meta-analysis. Leuk Lymphoma. 2014;55(12):2691–8.
83. Ahn JS, Kim HJ, Kim YK, Jung SH, Yang DH, Lee JJ, et al. Adverse prognostic effect of homozygous TET2 mutation on the relapse risk of acute myeloid leukemia in patients of normal karyotype. Haematologica. 2015;100(9):e351–3.
84. Bravo GM, Lee E, Merchan B, Kantarjian HM, García-Manero G. Integrating genetics and epigenetics in myelodysplastic syndromes: advances in pathogenesis and disease evolution. Br J Haematol. 2014;166(5):646–59.
85. Karimi M, Nilsson C, Dimitriou M, Jansson M, Matsson H, Unneberg P, et al. High-throughput mutational screening adds clinically important information in myelodysplastic syndromes and secondary or therapy-related acute myeloid leukemia. Haematologica. 2015;100(6):e223–5.
86. Damm F, Chesnais V, Nagata Y, Yoshida K, Scourzic L, Okuno Y, et al. BCOR and BCORL1 mutations in myelodysplastic syndromes and related disorders. Blood. 2013;122(18):3169–77.
87. Lindsley RC, Mar BG, Mazzola E, Grauman PV, Shareef S, Allen SL, et al. Acute myeloid leukemia ontogeny is defined by distinct somatic mutations. Blood. 2015;125(9):1367–76.
88. Thol F, Bollin R, Gehlhaar M, Walter C, Dugas M, Suchanek KJ, et al. Mutations in the cohesin complex in acute myeloid leukemia: clinical and prognostic implications. Blood. 2014;123(6):914–20.
89. Grossmann V, Tiacci E, Holmes AB, Kohlmann A, Martelli MP, Kern W, et al. Whole-exome sequencing identifies somatic mutations of BCOR in acute myeloid leukemia with normal karyotype. Blood. 2011;118(23):6153–63.
90. Cho YU, Jang S, Seo EJ, Park CJ, Chi HS, Kim DY, et al. Preferential occurrence of spliceosome mutations in acute myeloid leukemia with preceding myelodysplastic syndrome and/or myelodysplasia morphology. Leuk Lymphoma. 2015;56(8):2301–8.
91. Nardi V, Hasserjian RP. Genetic testing in acute myeloid leukemia and Myelodysplastic syndromes. Surg Pathol Clin. 2016;9(1):143–63.
92. Churpek JE, Marquez R, Neistadt B, Claussen K, Lee MK, Churpek MM, et al. Inherited mutations in cancer susceptibility genes are common among survivors of breast cancer who develop therapy-related leukemia. Cancer. 2016;122(2):304–11.
93. Shkreta L, Bell B, Revil T, Venables JP, Prinos P, Elela SA, Chabot B. Cancer-associated perturbations in alternative pre-messenger RNA splicing. Cancer Treat Res. 2013;158:41–94.
94. Moon H, Cho S, Loh TJ, Oh HK, Jang HN, Zhou J, et al. SRSF2 promotes splicing and transcription of exon 11 included isoform in Ron proto-oncogene. Biochim Biophys Acta. 2014;1839(11):1132–40.
95. Makishima H, Visconte V, Sakaguchi H, Jankowska AM, Abu Kar S, Jerez A, et al. Mutations in the spliceosome machinery, a novel and ubiquitous pathway in leukemogenesis. Blood. 2012;119(14):3203–10.
96. Peng J, Hasserjian RP, Tang G, Patel KP, Goswami M, Jabbour EJ, Garcia-Manero G, et al. Myelodysplastic syndromes following therapy with hypomethylating agents (HMAs): development of acute erythroleukemia may not influence assessment of treatment response. Leuk Lymphoma. 2016;57(4):812–9.
97. Hou HA, Liu CY, Kuo YY, Chou WC, Tsai CH, Lin CC, et al. Splicing factor mutations predict poor prognosis in patients with de novo acute myeloid leukemia. Oncotarget. 2016;7(8):9084–101.
98. Shao C, Yang B, Wu T, Huang J, Tang P, Zhou Y, et al. Mechanisms for U2AF to define 3′ splice sites and regulate alternative splicing in the human genome. Nat Struct Mol Biol. 2014;21(11):997–1005.
99. Ohgami RS, Ma L, Merker JD, Gotlib JR, Schrijver I, Zehnder JL, Arber DA. Next-generation sequencing of acute myeloid leukemia identifies the significance of TP53, U2AF1, ASXL1, and TET2 mutations. Mod Pathol. 2015;28(5):706–14.
100. Neumann M, Vosberg S, Schlee C, Heesch S, Schwartz S, Gökbuget N, et al. Mutational spectrum of adult T-ALL. Oncotarget. 2015;6(5):2754–66.

101. Yoshida K, Sanada M, Shiraishi Y, Nowak D, Nagata Y, Yamamoto R, et al. Frequent pathway mutations of splicing machinery in myelodysplasia. Nature. 2011;478(7367):64–9.
102. An HJ, Yoon DH, Kim S, Shin SJ, Huh J, Lee KH, Suh C. Blastic plasmacytoid dendritic cell neoplasm: a single-center experience. Ann Hematol. 2013;92(3):351–6.
103. Margueron R, Reinberg D. The Polycomb complex PRC2 and its mark in life. Nature. 2011;469(7330):343–9.
104. Morin RD, Johnson NA, Severson TM, Mungall AJ, An J, Goya R, et al. Somatic mutations altering EZH2 (Tyr641) in follicular and diffuse large B-cell lymphomas of germinal-center origin. Nat Genet. 2010;42(2):181–5.
105. Ernst T, Chase AJ, Score J, Hidalgo-Curtis CE, Bryant C, Jones AV, et al. Inactivating mutations of the histone methyltransferase gene EZH2 in myeloid disorders. Nat Genet. 2010;42(8):722–6.
106. Nikoloski G, Langemeijer SM, Kuiper RP, Knops R, Massop M, Tönnissen ER, et al. Somatic mutations of the histone methyltransferase gene EZH2 in myelodysplastic syndromes. Nat Genet. 2010;42(8):665–7.
107. Sashida G, Harada H, Matsui H, Oshima M, Yui M, Harada Y, et al. Ezh2 loss promotes development of myelodysplastic syndrome but attenuates its predisposition to leukaemic transformation. Nat Commun. 2014;5:4177.
108. Wang X, Dai H, Wang Q, Wang Q, Xu Y, Wang Y, et al. EZH2 mutations are related to low blast percentage in bone marrow and −7/del(7q) in de novo acute myeloid leukemia. PLoS One. 2013;8(4):e61341.
109. Wassmann K. Sister chromatid segregation in meiosis II: deprotection through phosphorylation. Cell Cycle. 2013;12(9):1352–9.
110. Solomon DA, et al. Mutational inactivation of STAG2 causes aneuploidy in human cancer. Science. 2011;333(6045):1039–43.
111. Solomon DA, Kim JS, Waldman T. Cohesin gene mutations in tumorigenesis: from discovery to clinical significance. BMB Rep. 2014;47:299–310.
112. Marcucci G, Metzeler KH, Schwind S, Becker H, Maharry K, Mrózek K, et al. Age-related prognostic impact of different types of DNMT3A mutations in adults with primary cytogenetically normal acute myeloid leukemia. J Clin Oncol. 2012;30(7):742–50.
113. Metzeler KH, Walker A, Geyer S, Garzon R, Klisovic RB, Bloomfield CD, et al. DNMT3A mutations and response to the hypomethylating agent decitabine in acute myeloid leukemia. Leukemia. 2012;26(5):1106–7.
114. Levis M, Small D. FLT3 tyrosine kinase inhibitors. Int J Hematol. 2005;82(2):100–7.
115. DeAngelo DJ, Stone RM, Heaney ML, Nimer SD, Paquette RL, Klisovic RB, et al. Phase 1 clinical results with tandutinib (MLN518), a novel FLT3 antagonist, in patients with acute myelogenous leukemia or high-risk myelodysplastic syndrome: safety, pharmacokinetics, and pharmacodynamics. Blood. 2006;108(12):3674–81.
116. Pemmaraju N, Kantarjian H, Ravandi F, Cortes J. FLT3 inhibitors in the treatment of acute myeloid leukemia: the start of an era? Cancer. 2011;117(15):3293–304.
117. Takahashi K, Kantarjian H, Pemmaraju N, Andreeff M, Borthakur G, Faderl S, et al. Salvage therapy using FLT3 inhibitors may improve long-term outcome of relapsed or refractory AML in patients with FLT3-ITD. Br J Haematol. 2013;161(5):659–66.
118. Weisberg E, Boulton C, Kelly LM, Manley P, Fabbro D, Meyer T, et al. Inhibition of mutant FLT3 receptors in leukemia cells by the small molecule tyrosine kinase inhibitor PKC412. Cancer Cell. 2002;1(5):433–43.
119. Stone RM, DeAngelo DJ, Klimek V, Galinsky I, Estey E, Nimer SD, et al. Patients with acute myeloid leukemia and an activating mutation in FLT3 respond to a small-molecule FLT3 tyrosine kinase inhibitor, PKC412. Blood. 2005;105(1):54–60.
120. Fischer T, Stone RM, Deangelo DJ, Galinsky I, Estey E, Lanza C, et al. Phase IIB trial of oral Midostaurin (PKC412), the FMS-like tyrosine kinase 3 receptor (FLT3) and multi-targeted kinase inhibitor, in patients with acute myeloid leukemia and high-risk myelodysplastic syndrome with either wild-type or mutated FLT3. J Clin Oncol. 2010;28(28):4339–45.

121. Kindler T, Lipka DB, Fischer T. FLT3 as a therapeutic target in AML: still challenging after all these years. Blood. 2010;116(24):5089–102.
122. Smith BD, Levis M, Beran M, Giles F, Kantarjian H, Berg K, et al. Single-agent CEP-701, a novel FLT3 inhibitor, shows biologic and clinical activity in patients with relapsed or refractory acute myeloid leukemia. Blood. 2004;103(10):3669–76.
123. Fathi AT, Chen YB. Treatment of FLT3-ITD acute myeloid leukemia. Am J Blood Res. 2011;1(2):175–89.
124. Zarrinkar PP, Gunawardane RN, Cramer MD, Gardner MF, Brigham D, Belli B, et al. AC220 is a uniquely potent and selective inhibitor of FLT3 for the treatment of acute myeloid leukemia (AML). Blood. 2009;114(14):2984–92.
125. Lee HK, Kim HW, Lee IY, Lee J, Lee J, Jung DS, et al. G-749, a novel FLT3 kinase inhibitor, can overcome drug resistance for the treatment of acute myeloid leukemia. Blood. 2014;123(14):2209–19.
126. Coombs CC, Tallman MS, Levine RL. Molecular therapy for acute myeloid leukaemia. Nat Rev Clin Oncol. 2016;13(5):305–18.
127. Brandwein JM, Hedley DW, Chow S, Schimmer AD, Yee KW, Schuh AC, et al. A phase I/II study of imatinib plus reinduction therapy for c-kit-positive relapsed/refractory acute myeloid leukemia: inhibition of Akt activation correlates with complete response. Leukemia. 2011;25(6):945–52.
128. Advani AS, Tiu R, Saunthararajah Y, Maciejewski J, Copelan EA, Sobecks R, et al. A phase 1 study of imatinib mesylate in combination with cytarabine and daunorubicin for c-kit positive relapsed acute myeloid leukemia. Leuk Res. 2010;34(12):1622–6.
129. Heidel F, Cortes J, Rücker FG, Aulitzky W, Letvak L, Kindler T, et al. Results of a multicenter phase II trial for older patients with c-kit-positive acute myeloid leukemia (AML) and high-risk myelodysplastic syndrome (HR-MDS) using low-dose Ara-C and Imatinib. Cancer. 2007;109(5):907–14.
130. Boissel N, Renneville A, Leguay T, Lefebvre PC, Recher C, Lecerf T, et al. Dasatinib in high-risk core binding factor acute myeloid leukemia in first complete remission: a French acute myeloid leukemia intergroup trial. Haematologica. 2015;100(6):780–5.
131. Rowinsky EK, Windle JJ, Von Hoff DD. Ras protein farnesyltransferase: a strategic target for anticancer therapeutic development. J Clin Oncol. 1999;17(11):3631–52.
132. Harousseau JL, Lancet JE, Reiffers J, Lowenberg B, Thomas X, Huguet F, et al. A phase 2 study of the oral farnesyltransferase inhibitor tipifarnib in patients with refractory or relapsed acute myeloid leukemia. Blood. 2007;109(12):5151–6.
133. Lancet JE, Gojo I, Gotlib J, Feldman EJ, Greer J, Liesveld JL, et al. A phase 2 study of the farnesyltransferase inhibitor tipifarnib in poor-risk and elderly patients with previously untreated acute myelogenous leukemia. Blood. 2007;109(4):1387–94.
134. Erba HP, Othus M, Walter RB, Kirschbaum MH, Tallman MS, Larson RA, et al. Four different regimens of farnesyltransferase inhibitor tipifarnib in older, untreated acute myeloid leukemia patients: north American intergroup phase II study SWOG S0432. Leuk Res. 2014;38(3):329–33.
135. Posch C, Moslehi H, Feeney L, Green GA, Ebaee A, Feichtenschlager V, et al. Combined targeting of MEK and PI3K/mTOR effector pathways is necessary to effectively inhibit NRAS mutant melanoma in vitro and in vivo. Proc Natl Acad Sci U S A. 2013;110(10):4015–20.
136. US National Library of Medicine. ClinicalTrials.gov [online], 2015. https://clinicaltrials.gov/ct2/show/NCT01907815?term=NCT01907815&rank=1.
137. Deshpande AJ, Chen L, Fazio M, Sinha AU, Bernt KM, Banka D, et al. Leukemic transformation by the MLL-AF6 fusion oncogene requires the H3K79 methyltransferase Dot1l. Blood. 2013;121(13):2533–41.
138. Daigle SR, Olhava EJ, Therkelsen CA, Basavapathruni A, Jin L, Boriack-Sjodin PA, et al. Potent inhibition of DOT1L as treatment of MLL-fusion leukemia. Blood. 2013;122(6):1017–25.
139. Daigle SR, Olhava EJ, Therkelsen CA, Majer CR, Sneeringer CJ, Song J, et al. Selective killing of mixed lineage leukemia cells by a potent small-molecule DOT1L inhibitor. Cancer Cell. 2011;20(1):53–65.

140. Stein EM, Tallman MS. Mixed lineage rearranged leukaemia: pathogenesis and targeting DOT1L. Curr Opin Hematol. 2015;22(2):92–6.
141. Lee SC, Dvinge H, Kim E, Cho H, Micol JB, Chung YR, et al. Erratum: modulation of splicing catalysis for therapeutic targeting of leukemia with mutations in genes encoding spliceosomal proteins. Nat Med. 2016;22(6):692.
142. Lee SC, Dvinge H, Kim E, Cho H, Micol JB, Chung YR, et al. Modulation of splicing catalysis for therapeutic targeting of leukemia with mutations in genes encoding spliceosomal proteins. Nat Med. 2016;22(6):672–8.
143. Rose D, Haferlach T, Schnittger S, Perglerová K, Kern W, Haferlach C. Subtype-specific patterns of molecular mutations in acute myeloid leukemia. Leukemia. 2017;31(1):11–7.
144. Ok CY, Patel KP, Garcia-Manero G, Routbort MJ, Fu B, Tang G, et al. Mutational profiling of therapy-related myelodysplastic syndromes and acute myeloid leukemia by next generation sequencing, a comparison with de novo diseases. Leuk Res. 2015;39(3):348–54.
145. Yang L, Rau R, Goodell MA. DNMT3A in haematological malignancies. Nat Rev Cancer. 2015;15(3):152–65.
146. Damm F, Bunke T, Thol F, Markus B, Wagner K, Göhring G, et al. Prognostic implications and molecular associations of NADH dehydrogenase subunit 4 (ND4) mutations in acute myeloid leukemia. Leukemia. 2012;26(2):289–95.

# Chapter 4
# Myelodysplastic Syndrome

**Shaoying Li and C. Cameron Yin**

## Introduction

Myelodysplastic syndromes (MDS) are a group of hematopoietic stem cell neoplasms defined by morphologic dysplasia in at least one lineage of myeloid, erythroid, and megakaryocytic cells and peripheral blood (PB) cytopenia(s), that is, ineffective bone marrow (BM) hematopoiesis, as well as an increased risk of progression to acute myeloid leukemia (AML) [1, 2]. Cytopenias are defined by the International Prognostic Scoring System (IPSS) for risk stratification of MDS as hemoglobin <10 g/dL, absolute neutrophil count (ANC) $<1.8 \times 10^9$/L, and platelets $<100 \times 10^9$/L [1, 3]; morphologic dysplasia needs to be present in at least 10% of each involved lineage and blasts in the BM or PB should be less than 20%.

With the introduction of next-generation sequencing into clinical practice, abundant recurrent somatic mutations have been identified which has advanced our understanding of MDS significantly. Here we discuss the epidemiology, etiology, morphology, immunophenotype, cytogenetic and molecular features, and prognosis of MDS with a focus on the cytogenetic and molecular features and the new 2016 updates of the World Health Organization (WHO) classification.

## Epidemiology, Etiology, and Clinical Features

MDS is a disease of older adults with a male predominance; the median age at diagnosis is 70–75 years. The population-based annual incidence is 3–5/100,000 persons which rises to 13.5 in those aged 65–69 years and to 63.6 in those aged

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≥85 years. The prevalence of MDS is estimated to be 60,000–170,000 in the USA [4].

From an etiology standpoint, MDS can be approached according to a subclassification as de novo MDS, therapy-related MDS, or an MDS of germ line predisposition. De novo MDS has no definitive etiology, but some factors can increase the risk of MDS such as benzene exposure above safe levels, smoking, ionizing radiation, or exposure to certain chemicals or solvents (such as pesticides) [5]. Therapy-related MDS (t-MDS), as self-illustrated by its name, occurs as a late complication of cytotoxic chemotherapy and/or radiation therapy for the treatment of a previous neoplastic or non-neoplastic entity. Other cases of MDS are associated with an underlying inherited disease, such as inherited BM failure syndrome (Fanconi anemia, Shwachman-Diamond syndrome, Diamond-Blackfan syndrome, dyskeratosis congenita) or Down syndrome. In some cases, more recently recognized monogenic inherited disorders (e.g., MDS with *RUNX1* or *GATA2* mutations) are apparent and are familial; these are discussed in a separate chapter [6, 7].

Clinically, most MDS patients present with cytopenia(s)-related symptoms. Anemia is the most frequent cytopenia and patients may be transfusion-dependent. Neutropenia and thrombocytopenia are less frequent. Unlike myeloproliferative neoplasms, splenomegaly is usually not present in patients with MDS.

## Morphology

In the BM, the myeloid, erythroid, and megakaryocytic lineages each have characteristic dysplastic changes (Fig. 4.1, Table 4.1) . Myeloid, erythroid, and megakaryocytic dysplasia are readily apparent on BM aspirate smears, and megakaryocytic dysplasia may also be easily identifiable on the core biopsy. In the PB, granulocytic dysplasia typically has similar features as in marrow specimens; erythrocytes may show a nonspecific macrocytic anemia and increased anisopoikilocytosis; and platelets may be giant in size and hypogranular.

The majority of MDS cases show a hypercellular marrow with or without an increase in blasts depending on the subtype. Abnormal localization of immature precursors (ALIP), scattered myeloid precursors abnormally localized away from trabecular bone, is also a feature seen in MDS. In high-grade MDS, aggregates or small clusters of blasts (>3–5 cells) can be seen by morphology or CD34 immunohistochemistry.

There are several morphologic subtypes of MDS. The 2008 WHO classification of MDS was based on three factors: (1) the blast count in BM and PB, (2) the lineage(s) involved by dysplasia, and (3) the presence or absence of ring sideroblasts. Based on the above three factors, adult MDS was subclassified into six subtypes: (1) refractory anemia with ring sideroblasts (RARS), (2) refractory cytopenia with unilineage dysplasia (RCUD), (3) refractory cytopenia with multilineage dysplasia

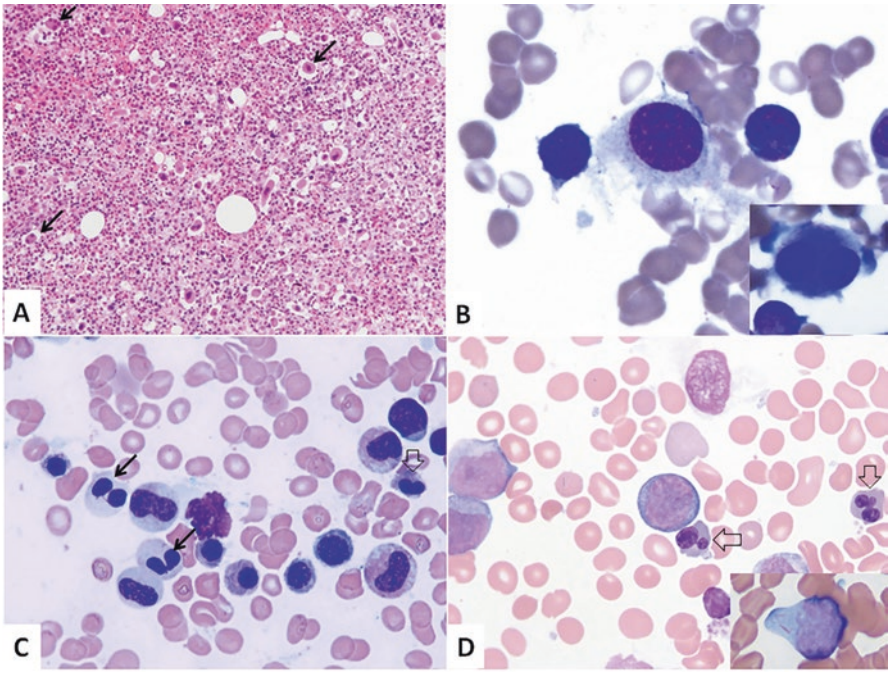


**Fig. 4.1** Morphologic features of MDS. A typical case of MDS shows a hypercellular bone marrow with marked megakaryocytic dysplasia ((**A**) core biopsy and (**B**) aspirate smear), granulocytic dysplasia ((**C**) *arrow*), and erythroid dysplasia ((**C**) *open arrow*). A different case shows marked erythroid dysplasia (*open arrow*) with increased blasts (**D**) and Auer rod in a blast ((**D**) insert)

**Table 4.1** Morphologic features of dysplasia

| | Nuclei | Cytoplasm | Both |
|---|---|---|---|
| Erythroid | Nuclear irregularity | Vacuolization | Megaloblastic changes |
| | Nuclear budding or bridging | Ring sideroblasts | |
| | Nuclear hyperlobation | Periodic acid-Schiff positivity | |
| | Bi-/multinucleation | | |
| | Karyorrhexis | | |
| Myeloid | Nuclear hypolobation (pseudo-Pelger-Huët, pelgeroid) | Hypogranularity/ agranularity | Unusual size |
| | Nuclear Hypersegmentation | Unusual hypergranularity | |
| | | Auer rods | |
| Megakaryocyte | Nuclear hypolobation | | Micromegakaryocytes |
| | Bi-/multinucleation (Nuclear lobe separation) | | |

(RCMD), (4) refractory anemia with excess blasts (RAEB-1 and RAEB-2), (5) MDS with isolated del(5q), and (6) MDS, unclassifiable (MDS-U). However, the dysplasia in a specific lineage did not always correlate with the cytopenic lineage observed [8–10]. As a consequence, the exact lineages of cytopenias have been taken out of the classification in the 2016 revision to the WHO classification which still includes six subtypes though names have changed (Table 4.2). Similar to the 2008 WHO classification, in the 2016 WHO classification, the presence of Auer rods in blasts is a feature of at least an MDS with excess blasts-2 regardless of blast count. No changes have been made to the childhood MDS with refractory cytopenia of childhood as a provisional entity. The PB and BM findings in each subtype, according to the 2016 WHO classification, are summarized in Table 4.2.

Several major changes in the 2016 revision need to be noted:

1. MDS with ring sideroblasts (MDS-RS): this category includes cases of RARS and RCMD with ring sideroblasts (RS) from the 2008 WHO classification. In general ≥15% of RS is needed. However, recent studies showed that *SF3B1* is frequently mutated in these cases and the percentage of RS is prognostically irrelevant [11–13]. Therefore, only ≥5% of RS is needed for cases with *SF3B1* mutation.
2. MDS with isolated del(5q): the 2016 revision allows one additional cytogenetic abnormality except −7 or −7q based on the findings that no adverse prognosis has been observed in such cases [14–16]. In addition, *TP53* mutation analysis is recommended to identify poor prognosis cases within this overall favorable subtype [17].
3. MDS-U: the 1% blasts in PB must be present in at least two separate occasions.
4. MDS with excess blasts (MDS-EB): This includes previous RAEB-1, RAEB-2, and acute erythroid leukemia, erythroid/myeloid subtype. The 2016 revision changed the denominator used to calculate blast percentage to all nucleated cells in BM, which results in cases previously within the "acute erythroid leukemia, erythroid/myeloid subtype" being reclassified into MDS-EB.

There are two special subtypes of MDS:

1. *Hypoplastic MDS*: Although most MDS BM is hypercellular, approximately 10% of MDS cases are hypocellular. Hypoplastic MDS has no prognostic significance but poses a diagnostic challenge as it is difficult to differentiate from aplastic anemia [18]. Careful history, morphology, immunophenotype, and molecular cytogenetic evaluation are needed in such cases.
2. *MDS with myelofibrosis*: Approximately 10% of MDS cases demonstrate significant myelofibrosis [19]. Most of these cases have increased blasts and have a poor prognosis. Immunohistochemical stains for CD34 are critical in such cases, generally due to the poor quality of bone marrow aspirate smears (hemodilution) secondary to fibrosis and therefore resulting in an underestimated blast count.

**Table 4.2** PB and BM findings in myelodysplastic syndromes (MDS)

| Subtype | PB cytopenias[a] | Dysplastic lineages | Blasts | | Auer rods | % (RS/BM erythroid) | |
|---|---|---|---|---|---|---|---|
| | | | PB | BM | | *SF3B1*−/unknown | *SF3B1*+ |
| *MDS with single lineage dysplasia (MDS-SLD)* | 1–2 | 1 | <1% | <5% | No | <15% | <5% |
| *MDS with multilineage dysplasia (MDS-MLD)* | 1–3 | 2–3 | <1% | <5% | No | <15% | <5% |
| *MDS with ring sideroblasts (MDS-RS)* | | | | | | | |
| MDS-RS-SLD | 1–2 | 1 | <1% | <5% | No | ≥15% | ≥5% |
| MDS-RS-MLD | 1–3 | 2–3 | <1% | <5% | No | ≥15% | ≥5% |
| *MDS with isolated del(5q)* | 1–2 | 1–3 | <1% | <5% | No | None/any | None/any |
| *MDS with excess blasts (MDS-EB)* | | | | | | | |
| MDS-EB-1 | 1–3 | 0–3 | 2–4% | 5–9% | No | None/any | None/any |
| MDS-EB-2 | 1–3 | 0–3 | 5–19% | 10–19% | Yes | None/any | None/any |
| *MDS, unclassifiable (MDS-U)*[b] | | | | | | | |
| With 1% blasts in PB | 1–3 | 1–3 | 1% | <5% | No | None/any | None/any |
| With SLD and pancytopenia | 3 | 1 | <1% | <5% | No | None/any | None/any |
| Based on defining cytogenetic abnormality | 1–3 | 0 | <1% | <5% | No | <15% | <15% |
| *Refractory cytopenia of childhood* | 1–3 | 1–3 | <2% | <5% | | None | None |

[a]Cytopenias are defined as hemoglobin <10 g/dL, absolute neutrophil count $<1.8 \times 10^9$/L, and platelets $<100 \times 10^9$/L. Monocytes should be $<1 \times 10^9$/L for all
[b]Include cases not qualified for any other subtypes

## Immunophenotype

Immunophenotyping is a useful ancillary test in the assessment of MDS. CD34 and CD117 immunohistochemistry and multicolor flow cytometry are important ancillary studies. An MDS flow cytometry workup can be very complex and include evaluation of blasts, hematogones, myelomonocytic cells, erythroid cells, and B and T cells. In general, flow cytometry analysis can identify aberrant CD34+ myeloblasts (altered expression intensity, lack of expression of normal markers, aberrant expression of T or B markers) which are relatively specific for BM stem cell neoplasms such as MDS. In addition, evaluation of an aberrant pattern of maturation or antigen expression in myelomonocytic cells, absence of hematogones, and decreased side scatter in granulocytes (reflects hypogranulation by morphology) are all helpful [20–23]. Though not formally used in the diagnosis of MDS, abnormalities observed by flow cytometry are especially useful for morphologic borderline cases and for differentiating dysplasia due to reactive etiologies or posttreatment recovery/regenerating BM from MDS.

## Cytogenetic and Molecular Findings

Cytogenetic studies are important in the diagnosis and risk stratification of MDS. Recent studies have shown that molecular aberrations also have critical impacts on the management of MDS, not only on diagnosis and prognosis but also on monitoring of response to therapy and identification of novel therapeutic targets.

Cytogenetic abnormalities have been identified in approximately 50–60% of de novo MDS and 80% of therapy-related MDS cases. Most of the recurrent clonal cytogenetic abnormalities in MDS are chromosomal gains or losses, such as −7, del(7q), −5, del(5q), and +8. Translocations are less frequent and, if present, are often unbalanced. Several widely accepted prognostic scoring systems, including the IPSS [3] (Table 4.3), WHO classification-based Prognostic Scoring System (WPSS) [24], MD Anderson Cancer Center Prognostic Scoring System (MDAPSS) [25], and the Revised IPSS (IPSS-R, Table 4.4) [26], all use karyotype in addition

**Table 4.3** International Prognostic Scoring System (IPSS) for myelodysplastic syndrome

| Prognostic variable | 0 | 0.5 | 1 | 1.5 | 2 |
|---|---|---|---|---|---|
| Cytopenia | 0–1 | 2–3 | – | – | – |
| Bone marrow blasts % | <5 | 5–10 | – | 11–19 | 20–29[a] |
| Karyotype | Good | Intermediate | Poor | | |

Cytopenia: hemoglobin <10 g/dL, absolute neutrophil count $<1.8 \times 10^9$/L, platelet count $<100 \times 10^9$/L

Karyotype: good: normal, −Y, del(5q), del(20q); intermediate: others; poor: chromosome 7 abnormalities, complex ≥3

*Risk groups*: low (0), intermediate-1 (0.5–1.0), intermediate-2 (1.5–2.0), high (≥2.5)

[a]This group is recognized as acute myeloid leukemia in the WHO classification

**Table 4.4** Revised International Prognostic Scoring System (IPSS-R) for myelodysplastic syndrome

| Prognostic variable | 0 | 0.5 | 1 | 1.5 | 2 | 3 | 4 |
|---|---|---|---|---|---|---|---|
| Karyotype | Very good | – | Good | – | Intermediate | Poor | Very poor |
| Bone marrow blasts % | ≤2 | – | >2 to <5 | – | 5–10 | >10 | – |
| Hemoglobin (g/dL) | ≥10 | – | 8 to <10 | <8 | – | – | – |
| Platelet ($\times 10^9$/L) | ≥100 | 50 to <100 | <50 | – | – | – | – |
| Absolute neutrophil ($\times 10^9$/L) | ≥0.8 | <0.8 | – | – | – | – | – |

Karyotype: very good: −Y, del(11q); good: normal, del(5q), del(12p), del(20q), double including del(5q); intermediate: del(7q), +8, +19, i(17q), any other single or double independent clones; poor: −7, inv(3)/t(3q)/del(3q), double including −7/del(7q), complex with 3 abnormalities; very poor: complex >3 abnormalities
*Risk groups*: very low (≤1.5), low (>1.5–3), intermediate (>3–4.5), high (>4.5–6), very high (>6)

**Table 4.5** Recurrent cytogenetic abnormalities listed in the WHO classification as presumptive evidence for myelodysplastic syndrome

| Type | Cytogenetic abnormalities |
|---|---|
| Unbalanced | −7 or del(7q), −5 or del(5q), i(17q) or t(17p), −13 or del(13q), del(11q), del(12p) or t(12p), del(9q), idic(X)(q13) |
| Balanced | t(11;16)(q23;p13.3), t(3;21)(q26.2;q22.1), t(1;3)(p36;q21.2), t(2;11)(p21;q23), inv(3)(q21q26.2), t(6;9)(p23;q34) |

In the setting of persistent cytopenias of undetermined origin, these abnormalities are considered presumptive evidence of MDS in the absence of definitive morphologic features

to blast percentage and number of cytopenias to stratify MDS patients. The WPSS adds transfusion dependence and WHO morphologic subclassification [24], and the MDAPSS also includes performance status, age, and prior treatment [25]. IPSS-R encompasses five-tiered cytogenetic prognostic subgroups, low BM blast percentage value, and degree of cytopenias and defines five rather than four major prognostic groups, very low, low, intermediate, high, and very high, with median survival ranging from 8.8 to 0.8 years [26] (Table 4.4). MDAPSS and IPSS-R have improved predictive value for survival and leukemic transformation and have shown to be a useful prognostic tool not only in untreated de novo MDS patients but also in secondary MDS and after therapy.

Some clonal cytogenetic aberrations, in the presence of refractory cytopenia without morphologic evidence of dysplasia, are considered presumptive evidence for MDS [2] (Table 4.5) . However, the abnormality must be demonstrated by conventional karyotyping, not by fluorescence in situ hybridization (FISH) or sequencing techniques according to the 2016 revision to the WHO classification [1]. It is recommended that these patients be followed carefully for emerging morphological evidence of MDS. However, −Y, +8, or del(20q) as the sole abnormality is not definitive for a diagnosis of MDS in the absence of morphological evidence of dysplasia.

Whereas classical karyotyping remains the cornerstone in the diagnostic workup of patients with MDS, targeted FISH studies for common chromosomal abnormalities

in MDS may be helpful if the karyotype fails or is insufficient. In addition, FISH provides increased sensitivity in monitoring response to therapy once a recurring abnormality has been identified.

Genome-wide analyses by high-throughput next-generation sequencing (NGS) have identified novel mutations with prognostic and therapeutic value. Recurrent mutations in more than 50 genes have been detected in MDS patients. Approximately 90% of MDS patients carry at least one mutation, with a median of 2–3 mutations detected per patient [27, 28]. Usually few [1–3] driver mutations are identified at initial presentation, with more passenger mutations that increase with disease progression [27, 28]. The most common mutations occur in genes involved in RNA splicing (*SF3B1*, *SRSF2*, *U2AF1*, and *ZRSR2*) [12, 29–31], DNA methylation (*TET2*, *DNMT3A*, *IDH1*, and *IDH2*) [32–37], and chromatin modification (*ASXL1* and *EZH2*) [38–40]. Other molecular abnormalities include genes involved in transcription regulation (*RUNX1*, *TP53*, *CEBPA*, *ETV6*, *BCOR*, and *SETBP1*) [41], signaling transduction pathway (*NRAS*, *KRAS*, *JAK2*, *FLT3*, CNL, and *NF1*) [41], and cohesin complexes (*STAG2* and *RAD21*) (Table 4.6) [41]. The occurrence of these

**Table 4.6** Recurrent gene mutations in myelodysplastic syndrome

| Gene | Function | Frequency (%) | Prognostic impact | Therapeutic application |
|---|---|---|---|---|
| *RNA splicing* | | | | |
| *SF3B1* | Spliceosome assembly | 15–30 (90 in RARS) | Favorable | None |
| *SRSF2* | Spliceosome assembly | 10–20 | Unfavorable | None |
| *U2AF1* | Spliceosome assembly | 5–10 | Unfavorable | None |
| *ZRSF2* | Spliceosome assembly | 5–10 | None | None |
| *DNA methylation* | | | | |
| *TET2* | Convert 5-mC to 5-hmC<br>Required for myelopoiesis | 20–30 | Unclear | DNA methyltransferase inhibitors |
| *DNMT3A* | DNA methyltransferase<br>Histone methylation<br>Transcription repression | 10 | Unfavorable | DNA methyltransferase inhibitors |
| *IDH1/2* | Convert isocitrate to α-KG<br>Regulate TET2 | 5 | Unfavorable | IDH1/2 inhibitors<br>DNA methyltransferase inhibitors |
| *Histone modification* | | | | |
| *ASXL1* | Histone methylation<br>Transcription repression | 10–20 | Unfavorable | HDAC inhibitors |
| *EZH2* | Histone methyltransferase<br>Transcription repression | 5–10 | Unfavorable | EZH2 inhibitors<br>HDAC inhibitors |
| *Transcription factor* | | | | |
| *RUNX1* | Regulate hematopoiesis | 10 | Unfavorable | None |
| *TP53* | Tumor suppressor, regulate cell cycle, apoptosis, DNA repair | 5–10 | Very unfavorable | None |

(continued)

**Table 4.6** (continued)

| Gene | Function | Frequency (%) | Prognostic impact | Therapeutic application |
|---|---|---|---|---|
| *CEBPA* | Regulate myelopoiesis | <5 | Unclear | None |
| *ETV6* | ETS transcription factor Required for hematopoiesis | <5 | Unfavorable | None |
| *BCOR* | BCL6 repressor | <5 | Unfavorable | None |
| *SETBP1* | DNA replication Cell division | <5 | Unfavorable | None |
| *Signal transduction* | | | | |
| *NRAS* | GTPase, oncogenic | 5 | Unfavorable | Farnesyl transferase inhibitors |
| *KRAS* | GTPase, oncogenic | <5 | Unfavorable | Farnesyl transferase inhibitors |
| *JAK2* | Tyrosine kinase activation | 5 (50 in RARS-t) | None | JAK2 inhibitor |
| *FLT3* | Tyrosine kinase activation | <5 | Unfavorable | FLT3 inhibitors |
| *CBL* | E3 ubiquitin ligase Regulate hematopoiesis | <5 | Unfavorable | None |
| *NF1* | Regulate hematopoiesis | <5 | Unfavorable | None |
| *Cohesin complex* | | | | |
| *STAG2* | Regulate separation of sister chromatids during cell division | 5–10 | Unfavorable | None |
| *RAD21* | Regulate separation of sister chromatids during cell division | <5 | None | None |

recurrent gene mutations and the fact that mutations within the same functional pathways often are mutually exclusive, whereas mutations from different functional pathways often occur together, underscore the importance of these pathways in MDS pathogenesis.

Somatic mutations in genes encoding core spliceosomal proteins and accessory regulatory splicing factors have been described in 45–80% of MDS cases [12, 29–31]. Interestingly, nearly all mutations occur as heterozygous missense rather than nonsense or frameshift mutations, suggesting that the mutations may confer an alteration of function, such as dominant negative activity, affecting RNA splicing [12, 31]. *SF3B1* mutations are most frequent and exhibit strong correlation with the presence of ring sideroblasts, seen in >90% of RARS and ~70% of RCMD-RS (now both collectively known as MDS-RS) and RARS associated with marked thrombocytosis (RARS-T; now known as MDS/MPN with ring sideroblasts and thrombocytosis) [12, 29, 31]. This is because mutant SF3B1 downregulates genes essential in the mitochondrial pathway, thus leading to mitochondrial iron overload and ineffective erythropoiesis [42]. *SF3B1* mutations are associated with a favorable prognosis and prolonged survival [30]. In contrast, mutations in *SRSF2* and *U2AF1* appear to

be enriched in more aggressive subtypes of MDS such as RAEBI and RAEBII [43]. *SRSF2* mutations have also been associated with *TET2* mutations and monocytosis [44].

Epigenetic silencing by promoter hypermethylation of several genes, including DNA repair genes, cell-cycle regulators, and apoptotic genes, represents one of the most important mechanisms underlying MDS pathogenesis. *TET2* is the most commonly mutated epigenetic regulator gene in MDS, seen in 20–30% MDS patients [34]. *TET2* mutations have been associated with favorable responses to hypomethylating agents [45]; however, their prognostic relevance in MDS remains unclear [46]. *DNMT3A* mutations occur in ~10% of MDS patients and correlate with unfavorable overall survival and a high risk of leukemic transformation [37]. A variety of *DNMT3A* mutations has been reported, with R882H as the most frequent [37]. Mutations affecting the isocitrate dehydrogenase genes, *IDH1* and *IDH2*, are reported in ~5% of MDS patients [35, 36]. *IDH1* and *IDH2* mutations are heterozygous and occur mostly at residues R132 in *IDH1* and R140 or R172 in *IDH2* [35, 36]. Mutant IDH1 and IDH2 proteins convert alpha-ketoglutarate (α-KG) to 2-hydroxyglutarate (2-HG), leading to epigenetic dysregulation of genes involved in leukemogenesis [47]. *IDH1* and *IDH2* mutations in MDS predict inferior overall survival [36]. ASXL1 regulates histone modification. *ASXL1* mutations (mostly in exon 12) occur in 10–20% of MDS cases and demonstrate a positive correlation with secondary leukemia and inferior overall survival [48]. EZH2, a histone-lysine N-methyltransferase, catalyzes the methylation of histone H3. *EZH2* mutations are observed in 5–10% of MDS cases and are associated with worse survival [49].

Understanding the molecular landscape of MDS has several clinical implications. Firstly, genetic testing at the time of initial evaluation can aid in establishing a diagnosis. In cases that meet morphologic criteria of MDS, typical somatic mutations strongly support the diagnosis. On the other hand, in patients with low-grade or borderline MDS and diploid karyotype, or patients with unexplained cytopenias but not meeting diagnostic criteria for MDS (referred to as "idiopathic cytopenia of undetermined significance," or "ICUS"), the identification of a mutation in an MDS-related gene may be a useful diagnostic adjunct. However, in patients with ICUS who bear mutations in MDS-related genes indicative of clonal hematopoiesis (referred to as "clonal cytopenia of undetermined significance", or "CCUS"), gene mutation data must be interpreted with caution. It is well recognized that mutations in MDS-related genes may be present in individuals who are either healthy or have minor cytopenias without evidence of MDS (referred to as "clonal hematopoiesis of indeterminate potential," or "CHIP" ), and the frequency increases with age, being very rare in people <40 years old, but reaches ~10% in people aged 70–80 [50]. The most commonly mutated genes include *DNMT3A*, *TET2*, *ASXL1*, *JAK2*, *TP53*, *SF3B1*, *SRSF2*, and *CBL*. Whereas the vast majority of individuals with CHIP never develop a hematologic neoplasm, they do have a 10–15-fold increased risk of developing a hematologic neoplasm, with an estimated risk of 0.5–1% per year. The high frequency of CHIP in the elderly population limits the diagnostic value of somatic mutations. Nevertheless, certain mutations, when used in an appropriate clinical setting, have been shown to be helpful diagnostic adjuncts. For example, an isolated

mutation in *DNMT3A*, *TET2*, or *ASXL1* in an elderly person who lacks cytopenias and morphologic evidence of dysplasia likely cannot be considered diagnostically helpful, but mutations in some of the less frequently mutated CHIP genes, especially when present more than one and at high allele frequencies, in younger patients with cytopenias, may raise the likelihood of MDS, and the absence of mutations in a sufficiently broad gene panel in patients with mild cytopenia and minimal dysplasia may render a diagnosis of MDS less likely.

Secondly, some gene mutations have shown correlations with clinicopathologic features and may be used in the subclassification of MDS. As mentioned above, *SF3B1* mutations are associated with MDS-RS (RARS and RCMD-RS) [12], whereas *SRSF2* and *TET2* mutations are associated with monocytosis [44]. Moreover, haploinsufficiency of the ribosomal protein gene *RPS14* has been correlated with the characteristic megakaryocyte morphology, anemia, response to lenalidomide, and favorable prognosis in patients with 5q− syndrome [51].

Thirdly, molecular aberrations will supplement current prognostic models in refining risk stratification and impacting treatment decisions. *SF3B1* mutations have been shown to predict a favorable prognosis [29]. In contrast, most of the MDS-related mutations have been associated with poor prognosis, with good reproducibility for at least five genes: *TP53*, *EZH2*, *ETV6*, *RUNX1*, and *ASXL1* [52]. Other common mutations that have been associated with poor clinical outcome include *DNMT3A* [37], *IDH1/IDH2* [36], *SRSF2* [30], and *FLT3* [53]. Additionally, an inverse correlation exists between the number of mutations and survival [50].

Next, periodic evaluation of mutation status of genes that have been found to be mutated in the initial diagnostic workup will aid in assessment of response to therapy and monitoring of minimal residual disease (MRD). MRD analysis allows for early detection of impending hematologic relapse and timely therapeutic intervention and therefore has significantly improved clinical outcome in hematopoietic neoplasms. Assessment of MRD requires molecular technologies with sufficient sensitivity to detect mutations with low allele burdens. Quantitative allele-specific PCR-based methods have been developed for single-nucleotide variants and small insertions in genes such as *DNMT3A*, *IDH1/IDH2*, *NPM1*, and *FLT3* and can detect the presence of a mutation as low as 0.01–0.001% of the template DNA [54]. However, such methods are limited by primer design and further complicated by clonal evolution. NGS-based assays have the potential to increase detection sensitivity (~1%), detect several mutations in a multiplex assay, and identify emerging clonal evolution. *NPM1* has been regarded as the most attractive molecular marker for monitoring MRD in acute myeloid leukemia due to its stability [55]; however, *NPM1* is only rarely mutated in MDS. On the contrary, *FLT3* internal tandem duplication (ITD) is generally regarded as a relatively unstable marker but may serve as a useful marker if used with caution [55]. The roles of other gene mutations in MRD monitoring await further exploration.

Finally, insight into the molecular pathogenesis of MDS has led to the development of novel therapeutic agents [56]. *DNMT3A* mutations may serve as specific biomarkers of positive response to DNA methyltransferase inhibitors. Farnesyl transferase inhibitors, such as tipifarnib and lonafarnib, represent a class of potent

inhibitors of RAS activation and are able to modulate multiple signaling pathways implicated in the pathogenesis of MDS. *TET2* mutations may predict a more favorable response to hypomethylating agents. Other examples include FLT3 inhibitors in *FLT3*-ITD-positive cases and IDH1/IDH2 inhibitors in *IDH1/IDH2*-mutated cases. With the rapid advances in molecular biology and drug development, it is hoped that more and more of these mutations may soon become targetable.

## Diagnosis and Prognosis

The diagnosis of MDS is mainly based on the following three criteria:

1. Persistent (usually >6 months) and significant PB cytopenia(s) (numbers defined at the introduction)
2. Significant morphologic dysplasia in one or more lineages seen in more than 10% of cells in each involved lineage in BM and/or blasts ≥5%
3. Cytogenetic abnormality, especially MDS-defining cytogenetic abnormality (Table 4.5) in the absence of morphologic dysplasia

A few challenges are present for MDS diagnosis. First and also the biggest challenge is that none of the above three categories is specific for MDS. Both cytopenias and morphologic dysplasia can be seen either in healthy individuals or in some reactive conditions such as nutritional deficiency (vitamin B12, folic, iron, and copper), zinc excess, alcoholism, infection, autoimmune disease, therapy with medication and/or radiation including growth factors, etc. [57–59]. The second challenge is the lack of reproducibility of 10% threshold of morphologic dysplasia even in experienced hematopathologists [59–62]. Last but not least, some congenital hematopoietic disorders and paroxysmal nocturnal hemoglobinuria (PNH) can demonstrate morphologic dysplasia. As such, a thorough history and physical examination and laboratory workup to exclude the above conditions are recommended before rendering a diagnosis of MDS.

In addition, cytogenetic, gene mutation and flow cytometry analyses are important ancillary studies which can aid in the diagnosis of MDS, especially in borderline cases to differentiate between MDS and reactive dysplasia and in posttreatment or posttransplant settings to differentiate between residual disease and recovery/regenerative process. However, these ancillary results need to be interpreted with caution and to correlate with clinical and morphologic findings, since none of the abovementioned aberrations is specific for a diagnosis of MDS. Patients with persistent cytopenias without morphologic dysplasia and MDS-defining cytogenetic abnormality (ICUS) should be followed up closely with complete blood cell count and cytogenetic monitoring.

The prognosis of MDS patients is variable with a median overall survival ranging from 5 years to less than 6 months [26]. Up to 30% of MDS cases progress to AML which usually have a poor clinical outcome. The prognosis of MDS depends on a lot of factors. Morphologically, MDS with isolated del(5q) without *TP53* mutation has

the best prognosis, while RAEB-2 has the worst prognosis. Several scoring systems (IPSS, WPSS, MDAPSS, and IPSS-R) that incorporate the BM blast count, degree of cytopenias, and cytogenetic abnormalities have been developed for MDS risk stratification and have been widely adopted (Table 4.3 and 4.4). More and more recurrent somatic mutations have been identified in MDS, and some of them also have a prognostic value as described in detail in the "Cytogenetic and Molecular Findings" section and in Table 4.5 and 4.6.

## Summary

In summary, MDS is a hematopoietic stem cell neoplasm predominantly affecting the elderly. A growing understanding of MDS-related somatic gene mutations and karyotypic abnormalities identified by NGS and other technologies has significantly shaped our understanding of the diagnosis, prognosis, and novel targeted therapies used in MDS. Despite the rapid advances in identifying MDS-related genes, the influence of these gene mutations on prognosis has not yet been taken into consideration in the current risk stratification schemes. While there is still a role for single gene testing in some clinical settings, multiplex testing of multiple genes using NGS technologies, standardization of consensus MDS gene panels, as well as optimization of ways to incorporate mutation data into revised prognostic schemes await to be defined by expert panels.

## References

1. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–405.
2. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW. WHO classification of tumours of haematopoietic and lymphoid tissues. 4th ed. Lyon: IARC; 2008.
3. Greenberg P, Cox C, LeBeau MM, et al. International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood. 1997;89(6):2079–88.
4. Cogle CR. Incidence and burden of the myelodysplastic syndromes. Curr Hematol Malig Rep. 2015;10(3):272–81.
5. Strom SS, Gu Y, Gruschkus SK, Pierce SA, Estey EH. Risk factors of myelodysplastic syndromes: a case-control study. Leukemia. 2005;19(11):1912–8.
6. Wong TN, Ramsingh G, Young AL, et al. Role of TP53 mutations in the origin and evolution of therapy-related acute myeloid leukaemia. Nature. 2015;518(7540):552–5.
7. West AH, Godley LA, Churpek JE. Familial myelodysplastic syndrome/acute leukemia syndromes: a review and utility for translational investigations. Ann N Y Acad Sci. 2014;1310:111–8.
8. Germing U, Strupp C, Giagounidis A, et al. Evaluation of dysplasia through detailed cytomorphology in 3156 patients from the Dusseldorf Registry on myelodysplastic syndromes. Leuk Res. 2012;36(6):727–34.
9. Maassen A, Strupp C, Giagounidis A, et al. Validation and proposals for a refinement of the WHO 2008 classification of myelodysplastic syndromes without excess of blasts. Leuk Res. 2013;37(1):64–70.

10. Verburgh E, Achten R, Louw VJ, et al. A new disease categorization of low-grade myelodysplastic syndromes based on the expression of cytopenia and dysplasia in one versus more than one lineage improves on the WHO classification. Leukemia. 2007;21(4):668–77.
11. Malcovati L, Karimi M, Papaemmanuil E, et al. SF3B1 mutation identifies a distinct subset of myelodysplastic syndrome with ring sideroblasts. Blood. 2015;126(2):233–41.
12. Papaemmanuil E, Cazzola M, Boultwood J, et al. Somatic SF3B1 mutation in myelodysplasia with ring sideroblasts. N Engl J Med. 2011;365(15):1384–95.
13. Patnaik MM, Hanson CA, Sulai NH, et al. Prognostic irrelevance of ring sideroblast percentage in World Health Organization-defined myelodysplastic syndromes without excess blasts. Blood. 2012;119(24):5674–7.
14. Germing U, Lauseker M, Hildebrandt B, et al. Survival, prognostic factors and rates of leukemic transformation in 381 untreated patients with MDS and del(5q): a multicenter study. Leukemia. 2012;26(6):1286–92.
15. Mallo M, Cervera J, Schanz J, et al. Impact of adjunct cytogenetic abnormalities for prognostic stratification in patients with myelodysplastic syndrome and deletion 5q. Leukemia. 2011;25(1):110–20.
16. Schanz J, Tuchler H, Sole F, et al. New comprehensive cytogenetic scoring system for primary myelodysplastic syndromes (MDS) and oligoblastic acute myeloid leukemia after MDS derived from an international database merge. J Clin Oncol. 2012;30(8):820–9.
17. Saft L, Karimi M, Ghaderi M, et al. p53 protein expression independently predicts outcome in patients with lower-risk myelodysplastic syndromes with del(5q). Haematologica. 2014;99(6):1041–9.
18. Orazi A, Albitar M, Heerema NA, Haskins S, Neiman RS. Hypoplastic myelodysplastic syndromes can be distinguished from acquired aplastic anemia by CD34 and PCNA immunostaining of bone marrow biopsy specimens. Am J Clin Pathol. 1997;107(3):268–74.
19. Lambertenghi-Deliliers G, Orazi A, Luksch R, Annaloro C, Soligo D. Myelodysplastic syndrome with increased marrow fibrosis: a distinct clinico-pathological entity. Br J Haematol. 1991;78(2):161–6.
20. Kern W, Haferlach C, Schnittger S, Alpermann T, Haferlach T. Serial assessment of suspected myelodysplastic syndromes: significance of flow cytometric findings validated by cytomorphology, cytogenetics, and molecular genetics. Haematologica. 2013;98(2):201–7.
21. Malcovati L, Hellstrom-Lindberg E, Bowen D, et al. Diagnosis and treatment of primary myelodysplastic syndromes in adults: recommendations from the European LeukemiaNet. Blood. 2013;122(17):2943–64.
22. Porwit A, van de Loosdrecht AA, Bettelheim P, et al. Revisiting guidelines for integration of flow cytometry results in the WHO classification of myelodysplastic syndromes-proposal from the International/European LeukemiaNet Working Group for Flow Cytometry in MDS. Leukemia. 2014;28(9):1793–8.
23. Tang G, Jorgensen LJ, Zhou Y, et al. Multi-color CD34(+) progenitor-focused flow cytometric assay in evaluation of myelodysplastic syndromes in patients with post cancer therapy cytopenia. Leuk Res. 2012;36(8):974–81.
24. Malcovati L, Germing U, Kuendgen A, et al. Time-dependent prognostic scoring system for predicting survival and leukemic evolution in myelodysplastic syndromes. J Clin Oncol. 2007;25(23):3503–10.
25. Kantarjian H, O'Brien S, Ravandi F, et al. Proposal for a new risk model in myelodysplastic syndrome that accounts for events not considered in the original International Prognostic Scoring System. Cancer. 2008;113(6):1351–61.
26. Greenberg PL, Tuechler H, Schanz J, et al. Revised international prognostic scoring system for myelodysplastic syndromes. Blood. 2012;120(12):2454–65.
27. Bejar R, Steensma DP. Recent developments in myelodysplastic syndromes. Blood. 2014;124(18):2793–803.
28. Papaemmanuil E, Gerstung M, Malcovati L, et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. Blood. 2013;122(22):3616–27; quiz 3699.

29. Malcovati L, Papaemmanuil E, Bowen DT, et al. Clinical significance of SF3B1 mutations in myelodysplastic syndromes and myelodysplastic/myeloproliferative neoplasms. Blood. 2011;118(24):6239–46.
30. Thol F, Kade S, Schlarmann C, et al. Frequency and prognostic impact of mutations in SRSF2, U2AF1, and ZRSR2 in patients with myelodysplastic syndromes. Blood. 2012;119(15):3578–84.
31. Yoshida K, Sanada M, Shiraishi Y, et al. Frequent pathway mutations of splicing machinery in myelodysplasia. Nature. 2011;478(7367):64–9.
32. Delhommeau F, Dupont S, Valle VD, et al. Mutation in TET2 in myeloid cancers. N Engl J Med. 2009;360(22):2289–301.
33. Jankowska AM, Szpurka H, Tiu RV, et al. Loss of heterozygosity 4q24 and TET2 mutations associated with myelodysplastic/myeloproliferative neoplasms. Blood. 2009;113(25):6403–10.
34. Langemeijer SM, Kuiper RP, Berends M, et al. Acquired mutations in TET2 are common in myelodysplastic syndromes. Nat Genet. 2009;41(7):838–42.
35. Marcucci G, Maharry K, Wu YZ, et al. IDH1 and IDH2 gene mutations identify novel molecular subsets within de novo cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B study. J Clin Oncol. 2010;28(14):2348–55.
36. Patnaik MM, Hanson CA, Hodnefield JM, et al. Differential prognostic effect of IDH1 versus IDH2 mutations in myelodysplastic syndromes: a Mayo Clinic study of 277 patients. Leukemia. 2012;26(1):101–5.
37. Walter MJ, Ding L, Shen D, et al. Recurrent DNMT3A mutations in patients with myelodysplastic syndromes. Leukemia. 2011;25(7):1153–8.
38. Boultwood J, Perry J, Pellagatti A, et al. Frequent mutation of the polycomb-associated gene ASXL1 in the myelodysplastic syndromes and in acute myeloid leukemia. Leukemia. 2010;24(5):1062–5.
39. Gelsi-Boyer V, Trouplin V, Adelaide J, et al. Mutations of polycomb-associated gene ASXL1 in myelodysplastic syndromes and chronic myelomonocytic leukaemia. Br J Haematol. 2009;145(6):788–800.
40. Nikoloski G, Langemeijer SM, Kuiper RP, et al. Somatic mutations of the histone methyltransferase gene EZH2 in myelodysplastic syndromes. Nat Genet. 2010;42(8):665–7.
41. Pellagatti A, Boultwood J. The molecular pathogenesis of the myelodysplastic syndromes. Eur J Haematol. 2015;95(1):3–15.
42. Visconte V, Rogers HJ, Singh J, et al. SF3B1 haploinsufficiency leads to formation of ring sideroblasts in myelodysplastic syndromes. Blood. 2012;120(16):3173–86.
43. Damm F, Kosmider O, Gelsi-Boyer V, et al. Mutations affecting mRNA splicing define distinct clinical phenotypes and correlate with patient outcome in myelodysplastic syndromes. Blood. 2012;119(14):3211–8.
44. Malcovati L, Papaemmanuil E, Ambaglio I, et al. Driver somatic mutations identify distinct disease entities within myeloid neoplasms with myelodysplasia. Blood. 2014;124(9):1513–21.
45. Bejar R, Lord A, Stevenson K, et al. TET2 mutations predict response to hypomethylating agents in myelodysplastic syndrome patients. Blood. 2014;124(17):2705–12.
46. Smith AE, Mohamedali AM, Kulasekararaj A, et al. Next-generation sequencing of the TET2 gene in 355 MDS and CMML patients reveals low-abundance mutant clones with early origins, but indicates no definite prognostic value. Blood. 2010;116(19):3923–32.
47. Gross S, Cairns RA, Minden MD, et al. Cancer-associated metabolite 2-hydroxyglutarate accumulates in acute myelogenous leukemia with isocitrate dehydrogenase 1 and 2 mutations. J Exp Med. 2010;207(2):339–44.
48. Thol F, Friesen I, Damm F, et al. Prognostic significance of ASXL1 mutations in patients with myelodysplastic syndromes. J Clin Oncol. 2011;29(18):2499–506.
49. Bejar R, Stevenson KE, Caughey BA, et al. Validation of a prognostic model and the impact of mutations in patients with lower-risk myelodysplastic syndromes. J Clin Oncol. 2012;30(27):3376–82.
50. Steensma DP, Bejar R, Jaiswal S, et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. Blood. 2015;126(1):9–16.

51. Boultwood J, Pellagatti A, McKenzie AN, Wainscoat JS. Advances in the 5q- syndrome. Blood. 2010;116(26):5803–11.
52. Bejar R, Stevenson K, Abdel-Wahab O, et al. Clinical effect of point mutations in myelodysplastic syndromes. N Engl J Med. 2011;364(26):2496–506.
53. Shih LY, Huang CF, Wang PN, et al. Acquisition of FLT3 or N-ras mutations is frequently associated with progression of myelodysplastic syndrome to acute myeloid leukemia. Leukemia. 2004;18(3):466–75.
54. van der Velden VH, Hochhaus A, Cazzaniga G, Szczepanski T, Gabert J, van Dongen JJ. Detection of minimal residual disease in hematologic malignancies by real-time quantitative PCR: principles, approaches, and laboratory aspects. Leukemia. 2003;17(6):1013–34.
55. Palmisano M, Grafone T, Ottaviani E, Testoni N, Baccarani M, Martinelli G. NPM1 mutations are more stable than FLT3 mutations during the course of disease in patients with acute myeloid leukemia. Haematologica. 2007;92(9):1268–9.
56. Garcia-Manero G. Myelodysplastic syndromes: 2015 Update on diagnosis, risk-stratification and management. Am J Hematol. 2015;90(9):831–41.
57. Halfdanarson TR, Kumar N, Li CY, Phyliky RL, Hogan WJ. Hematological manifestations of copper deficiency: a retrospective review. Eur J Haematol. 2008;80(6):523–31.
58. Kim M, Lee SE, Park J, et al. Vitamin B(12)-responsive pancytopenia mimicking myelodysplastic syndrome. Acta Haematol. 2011;125(4):198–201.
59. Parmentier S, Schetelig J, Lorenz K, et al. Assessment of dysplastic hematopoiesis: lessons from healthy bone marrow donors. Haematologica. 2012;97(5):723–30.
60. Della Porta MG, Travaglino E, Boveri E, et al. Minimal morphological criteria for defining bone marrow dysplasia: a basis for clinical implementation of WHO classification of myelodysplastic syndromes. Leukemia. 2015;29(1):66–75.
61. Font P, Loscertales J, Benavente C, et al. Inter-observer variance with the diagnosis of myelodysplastic syndromes (MDS) following the 2008 WHO classification. Ann Hematol. 2013;92(1):19–24.
62. Senent L, Arenillas L, Luno E, Ruiz JC, Sanz G, Florensa L. Reproducibility of the World Health Organization 2008 criteria for myelodysplastic syndromes. Haematologica. 2013;98(4):568–75.

# Chapter 5
# Chronic Myeloid Leukemia, *BCR-ABL1* Positive

**Ren Ching Wang and Chung-Che (Jeff) Chang**

## Introduction

Chronic myeloid leukemia (CML) is a clonal proliferative disorder of abnormal pluripotent bone marrow stem cells. It is consistently associated with a reciprocal translocation between the long arms of chromosomes 9 and 22, t(9;22)(q34;q11), also named the Philadelphia (Ph) chromosome, which fuses the breakpoint cluster (*BCR*) gene to the Abelson murine leukemia viral oncogene homolog 1 (*ABL1*)gene producing the *BCR-ABL1* fusion. This fusion provides a unique biomarker for diagnosis as well as for monitoring residual disease during treatment. The fusion protein plays an important role not only in the pathogenesis of CML, by uncontrolled active tyrosine kinase activity, but also is an ideal target for therapy. Before the introduction of targeted therapy with tyrosine kinase inhibitors (TKIs), the median survival was 5–7 years. Now, in the current era of target TKI therapies, CML has no longer a universally fatal disease and instead a chronic medically manageable disorder with a near normal lifespan with appropriate treatment.

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### *Epidemiology*

Although CML can occur at any age, it is still a disease of the elderly. The median age at diagnosis is in the fifth and sixth decades of life. The incidence of CML has been stable over the years, at 0.7–1.0 cases per 100,000 people per year, and a slight male predominance with male/female ratio of 1.2–1.7. With the dramatic improvement in survival of these patients, the prevalence is not well known but due to increased survival, has been estimated to be increasing, now at 10–12 cases per 100,000 people [1].

### *Etiology*

While it is understood that the *BCR-ABL1* translocation is essential and necessary for the pathogenesis of CML, its cause is most frequently unclear. Radiation exposure is an established risk factor [2], and smoking is also believed to increase the risk of disease [3]. No known genetic predispositions are currently known.

### *Clinical Features*

CML is a triphasic disease with an initial chronic phase (CP), followed by an accelerated phase (AP) and subsequently blast phase (BP). Most patients are diagnosed in the chronic phase and common clinical symptoms include fever, fatigue, night sweats, malaise, body weight loss, and left upper quadrant pain. Splenomegaly is seen in 30–50% of patients with occasional hepatomegaly. However, 20–40% of patients may be asymptomatic and are incidentally identified with a leukocytosis during studies for other purposes. Before the availability of TKI treatment, the rate of CP-CML progression to BP-CML was 5% in the first year and 20–25% in each year thereafter [4, 5]. Following the introduction of TKI treatment, for those patients who have achieved major molecular remission, there is virtually no progress to AP and BP [6].

## Morphology and Immunophenotyping

In the chronic phase, the peripheral blood cell count usually shows a prominent leukocytosis due to markedly increased neutrophils and left-shifted myeloid cell; so-called myelocyte bulge is frequently seen. Additionally, basophilia is common and relatively specific for differentiating CML from a leukemoid reaction; basophilia is also less frequent in other *BCR-ABL1*-negative myeloproliferative

neoplasms. Due to a generalized increased of leukocytes, an absolute monocytosis may be noted; however, the percentage of monocytes is typically less than 3%, and blasts are usually less than 2%. Laboratory data frequently shows low leukocyte alkaline phosphatase (LAP) score.

The bone marrow is hypercellular and nearly devoid of adipose tissue due to a striking proliferation of the granulocytic lineage. An increased thickness of paratrabecular immature myeloid precursors is a common finding, from normally two to three layers of immature precursors to more than five layers in CML. Dysplasia in the granulocytic or erythroid lineage should be minimal to absent. The myeloid to erythroid ratio is markedly increased, frequently greater than 10:1. The morphologic changes of megakaryocytes are a hallmark feature of CML, known as "micromegakaryocytes" or "dwarf megakaryocytes," small megakaryocytes with hypolobated nuclei (Fig. 5.1). Mild to moderate reticulin fibrosis may be also noted in the initial stage of CML (Fig. 5.2). Occasionally, micromegakaryocytes may

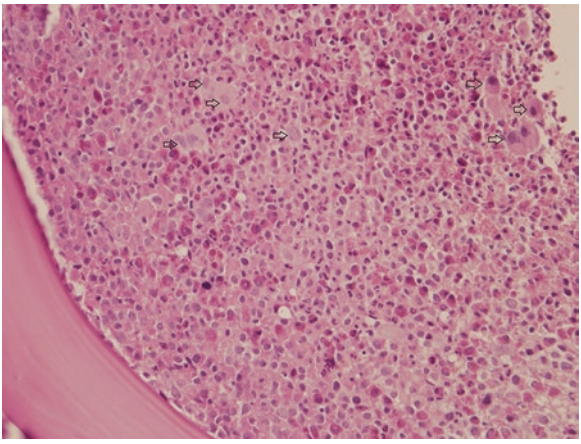

**Fig. 5.1** 400× HE. Hypercellular marrow with nearly no fat content with markedly increased myeloid to erythroid ratio, over 10:1. *Arrows* highlights the micromegakaryocytes, a characteristic finding of chronic myeloid leukemia. Sometimes, increased eosinophils and eosinophilic precursors may be observed

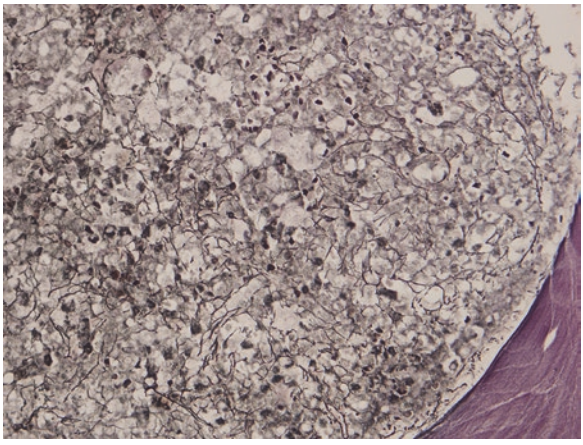

**Fig. 5.2** 400× reticulin stain. Diffuse mild reticulin fibrosis in bone marrow biopsy of CML

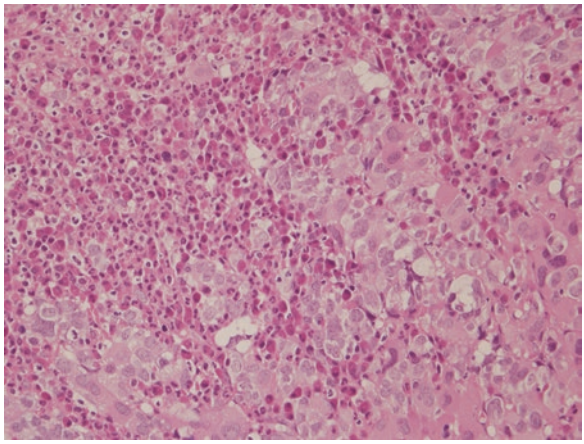

**Fig. 5.3** 200× HE. Overt proliferation of micromegakaryocytes in patient with CML. The proliferated micromegakaryocytes show large sheet-like pattern in the lower part of the image

show a striking proliferation with a sheet-like pattern (Fig. 5.3). Marrow histiocytes may resemble Gaucher cells or so-called "sea-blue" histiocytes due to phagocytosis of excess phospholipids released from the granulocytic proliferation and high cellular turnover. The proportion of blasts in chronic phase of CML is usually less than 5% of all nucleated cells.

In the AP, the proportion of blasts are increased but do not exceed 20% or 30% of cells in the bone marrow and/or peripheral blood based on criteria outlined by different organization. Table 5.1 summarizes the detailed criteria for defining AP and BP by the WHO [7, 8], MD Anderson Cancer Center (MDACC), and European Leukemia Net criteria [9]. In addition to hematologic, morphologic, and cytogenetic parameters, the recently published 2016 revision of WHO classification of hematopoietic neoplasms [8] added provisional criteria of AP-CML based on the treatment response to TKIs (Table 5.1). Additionally, because the onset of lymphoid BP may be quite sudden, the detection of any bona fide lymphoblasts in the blood or marrow should raise concern for a possible impending lymphoid BP and prompt additional laboratory and genetic studies to exclude this possibility.

The criteria for BP isn't changed in WHO 2016, and BP may be diagnosed when (1) blasts are equal to or greater than 20% of cells in the bone marrow or peripheral blood or/and (2) there is an extramedullary proliferation of myeloblasts or lymphoblasts. Common sites for extramedullary proliferations include the skin, lymph node, bone, and central nervous system. Additionally, the accumulation of blasts in the core biopsy, even if only in a single intertrabecular area filled with blasts in the background of chronic phase, warrants the diagnosis of blast phase. European LeukemiaNet and MDACC are similar, but the blast cutoff is higher at ≥30% blasts in the peripheral blood or bone marrow and/or an extramedullary blast proliferation outside of the spleen.

**Table 5.1** Criteria for accelerated phase and blast phase of CML by various organizations

| **Accelerated phase** | **WHO 2016 criteria** | **European LeukemiaNet criteria** | **MDACC criteria** |
|---|---|---|---|
| Blasts in PB and/or BM | 10–19% | 15–29%;<br>or (myeloblasts plus promyelocytes) ≧30% | 15–29%;<br>or (myeloblasts plus promyelocytes) ≧30% |
| Peripheral basophilia | ≧20% of total WBC | ≧20% of total WBC | ≧20% of total WBC |
| Platelet count | <100 × $10^9$/L unrelated to therapy; or >1000 × $10^9$/L, unresponsive to therapy | ≦100 × $10^9$/L, unrelated to therapy | ≦100 × $10^9$/L unrelated to therapy |
| White cell count or splenomegaly | Persistent leukocytosis (>10 × $10^9$/L) and/or persistent or increasing splenomegaly, unresponsive to therapy | – | – |
| Cytogenetic finding | 1. Additional clonal chromosomal abnormalities in Ph+ cells at diagnosis[a]<br>2. Any new clonal chromosomal abnormality in Ph+ cells occurring during therapy | Clonal evolution (CCA/Ph+, major route, on treatment)[a] | Clonal evolution |
| "Provisional" criteria based on response-to-TKI treatment | 1. Hematologic resistance to the first TKI (or failure to achieve a complete hematologic response[b] to the first TKI) or<br>2. Any hematological, cytogenetic, or molecular indications of resistance to 2 sequential TKIs or<br>3. Occurrence of 2 or more mutations in BCR-ABL1 during TKI therapy | – | – |
| **Blast phase** | **WHO 2016 criteria** | **European LeukemiaNet criteria** | **MDACC/International Bone Marrow Transplant Registry criteria** |
| Immature blasts in PB and/or BM | ≧20% | ≧30% | ≧30% |
| Or | Extramedullary (except spleen) proliferation of myeloblasts or lymphoblasts; or intertrabecular area(s) filled by blasts in marrow core biopsy | Extramedullary (except spleen) proliferation of myeloblasts or lymphoblasts | Extramedullary infiltration of leukemic cells |

[a]CCA (clonal chromosomal abnormalities)/Ph+, include "major route" abnormalities (second Ph, trisomy 8, isochromosome 17q, trisomy 19), complex karyotype, or abnormalities of 3q26.2

[b]Complete hematologic response: WBC <10 × $10^9$/L; platelet count <450 × $10^9$/L, no immature granulocytes in the differential, and spleen non-palpable

### *Immunophenotyping of Blasts by Flow Cytometry and/or Immunohistochemistry*

Flow cytometry and/or immunohistochemistry studies are essential for lineage assignment. The phenotype of blasts occurring in BP-CML can be lymphoid lineage (20–30% cases, Fig. 5.4), myeloid lineage (70% cases, Fig. 5.5), biphenotypic, or undifferentiated (rarely). Phenotypic markers indicative of myeloid lineage differentiation are myeloperoxidase (MPO), CD11b, CD13, and CD33. Rarely, the blasts may have erythroid differentiation with expression of glycophorin (CD235), CD71 (bright), and CD36 (thrombospondin receptor) or megakaryocytic differentiation with expression of CD41 (glycoprotein IIb-IIIa) and CD61 (glycoprotein IIIa). Markers for B-cell linage blasts are CD19, CD22, and cytoplasmic CD79 and for T-cell linage blasts are CD1a, CD2, cytoplasmic CD3, CD5, CD7, and dual expression or the absence of CD4 and CD8 [10, 11]. Regardless of lineage differentiation, blasts commonly express CD34. The myeloblasts often express CD117, and the lymphoblasts frequently express nuclear TdT. For details about definitive lineage assignment using immunophenotypic markers, please refer to Chap. 3.

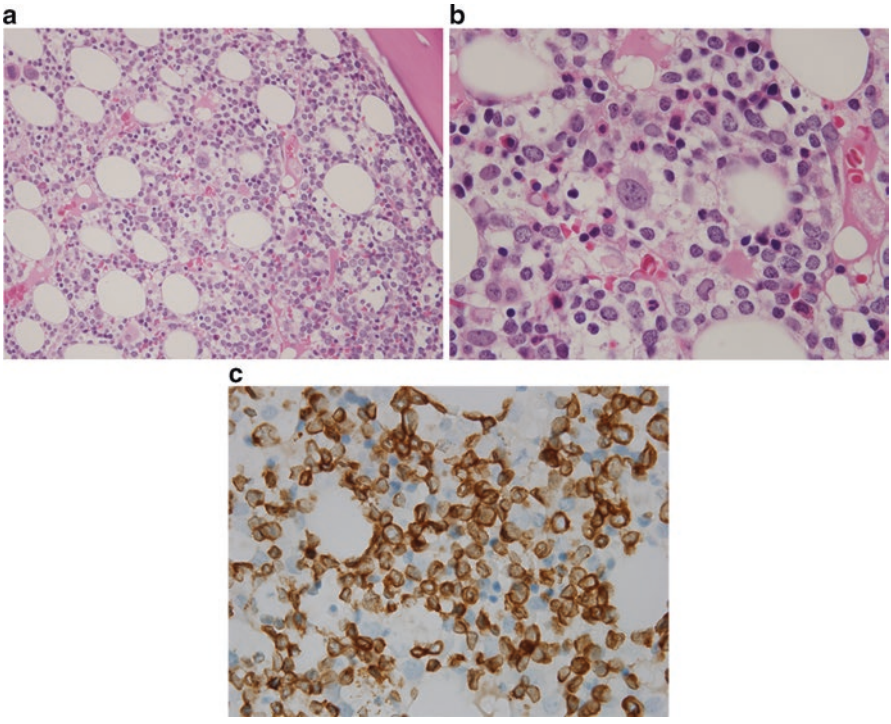


**Fig. 5.4** (**a**) 400× HE, (**b**) 1000× HE, (**c**) 1000× IHC for CD3. Blast (T-lymphoblastic) phase of CML. Increased blasts with dwarf megakaryocytes in the background (**a**, **b**). These immature blasts show T lineage markers, CD3 (**c**), CD4, and CD8 but negative to MPO and CD117 (not shown)

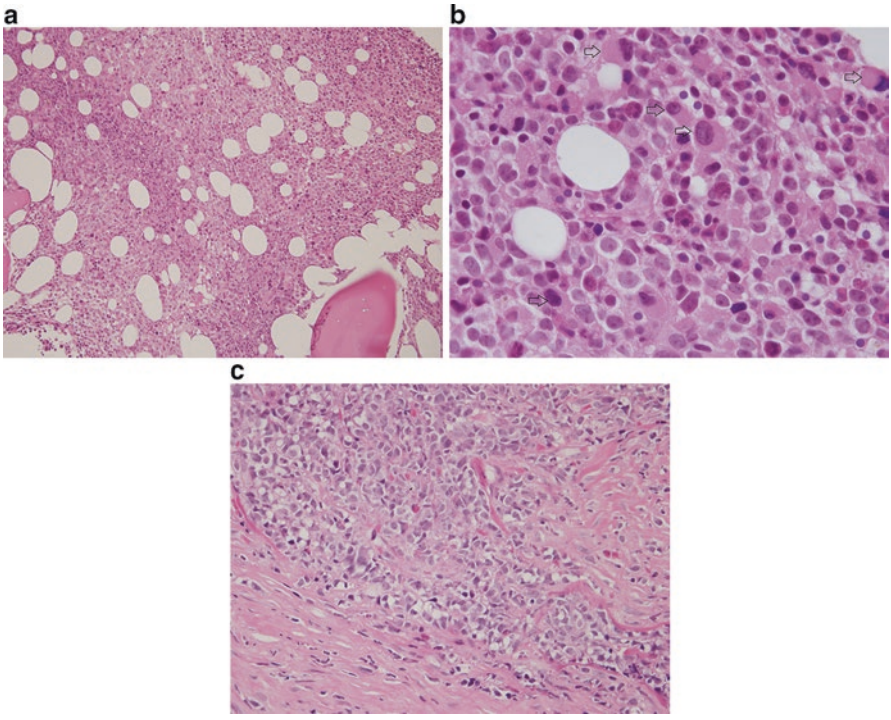


**Fig. 5.5** (**a**) 400× HE, (**b**) 1000× HE, (**c**) 400× HE. Blast (myeloid) phase of CML. Increased blasts with dwarf megakaryocytes in the background in bone marrow (**a**, **b**). Sometimes, blast phase presenting as myeloid sarcoma in soft tissue (**c**)

## Cytogenetics and Molecular Findings

All patients with CML have chromosomal abnormality, t(9;22)(q34;q11), *BCR-ABL1*. This translocation results in a fusion gene of *BCR* on chromosome 22 and *ABL1* on chromosome 9. Most cases can be recognized by routine karyotyping as the Ph chromosome. In some instances, especially for cases with crypt translocations that cannot be detected by conventional karyotyping, FISH or RT-PCR (reverse transcription polymerase chain reaction) may be necessary.

The locations of the *BCR* and *ABL1* genomic breakpoints are variable. Previous sequencing of the *BCR* showed five exons (b1–b5), with the most frequent breakpoints being between b2 and b3 or b3 and b4. The breakpoints within the *ABL1* gene always result in fusion upstream of the second *ABL1* exon, so the fusions were originally known as b2a2 or b3a2. Later, b1–b5 exons were shown to be exons 12–16 of a much larger gene, also named *BCR*, and these foci also referred to as the major breakpoint cluster region (M-bcr). Hence the translocation of b2a2 and b3a2 became e13a2 and e14a2; they generate a 210-kDa protein (p210 BCR-ABL1). Figure 5.6 illustrates the configuration of *BCR* and *ABL1* fusion gene.

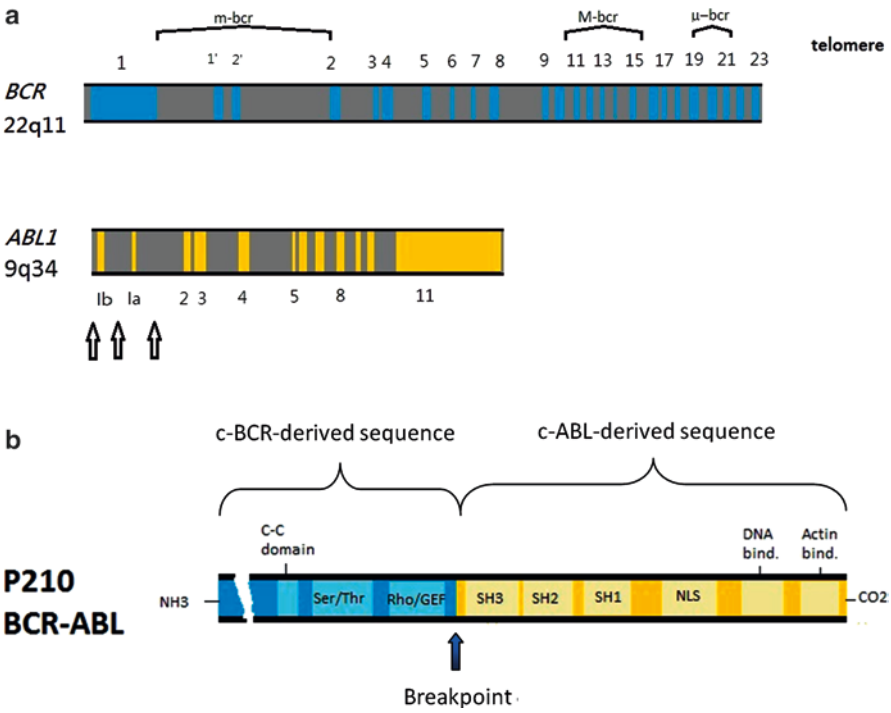


**Fig. 5.6** (**a**), Schematic representation of the *ABL1* and *BCR* genes and the *BCR-ABL1* kinase. *BCR* contains 23 exons. The three main breakpoint cluster regions (*m-bcr*, *M-bcr*, and *μ-bcr*) in *BCR* are presented. *ABL1* contains two alternative first exons (*1b* and *1a*). The *arrows* represent the breakpoints within *ABL1*. (The diagrams are not to scale). (**b**), The BCR-ABL protein contains the dimerization or coiled-coil (C-C) domain, Ser/Thr kinase domain, and the Rho/GEF domain of BCR, as well as the SH-domains, nuclear localization signal (NLS), DNA-binding, and actin-binding domains from ABL (the diagrams are not to scale)

There are two other minor isoforms of fusion proteins generated by different *BCR-ABL1* translocated foci. When the *BCR* breakpoint localizes in minor breakpoint cluster region (or m-bcr leading to fusion transcript of e1a2), it produces a 190-KDa protein (p190 BCR-ABL1). When the breakpoint of BCR gene is in the 3′ end of the gene, named as μ-bcr, it generates a e19a2 fusion transcript leading to a 230-kDa fusion protein (p230 BCR-ABL1).

Different isoforms of BCR-ABL1 fusion proteins show greater incidences in subtypes of leukemia: p210 with 90% of chronic myeloid leukemia (CML), p190 is associated with 20–30% of acute lymphoblastic leukemia (ALL), and p230 with a morphologic variant of CML which is distinct in that mature neutrophils predominate without significant left shift. However, there are overlaps among these associations, for example, p210 also occurs in Ph+ ALL, and p190 occurs in 2–3% of CMLs [12]. Different isoforms of the *BCR-ABL1* translocation are also associated with different clinical features and treatment response. For example, in CML patients with p190 BCR-ABL1, monocytosis is a common finding [13], and the treatment response to tyrosine kinase inhibitors (TKI) is inferior [14].

Although *BCR-ABL1* translocation is the diagnostic hallmark genetic finding of CML, it is not entirely specific for CML. Some proportion of health persons harbors very low level of BCR-ABL1 fusion transcripts in the peripheral blood [15]. As discussed above, it also occurs in acute lymphoblastic leukemia and in rare cases of de novo acute myeloid leukemia and myeloproliferative neoplasms. A new provisional category of AML with BCR-ABL1 is added to WHO 2016 classification to recognize the rare de novo AML cases that may benefit from tyrosine kinase inhibitor therapy. The diagnostic distinction between de novo AML with BCR-ABL1 and blast transformation of CML is very difficult without adequate clinical information. Of note, preliminary data suggest that deletion of antigen receptor genes (IGH, TCR), IKZF1, and/or CDKN2A may support a diagnosis of de novo disease versus blast phase of CML.

Recent studies using next-generation sequencing (NGS) technology have revealed that somatic mutations of some genes, such as TET2 (TET oncogene family number 2), IDH1 and IDH2 (isocitrate dehydrogenase 1 and 2), RUNX1, DNMT3, ASXL1 (additional sex combs-like 1) reported in BCR-ABL1-negative myeloid neoplasms, are also found in BCR-ABL1-positive CML with variable incidence [16, 17]. Furthermore, mutations of some genes such as EZH2, TP53, U2AF1, and ZRSR2 appear to specifically occur in the Ph-negative clones in patients who developed clonal cytogenetic abnormalities in Ph-negative cells during treatment with tyrosine kinase inhibitors (TKI) [17]. Whether these additional gene mutations play roles in disease initiation or progression needs further investigation.

The BCR-ABL1 fusion protein shows deregulated tyrosine kinase activity and acts as an onco-protein-activating signaling transduction pathways that lead to transformation. Structurally, BCR-ABL1 contains multiple domains (Fig. 5.6b). The ABL1 protein is made up of Src-homology (SH3 and SH2) domains, tyrosine kinase domain, DNA-binding domain, actin-binding domain, nuclear localization signals, and nuclear export signal. The BCR protein contains a coiled-coil oligomerization domain, serine/threonine kinase domain, pleckstrin homology (PH) domain, Dbl/cdc24 guanine nucleotide exchange factor homology domain, several serine/threonine and tyrosine phosphorylation sites, and binding sites for the ABL SH2 domain, Grb2, and 14-3-3 proteins [18]. A complex network of signal transduction pathways, including Jak/STAT, PI3K/Akt, and Ras/MEK, is hijacked by BCR-ABL1; the net results are promoting cell growth/survival and inhibiting cell death leading to CP-CML [19].

The exact mechanisms of transition from chronic phase to accelerated/blast phase (AP/BP) are currently unknown. It is proposed that the progenitor cells in AP/BP show more stem cell-like characteristics compared to chronic phase progenitors, i.e., gaining more self-renewal capacity, more immature in differentiation, and more uncontrolled proliferation. Additional hits of chromosomal anomalies play important roles in the progression of CML, such as trisomy 8, a second Ph-chromosome, and isochromosome 17q. Additionally, alterations of tumor suppressor genes and oncogenes, such as the *MYC* proto-oncogene, *TP53*, etc. also contribute to the progression of CML. Diminished efficacy of TKIs during transformation to AP/BP also suggested the reduced reliance on *BCR-ABL1* activity in the presence of other mutations [19].

## Therapy, Prognosis, and Monitoring

Tyrosine kinase inhibitors (TKI) have become the standard of therapy for CML. These function by binding directly to the BCR-ABL1 fusion protein thereby inhibiting its activity. Imatinib was the first TKI used to treat CML in the late 1990s and early 2000s and prolonged overall survival in CP-CML to over 87% of patients alive at a follow-up time of 8 years. In AP-CML, the estimated 8-year survival rate has also increased to 75%. However, in BP-CML, the median survival among patients remains at 7 months [20]. Of note, the median survival for myeloid blasts crisis is inferior to lymphoid blasts crisis [18]. Newer generations TKIs such as nilotinib, dasatinib, bosutinib, and ponatinib have been developed and function similarly.

Regarding risk assessment, the Sokal [21], Hasford (Euro) [22], and EUTOS [23] scores are commonly used. Table 5.2 summarizes their calculations which depend on multiple clinical and laboratory findings such as age, spleen size, platelet count, peripheral blood blast counts, eosinophils, and basophils percentages. Of note, the EUTOS score is superior than Sokal and Hasford score in risk assessment in the era of TKI therapy, since the latter two scores were developed before TKI treatment.

### *Monitoring Therapy Response*

After initiation of treatment, it is important to follow patients with appropriate tests to ensure optimal treatment outcomes and to adjust treatment scheme if optimal outcomes are not achieved.

**Table 5.2** Calculation of relative risk

| Study | Calculation | Results |
|---|---|---|
| Sokal score [21] | Exp 0.0116 × (age – 43.4) + 0.0345 × (spleen – 7.51) + 0.188 × [(platelet count ÷ 700)$^2$ – 0.563] + 0.0887 × (blast cells – 2.10) | Low risk: <0.8<br>Intermediate risk: 0.8–1.2<br>High risk: >1.2 |
| Hasford score (or Euro score) [22] | 0.666 when age ≧50 y + (0.042 × spleen) + 1.0956 when platelet count >1500 × 10$^9$L + (0.0584 × blast cells) + 0.20399 when basophils >3% + (0.0413 × eosinophils) × 100 | Low risk: ≦780<br>Intermediate risk: 781–1480<br>High risk: >1480 |
| EUTOS risk score [23] | Spleen × 4 + basophils × 7 | Low risk: ≦87<br>High risk: >87 |

All values must be collected before any treatment
Age is given in years. Spleen is given in centimeters below the costal margin (maximum distance). Blast cells, eosinophils, and basophils are given in percent of peripheral blood differential
To calculate Sokal and Euro risk score, go to http://www.leukemia-net.org/content/leukemias/cml/cml_score/index_eng.html
To calculate EUTOS risk score, go to http://www.leukemia-net.org/content/leukemias/cml/eutos_score/index_eng.html
For on-line calculation of three scores, go to http://bloodref.com/myeloid/cml/sokal-hasford

**Table 5.3** The definition of various degrees of treatment response in CML

| | Criteria |
|---|---|
| Complete Hematologic response | (i) Complete normalization of peripheral blood counts with leukocyte count <10 × 109/L<br>(ii) Platelet count <450 × 109/L<br>(iii) No immature cells, such as myelocyte, promyelocytes, or blasts in peripheral blood<br>(iv) No signs and symptoms of disease with disappearance of palpable splenomegaly |
| Cytogenetic response by metaphase karyotyping | *At least 20 cells analyzed* |
| Complete cytogenetic response (CCyR) | No Ph-positive metaphases |
| Partial cytogenetic response (PCyR) | 1–35% Ph-positive metaphases |
| Major cytogenetic response | 0–35% Ph-positive metaphases (complete + partial) |
| Minor cytogenetic response | >35% Ph-positive metaphases |
| Molecular response by RQ-PCR | International scale (IS) defined as the ratio of *BCR-ABL1* transcripts to *ABL1* transcripts |
| Early molecular response (EMR) | *BCR-ABL1* transcripts ≤ 10% by QPCR (IS) at 3 and 6 months |
| Major molecular response (MMR) | *BCR-ABL1* expression of ≦0.1% *BCR-ABL1* IS or if IS not available, ≥3 log reduction of *BCR-ABL1* transcript from baseline |
| Complete molecular response (CMR)[a] | No detectable *BCR-ABL1* mRNA by QPCR (IS) using an assay with a sensitivity of at least 4.5 logs below the standardized baseline |
| Deep molecular response (MR) $MR^{4.0}$ | (i) Detectable disease with <0.01% *BCR-ABL1* IS<br>(ii) Undetectable disease in cDNA with>10,000 *ABL1* transcripts |
| $MR^{4.5}$ | (i) Detectable disease with <0.0032% *BCR-ABL1* IS<br>(ii) Undetectable disease in cDNA with>32,000 *ABL1* transcripts in the same volume of cDNA used to test for *BCR-ABL1* |
| Relapse | (i) Any sign of loss of response (defined as hematologic or cytogenetic relapse)<br>(ii) 1-log increase in *BCR-ABL1* transcript levels with loss of MMR should prompt bone marrow evaluation for loss of CcyR but is not itself defined as relapse |

[a]CMR is variably described in the literature and is best defined by the assay's level of sensitivity (e.g., MR 4.5). In practice, the term complete molecular response should be avoided and substituted with the term *molecularly undetectable leukemia*, with specification of the number of the control gene transcript copies

The criteria for various degrees of treatment outcomes (responses), including hematologic, cytogenetic, and molecular, are summarized in Table 5.3 [9, 24]. Of note, the term "complete molecular response" should be avoided and substituted with the term "molecularly undetectable leukemia," with specification of the copy number of the control gene transcripts. Table 5.4 shows the timeline and criteria for optimal response, warning (suboptimal) response and treatment failure after initiation of treatment.

**Table 5.4** Recommendation for cytogenetic studies, RT-PCR studies, and BCR-ABL kinase domain mutation analysis suggested by NCCN guideline [24]

| Tests | Recommendation |
|---|---|
| Bone marrow cytogenetics | 1. At diagnosis to establish the disease phase[a]<br>2. At 3 and 6 months from initiation of therapy if QPCR using IS is not available to assess response to TKI therapy<br>3. At 12 months from initiation of therapy, if CCyR or MMR is not achieved[b]<br>4. 1-log increased in BCR-ABL1 transcript levels without MMR. |
| Quantitative RT-PCR (QPCR) using international scale (IS) | 1. At diagnosis<br>2. Every 3 months after initiating treatment<br>3. After CcyR has been achieved, every 3 months for 2 years and every 3–6 months thereafter[c] |
| BCR-ABL kinase domain mutation analysis | Chronic phase<br>1. Inadequate initial response to TKI therapy[d]<br>2. Any sign of loss of response (defined as hematologic or cytogenetic relapse)<br>3. 1-log increased in *BCR-ABL1* transcript levels and loss of MMR<br>Disease progression to accelerated or blast phase |

[a]If collection of bone marrow is not feasible, FISH on a peripheral blood specimen using dual probes for the BCR and ABL gene is an acceptable method of confirming the diagnosis
[b]Absence pf MMR in the presence of a CCyR is not considered a treatment failure
[c]If there is 1-log increased in *BCR-ABL1* transcript levels with MMR, QPCR analysis should be repeated in 1–3 months
[d]Lack of PCyR or *BCR-ABL1* transcripts >10% (IS) at 3 and 6 months or less than a CCyR or *BCR-ABL1* transcripts >1% (IS) at 12 months

**Table 5.5** Summary of the most appropriate alternative therapeutic options based on the *BCR-ABL1* KD mutation status

| Mutation | Frequency of mutation | Suggested alternative therapeutic options |
|---|---|---|
| T315I | 15% | Ponatinib or HSCT |
| V299L, T315A, and F317L/V/I/C | <2%/<2%/4% | Consider nilotinib rather than dasatinib |
| Y253H, E255K/V, and F359V/C/I | 12%/18%/6% | Consider dasatinib rather than nilotinib |
| Any other mutation | 30 to 40% | Consider high-dose imatinib[a] or dasatinib or nilotinib |

HSCT indicates hematopoietic stem cell transplantation
[a]No sufficient data on dose escalation available to indicate if mutations with lower $IC_{50}$ values are sensitive to high-dose imatinib

The NCCN guideline [24] for monitoring patients on TKI treatment with different test is summarized in Table 5.5. Briefly, the suggested tests for therapy response evaluation are (i) cytogenetics analysis (chromosome karyotyping of at least 20 metaphase cells) to assess the degree of cytogenetic response (CyR) and to evaluate the possibility of clonal evolution at time of relapse or disease progression,

(ii) real-time quantitative reverse transcription polymerase chain reaction (RQ-PCR) using the international scale (IS) to evaluate the degree of molecular response, and (iii) BCR-ABL kinase domain mutation test at time of disease progression or suboptimal treatment responses. IS is defined as the ratio of *BCR-ABL1* transcripts to *ABL1* transcripts, or other internationally recognized control transcripts, and it is reported as *BCR-ABL1* percentage on a log scale, where 10%, 1%, 0.1%, 0.01%, 0.0032%, and 0.001% correspond to a decrease of 1, 2, 3, 4, 4.5, and 5 logs, respectively, below the standard baseline that was used in the IRIS study [25]. Additional guideline for monitoring the responses to TKI by European LeukemiaNet can be found in Reference [9].

Some CML patients become resistant to TKI therapy and fail to respond or show disease progression. TKI resistance occurs due to *BCR-ABL1* amplification, kinase domain (KD) mutations, and clonal evolution. Most frequently, TKI resistance is caused by single amino acid mutation in the BCR-ABL1 protein that changes its' basic structure and prevents further binding of TKIs. Direct (Sanger) sequencing is the method ELN recommends for BCR-ABL1 KD mutation analysis; it allows detection of mutations present in 20% of Ph+ cells. However, NGS has been shown to be a more sensitive method to detect low level BCR-ABL1 mutation with the analytical sensitivity about 1% [26]. NGS can help in early detection of emerging BCR-ABL1 mutation, 2–11 months earlier than conventional sequencing. In cases who later failed with first-line TKI therapy, NGS detects TKI-resistant mutations as early as at the time of major or deeper molecular response. Using NGS to detect the low level of BCR-ABL1 kinase domain mutation may allow early shift to more appropriate TKI therapy to further improve patient outcome [27].

The NCCN guideline [24] for BCR-ABL1 kinase domain mutation testing is summarized in Table 5.4. The European LeukemiaNet recommends BCR-ABL1 KD mutation analysis in the following situations: (1) fresh cases of CML initially presenting in accelerated phase or blast phase; (2) cases with treatment failure, or increased *BCR-ABL1* transcript levels leading to MMR loss, or any case of suboptimal response during the first-line imatinib therapy; (3) cases with hematologic or cytogenetic failure during the second-line dasatinib or nilotinib therapy [28].

BCR-ABL1 KD mutation analysis is essential for adjusting therapeutic TKI usage (Table 5.5). However, the possibility for CML patients in chronic phase to have BCR-ABL1 KD mutation is very low; KD mutations are found only in less than 3% of CP-CML patients [29]. Therefore, screening for KD mutation is not recommended in initial diagnosis in CP-CML patients [28].

Of note, clonal cytogenetic abnormalities (most frequently trisomy 8) in Ph-negative cells have been noted in a few patients during TKI treatment. The clinical significance of such abnormalities is uncertain. Only rare patients with such abnormalities develop a myelodysplastic syndromes or acute myeloid leukemia from the clonal cytogenetically abnormal cells, and some of these abnormalities may be transient.

## Conclusion

CML is characterized by *BCR-ABL1* fusion gene leading to the production of the BCL-ABL1 fusion protein which plays a critical role in the pathogenesis of CML and serves as a characteristic biomarker for diagnosis. Understanding the molecular pathogenesis of CML has resulted in the development of various TKI. The success of treating CML with targeted therapies (TKIs) against the BCR-ABL1 fusion protein is a prototypical example of successful precision medicine and changed CML from a universally deadly disease to a chronic disease that can be well controlled/cured with appropriate treatment and careful monitoring of disease burden with appropriate molecular tests.

## References

1. Höglund M, Sandin F, Simonsson B. Epidemiology of chronic myeloid leukaemia: an update. Ann Hematol. 2015;94:S241–7.
2. Ichimaru M, Tomonaga M, Amenomori T, Matsuo T. Atomic bomb and leukemia. J Radiat Res. 1991;32(Suppl):162–7.
3. Musselman JR, Blair CK, Cerhan JR, Nguyen P, Hirsch B, Ross JA. Risk of adult acute and chronic myeloid leukemia with cigarette smoking and cessation. Cancer Epidemiol. 2013;37(4):410–6.
4. Inbal AA. Retrospective study of patients with chronic myeloid leukemia diagnosed and treated at the Chaim Sheba Medical Center during the years 1966-76. Isr J Med Sci. 1978;14:1259–64.
5. Kardinal CG, Bateman JR, Weinter J. Chronic granulocytic leukemia: review of 536 cases. Arch Intern Med. 1976;136:305–13.
6. Hughes TP, Hochhaus A, Branford S, Müller MC, Kaeda JS, Foroni L, Druker BJ, Guilhot F, Larson RA, O'Brien SG, Rudoltz MS, Mone M, Wehrle E, Modur V, Goldman JM, Radich JP, IRIS investigators. Long-term prognostic significance of early molecular response to imatinib in newly diagnosed chronic myeloid leukemia: an analysis from the International Randomized Study of Interferon and STI571 (IRIS). Blood. 2010;116(19):3758–65.
7. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW. WHO classification of tumours of haematopoietic and lymphoid tissue Lyon, France: IARC Press. 2008.
8. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, Bloomfield CD, Cazzola M, Vardiman JW. The 2016 revision to the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–405.
9. Baccarani M, Deininger MW, Rosti G, et al. European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. Blood. 2013;122(6):872–84.
10. Jennings CD, Foon KA. Recent advances in flow cytometry: application to the diagnosis of hematologic malignancy. Blood. 1997;90(8):2863–92.
11. Béné MC, Nebe T, Bettelheim P, Buldini B, Bumbea H, Kern W, Lacombe F, Lemez P, Marinov I, Matutes E, Maynadié M, Oelschlagel U, Orfao A, Schabath R, Solenthaler M, Tschurtschenthaler G, Vladareanu AM, Zini G, Faure GC, Porwit A. Immunophenotyping of acute leukemia and lymphoproliferative disorders: a consensus proposal of the European LeukemiaNet Work Package 10. Leukemia. 2011;25:567–74.
12. Melo JV. The diversity of BCR–ABL fusion proteins and their relationship to leukemia phenotype. Blood. 1996;88:2375.
13. Ohsaka A, Shiina S, Kobayashi M, Kudo H, Kawaguchi R. Philadelphia chromosome-positive chronic myeloid leukemia expressing p190(BCR-ABL). Intern Med. 2002;41(12):1183–7.
14. Verma D, Kantarjian HM, Jones D, Luthra R, Borthakur G, Verstovsek S, Rios MB, Cortes J. Chronic myeloid leukemia (CML) with $P190^{BCR-ABL}$: analysis of characteristics, outcomes, and prognostic significance. Blood. 2009;114:2232–5.

15. Song J, Mercer D, Xiaofeng H, Liu H, Li MM. Common leukemia- and lymphoma-associated genetic aberrations in healthy individuals. J Mol Diagn. 2011;13:213–9.
16. Roche-Lestienne C, Marceau A, Labis E, Nibourel O, Coiteux V, Guilhot J, Legros L, Nicolini F, Rousselot P, Gardembas M, Helevaut N, Frimat C, Mahon F-X, Guilhotand F, Preudhomme C. Mutation analysis of TET2, IDH1, IDH2 and ASXL1 in chronic myeloid leukemia. Leukemia. 2011;25(10):1661–5.
17. Schmidt M, Rinke J, Schafer V, Schnittger S, Kohlmann A, Obstfelder E, Kunert C, Ziermann J, Winkelmann N, Eigendorff E, Haferlach T, Haferlach C, Hochhaus A, Ernt T. Molecular-defined clonal evolution in patients with chronic myeloid leukemia independent of the BCR-ABL status. Leukemia. 2014;28:2292–9.
18. Advani AS, Pendergast AM. Millennium Review. *Bcr–Abl* variants: biological and clinical aspects. Leuk Res. 2002;26:713–20.
19. Chereda B, Melo JV. Natural course and biology of CML. Ann Hematol. 2015;94(Suppl 2):S107–21.
20. Kantarjian H, O'Brien S, Jabbour E, Garcia-Manero G, Quintas-Cardama A, Shan J, Rios MB, Ravandi F, Faderl S, Kadia T, Borthakur G, Huang X, Champlin R, Talpaz M, Cortes J. Improved survival in chronic myeloid leukemia since the introduction of imatinib therapy: a single-institution historical experience. Blood. 2012;119(9):1981–7.
21. Sokal JE, Cox EB, Baccarani M, Tura S, Gomez GA, Robertson JE, et al. Prognostic discrimination in "good-risk" chronic granulocytic leukemia. Blood. 1984;63:789–99.
22. Hasford J, Pfirrmann M, Hehlmann R, Allan NC, Baccarani M, Kluin-Nelemans JC, et al. A new prognostic score for survival of patients with chronic myeloid leukemia treated with interferon alfa. J Natl Cancer Inst. 1998;90:850–8.
23. Hasford J, Baccarani M, Hoffmann V, Guilhot J, Saussele S, Rosti G, et al. Predicting complete cytogenetic response and subsequent progression-free survival in 2060 patients with CML on imatinib treatment: the EUTOS score. Blood. 2011;118:686–92.
24. Radich JP, Deininger M, Abbound CN, Altman JK, Barta SK, Berman E, Curtin P, DeAngelo DJ, Devine S, Gotlib J, Hagelstrom RT, Hobbs G, Jagasia M, Kantarjian HM, Moore JO, Ontiveros E, Pallera A, Quiery A, Reddy VVB, Rose MG, Shah NP, Smith BD, Snyder DS, Sweet KL, Gregory K, Sundar H. NCCN clinical practice guildlines in oncology (NCCN guilelines) chronic myelogenous leukemia. Version I. 2016. NCCN.org.
25. Branford S, Fletcher L, Cross NCP, Müller MC, Hochhaus A, Kim D-W, Radich JP, Saglio G, Pane F, Kamel-Reid S, Wang YL, Press RD, Lynch K, Rudzki Z, Goldman JM, Hughes T. Desirable performance characteristics for BCR-ABL measurement on an international reporting scale to allow consistent interpretation of individual patient response and comparison of response rates between clinical trials. Blood. 2008;112:3330–8.
26. Erbilgin Y, Eskazan AE, Ng OH, Salihoglu A, Elverdi T, Firtina S, Tatonyan SC, Aydin SO, Ar MC, Baslar Z, Sayitoglu M, Ferhanoglu AB, Aydin Y, Ozbek U, Soysal T. Next-generation sequencing of the BCR-ABL1 kinase domain may be beneficial in decision making among chronic myeloid leukemia patients with tyrosine kinase inhibitor resistance. Blood. 2013;122(21):384.
27. Polakova KM, Kulvait V, Benesova A, Linhartova J, Klamova H, Jaruskova M, de Benedittis C, Haferlach T, Baccarani M, Martinelli G, Stopka T, Ernst T, Hochhaus A, Kohlmann A, Soverini S. Next-generation deep sequencing improves detection of BCR-ABL1 kinase domain mutations emerging under tyrosine kinase inhibitor treatment of chronic myeloid leukemia patients in chronic phase. J Cancer Res Clin Oncol. 2015;141(5):887–99.
28. Soverini S, Hochhaus A, Nicolini FE, Gruber F, Lange T, Saglio G, Pane F, Martin CM, Ernst T, Rosti G, Porkka K, Baccarani M, Cross NCP, Martinelli G. BCR-ABL kinase domain mutation analysis in chronic myeloid leukemia patients treated with tyrosine kinase inhibitors: recommendations from an expert panel on behalf of European LeukemiaNet. Blood. 2011;118(5):1208–15.
29. Khorashad JS, de Lavallade H, Apperley JF, Milojkovic D, Reid AG, Bua M, Szydlo R, Olavarria E, Kaeda J, Goldman JM, Marin D. Finding of kinase domain mutations in patients with chronic phase chronic myeloid leukemia responding to imatinib may identify those at high risk of disease progression. J Clin Oncol. 2008;26(29):4806–13.

# Chapter 6
# Updates in Polycythemia Vera

**Vidya Nagrale, Randall Olsen, and Youli Zu**

Polycythemia vera (PV) is a chronic myeloproliferative neoplasm (MPN) of hematopoietic stem cells (HSC, predominantly characterized by an increase in erythrocyte mass with trilineage proliferation [1]. The blood, bone marrow, spleen, and liver are variably involved during progression through the two phases of disease progression: (1) a proliferative or polycythemic phase with increased red blood cells (RBCs); and (2) a postpolycythemic or spent phase with marked cytopenias, extramedullary hematopoiesis, hypersplenism, and myelofibrosis (MF). Hemorrhage and thrombosis are the primary clinical manifestations; the disease progresses to acute leukemia in a subgroup of patients (10–15%) who are older, with high white blood cell (WBC) count, and in those with additional somatic mutations other than Janus kinase 2 (*JAK2*) mutations [2]. The differential diagnosis includes secondary polycythemia and other chronic MPNs. Vaquez and Osler [3–6] first reported PV associated with erythremia and polycythemia, respectively. The concept of chronic myeloproliferative disorders (MPDs) emerged during the 1950s when PV was categorized with chronic myeloid leukemia, essential thrombocythemia (ET), and chronic idiopathic myelofibrosis (CIMF) based on common clinicopathological features [7]. The Polycythemia Vera Study Group (PVSG) was then formed to define the diagnostic criteria for this entity. PVSG recommendations remained the gold standard for many years [8, 9]. During the 1970s, considerable advancements in understanding PV were made due to the improvement in laboratory techniques and evolvement of scientific perspective on neoplasia. Several studies evaluating various markers such as X-chromosome inactivation, glucose-6-phosphate dehydrogenase isoenzymes, and DNA methylation demonstrated that PV arises from clonal transformation of a single hematopoietic stem cell (HSC) [10–16]. However, consensus

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with PVSG remained until 2001 when the World Health Organization (WHO) reclassified myeloproliferative disorders as neoplastic – renaming them as MPNs and modernized the diagnostic criteria using a comprehensive clinicopathological approach [17]. A point mutation in *JAK2*, resulting in substitution of valine for phenylalanine (V617F), was identified in a significant number of PV patients [18–25]. In 2008, WHO incorporated the *JAK2* mutation as one of the major diagnostic criteria [1], and in 2016, WHO lowered the cutoff values for hemoglobin and recognized the importance of bone marrow morphology (Table 6.1) [26]. Additionally, the mutually exclusive MPL proto-oncogene, thrombopoietin receptor (*MPL)* and calreticulin (*CALR)* mutations were associated with ET and primary myelofibrosis (PMF) [26, 27]. *MPL* and *CALR* mutations practically exclude PV.

**Table 6.1** Classification Criteria of PV

| | | [a]PVSG | 2001 WHO | 2008 WHO | 2016 WHO |
|---|---|---|---|---|---|
| Hemoglobin, g/dL | **A1** | | 18.5 g/dL in men 16.5 g/dL in women | >18.5 g/dL in men >16.5 g/dL in women | > 16.5 g/dL in men > 16.0 g/dL in women |
| Red cell mass (RCM) | | 36 mL/kg in men; 32 mL/kg in women | OR Increased[b] | OR Increased[b] | OR Increased[b] |
| Hematocrit (%) | | | | OR Increased[c] | OR >49% in men >48% in women |
| Arterial oxygen saturation | | **A2** ≥ 92% | – | – | – |
| No evident cause of secondary erythrocytosis | | | **A2** | – | – |
| Splenomegaly | | **A3** | **A3** | – | – |
| Clonal genetic abnormality | | | **A4**: other than[d] Ph chromosome or BCR/ABL fusion gene in marrow cells | **A2:** presence of JAK2 V617F or functionally similar JAK2 exon 12 mutation | **A3**: JAK2 V617F or JAK2 exon12 mutation |
| Endogenous erythroid colony formation | | – | **A5** | **A3** | – |
| Subnormal serum erythropoietin level | | – | **B4** | **A2** | **B1** |
| White blood cell count >12 × 109/L | | **B2** | **B2** | – | – |

(continued)

**Table 6.1** (continued)

| | [a]PVSG | 2001 WHO | 2008 WHO | 2016 WHO |
|---|---|---|---|---|
| Bone marrow (BM) histology | – | **B3:** panmyelosis with prominent erythroid and megakaryocytic proliferation | **B1:** panmyelosis with prominent trilineage proliferation | **A2**: panmyelosis with prominent trilineage proliferation with mature megakaryocytes of varying sizes |
| Increased leukocyte alkaline phosphatase >100 U/L | **B3** | – | – | – |
| Serum vitamin B12 concentration greater than 900 pg/mL or binding capacity >2200 pg/mL | **B3** | – | – | – |
| Requirements for diagnosis of PV | **A1 + A2 + A3** OR **A1 + A2 + any 2 from B** | **A1 + A2 and any other A** OR **A1 + A2 and any 2 from B** | **A1 + A2 and any one B** OR **A1 + any 2 from0 B** | [e]**A1 + A2 + A3** OR [e]**A1 + A2 + B** |

[a]*PVSG* Polycythemia Vera Study Group, *WHO* World Health Organization; **A** = major criterion; **B** = minor criterion

[b]RCM > 25% above mean normal predicted value) or

[c]Hb or Hct > 99th percentile of method-specific reference range for age, sex, altitude of residence or Hb > 17 g/dl men or 15 g/dl women if associated with a sustained increase of 2 g/dl or more from baseline that cannot be attributed to correction of iron deficiency

[d]*Ph* Philadelphia chromosome

[e]Exception: A2 is not required in 2016 criterion when there is sustained absolute erythrocytosis, Hb >18.5 g/dL in men (Hct 55.5%) or >16.5 g/dL in women (HCt 49.5%) if criterion A3 and B are present

## Epidemiology

A recent meta-analysis reported an annual worldwide incidence of PV ranging from 0.01 to 2.61 per million per year in various studies, and a pooled incidence rate of 0.84 per million per year [28]. PV has been reported in patients from all ethnic backgrounds, and is more common among Europeans and North Americans. Apparent geographic variation is possibly influenced by other heterogeneous variables [28]. Although several studies suggest a slightly higher incidence in males than females, a recent meta-analysis did not support a gender difference [1]. In general, PV is a disease encountered in older individuals, occurring most frequently between 50 and 70 years of age [1]; occurrences during childhood and adolescence are exceptionally rare [28]. Familial cases have been documented, but their significance remains uncertain.

## Clinical Manifestations

Patients with PV may be identified by chance when they present with nonspecific symptoms such as mild hypertension, fatigue, intense itching, and headache [29]. Symptoms of hyperviscosity associated with increased red cell mass (RCM) and thrombocytosis may cause the clinician to first suspect PV. Other presenting conditions include arterial and venous thrombosis with cardiovascular events, Budd–Chiari syndrome, or mesenteric ischemia, bleeding complications from nonspecific ecchymoses, epistaxis to major hemorrhage, hypertension, headache, dizziness, visual disturbances, vertigo, tinnitus, claudication, and erythromelalgia [30–32]. Another distinctive symptom of PV is pruritus, which occurs after exposure to warm water, resulting from increased histamine release from activated basophils and functionally different mast cells [33]; the classic symptom is intense itching following bathing. Gouty arthritis and hepatosplenomegaly may also be observed. Leukemic transformation often follows progression to end-stage disease (relative risk 1.4–6.3).

## Diagnostic Criteria

Considerable advances in the diagnostic criteria for PV were reported as the scientific community made new discoveries bearing on PV biology (Table 6.1). Presently, molecular testing is standard for PV diagnosis. The PVSG was originally formed with the support of the National Cancer Institute to evaluate treatment modalities. Eligibility criteria for entering PV clinical trials were defined, and these became known as the PVSG diagnostic criteria [8, 9], which remained the gold standard for decades [34, 35]. In 2001, the WHO classification for PV was published [17]. This classification represented a modernized approach for diagnosis and took into consideration several new laboratory studies. These studies included in vitro bone marrow endogenous erythroid colony (EEC) formation, the presence of clonal genetic abnormalities other than the Philadelphia chromosome (*BCR-ABL1* fusion gene and/or protein), revised histological findings for bone marrow, and low serum erythropoietin (EPO) levels (Table 6.1). The 2008 WHO further revised the adapted classification by incorporating molecular criteria for the presence of *JAK2* V617F, additional *JAK2* exon 12 variants, or other functionally similar mutations. The revised 2016 WHO lowered the diagnostic cutoff of hemoglobin to >16.5 g/dL from >18.5 g/dL in men, and >16.0 g/dL from 16.5 g/dL in women (Table 6.1). Moreover, bone marrow morphological examination has been established as a major mandatory criterion for diagnosis of PV. The practical utility of this morphological criterion stems from its reproducibility, the diagnostic features present in both overt and latent PV, and its ability to distinguish PV from JAK2V617F-positive ET [26, 36–39].

### *Red Blood Cell Mass, Hemoglobin, and Hematocrit*

The RBC mass is determined by comparing the total blood volume to the plasma volume. Originally, it was considered a sensitive marker for PV, but subsequent studies reported a relatively low predictive value as several confirmed PV cases were demonstrated to have a lower RBC mass than the reference limit [40, 41]. An actual measurement of RBC mass is no longer performed in daily clinical laboratory practice due to its high cost, labor intensity and suboptimal diagnostic accuracy [36, 41]. The 2016 revision to the WHO added a clearly-defined gender-specific criterion for hematocrit in addition to lowering the diagnostic cutoff of hemoglobin (Table 6.1). This allows diagnosis of PV in patients with an actual increase in RCM above 25% from the mean predicted value with borderline increases in hemoglobin and hematocrit; these cases were missed with the previous WHO classification. Diagnostically, this higher sensitivity captures a group that may be in the latent or prepolycythemic phase of PV. These patients are typically identified as younger males with high platelet and low white blood cell (WBC) counts; they are more prone to arterial thrombosis with poor overall survival when compared to those with apparent PV [36, 38, 39, 42, 43]. Studies have shown the benefit of early treatment in this clinically latent group that could have latent PV or a disease with different biology and prolonged latency [43, 44]. Additionally, the new guidelines assist in distinguishing PV from *JAK2* V617F-positive ET; this distinction has therapeutic and prognostic implications because PV has lower overall survival rates and higher risk of leukemic transformation [38, 45, 46]. It is notable that maintaining a hematocrit below 45% is beneficial in preventing thrombotic complications [44].

### *Bone Marrow Pathology*

Bone marrow morphology is a major mandatory criterion in the 2016 revision to the WHO. It has been duly acknowledged despite the fact that it was not included in the original PVSG criteria and was a minor criterion in the 2001 and 2008 WHO. In PV, the bone marrow should be hypercellular (Fig. 6.1a) with respect to patient age, with proliferation in all three lineages (panmyelosis – erythroid, myeloid, and megakaryocytic proliferation). Megakaryocytes display the most distinguishing features. In PV, megakaryocytes appear mature, but are pleomorphic with variability in size. Based on the WHO criteria, concordance rates for morphology of PV range between 71% and 82% among different pathologists. The utility of bone marrow morphology in cases of overt PV has been established [1, 47]. However, cases of "prodromal/early PV" have been recognized, and it is necessary to distinguish them from the clinical phenotype for ET. A large study showed that all the patients with *JAK2*-mutated PV, including overt and "masked PV" (mPV), met the 2008 WHO bone marrow morphology criteria [36, 37]. This supports the clinical utility of histological examination in patients with borderline or mild increases in RBC mass.

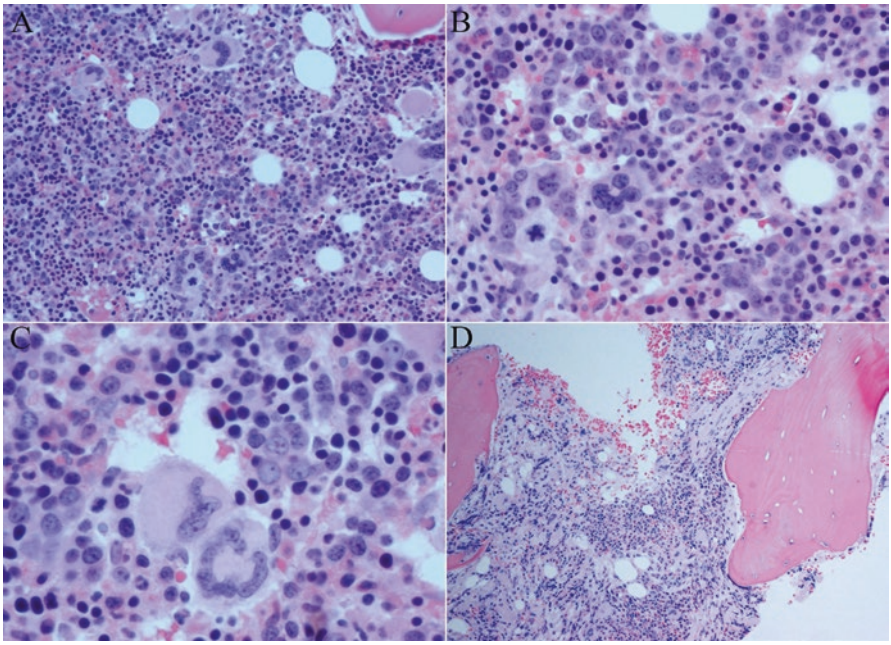


**Fig. 6.1** Bone marrow biopsy of polycythemia vera. (**a**) Hypercellularity (hematoxylin–eosin, original magnification ×200). (**b**) Prominent erythroid as well as myeloid hyperplasia (hematoxylin–eosin, original magnification ×400). (**c**) Loosely clustered pleomorphic megakaryocytes with hyperlobulation (hematoxylin–eosin, original magnification ×600). (**d**) Fibrosis change in post-polycythemic stage (hematoxylin–eosin, original magnification ×100)

Several large histopathological studies demonstrated that PV could be differentiated from secondary polycythemia in approximately 96% of patients who presented with mild to significant erythrocytosis [47–49]. PV specimens typically lack a prominent inflammatory stromal reaction, including only sparse numbers of perivascular plasma cells, eosinophils, hemosiderin-laden macrophages, and cellular debris.

The natural progression of PV involves a latent/prodromal phase, followed by the overt polycythemic phase and the "spent" or postpolycythemic myelofibrosis phase (post-PV MF) [1]. In the latent or prepolycythemic phase, bone marrow findings are similar to those in the polycythemic phase [36, 37]. Findings are specific and do not overlap with secondary erythrocytosis [1, 47]. In the polycythemic phase, the bone marrow is hypercellular with trilineage proliferation (panmyelosis). Hyperplastic normoblastic erythropoiesis predominates, but maturing granulopoiesis and megakaryopoiesis without dysplasia remain intact (Fig. 6.1a–c). In comparison, in the postpolycythemic or spent phase, erythropoiesis is markedly decreased, granulocyte maturation is delayed, and pleomorphic megakaryocytes without atypia are frequently observed. Overlapping features are typically observed with advancement of the disease, and MF progresses from a dense meshwork of reticulin with thickened sinuses (sinus wall sclerosis) to deposition of coarse collagen bundles to end-

stage myeloid scarring (Fig. 6.1d). More than 10% blasts in the peripheral blood or bone marrow herald disease acceleration, whereas 20% or higher is regarded as acute leukemia [1].

### *Cytogenetic Abnormalities*

Absence of the Philadelphia chromosome or the *BCR-ABL1* fusion product is essential for exclusion of chronic myeloid leukemia, *BCR-ABL1* positive. Various mutations in the hematopoietic progenitor cells of PV patients have been detected by conventional cytogenetic and fluorescence in situ hybridization methods, and these abnormalities have been shown to accumulate over time [50–53]. Fewer than 20% of cases have an identifiable cytogenetic clone at diagnosis, whereas more than 80–90% have one at 10 years [54–57]. The most frequent genetic aberrations include deletion or translocation of chromosome 20, trisomy 8, and trisomy 9. Abnormalities in 13q, 5q, 7q, 1q, 5, and 7 are less common. Because similar karyotypes are also observed in patients with other MPNs, they do not convey specific data. Although complex karyotypes are more common in PV with transformation, a significant number of patients with fibrotic transformation have a normal diploid karyotype [54, 57–59]. Hence, cytogenetic abnormalities are not necessarily a good predictor for post-PV MF transformation. In several studies, the transformation was not found to be associated with mutant *JAK2* V617F burden [57, 60]. However, a recent study identified significant impact of *JAK2* V617F mutant allele burden on fibrotic transformation [61]. The group demonstrated that when *JAK2* V617F is regarded as a continuous variable, a difference of 10% in mutant allele burden between two subjects increases the risk of post-PV MF by 40% in the subject with a higher mutant allele burden [61]. Currently, the use of the mutant allele burden to guide treatment strategies is under investigation.

### *Serum EPO Levels*

The vast majority of PV patients express very low levels of serum EPO (Table 6.1). This is a key diagnostic feature that differentiates PV from other polycythemic conditions [62, 63]. The molecular mechanism causing low EPO levels in PV is likely due to normal compensatory feedback loops in the setting of elevated erythropoiesis.

## Molecular Pathogenesis and JAK2 V617F Mutation

The most significant discovery in *BCR-ABL1* negative MPNs has been the presence of an acquired *JAK2* V617F mutation in the *JAK2* gene on chromosome 9p24 (Table 6.2). It has been established as a driver mutation in MPN mouse models

**Table 6.2** Mutations in PV

| Gene | Location | Function | Type of mutation | Frequency (%) | Prognostic significance |
|---|---|---|---|---|---|
| *JAK2* | 9p24 exon 14 | Tyrosine kinase, protein coding | Point mutation at codon 617, Gain of function | 96–99 | Allele burden single most risk factor for thrombosis; higher allele burdens are more responsive to ruxolitinib therapy |
| | 9p24 exon 12 | | Across multiple codons from 533 to 547 as substitutions, deletions, duplications or insertions, gain of function | 3 | Prognosis is similar to PV due to JAK2 V617F mutation |
| *MPL* | 1p34 exon 10 | Thrombopoietin receptor, signaling JAK-STAT pathway | Single amino acid substitution at codon 515, loss of function | Rare (0) | – |
| *CALR* | 19p13 exon 9 | Calcium binding protein associated with endoplasmic reticulum, transcriptional regulator | Insertions, deletions, indels with frameshift, resulting in truncated protein | Rare (0) | – |
| *TET2* | 4q24 all codons | Epigenetic transcriptional regulator, hydroxymethylation | Insertions, deletions, nonsense; loss of function | 10–20 | Lower[a] OS; often acquired at the time of[b] LT |
| *ASXL1* | 20q11 exon 13 | Encodes chromatin binding protein | Frameshift and stop, loss of function | 2–10 | May indicate fibrotic transformation |
| *DNMT3A* | 2p23 exons 7–23 | Epigenetic modification, methylation of histones | Inhibits differentiation | 5–10 | Higher risk of[b] LT |
| *EZH2* | 7q 35 all codons | Epigenetic modifier, transcriptional repressor via histone methylations | Loss of function | ≤2 | Infrequent[b] LT |

| | | | | | |
|---|---|---|---|---|---|
| *IDH1/2* | 2q33/15q26 | Cell cycle, metabolism | Gain of function | ≤2 | Decreased leukemia free survival |
| *TP53* | 17p13.1 | Cell cycle, apoptotic tumor suppressor | Loss of function | ≤2 | Frequent[b] LT |
| *SOCS1* | 16p13.2 | E3 ubiquitin ligase, negative regulation | Methylation, loss of function | ≤2 | Unclear |
| *LNK (SH2B3)* | 12q24 exon 2 | Encodes adaptor protein directly binding to phosphorylated JAK2 | Loss of negative regulation function | ≤2 | Unclear |
| *CBL* | 11q23 exons 8–9 | E3 ubiquitin ligase, negative regulation | Loss of negative regulation function | Rare | Infrequent[b] LT |
| *SF3B1* | 2q23 exons 12–16 | Splicing factor 3b subunit 1 | Loss of function | Rare | Infrequent[b] LT |
| *IKZF* | 7p12 | Encodes transcription factor, lymphopoiesis | Deletion | Not reported | Unclear |
| *NRAS* | 1p13.2 | GTPase | Gain of function | ≤2 | Possibly associated with LT |
| *SRSF2* | 17q21 exon 1 | Splicing factor | Unclear | Rare | Decreased OS |

[a]*OS* Overall survival
[b]*LT* Leukemic transformation

[64, 65]. *JAK2* V617F is the most frequent molecular abnormality found in almost all patients (>95%) with PV, and was independently identified by four different groups [18, 20, 21, 66]. A guanine to cytosine mutation occurs in codon 617, which resides in the JH2 pseudokinase domain of exon 14, resulting in a valine to phenylalanine substitution (V617F) [20, 21, 66]. The *JAK2* V617F is a somatic Class I mutation, which modifies growth factor signaling [67]. Damage in the JAK2 JH2 domain results in loss of autoregulatory inhibition of JH1 kinase exerted via JH2 pseudokinase, leading to phosphorylation of the JAK2 tyrosine kinase and its constitutive activation, with downstream activation of JAK-signal transducer and activator of transcription (STAT)/RAS/MAPK/PI3K/AKT pathways (Fig. 6.2). This results in myeloid proliferation, which occurs at decreased levels or in the absence of cytokines [69].

It is known that cytoplasmic domains of cytokine receptors are docked to JAK2 dimers, one of the Janus kinases, a nonreceptor tyrosine kinase. At the $NH_2$ terminus, protein 4.1, ezrin, radixin, and moesin (FERM) and SH2 domains secure JAK2 to the cytokine receptors. The JH1 kinase domain is located at the COOH terminal, adjacent to the JH2 pseudokinase domain (Fig. 6.3) [69]. The classical view of the JAK-STAT (signal transducer and activator of transcription proteins) pathway activation is the following: binding of cytokine to the receptor brings the JAK2 molecules into proximity, cross-activation leading to phosphorylation of their tyrosine kinase residues; subsequently, this causes phosphorylation and dimerization of STATs, which translocate to the nucleus for further transcriptional downstream activity. However, recent X-ray crystallographic studies, conducted by molecular dynamic simulations, support a contrary currently prevalent view [64]. Studies on the growth hormone (GH) receptor-JAK2 activation have showed that the cytoplasmic regions of the receptor are in proximity in the basal state. Binding of GH causes activation of the JAK2 receptor; the cytoplasmic regions of the receptor crossover resulting in separation of JH2 pseudokinase domains. This change eliminates the autoinhibitory effect of the JH2 pseudokinase on the JH1 kinase of the other JAK2 in the dimer, thus bringing the JH1 kinase into close proximity with JH2, which results in JAK2 phosphorylation at multiple amino acid residues through unknown mechanisms [70].

Crystallographic modeling shows that the V617F mutation causes destabilization of the JH2-SH2 linker, which then destabilizes the JH2-JH1 interaction (Fig. 6.4) [64, 71]. It is also postulated that the V617F mutation may overstabilize the positive regulation mediated by JH2 by an unknown molecular mechanism [64]. The V617F mutation straightens and rigidifies the αC helix within the JH2 domain [64, 72, 73], whereas the wild type (WT) allele has a kink without considerable structural difference [64, 73]. It has been shown that JAK2 JH2 binds adenosine triphosphate (ATP) and phosphorylates negative regulatory sites involved in JAK2 activity [64, 74]. Understanding these mechanisms may provide potential therapeutic targets such as αC, ATP binding pockets; for example novel small molecules can inhibit JAK2 V617F activity without affecting the activity of the WT allele [64]. Currently, the approved JAK2 inhibitors, such as ruxolitinib are type I; these bind to the ATP binding pocket in the kinase domain in its active configuration, thus also

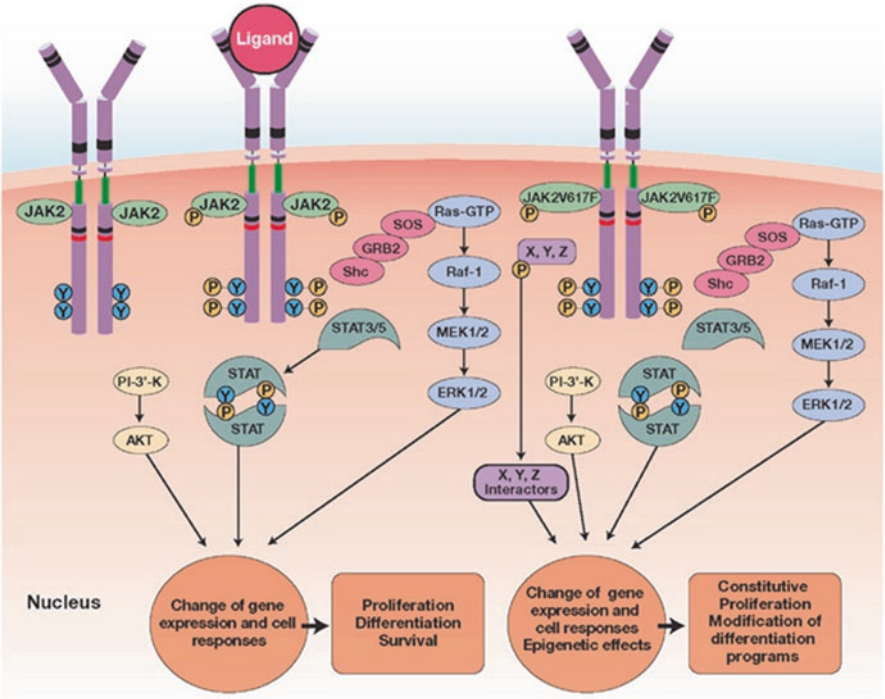


**Fig. 6.2** Cytokine receptors exist on the cell surface in an inactive state bound to JAKs via their cytosolic domains. The binding of a specific ligand induces a conformational change in the preformed dimer, leading to tyrosine phosphorylation and cross-activation of JAKs, which phosphorylate intracellular receptor tyrosine residues (Y-P). In turn, the phosphorylated residues attract signaling adaptor proteins that recognize specific tyrosine phosphorylated sequences. Various adaptor proteins become substrates of JAKs, triggering signaling cascades. Cytokine receptors are linked to the STAT, Ras–MAPK, and phosphatidylinositol-3′-kinase (PI3K)–AKT pathways, which converge at the nucleus and regulate gene expression. *Left*, unliganded inactive cytokine receptor; *middle*, ligand-activated receptor, which is transient and induces anti-apoptotic, proliferative, and differentiation signals. *Right*, unliganded receptor that is constitutively active because of the attachment of JAK2 V617F, a constitutively active JAK, and is therefore recapitulating the cytokine-induced pathway, although in a persistent manner. Novel signaling molecules (X, Y, and Z) become substrates of activated JAK, initiating novel interactions (X, Y, and Z interactors) that change gene expression and/or induce novel epigenetic events (With permission from Vainchenker et al. [68])

affecting WT JAK2. Novel more effective JAK2 type II inhibitors, causing actual decrease in the mutant allele load, are currently developed; these inhibitors bind in a similar fashion to the ATP binding pocket in the kinase domain, but in its inactive configuration [64].

Among the various downstream activators, *STAT5* is also a crucial actor in disease pathogenesis. Hence, there is an opportunity to therapeutically abrogate dysregulation of its transcriptional targets [73]. These targets are PIM kinases, c-MYC and JUNB, CYCLIN D2, P27KIP, CDC25A, PU.1, ID1, BCL-XL, MCL-1, and RAD51 [73]. However, Kouzarides et al. showed that JAK2 alone can be localized

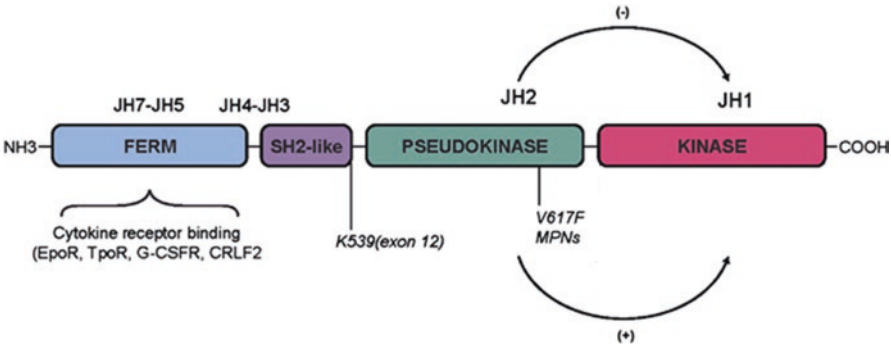


**Fig. 6.3** Domain structure of JAK2. JAK2 contains a tyrosine kinase domain (JAK homology 1 (JH1)), a pseudokinase domain (JH2), SH2-like domain, and a domain that resembles protein 4.1, ezrin, radixin and moesin (FERM). The latter domain is responsible for attachment to the cytosolic domains of cytokine receptors. The pseudokinase domain, JH2, functionally prevents the activation of the kinase domain, JH1 (*curved arrow on the top*). In contrast, mutations in the pseudokinase domain (*red*) lead to activation (*arrow on the bottom*) of JH1. The V617F mutation has been identified primarily in MPNs

in the nucleus and phosphorylate histone Y41, and increase expression of oncogenes such as *MYC* and *LMO2* [75]. One of the mechanisms of cytokine hypersensitivity of JAK2 V617F mutant cells and disease progression is attributed to upregulation of La auto-antigen with resultant p53 inactivation [73, 76]. The *JAK2* V617F mutation simulates fibroblastic growth and proliferation by increasing oncostatin M expression (OSM), a profibrogenic cytokine stimulator of bone marrow fibroblasts. Pathogenic hypercytokinemia can be controlled by potentially targeting OSM [73, 77]. Mutant JAK2 binds more strongly, phosphorylates and downregulates activity of PRMT5, an arginine methyltransferase JAK binding protein. Abnormal histone methylation affects chromatid remodeling, increases hematopoietic stem cell progenitor population, myeloproliferation, and erythroid differentiation [73, 78]. These insights in the pathogenesis provide possible therapeutic strategies.

The *JAK2* V617F mutation is found in MPNs associated with PV (>95%), essential thrombocythemia (50%), PMF (50%) or various other disorders. It is also seen in refractory anemia with ringed sideroblasts associated with marked thromobocytosis (~50%), and rarely in other myelodysplastic syndromes (MDS), MDS/MPNs such as chronic myelomonocytic leukemia (CMML), juvenile myelomonocytic leukemia, hypereosinophilic syndrome, and systemic mastocytosis. The *JAK2* V617F mutation is consistently absent in all secondary bone marrow conditions, unrelated leukemias, and healthy controls [79]. However, screening for the *JAK2* V617F mutation is the first diagnostic test for PV in the appropriate clinicopathological context.

The *JAK2* V617F mutation is the major diagnostic criterion for *BCR-ABL1* negative MPNs (PV, ET, and MF). The three MPNs have different phenotypes, thus this single mutation associated with different phenotypes is of great interest in

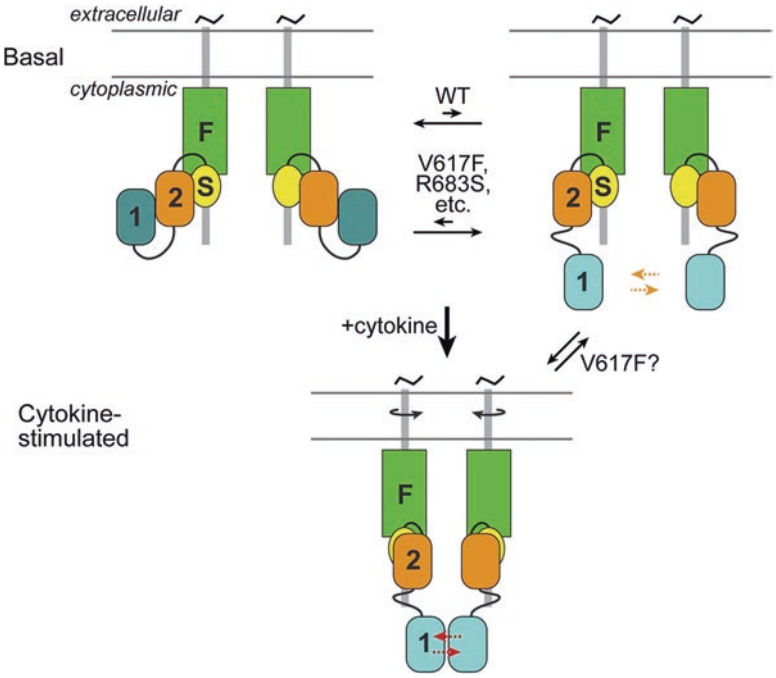


**Fig. 6.4** Schematic of JAK2 activation. Top: In the basal state, two JAK2 molecules associate via their N-terminal band 4.1, ezrin, radixin, moesin (FERM or F) and SH2 (S) domains with predimerized type I cytokine receptors. The autoinhibitory interaction between JH2 [2] and JH1 [1] sequesters JH1 from each other and stabilizes an inactive state of JH1 (*dark cyan*) (*left*). This is in equilibrium with a state (*right*) in which JH1 is disengaged from JH2 (JH1 *cyan*, higher activity), which increases the probability of trans-phosphorylation (*orange arrows*) on the activation loop of JH1. By destabilizing the autoinhibitory interaction, pathogenic mutations, such as V617F, shift the equilibrium to the partially active state. *Bottom*: Cytokine binding to the extracellular region of the cytokine receptors induces a structural rearrangement in the cytoplasmic region (possibly through rotation of the transmembrane helices), which greatly facilitates (*red arrows*) JH1 trans-phosphorylation. JH2 is also necessary for cytokine-induced JAK2 activation, but the molecular interaction(s) responsible for this is not known (and not shown). As indicated, in addition to destabilizing the autoinhibitory interaction, V617F might promote the JH2-mediated positive interaction normally induced by cytokine (or possibly promote an interaction distinct from the cytokine-mediated one)

pathogenesis. Mutations primarily occur at the multipotent HSC level, and are found in granulocytic, erythroid, megakaryocytic, as well as lymphoid lineages [80]. Molecular analysis showed that the *JAK2* V617F mutation enables and perpetuates clonal proliferation distorted towards phenotypic erythrocytosis in PV [81], whereas myeloid differentiation is seen in MF [82, 83]. The same *JAK2* V617F mutation may affect subsets of HSCs with distinct anomalous differentiation to a specific MPN [80]. Preference to develop into PV may be modulated by quantitative differences in the JAK2 type. In murine engraftment experiments, James et al. found that engrafted PV primarily presents with *JAK2* WT with a small proportion that is

heterozygous for the *JAK2* V617F mutation. The authors also found an overwhelming number of *JAK2* V617F mutations that are homozygous are seen in post-PV MF or PMF. Apart from the *JAK2* V617F mutation, additional genetic or other changes and increased responsiveness towards cytokine stimulation of *JAK2* V617F-committed progenitors and HSCs (especially in PMF) may play a role in disease pathogenesis [83, 84]. Another study suggested that PV phenotype is a function of its allelic profile and mutation burden. This study suggested that clone sizes for the *JAK2* V617F mutation are small and heterozygous in PV and ET, whereas early progenitors in heterozygous *JAK2* V617F PV are more erythropoietin-sensitive, dedicated and committed as compared to those in ET. Homozygous PV was found to be a minor subset, whereas progenitors were found to be erythropoietin-independent with preferential proliferation occurring at the terminal stage of differentiation. No homozygous clones were found in ET [85]. In a different study, microsatellite mapping and analysis of clonality in the earliest erythroid progenitors of PV and ET demonstrated that both PV and ET acquire homozygous clones, but PV has a dominant single homozygous mutant subclone 8–85 times higher than the minor mutant subclones. These findings show that the homozygous subclone is a driver for erythrocytosis and hence PV phenotype. Additional genetic or epigenetic changes may also play a role in progression of the disease [86]. Overall, the prevailing view is that *JAK2* V617F alone is not a strong driver mutation with small clone sizes and can remain clinically stable disease over years [73, 85, 87].

Homozygous *JAK2* V617F has been detected in approximately 30% of patients with PV [18, 19, 21, 88]. Homozygosity in PV is associated with older age groups, higher hemoglobin and leukocyte counts, splenomegaly, thrombosis and cardiovascular events, and symptomatic disease with consequent therapy [73, 89]. Chromosome 9p with the mutation *JAK2* V617F is frequently involved in loss of heterozygosity (LOH at 9p) in PV [20, 90]. Thus, the lost allele 9p with the *JAK2* V617F mutation is duplicated by mitotic recombination which itself likely causes LOH at 9p and its duplication by uniparental disomy (UPD), resulting in two copies of *JAK2* V617F allele in the affected cell [20, 73, 90]. This change confers variability, heterogeneity, variable levels of mutant allele with dosage effect, with a progression from heterozygosity to homozygosity in clinical presentation of PV [89]. As discussed earlier, homozygosity appears to be a driver of erythrocytosis (PV phenotype) and adverse prognostic variables. The *JAK2* V617F mutant allelic burden, quantified by PCR, is routinely measured in granulocytes. Generally, mutant allele burdens lower than 50% and higher than 50% are reported as heterozygous and homozygous, respectively. This routinely used terminology can cause confusion and deception regarding mutant *JAK2* V617F genotype because the mutant can comprise either sole or combinations of heterozygous, hetero-homozygous or homozygous cell populations [91]. Nevertheless, it has been demonstrated that a higher allelic load in granulocytes (>50%) corresponds to the adverse effects seen in homozygous population, helps risk stratification by molecular quantification, and is a threshold for initiation of therapy with JAK2 inhibitors [73, 89, 92]. Low allelic burdens can be managed with phlebotomy and aspirin to reduce risk of thrombosis [92].

The mutation *JAK2* V617F appears to confer a slower rate of disease progression, a higher risk for thrombotic or hemorrhagic complications, and an increased degree of MF. In addition, homozygous versus heterozygous clones for *JAK2* V617F may confer a poorer overall prognosis [89]. Several studies showed the absence of correlation between *JAK2* V617F allelic burden and fibrotic risk of transformation [57, 60].

The mutant allelic burden is the single most important factor for risk of thrombosis irrespective of phenotype of *JAK2* V617F disease. Rates of thrombosis increase with increasing allele burden and in particular, the risk increases with >50% *JAK2* V617F [93].

Interestingly, higher *JAK2* V617F mutant allele burdens have been found to be responsive to ruxolitinib therapy [94]. Serial measurements can help predict evolution of disease with increasing mutant allele burdens [95], assess effectiveness of therapy and clinical remission, or guide adoptive immunotherapy such as donor lymphocyte infusion [91, 96].

## Methods for Detection of JAK2V671F and Its Allele Burden

Detection of the *JAK2* V617F mutation is diagnostic of PV in the appropriate clinicopathological setting. Limited information is available on the clinical significance of a specific quantitative allele burden. The majority of research studies examining *JAK2* in PV patient specimens have utilized various DNA sequencing platforms to identify the mutated allele in the purified granulocyte fraction [18–21]. DNA sequencing is routinely and successfully used in many labs, and is considered the "gold standard" [97]. Direct sequencing has low analytical sensitivity (~20%), is time-consuming, and technically demanding [18, 97, 98]. High throughput techniques for targeted massive parallel/next generation sequencing (MPS/NGS) are preferred as they can detect all *JAK2* mutations, and are not limited to V617F and non-driver mutations affecting prognosis [2]. Most molecular diagnostic laboratories have instrumentation and technical expertise readily available to perform real-time polymerase chain reaction (RT-PCR) [95].

It is important to consider pre-analytical variables of the specimen before choosing an assay [79]. Several PCR-based assays for rapid detection of *JAK2* V617F in peripheral blood, bone marrow aspirate, and paraffin-embedded tissue specimens have been studied and compared [95, 99, 100]. A small amount of peripheral blood (2–10 ml) in Ethylenediaminetetraacetic acid (EDTA) is adequate for genomic DNA extraction, and can be frozen for further analysis. Bone marrow aspirate is acceptable [101] and unstained (preferred) unfixed slides [102] can be used for this purpose. Purification of granulocytes for DNA extraction by Ficoll gradient centrifugation and the amount of nucleic acid template depend on the sensitivity and type of assay, respectively [79].

The JAK2 Mutation Working Group of the Association for Molecular Pathology (AMP) and the MPNs and MPN-related congenital disease (MPNr) European Network (MPN and MPNr-EuroNet) suggest that suitable assays should have a low

limit of detection (at least 1% for diagnosis and 0.1% for residual disease monitoring), nearly 100% specificity at the above detection levels, high reproducibility, and transferability between laboratories [91, 103]. Low levels of *JAK2* V617F allele identification should be interpreted carefully. Less than 1% mutant allele burden and very low levels (<0.1%) have been found in ET and in healthy individuals, respectively. Repeated testing after 3–6 months is warranted in these cases [79, 91].

Studies have shown that various PCR assays to detect the *JAK2* V617F mutation are in agreement when the mutant allele load is higher [97, 104]. Overall, allele-specific quantitative PCR (qAS-PCR) or the amplification refractory mutation system PCR (ARMS-PCR) are the most reliable and sensitive assays (~ analytical sensitivity 0.1–5%) [103, 105, 106]. A clinically significant and pathogenic mutant allele burden is found to be in the range 1–3% [79, 107]. Other methods such as high-resolution melting PCR (HRM-PCR) and melting curve analysis display 1–5% analytical sensitivity [79], which is equivalent to that of ARMS-PCR. However, HRM-PCR is easy to perform because it involves a single amplification step versus ARMS-PCR, which amplifies WT and mutant alleles in two separate consecutive reactions [97]. Real time allele-specific PCR with sensitivity of 0.01–1% can be used to assess minimal residual disease and response to JAK2 inhibitor therapy [79, 96, 106, 108]. Similar to direct sequencing, pyrosequencing lacks optimal sensitivity [103]. Digital PCR, which is technically less demanding, has been found to have analytical sensitivity equivalent to that of real time AS-qPCR [109]. Point of care microchips with visual assays based on microfluidics have also been developed and are potentially helpful in limited resource settings [110].

## *JAK2* Exon 12 Mutations

*JAK2* exon 12 mutations are present in approximately 3% of PV patients; they occur across multiple codons – from 533 to 547 – as substitutions, deletions, duplications or insertions, near the pseudokinase domain, in the linker region between the pseudokinase and SH2 domains. The most common mutation in *JAK2* exon 12 (23–30%) is an in-frame deletion of six nucleotides at codons 542 and 543 (N542-E543del). Other mutations encountered in exon 12 are R541-E543delinsK, E543_D544del, F537_K539delinsL, and K539 L. Each mutation occurs with an approximate frequency 10% [91, 103, 111]. Exon 12 mutations result in erythrocytosis phenotype at a younger age (~50 years of age) involving higher hemoglobin and hematocrit *vis-a -vis JAK2* V617F type, normal WBC and platelet counts with low serum erythropoietin, erythroid hyperplasia in bone marrow with rare trilineage expansion, variable megakaryocytic morphology (ranging from small to medium or large megakaryocytes with mono or hyperlobated nuclei). Transformation to leukemia or MF is a rare occurrence, and patients with *JAK2* exon 12 mutated PV have a good prognosis with nearly normal life expectancy [91, 92, 103, 112]. The molecular mechanisms of *JAK2* exon 12 mutations are similar to that of mutation *JAK2*

V617F, with cytokine independent proliferation, and JAK2 inhibitors capable of targeting the pathway. The consequences of these founding mutations on the signaling cascade downstream of JAK2 (increased ERK1 and ERK2) are distinct from those with *JAK2* V617F [111]. An improved course of the disease is expected with higher proportions of stable heterozygous colonies and small amounts or absence of homozygous clones over time [92, 111, 113]. However, in a multicenter study, it was found that PV due to *JAK2* exon 12 mutations initially presents with isolated erythrocytosis, but its subsequent behavior, risk stratification and prognosis are similar to PV due to the *JAK2* V617F mutation [114]. Direct sequencing has limited diagnostic importance if peripheral blood with low mutant allele burden is used. Several *JAK2* exon 12 mutations exist (at least 27 are known), precluding the choice of targeted AS-PCR despite its superior detection sensitivity (10%) [115]. HRM analysis, locked nucleic acid (LNA)-clamped fragment analysis, melting curve assay, dHPLC (denaturing high-pressure liquid chromatography) are used to screen for this mutation. AS-qPCR has a sensitivity of 0.01% for 10 of the most frequent *JAK2* exon mutant alleles (overall 80% for *JAK2* exon 12) [79, 103].

## Latest Somatic Mutations

Molecular analyses have unraveled numerous somatic mutations in MPN with *JAK2*, *MPL*, and *CALR* established as driver mutations (Table 6.2). Additional somatic mutations are "nondriver" mutations, nonspecific for MPNs as they can be found in myeloid malignancies and normal elderly individuals [68]. It has been found that, as a group MPNs have a low frequency of somatic mutations, namely ~0.2 somatic mutations per Mb or 1 somatic mutation per 45 patient years, with most mutations being present early on when the disease is detected [116]. These mutations provide prognostic rather than diagnostic information and some of them occur synergistically or together with the driver mutation, thus providing fertile ground for disease initiation or progression. The number of somatic mutations is a predictor of overall survival and leukemic transformation [2, 116]. The median number of somatic mutations in PV is less than that in PMF (6.5 vs 13 per patient) [117]. Rarely *CALR* mutations have been described in *JAK2* negative PV [118]. The typical triple negative (TN) mutation profile encountered in ET and PMF (overall 10–15%) is essentially not seen in PV; in theory, this could be due to undetected *JAK2*, especially *JAK2* exon 12 variants, or other mutations leading to erythrocytosis. Recent findings on selected mutations are detailed in the next paragraph.

In PV, *TET2* mutations are predominantly found to occur as pre *JAK2* V617F event providing self-renewal and survival advantage to the *JAK2* mutated clone for myeloproliferation with no apparent effect on transformation [116]. Dual concomitant or secondary *TET2* mutations in a disease with *JAK2* V617F mutation are less frequent [116]; but are connected with lower overall survival due to increasing intensity of myeloproliferation and extra-medullary hematopoiesis, and are often acquired at the time of leukemic transformation [119]. TET2 loss may limit

therapeutic efficacy of interferon (IFN)-α as IFN-α acts by depleting the *JAK2* V617F mutated HSCs [120].

*ASXL1* mutations have a relatively low frequency in PV (~2–10%) versus ET (5–10%) and PMF (13–26%), and post PV/ET MF (22–39%) [121]. A study showed that patients with ASXL1 loss of function had significantly lower hemoglobin levels than those without the mutation [116]. The same study noted that despite no specific temporal relation with JAK2 mutation, *ASXL1* mutations were often acquired as a pre *JAK2* event [116]. Loss of ASXL1 is an independent negative prognostic marker in PMF, MDS, CMML [122, 123], and may indicate fibrotic transformation in PV [2]. It was recently shown that ASXL1 has a role in effective erythropoiesis and effective maturation of erythroids; its loss leads to anemia, and anemia confers poor prognosis in MDS, CMML, and PMF [122, 124].

The *EZH2* gene is involved in DNA methylation [116], and its mutations are thought to act synergistically with *JAK2* V617F and result in MPNs [125]. Murine experiments show that heterozygous loss in conjunction with *JAK2* V617F leads to PV, while homozygous mutations result in MF with higher platelet and WBC counts without intermediate erythrocytosis. Experimentally deletion of *EZH2* in mice without *JAK2* V617F mutation resulted in thrombocytosis. Additional studies are necessary to determine the efficacy and suitability of EZH2 inhibitors in MPN, as it is thought to have a tumor suppressor effect.

*DNMT3A* loss is more frequently associated with AML and leukemic transformation in MPN, possibly by upregulating genes such as *RUNX1* and *GATA3* [126]. *DNMT3A* mutations by inhibiting differentiation may provide the circumstance for HSC proliferation in the presence of the *JAK2* mutation with limited self-renewal [127]. The *DNMT3A* R882 mutation has been found to induce leukemia in animal models in presence of NRAS mutation by affecting DNA methylation apparatus [128].

IDH1/2 are enzymes in the tricarboxylic acid (TCA) cycle and fatty acid synthesis producing metabolites involved in epigenetic regulation of progenitor cell differentiation. Although they are less frequently mutated in PV as compared to other MPNs, one study has shown that leukemia-free survival was adversely affected by *IDH2* mutations in PV patients [2]. In the same study, *SRSF2* mutation was found to affect OS, *RUNX1* mutation affected OS and leukemia-free survival among PV patients.

## Clinical Management of PV

Contemporary management of PV is driven by the presence of major risk factors of thrombosis and a history of thrombosis in PV patients [129, 130]. Thrombosis prevention with low-dose aspirin, and management of cardiovascular risk factors is recommended for all PV patients. Phlebotomy remains a key intervention with a target hematocrit less than 45% in patients with PV. Chemotherapy with hydroxyurea should be considered as a first line of therapy depending on the history of

thrombosis, and for patients over 60 years of age. Interferon-α can be used in younger patients as the first line of therapy due to the absence of leukemogenic effect when compared to hydroxyurea, and its ability to induce clinical, hematological and molecular responses with persistence of response after therapy cessation [130]. However, additional mutations such as loss of *TET2* may limit therapeutic efficacy of IFN-α. Failure to respond to the first line of therapy, which is seen in ~16–24% of patients with progressive splenomegaly, leukocytosis, or thrombocytosis may warrant use of ruxolitinib, a JAK2 inhibitor. Although ruxolitinib reduces spleen volume, and alleviates constitutional symptoms, it does not appear to alter the natural course of the disease. Prognostic models developed for PMF cannot be accurately applied to post-PV-MF [130]. However, bone marrow transplantation can be considered for younger patients who rapidly progress to MF or leukemia [129, 130].

## Conclusions

Because the identification of *JAK2* V617F as its driver mutation in 2005, tremendous strides have been made by the scientific community in understanding the pathogenesis of PV. The 2016 WHO classification has updated disease diagnostic criteria, which significantly improves sensitivity to identify patients with PV and distinguish them from patients with other MPNs, thus offering benefit of specific treatment to reducing morbidity and mortality. In addition, molecular dynamic simulations are revealing pathogenic mechanisms involved in PV development, helping to identify new therapeutic targets for precision medicine that will have no or less adverse effects in patients. Moreover, new somatic mutations in PV are being identified through modern sequencing techniques. These mutations have an interesting interplay with *JAK2* V617, providing prognostication of disease evolution and transformation, and thus guiding personalized therapy in patients with PV.

## References

1. Swerdllow S, Campo E, Harris NL. WHO classification of tumours of haematopoietic and lymphoid tissues. Lyon: IARC Press; 2008.
2. Tefferi A, Lasho TL, Finke C, Elala Y, Barraco D, Hanson CA, et al. Targeted next-generation sequencing in polycythemia vera and essential thrombocythemia. Blood. 2015;126(23):354.
3. Vaquez H. Sur une forme spéciale de cyanose s' accompagnant d'hyperglobulie excessive et persistante. CR Soc Biol (Paris). 1892;44:384–8.
4. Osler W. Chronic cyanosis, with polycythemia and enlarged spleen: a new clinical entity. Am J Med Sci. 1903;126(2):187–201.
5. Steensma DP. Polycythemia vera: plethora, from prehistory to present. Curr Hematol Rep. 2005;4(3):230–4.
6. Berlin NI, Wasserman LR. Polycythemia vera: a retrospective and reprise. J Lab Clin Med. 1997;130(4):365–73.

7. Dameshek W. Some speculations on the myeloproliferative syndromes. Blood. 1951;6(4):372–5.
8. Wasserman LR. The treatment of polycythemia. A panel discussion. Blood. 1968;32(3):483–7.
9. Berlin NI. Diagnosis and classification of the polycythemias. Semin Hematol. 1975;12(4): 339–51.
10. Skoda R, Prchal JT. Chronic myeloproliferative disorders – introduction. Semin Hematol. 2005;42(4):181–3.
11. Adamson JW, Fialkow PJ, Murphy S, Prchal JF, Steinmann L. Polycythemia vera: stem-cell and probable clonal origin of the disease. N Engl J Med. 1976;295(17):913–6.
12. Skoda R, Prchal JT. Lessons from familial myeloproliferative disorders. Semin Hematol. 2005;42(4):266–73. Elsevier.
13. Beutler E, Collins Z, Irwin LE. Value of genetic variants of glucose-6-phosphate dehydrogenase in tracing the origin of malignant tumors. N Engl J Med. 1967;276(7):389–91.
14. Prchal JT, Guan Y. A novel clonality assay based on transcriptional analysis of the active X chromosome. Stem Cells. 1993;11(S1):62–5.
15. Prchal JT, Guan YL, Prchal JF, Barany F. Transcriptional analysis of the active X-chromosome in normal and clonal hematopoiesis. Blood. 1993;81(1):269–71.
16. Prchal JT. Polycythemia vera and other primary polycythemias. Curr Opin Hematol. 2005;12(2):112–6.
17. Jaffe ES. Pathology and genetics of tumours of haematopoietic and lymphoid tissues. Lyon: IARC; 2001.
18. Baxter EJ, Scott LM, Campbell PJ, East C, Fourouclas N, Swanton S, et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Lancet. 2005;365(9464):1054–61.
19. James C, Ugo V, Le Couédic J, Staerk J, Delhommeau F, Lacout C, et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. Nature. 2005;434(7037):1144–8.
20. Kralovics R, Passamonti F, Buser AS, Teo SS, Tiedt R, Passweg JR, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med. 2005;352(17):1779–90.
21. Levine RL, Wadleigh M, Cools J, Ebert BL, Wernig G, Huntly BJ, et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. Cancer Cell. 2005;7(4):387–97.
22. Steensma DP, Dewald GW, Lasho TL, Powell HL, McClure RF, Levine RL, et al. The JAK2 V617F activating tyrosine kinase mutation is an infrequent event in both "atypical" myeloproliferative disorders and myelodysplastic syndromes. Blood. 2005;106(4):1207–9.
23. Jones AV, Kreil S, Zoi K, Waghorn K, Curtis C, Zhang L, et al. Widespread occurrence of the JAK2 V617F mutation in chronic myeloproliferative disorders. Blood. 2005;106(6):2162–8.
24. Tefferi A, Gilliland DG. JAK2 in myeloproliferative disorders is not just another kinase. Cell Cycle. 2005;4(8):4053–6.
25. Tefferi A, Gary Gilliland D. The JAK2 V617F tyrosine kinase mutation in myeloproliferative disorders: status report and immediate implications for disease classification and diagnosis. Mayo Clin Proc. 2005;80(7):947–58. Elsevier.
26. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–405.
27. Klampfl T, Gisslinger H, Harutyunyan AS, Nivarthi H, Rumi E, Milosevic JD, et al. Somatic mutations of calreticulin in myeloproliferative neoplasms. N Engl J Med. 2013;369(25):2379–90.
28. Titmarsh GJ, Duncombe AS, McMullin MF, O'Rorke M, Mesa R, Vocht F, et al. How common are myeloproliferative neoplasms? A systematic review and meta-analysis. Am J Hematol. 2014;89(6):581–7.
29. Passamonti F. How I treat polycythemia vera. Blood. 2012;120(2):275–84.
30. Michiels J, Berneman Z, Schroyens W, van Urk H. Aspirin-responsive painful red, blue, black toe, or finger syndrome in polycythemia vera associated with thrombocythemia. Ann Hematol. 2003;82(3):153–9.

31. Michiels JJ. Erythromelalgia and vascular complications in polycythemia vera. Semin Thromb Hemost. 1997;23(5):441–54.
32. Tefferi A, Rumi E, Finazzi G, Gisslinger H, Vannucchi AM, Rodeghiero F, et al. Survival and prognosis among 1545 patients with contemporary polycythemia vera: an international study. Leukemia. 2013;27(9):1874–81.
33. Saini KS, Patnaik MM, Tefferi A. Polycythemia vera-associated pruritus and its management. Eur J Clin Investig. 2010;40(9):828–34.
34. Michiels JJ, Juvonen E. Proposal for revised diagnostic criteria of essential thrombocythemia and polycythemia vera by the Thrombocythemia Vera Study Group. Semin Thromb Hemost. 1997;23:339–47. Copyright© 1997 by Thieme Medical Publishers, Inc.
35. Murphy S. Diagnostic criteria and prognosis in polycythemia vera and essential thrombocythemia. Semin Hematol. 1999;36(1 Suppl 2):9–13.
36. Barbui T, Thiele J, Gisslinger H, Finazzi G, Carobbio A, Rumi E, et al. Masked polycythemia vera (mPV): results of an international study. Am J Hematol. 2014;89(1):52–4.
37. Barbui T, Thiele J, Vannucchi AM, Tefferi A. Myeloproliferative neoplasms: morphology and clinical practice. Am J Hematol. 2016;91(4):430–3.
38. Barbui T, Thiele J, Kvasnicka H, Carobbio A, Vannucchi A, Tefferi A. Essential thrombocythemia with high hemoglobin levels according to the revised WHO classification. Leukemia. 2014;28(10):2092–4.
39. Barbui T, Thiele J, Vannucchi AM, Tefferi A. Rationale for revision and proposed changes of the WHO diagnostic criteria for polycythemia vera, essential thrombocythemia and primary myelofibrosis. Blood Cancer J. 2015;5:e337.
40. Michiels JJ. Bone marrow histopathology and biological markers as specific clues to the differential diagnosis of essential thrombocythemia, polycythemia vera and prefibrotic or fibrotic agnogenic myeloid metaplasia. Hematol J. 2004;5(2):93–102.
41. Sirhan S, Fairbanks VF, Tefferi A. Red cell mass and plasma volume measurements in polycythemia. Cancer. 2005;104(1):213–5.
42. Lussana F, Carobbio A, Randi ML, Elena C, Rumi E, Finazzi G, et al. A lower intensity of treatment may underlie the increased risk of thrombosis in young patients with masked polycythaemia vera. Br J Haematol. 2014;167(4):541–6.
43. Barbui T, Thiele J, Carobbio A, Gisslinger H, Finazzi G, Rumi E, et al. Masked polycythemia vera diagnosed according to WHO and BCSH classification. Am J Hematol. 2014;89(2):199–202.
44. Marchioli R, Finazzi G, Specchia G, Cacciola R, Cavazzina R, Cilloni D, et al. Cardiovascular events and intensity of treatment in polycythemia vera. N Engl J Med. 2013;368(1):22–33.
45. Barbui T, Thiele J, Carobbio A, Guglielmelli P, Rambaldi A, Vannucchi AM, et al. Discriminating between essential thrombocythemia and masked polycythemia vera in JAK2 mutated patients. Am J Hematol. 2014;89(6):588–90.
46. Tefferi A. Polycythemia vera and essential thrombocythemia: 2013 update on diagnosis, risk-stratification, and management. Am J Hematol. 2013;88(6):507–16.
47. Thiele J, Kvasnicka H. Diagnostic impact of bone marrow histopathology in polycythemia vera (PV). Histol Histopathol. 2005;20(1):317–28.
48. Thiele J, Kvasnicka HM, Orazi A. Bone marrow histopathology in myeloproliferative disorders—current diagnostic approach. Semin Hematol. 2005;42(4):184–95. Elsevier.
49. Thiele J, Kvasnicka H, Muehlhausen K, Walter S, Zankovich R, Diehl V. Polycythemia rubra vera versus secondary polycythemias. A clinicopathological evaluation of distinctive features in 199 patients. Pathol Res Pract. 2001;197(2):77–84.
50. Najfeld V, Montella L, Scalise A, Fruchtman S. Exploring polycythaemia vera with fluorescence in situ hybridization: additional cryptic 9p is the most frequent abnormality detected. Br J Haematol. 2002;119(2):558–66.
51. Gribble S, Reid A, Bench A, Huntly B, Grace C, Green A, et al. Molecular cytogenetics of polycythaemia vera: lack of occult rearrangements detectable by 20q LSP screening, CGH, and M-FISH. Leukemia. 2003;17(7):1419–21.

52. Busson M, Romana S, Khac FN, Bernard O, Berger R. Cryptic translocations involving chromosome 20 in polycythemia vera. Ann Genet. 2004;47(4):365–71. Elsevier.
53. Zamora L, Espinet B, Florensa L, Besses C, Woessner S, Serrano S, et al. Is fluorescence in situ hybridization a useful method in diagnosis of polycythemia vera patients? Cancer Genet Cytogenet. 2004;151(2):139–45.
54. Diez-Martin JL, Graham DL, Petitt RM, Dewald GW. Chromosome studies in 104 patients with polycythemia vera. Mayo Clin Proc. 1991;66(3):287–99. Elsevier.
55. Bacher U, Haferlach T, Kern W, Hiddemann W, Schnittger S, Schoch C. Conventional cytogenetics of myeloproliferative diseases other than CML contribute valid information. Ann Hematol. 2005;84(4):250–7.
56. Bench AJ, Pahl HL. Chromosomal abnormalities and molecular markers in myeloproliferative disorders. Semin Hematol. 2005;42(4):196–205. Elsevier.
57. Sever M, Quintás-Cardama A, Pierce S, Zhou L, Kantarjian H, Verstovsek S. Significance of cytogenetic abnormalities in patients with polycythemia vera. Leuk Lymphoma. 2013;54(12):2667–70.
58. Gangat N, Strand J, Li C, Wu W, Pardanani A, Tefferi A. Leucocytosis in polycythaemia vera predicts both inferior survival and leukaemic transformation. Br J Haematol. 2007;138(3):354–8.
59. Boiocchi L, Mathew S, Gianelli U, Iurlo A, Radice T, Barouk-Fox S, et al. Morphologic and cytogenetic differences between post-polycythemic myelofibrosis and primary myelofibrosis in fibrotic stage. Mod Pathol. 2013;26(12):1577–85.
60. Gangat N, Strand J, Lasho TL, Finke CM, Knudson RA, Pardanani A, et al. Cytogenetic studies at diagnosis in polycythemia vera: clinical and JAK2V617F allele burden correlates. Eur J Haematol. 2008;80(3):197–200.
61. Passamonti F, Rumi E, Pietra D, Elena C, Boveri E, Arcaini L, et al. A prospective study of 338 patients with polycythemia vera: the impact of JAK2 (V617F) allele burden and leukocytosis on fibrotic or leukemic disease transformation and vascular complications. Leukemia. 2010;24(9):1574–9.
62. Mossuz P, Girodon F, Donnard M, Latger-Cannard V, Dobo I, Boiret N, et al. Diagnostic value of serum erythropoietin level in patients with absolute erythrocytosis. Haematologica. 2004;89(10):1194–8.
63. Birgegård G, Wide L. Serum erythropoietin in the diagnosis of polycythaemia and after phlebotomy treatment. Br J Haematol. 1992;81(4):603–6.
64. Silvennoinen O, Hubbard SR. Molecular insights into regulation of JAK2 in myeloproliferative neoplasms. Blood. 2015;125(22):3388–92.
65. Li J, Kent DG, Chen E, Green AR. Mouse models of myeloproliferative neoplasms: JAK of all grades. Dis Model Mech. 2011;4(3):311–7.
66. Ugo V, Marzac C, Teyssandier I, Larbret F, Lecluse Y, Debili N, et al. Multiple signaling pathways are involved in erythropoietin-independent differentiation of erythroid progenitors in polycythemia vera. Exp Hematol. 2004;32(2):179–87.
67. Viny AD, Levine RL. Genetics of myeloproliferative neoplasms. Cancer J. 2014;20(1):61–5.
68. Vainchenker W, Delhommeau F, Constantinescu SN, Bernard OA. New mutations and pathogenesis of myeloproliferative neoplasms. Blood. 2011;118(7):1723–35.
69. Vainchenker W, Constantinescu S. JAK/STAT signaling in hematological malignancies. Oncogene. 2013;32(21):2601–13.
70. Brooks AJ, Dai W, O'Mara ML, Abankwa D, Chhabra Y, Pelekanos RA, et al. Mechanism of activation of protein kinase JAK2 by the growth hormone receptor. Science. 2014;344(6185):1249783.
71. Shan Y, Gnanasambandan K, Ungureanu D, Kim ET, Hammarén H, Yamashita K, et al. Molecular basis for pseudokinase-dependent autoinhibition of JAK2 tyrosine kinase. Nat Struct Mol Biol. 2014;21(7):579–84.
72. Bandaranayake RM, Ungureanu D, Shan Y, Shaw DE, Silvennoinen O, Hubbard SR. Crystal structures of the JAK2 pseudokinase domain and the pathogenic mutant V617F. Nat Struct Mol Biol. 2012;19(8):754–9.

73. Chen E, Mullally A. How does JAK2V617F contribute to the pathogenesis of myeloproliferative neoplasms? Hematology Am Soc Hematol Educ Program. 2014;2014(1):268–76.
74. Hammaren HM, Ungureanu D, Grisouard J, Skoda RC, Hubbard SR, Silvennoinen O. ATP binding to the pseudokinase domain of JAK2 is critical for pathogenic activation. Proc Natl Acad Sci U S A. 2015;112(15):4642–7.
75. Dawson MA, Bannister AJ, Göttgens B, Foster SD, Bartke T, Green AR, et al. JAK2 phosphorylates histone H3Y41 and excludes HP1&agr; from chromatin. Nature. 2009;461(7265):819–22.
76. Nakatake M, Monte-Mor B, Debili N, Casadevall N, Ribrag V, Solary E, et al. JAK2V617F negatively regulates p53 stabilization by enhancing MDM2 via La expression in myeloproliferative neoplasms. Oncogene. 2012;31(10):1323–33.
77. Hoermann G, Cerny-Reiterer S, Herrmann H, Blatt K, Bilban M, Gisslinger H, et al. Identification of oncostatin M as a JAK2 V617F-dependent amplifier of cytokine production and bone marrow remodeling in myeloproliferative neoplasms. FASEB J. 2012;26(2):894–906.
78. Liu F, Zhao X, Perna F, Wang L, Koppikar P, Abdel-Wahab O, et al. JAK2V617F-mediated phosphorylation of PRMT5 downregulates its methyltransferase activity and promotes myeloproliferation. Cancer Cell. 2011;19(2):283–94.
79. Bench AJ, White HE, Foroni L, Godfrey AL, Gerrard G, Akiki S, et al. Molecular diagnosis of the myeloproliferative neoplasms: UK guidelines for the detection of JAK2 V617F and other relevant mutations. Br J Haematol. 2013;160(1):25–34.
80. Ishii T, Zhao Y, Sozer S, Shi J, Zhang W, Hoffman R, et al. Behavior of CD34 cells isolated from patients with polycythemia vera in NOD/SCID mice. Exp Hematol. 2007;35(11):1633–40.
81. Jamieson CH, Gotlib J, Durocher JA, Chao MP, Mariappan MR, Lay M, et al. The JAK2 V617F mutation occurs in hematopoietic stem cells in polycythemia vera and predisposes toward erythroid differentiation. Proc Natl Acad Sci U S A. 2006;103(16):6224–9.
82. Xu M, Bruno E, Chao J, Ni H, Lindgren V, Nunez R, et al. The constitutive mobilization of bone marrow-repopulating cells into the peripheral blood in idiopathic myelofibrosis. Blood. 2005;105(4):1699–705.
83. James C, Mazurier F, Dupont S, Chaligne R, Lamrissi-Garcia I, Tulliez M, et al. The hematopoietic stem cell compartment of JAK2V617F-positive myeloproliferative disorders is a reflection of disease heterogeneity. Blood. 2008;112(6):2429–38.
84. James C. The JAK2V617F mutation in polycythemia vera and other myeloproliferative disorders: one mutation for three diseases? Hematology Am Soc Hematol Educ Program. 2008;2008(1):69–75. ASH Education Program Book.
85. Dupont S, Masse A, James C, Teyssandier I, Lecluse Y, Larbret F, et al. The JAK2 617V>F mutation triggers erythropoietin hypersensitivity and terminal erythroid amplification in primary cells from patients with polycythemia vera. Blood. 2007;110(3):1013–21.
86. Godfrey AL, Chen E, Pagano F, Ortmann CA, Silber Y, Bellosillo B, et al. JAK2V617F homozygosity arises commonly and recurrently in PV and ET, but PV is characterized by expansion of a dominant homozygous subclone. Blood. 2012;120(13):2704–7.
87. Gale RE, Allen AJ, Nash MJ, Linch DC. Long-term serial analysis of X-chromosome inactivation patterns and JAK2 V617F mutant levels in patients with essential thrombocythemia show that minor mutant-positive clones can remain stable for many years. Blood. 2007;109(3):1241–3.
88. Li J, Kent DG, Godfrey AL, Manning H, Nangalia J, Aziz A, et al. JAK2V617F homozygosity drives a phenotypic switch in myeloproliferative neoplasms, but is insufficient to sustain disease. Blood. 2014;123(20):3139–51.
89. Tefferi A, Lasho TL, Schwager SM, Strand JS, Elliott M, Mesa R, et al. The clinical phenotype of wild-type, heterozygous, and homozygous JAK2V617F in polycythemia vera. Cancer. 2006;106(3):631–5.
90. Kralovics R, Guan Y, Prchal JT. Acquired uniparental disomy of chromosome 9p is a frequent stem cell defect in polycythemia vera. Exp Hematol. 2002;30(3):229–36.

91. Gong JZ, Cook JR, Greiner TC, Hedvat C, Hill CE, Lim MS, et al. Laboratory practice guidelines for detecting and reporting JAK2 and MPL mutations in myeloproliferative neoplasms: a report of the Association for Molecular Pathology. J Mol Diagn. 2013;15(6):733–44.
92. Michiels JJ, Tevet M, Trifa A, Niculescue-Mizil E, Lupu Aladareanu A, Bumbea H, et al. 2016 WHO clinical molecular and pathological criteria for classification and staging of myeloproliferative neoplasms (MPN) caused by MPN driver. Maedica J Clin Med. 2016;11(1):5–25
93. Barbui T, Falanga A. Molecular biomarkers of thrombosis in myeloproliferative neoplasms. Thromb Res. 2016;140(Suppl 1):S71–5.
94. Barosi G, Klersy C, Villani L, Bonetti E, Catarsi P, Poletto V, et al. JAK2V617F allele burden ⩾50% is associated with response to ruxolitinib in persons with MPN-associated myelofibrosis and splenomegaly requiring therapy. Leukemia. 2016;30(8):1772–5.
95. Olsen RJ, Tang Z, Farkas DH, Bernard DW, Zu Y, Chang C. Detection of the JAK2 V617F mutation in myeloproliferative disorders by melting curve analysis using the lightcycler system. Arch Pathol Lab Med. 2006;130(7):997–1003.
96. Kroger N, Badbaran A, Holler E, Hahn J, Kobbe G, Bornhauser M, et al. Monitoring of the JAK2-V617F mutation by highly sensitive quantitative real-time PCR after allogeneic stem cell transplantation in patients with myelofibrosis. Blood. 2007;109(3):1316–21.
97. Didone A, Nardinelli L, Marchiani M, Ruiz ARL, de Costa L, Lais A, Lima IS, et al. Comparative study of different methodologies to detect the JAK2 V617F mutation in chronic BCR-ABL1 negative myeloproliferative neoplasms. Pract Lab Med. 2016;4:30–7.
98. Frantz C, Sekora DM, Henley DC, Huang CK, Pan Q, Quigley NB, et al. Comparative evaluation of three JAK2V617F mutation detection methods. Am J Clin Pathol. 2007;128(5):865–74.
99. Horn T, Kremer M, Dechow T, Pfeifer WM, Geist B, Perker M, et al. Detection of the activating JAK2 V617F mutation in paraffin-embedded trephine bone marrow biopsies of patients with chronic myeloproliferative diseases. J Mol Diagn. 2006;8(3):299–304.
100. Campbell PJ, Baxter EJ, Beer PA, Scott LM, Bench AJ, Huntly BJ, et al. Mutation of JAK2 in the myeloproliferative disorders: timing, clonality studies, cytogenetic associations, and role in leukemic transformation. Blood. 2006;108(10):3548–55.
101. Larsen TS, Pallisgaard N, Møller MB, Hasselbalch HC. Quantitative assessment of the JAK2 V617F allele burden: equivalent levels in peripheral blood and bone marrow. Leukemia. 2008;22(1):194–5.
102. Jones AV, Silver RT, Waghorn K, Curtis C, Kreil S, Zoi K, et al. Minimal molecular response in polycythemia vera patients treated with imatinib or interferon alpha. Blood. 2006;107(8):3339–41.
103. Langabeer SE, Andrikovics H, Asp J, Bellosillo B, Carillo S, Haslam K, et al. Molecular diagnostics of myeloproliferative neoplasms. Eur J Haematol. 2015;95(4):270–9.
104. Cankovic M, Whiteley L, Hawley RC, Zarbo RJ, Chitale D. Clinical performance of JAK2 V617F mutation detection assays in a molecular diagnostics laboratory: evaluation of screening and quantitation methods. Am J Clin Pathol. 2009;132(5):713–21.
105. Lippert E, Girodon F, Hammond E, Jelinek J, Reading NS, Fehse B, et al. Concordance of assays designed for the quantification of JAK2V617F: a multicenter study. Haematologica. 2009;94(1):38–45.
106. Jovanovic JV, Ivey A, Vannucchi AM, Lippert E, Leibundgut EO, Cassinat B, et al. Establishing optimal quantitative-polymerase chain reaction assays for routine diagnosis and tracking of minimal residual disease in JAK2-V617F-associated myeloproliferative neoplasms: a joint European LeukemiaNet/MPN&MPNr-EuroNet (COST action BM0902) study. Leukemia. 2013;27(10):2032–9.
107. Mason J, Akiki S, Griffiths MJ. Pitfalls in molecular diagnosis in haemato-oncology. J Clin Pathol. 2011;64(4):275–8.
108. Denys B, El Housni H, Nollet F, Verhasselt B, Philippé J. A real-time polymerase chain reaction assay for rapid, sensitive, and specific quantification of the JAK2V617F mutation using a locked nucleic acid-modified oligonucleotide. J Mol Diagn. 2010;12(4):512–9.
109. Kinz E, Leiherer A, Lang A, Drexel H, Muendlein A. Accurate quantitation of JAK2 V617F allele burden by array-based digital PCR. Int J Lab Hematol. 2015;37(2):217–24.

110. Wang H, Liu W, Xu X, Kang Z, Li S, Wu Z, et al. Toward point-of-care testing for JAK2 V617F mutation on a microchip. J Chromatogr A. 2015;1410:28–34.
111. Scott LM, Tong W, Levine RL, Scott MA, Beer PA, Stratton MR, et al. JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. N Engl J Med. 2007;356(5):459–68.
112. Tefferi A, Pardanani A. Myeloproliferative neoplasms: a contemporary review. JAMA Oncol. 2015;1(1):97–105.
113. Pardanani A, Lasho T, Finke C, Hanson CA, Tefferi A. Prevalence and clinicopathologic correlates of JAK2 exon 12 mutations in JAK2V617F-negative polycythemia vera. Leukemia. 2007;21(9):1960–3.
114. Passamonti F, Elena C, Schnittger S, Skoda RC, Green AR, Girodon F, et al. Molecular and clinical features of the myeloproliferative neoplasm associated with JAK2 exon 12 mutations. Blood. 2011;117(10):2813–6.
115. Cazzola M. Somatic mutations of JAK2 exon 12 as a molecular basis of erythrocytosis. Haematologica. 2007;92(12):1585–9.
116. Lundberg P, Karow A, Nienhold R, Looser R, Hao-Shen H, Nissen I, et al. Clonal evolution and clinical correlates of somatic mutations in myeloproliferative neoplasms. Blood. 2014;123(14):2220–8.
117. Nangalia J, Massie CE, Baxter EJ, Nice FL, Gundem G, Wedge DC, et al. Somatic CALR mutations in myeloproliferative neoplasms with nonmutated JAK2. N Engl J Med. 2013;369(25):2391–405.
118. Broseus J, Park JH, Carillo S, Hermouet S, Girodon F. Presence of calreticulin mutations in JAK2-negative polycythemia vera. Blood. 2014;124(26):3964–6.
119. Abdel-Wahab O, Manshouri T, Patel J, Harris K, Yao J, Hedvat C, et al. Genetic analysis of transforming events that convert chronic myeloproliferative neoplasms to leukemias. Cancer Res. 2010;70(2):447–52.
120. Kiladjian JJ, Masse A, Cassinat B, Mokrani H, Teyssandier I, le Couedic JP, et al. Clonal analysis of erythroid progenitors suggests that pegylated interferon alpha-2a treatment targets JAK2V617F clones without affecting TET2 mutant cells. Leukemia. 2010;24(8):1519–23.
121. Abdel-Wahab O, Tefferi A, Levine RL. Role of TET2 and ASXL1 mutations in the pathogenesis of myeloproliferative neoplasms. Hematol Oncol Clin North Am. 2012;26(5):1053–64.
122. Vannucchi AM, Guglielmelli P, Rotunno G, Pascutto C, Pardanani A, Ferretti V, et al. Mutation-enhanced international prognostic scoring system (MIPSS) for primary myelofibrosis: an AGIMM & IWG-MRT project. Blood. 2014;124(21):405.
123. Patnaik MM, Lasho TL, Vijayvargiya P, Finke C, Hanson CA, Ketterling RP, et al. Prognostic interaction between ASXL1 and TET2 mutations in chronic myelomonocytic leukemia. Blood Cancer J. 2016;6(1):e385.
124. Shi H, Yamamoto S, Sheng M, Bai J, Zhang P, Chen R, et al. ASXL1 plays an important role in erythropoiesis. Sci Rep. 2016;6:28789.
125. Shimizu T, Kubovcakova L, Nienhold R, Zmajkovic J, Meyer SC, Hao-Shen H, et al. Loss of Ezh2 synergizes with JAK2-V617F in initiating myeloproliferative neoplasms and promoting myelofibrosis. J Exp Med. 2016;213(8):1479–96.
126. Challen GA, Sun D, Jeong M, Luo M, Jelinek J, Berg JS, et al. Dnmt3a is essential for hematopoietic stem cell differentiation. Nat Genet. 2012;44(1):23–31.
127. Nangalia J, Grinfeld J, Green AR. Pathogenesis of myeloproliferative disorders. Annu Rev Pathol Mech Dis. 2016;11:101–26.
128. Lu R, Wang P, Parton T, Zhou Y, Chrysovergis K, Rockowitz S, et al. Epigenetic perturbations by Arg882-mutated DNMT3A potentiate aberrant stem cell gene-expression program and acute leukemia development. Cancer Cell. 2016;30(1):92–107.
129. Tefferi A. Myeloproliferative neoplasms: a decade of discoveries and treatment advances. Am J Hematol. 2016;91(1):50–8.
130. Besses C, Alvarez-Larrán A. How to treat essential thrombocythemia and polycythemia vera. Clin Lymphoma Myeloma Leuk. 2016;16:S114–23.

# Chapter 7
# Essential Thrombocythemia

**April A. Ewton and Rachel E. Donohue**

## Introduction

Essential thrombocythemia (ET) is classified as a chronic myeloproliferative neoplasm (MPN) involving the megakaryocytic lineage [1]. It was first described by Austrian pathologists, Epstein and Goedel, in 1934 [2], and the definition has evolved over the years. By the most recent diagnostic criteria (Table 7.1), ET is characterized by thrombocytosis $\geq 450 \times 10^9$/L in the peripheral blood; a bone marrow showing increased numbers of enlarged, mature megakaryocytes with hyperlobulated nuclei; no fibrosis or rare grade 1 reticulin fibrosis; exclusion of other myeloid neoplasms; and the presence of mutations involving Janus kinase 2 (*JAK2*), calreticulin (*CALR*), or thrombopoietin receptor, also known as myeloproliferative leukemia virus oncogene (*MPL*) [3].

### *Epidemiology*

The overall age-adjusted incidence rate for ET is 9.6 per one million persons per year. Differences are seen in incidence rates for Blacks (11.5), Caucasians (9.7), and Hispanics (6.4). The median age at diagnosis is 68 years. ET is rarely seen in children, and incidence rates increase exponentially with increasing age. There is a female predilection, with a M:F ratio of 0.8:1, most prominent in women <60 years of age [4].

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**Table 7.1** World Health Organization essential thrombocythemia diagnostic criteria

| |
|---|
| *Major criteria* |
| 1. Platelet count ≥450 × $10^9$/L |
| 2. Bone marrow biopsy showing proliferation mainly of the megakaryocyte lineage with increased numbers of enlarged, mature megakaryocytes with hyperlobulated nuclei. No significant increase or left shift in neutrophil granulopoiesis or erythropoiesis and very rarely minor (grade 1) increase in reticulin fibers |
| 3. Not meeting WHO criteria for *BCR-ABL1*$^+$ CML, PV, PMF, myelodysplastic syndromes, or other myeloid neoplasms |
| 4. Presence of *JAK2, CALR,* or *MPL* mutation |
| *Minor criterion* |
| Presence of a clonal marker or absence of evidence for reactive thrombocytosis |

With permission from Arber et al. [3]
Diagnosis of ET requires meeting all four major criteria or the first three major criteria and the minor criterion

### *Clinical Features*

Up to 50% of patients can be asymptomatic at time of diagnosis, while others may present with vasomotor symptoms, thrombosis, or hemorrhage. Common patient complaints include difficulty sleeping, abdominal discomfort, dizziness/vertigo/lightheadedness, sweats, numbness/tingling in hands/feet and less commonly pruritus, bruising, and fatigue [5].

## Morphology

The 2016 World Health Organization (WHO) diagnostic criteria for ET have been updated to include the mutation status of *CALR* and *MPL* (in addition to *JAK2*, included in the 2008 classification), while the morphological criteria have remained essentially the same.

While morphology has always been an indispensable component of the diagnoses of MPNs, the new criteria emphasize the importance of distinguishing prefibrotic/early primary myelofibrosis (pre-PMF) from ET. Absence of fibrosis or only minimal fibrosis (grade 1) is acceptable for a diagnosis of ET. Although this was implied by the 2008 exclusion criteria for PMF and stated in the footnote, the degree of allowable fibrosis is now directly stated under the major criteria heading of the 2016 classification [3]. This distinction is important clinically, as true patients with ET can survive up to 7 years longer than those with pre-PMF [6]. Pre-PMF has a significantly worse prognosis than ET [6–8]. Compared with pre-PMF, patients with true ET have a lower risk of progression to acute leukemia and high-grade fibrosis, superior overall survival, and higher risk of bleeding complications [7].

## *Peripheral Blood*

The major finding in the peripheral blood is marked thrombocytosis. Platelets can vary in size and shape with occasional bizarre forms. The white blood cell count and differential are typically within normal limits, although they may be borderline high. Red blood cells are typically normochromic, normocytic [1].

## *Bone Marrow*

The bone marrow biopsy is normocellular or shows only a slight increase in age-matched cellularity (Fig. 7.1). Only the megakaryocytic lineage shows increased proliferation. Megakaryocytes are enlarged and increased in number, with mature morphology and hyperlobulated nuclei (staghorn appearance) (Fig. 7.2). Neutrophil

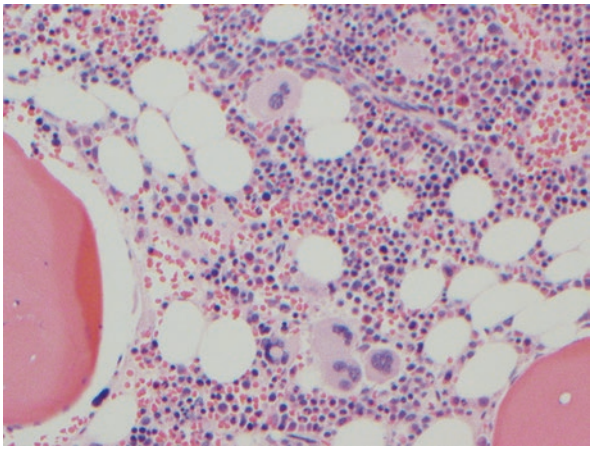

**Fig. 7.1** Bone marrow biopsy, normocellular for age with increased megakaryocytes in essential thrombocythemia (Hematoxylin–Eosin, original magnification ×200)

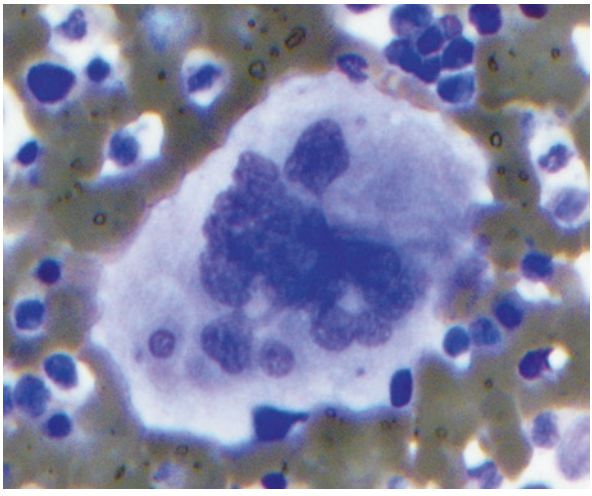

**Fig. 7.2** Large, mature megakaryocyte with hyperlobulated (staghorn) nucleus in essential thrombocythemia (Wright–Giemsa, original magnification ×500)

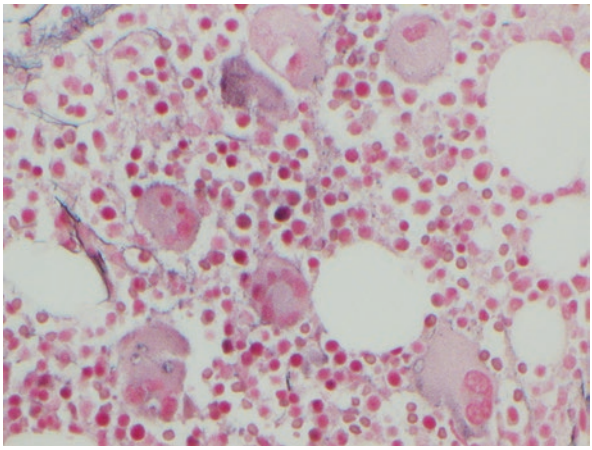

**Fig. 7.3** Bone marrow biopsy with no increase in reticulin fibrosis (grade MF-0) in essential thrombocythemia (reticulin, original magnification ×400). Absence of reticulin fibers, bone marrow core biopsy, reticulin stain, 100×

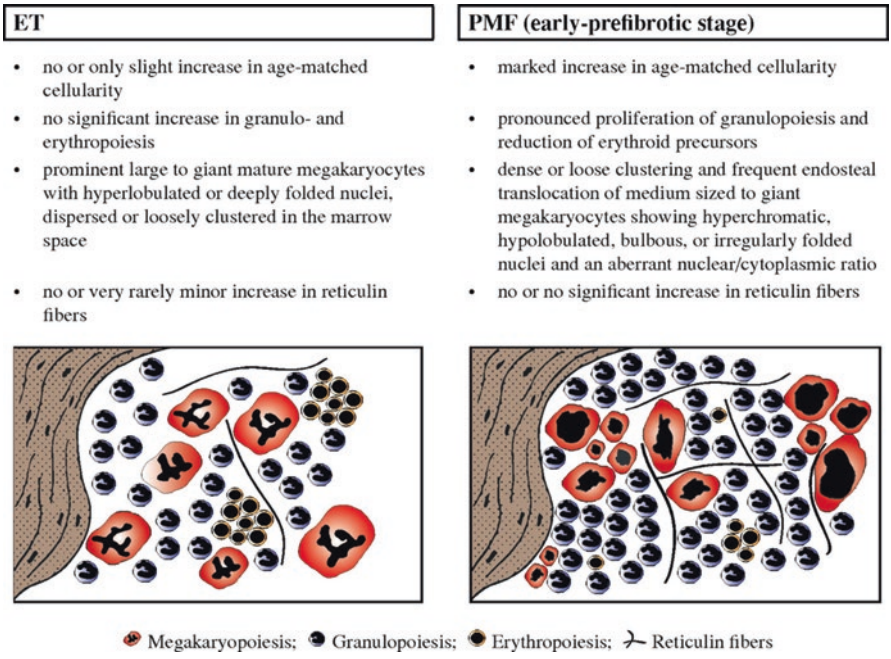


**Fig. 7.4** Diagnostic criteria of distinctive value regarding WHO-defined ET (*left*) versus early-prefibrotic stage of PMF (*right*), including standardized morphological features (see Table 7.1 for more details), allowing the generation of characteristic histological bone marrow patterns (Used with permission from Thiele et al. [6])

granulopoiesis and erythropoiesis are not significantly increased or left shifted. Reticulin fibrosis is absent, or very rarely there is minor (grade 1) increase in reticulin fibers [3, 6, 9] (Fig. 7.3). When differentiating ET from pre-PMF, it is important to note that ET lacks marrow hypercellularity, granulocytic proliferation, significant megakaryocyte clustering, and cloud-like or hyperchromatic megakaryocytes (Fig. 7.4).

## Molecular Features

Research efforts in the last 10 years have resulted in a much better understanding of the molecular pathogenesis of ET and other *BCR/ABL1*-negative MPNs. Cytogenetic abnormalities occur in less than 5% of ET, and no specific recurrent abnormalities have been documented. In contrast, approximately 90% of ET cases carry driver mutations in one of three genes.

Three main driver mutations have now been well characterized in terms of their incidence and clinical effects. *JAK2* is the most commonly mutated gene in MPNs, followed by *CALR* and *MPL*. These driver mutations ultimately result in overproduction of one or more cell lineages. All three driver mutations activate the Janus kinase 2/signal transducer and activator of transcription (JAK/STAT) signaling pathway. In the case of *JAK2* and *MPL* mutations, the mechanisms of action involve constitutive activation of their mutated counterparts, while mutated CALR has been shown to activate the thrombopoietin receptor, MPL [10]. *JAK2* mutation status was included in the 2008 WHO criteria for a diagnosis. In addition to *JAK2*, the 2016 classification includes mutation status of *MPL* and *CALR*. The clinical features of ET are influenced by which gene is mutated, as discussed further below (Table 7.2).

The first recurrent mutation identified in all three BCR/ABL-negative MPNs was *JAK2* V617F, discovered in 2005 by four groups [11–14]. About 50–60% of ET

**Table 7.2** Clinical and laboratory features of 299 patients with essential thrombocytopenia stratified by gene mutation

| Variables | *JAK2*-mutated ($n$ = 159) | *CALR*-mutated ($n$ = 95) | *MPL*-mutated ($n$ = 8) | Triple-negative ($n$ = 37) |
|---|---|---|---|---|
| Age in years; median (range) | 59 (16–88) | 47 (15–91) | 66 (57–85) | 42 (16–81) |
| Age >65 years | 42% | 27% | 50% | 22% |
| Females | 65% | 48% | 38% | 73% |
| Hemoglobin g/dL; median (range) | 14.3 (9.8–17.9) | 13.3 (6.9–16.4) | 12.9 (9.0–15.8) | 13 (8.4–15.9) |
| Leukocytes × $10^9$/L; median (range) | 10.0 (3.9–53.4) | 8.6 (3.3–32.6) | 7.0 (4.0–17.7) | 7.2 (2.8–12.6) |
| Platelets × $10^9$/L; median (range) | 960 (500–3000) | 1082 (454–3460) | 969 (685–2249) | 1000 (557–3300) |
| Leukocytes ≥ 11 × $10^9$/L | 39% | 32% | 25% | 16% |
| Platelets > 1000 × $10^9$/L | 45% | 62% | 50% | 54% |
| Microcirculatory symptoms | 21% | 7% | 13% | 32% |
| Postdiagnosis thrombosis | 26% | 18% | 38% | 11% |
| Deaths (maximum follow-up) | 52% (44 years) | 44% (34 years) | 88% (21 years) | 27% (36 years) |
| Leukemic conversions | 5% | 8% | 25% | 0% |
| Fibrotic progression | 8% | 12% | 38% | 5% |

Adapted from Tefferi et al. [47]; with permission

cases harbor a *JAK2* V617F mutation [15]. JAK2 is a protein tyrosine kinase. In normal megakaryocytes, binding of thrombopoietin to the thrombopoietin receptor results in JAK2 autophosphorylation, recruitment of STAT, and phosphorylation of STAT. The phosphorylated STAT dimerizes and moves to the nucleus, where it activates transcription of genes resulting in proliferation of platelets. The *JAK2* V617F mutation deactivates the repressor pseudokinase domain of JAK2, resulting in activation of JAK2 and downstream signaling pathways in the absence of the appropriate activating ligand.

The percentage of cells with the *JAK2* V617F mutation, or allele burden, affects the clinical features of the disease. Cells homozygous for *JAK2* V617F mutation also contribute to a higher allele burden. Low *JAK2* V617F allele burden is typically seen in ET and pre-PMF, with intermediate levels in polycythemia vera (PV), high levels in fibrotic PMF, and very high levels seen in post-PV MF. The percentage of*JAK2* V617F homozygous granulocytes is also higher in PV and PMF than in ET [16]. Some MPN experts suggest that ET and PV may be different stages of the same disease, with ET representing early, low allelic burden disease, and PV and PMF representing a higher allelic burden later in the disease course (Fig. 7.5) [17]. Recent studies suggest a diagnosis of true ET is unusual in patients with a *JAK2* V617F allele burden over 50% [18]. Increased *JAK2* V617F allele burden in ET is associated with increased splenomegaly, microvessel disease, higher leukocyte count, and history of thrombosis [19, 20]. Although less than 5% of patients with ET are homozygous for *JAK2* V617F, homozygosity also shows correlation with clinical features. Patients with ET homozygous for *JAK2* V617F are more likely than heterozygotes to have splenomegaly (73% vs 28%), cardiovascular events (43% vs 12%), and progression to myelofibrosis (14% vs 5%) [21].

The second most commonly mutated gene in ET is calreticulin (*CALR*). In 2013, two groups discovered *CALR* mutations in the majority of patients with *JAK2*-negative ET [22, 23]. About 20–25% of ET cases have *CALR* mutations [15]. CALR normally acts as a protein chaperone that helps newly synthesized proteins fold properly in the endoplasmic reticulum (ER). CALR is also a calcium ion ($Ca^{2+}$) transporter that regulates $Ca^{2+}$ levels between the ER and the cytoplasm of cells. The carboxy end of CALR is enriched in negatively charged amino acids that promote binding of $Ca^{2+}$. The carboxy terminal also has a four amino acid sequence – lysine, aspartic acid, glutamic acid, leucine (KDEL) – that acts as a signal for CALR to be retained in the ER. *CALR* mutations include more than 50 different insertions and deletions (indels). These indels result in altered charge of the carboxy end of the CALR protein, which is the primary calcium-binding domain. The two most common *CALR* mutations are classified as type 1 (a 52 base pair deletion in exon 19) and type 2 (a 5 base pair insertion in exon 19). Type 1 *CALR* mutation is seen in approximately 50% of *CALR*-mutated ET cases, while type 2 is seen in approximately 30%. Type 1-like mutations result in a loss of the majority of the negatively charged amino acids in the calcium-binding region of CALR, and type 2-like mutations result in loss of approximately half the positively charged amino acids in this region. A third group, seen in approximately 10% of cases, includes indels that are typically classified as type 1-like and type 2-like based on the expected change in charge of the mutated CALR carboxy terminus [24].

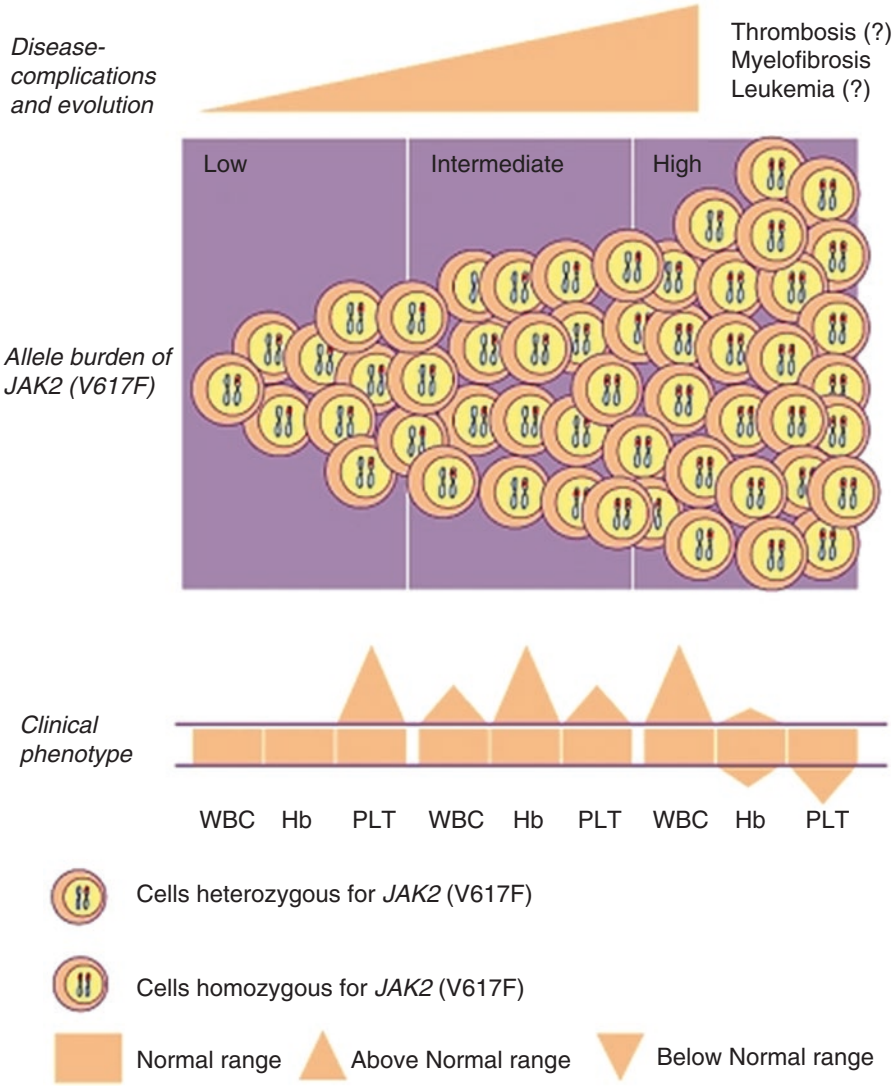


**Fig. 7.5** Schematic representation of *JAK2* (V617F) allele burden (*middle panel*) and its relationship with clinical phenotype (*bottom panel*), and disease complications (*top panel*). At low levels of mutant allele, the clinical phenotype is dominated by thrombocytosis, at intermediate levels by erythrocytosis, and at higher levels by leukocytosis. Among complications, current evidence indicates a relationship between allele burden and evolution into myelofibrosis (Used with permission from Passamonti et al. [17])

Compared with *JAK2* V617F-positive ET, *CALR*-mutated patients with ET present at a younger age and have a lower risk of thrombosis and higher platelet counts [25]. Within the group of CALR-mutated cases, type 1 mutations are more frequently associated with features of PMF at presentation than type 2 mutated cases. ET type 1 mutated cases have a higher risk of progression to post-ET MF than type 2 mutated cases. In contrast, patients with type 2 *CALR* mutations more frequently have features of ET at presentation as well as very high platelet counts and lower risk for thrombosis [24]. Increased *CALR* mutant allele burden has been demonstrated in post-ET MF [26].

Approximately 3–5% of ET cases have *MPL* mutations involving exon 515. These include W515L, W515K, W515A, W515S, and W515R [27]. These mutations result in constitutive activation of the JAK-STAT pathway via the thrombopoietin receptor encoded by *MPL*, as well as increased sensitivity of the receptor to thrombopoietin. *MPL*-mutated patients with ET tend to be older and have a higher rate of progression to fibrosis and acute myeloid leukemia (AML).

Approximately 10% of MPNs have none of the three main driver mutations and are referred to as triple-negative MPNs. A small subset of these "triple-negative" cases has been shown to have novel mutations in *JAK2* or *MPL*. The remaining cases have as yet unidentified abnormalities or rare mutations. Whole exome sequencing of triple-negative ET cases in one study uncovered the following mutations: *JAK2* G571S (germline), *ITGAV* R333H, *WBSCR28* A201T, and loss of chromosome 4q [27]. Lymphocyte-specific adapter protein (*LNK*) also known as src homology *2B3SH2B* adapter protein 3 (*SH2B3*) is mutated in rare cases of ET [28].

In addition to driver mutations, many patients with ET harbor mutations in other genes involved in epigenetic modification, RNA splicing, and cell signaling pathways (Table 7.3). Data regarding the frequency of these nondriver mutations in ET specifically are difficult to extract from the literature due to the inclusion of mixed cases of MPNs and small numbers of cases in most series; however, in one study including 69 patients with ET, 62% had only a single driver mutation, 22% had one additional mutation, and 3% had two additional mutations. The most commonly mutated nondriver genes in ET were *DNMT3A*, *TET2*, and *TP53* [29]. *TET2* was reported to occur in approximately 5% of ET in one study [30]. In another study, 15% of patients with ET had *TET2* mutations, 12.5% had *ASXL1* mutations, and less than 1% of patients had mutations in *SRSF2*, *SF3B1*, *IDH1*, *IDH2* or *GATA1* [31]. Single ET cases had mutations involving each of the following genes: *ASXL1*, *EXH2*, *CUX1*, *PIK3R2*, *SH2B3*, and del7q [29].

The order of acquisition of gene mutations also affects the clinical features of disease. Calreticulin mutations generally occur before other mutations. *JAK2*-positive cases may have mutations in *TET2* and/or *DNMT3A* that generally occur before *JAK2* V617F [29].

Cases in which *TET2* or *DNMT3A* mutations occur before *JAK2* mutations are more frequently associated with clinical features of ET, while cases carrying one of these mutations with *JAK2* mutated first are reportedly more frequently associated with features of PV [25].

**Table 7.3** Acquired mutations in sporadic essential thrombocythemia

| Gene | Mutation location | Genomic location | Protein | Frequency of mutation |
|---|---|---|---|---|
| *JAK2* | V617F exon 14 | 9p24 | JAK2 | 50–60% |
| *JAK2* | Various indels, exon 12 | 9p24 | Jak2 | Rare |
| MPL | W515 K/L/A S505 N | 1p34 | TpoR | 3–5% |
| CBL | Point mutations, exons 8 and 9 | 11q23 | CBL | Rare |
| TET2 | Mutations across gene | 4q24 | TET2 | 5% |
| SH2B3 | Various mutations, mainly exon 2 | 12q24 | LNK | 3–6% |
| ASXL1 | Mutations across gene | 20q11 | ASXL1 | 2–5% |
| EZH2 | Various mutations across gene | 7q36 | EZH2 | 1% |
| DNMT3A | Mutations across gene | 2p23 | DNMT3A | 1–5% |
| IDH1/IDH2 | Mainly IDH1 R132 or IDH2 R140 | 2q23/15q26 | IDH1/IDH2 | Rare |

Adapted with permission from Jones et al. [48]

Finally, host factors may also predispose patients to ET. Several single nucleotide polymorphisms (SNPs) have been shown to be associated with increased risk of developing MPNs. Compared with other SNPs, the 46/1 haplotype of *JAK2*, found in approximately 50% of healthy Caucasians, carries three to four times the risk of developing a MPN, not only mutated *JAK2* but also wild-type *JAK2* [32, 33]. The risk of ET in first-order relatives of 46/1 *JAK2* patients with ET is increased 12 times [33]. Other less common *JAK2* SNPs have also been associated with increased risk for ET [34]. Additional genes with SNPs associated with increased risk of ET and other Ph-negative MPNs include telomerase reverse transcriptase (*TERT*), *TET2*, and *SH2B* adapter protein 3 (*SH2B3*), also known as *LNK*, *HBS1L/MYB*, and *MECOM* [35, 36].

## Prognosis and Therapy

Prognosis in ET is determined by multiple clinical factors, including patient's age, hematologic parameters, mutation status, and type of previous therapy. The international prognostic score for essential thrombocythemia (IPSET-thrombosis) is the current standard for risk stratification in patients with ET and is based on age, history of thrombosis, and *JAK2* V617F mutation status [37–39]. In this model, patients' risk of thrombosis is stratified as follows: very low = age $\leq$ 60, JAK2-negative, no prior thrombosis; low = age $\leq$ 60, *JAK2*-positive, no prior thrombosis; intermediate = age > 60, *JAK2*-negative, no prior thrombosis; and high = prior thrombosis or age > 60 and *JAK2*-positive. The model predicts the risk of vascular events in patients with ET, ranging from very low risk, 0.44% patients per year, to high risk, 4.17% patients per year.

The rates of overall survival and disease progression vary in different studies, most likely due to data collected in studies including a mixed population of patients

diagnosed as ET that include pre-PMF patients. When taken as a pure population, patients with ET have rates of progression to post-ET MF of less than 1% at 5 and 10 years and approximately 9% at 15 years. The rate of progression of ET to AML is less than 1% at 5 and 10 years and approximately 2% at 20 years. Death rates are approximately 3% at 5 years, 5% at 10 years, and 25% at 15 years [11].

Risk factors for progression of ET to MF include older age, anemia, and absence of *JAK2* V6178F. Risk factors for progression of ET to AML include history of thrombosis and extreme thrombocytosis. Risk factors for death include older age, leukocytosis greater than $11 \times 10^9$/L, hemoglobin less than 12 g/dL, and history of thrombosis [11].

As described above, there are differences in the clinical features of ET based on the driver mutation causing the disease. Some molecular features have also been found to be correlated with prognosis. Overall survival is similar in *JAK2*-mutated and *CALR*-mutated cases, but is inferior in patients with MPL mutations and better in triple-negative cases [39]. However, patients with *MPL* mutations are older at presentation, accounting for the poor survival in that group [39]. Of the common nondriver mutations, *SRSF2* is associated with inferior survival in post-ET MF, whereas *EZH2*, *ASXL1*, *IDH1*, an *IDH2* mutations had no effect on survival [26]. Mutations in *TP53* are often seen at the time of leukemic transformation [40].

Treatment is primarily directed toward lowering the platelet count and decreasing the risk of thrombosis and bleeding complications. Treatment is tailored to each patient's risk profile. Patients are classified as high risk or low risk based on age and history of thrombosis. High-risk patients are those 60 years of age and older and/or those with a history of thrombosis. Low-risk patients are those under 60 with no history thrombosis. Secondary risk factors that may also be considered when selecting a treatment regimen are *JAK2* V617 status and the presence or absence of cardiovascular risk factors (diabetes, hypertension, and smoking history). The presence of either or both of these risk factors is associated with increased risk of thrombosis and may indicate a need for more aggressive therapy [41].

First-line therapy for all patients with ET is once-daily, low-dose aspirin unless contraindicated. In addition, cytoreductive treatment is recommended for patients 60 years and older and for patients with a history of arterial thrombosis with *JAK2* V617 positivity and/or the presence of cardiovascular risk factors. Patients with a history of venous thrombosis may receive systemic anticoagulation in addition to cytoreductive therapy and aspirin.

Cytoreductive agents frequently used to treat ET include hydroxyurea (HU), anagrelide, and pegylated interferon alpha-2a (PEG-IFNα-2a). HU is the frontline cytoreductive drug most often used. HU treatment has been shown to decrease platelet counts and risk of thrombosis.

PEG-IFNα-2a or anagrelide may be used in patients who do not tolerate HU. Long-term IFNα treatment has been shown to induce complete hematologic remission in 77% of patients with ET and complete molecular remission in 17% of patients with ET. In one study, the efficacy of PEG-IFNα-2a to achieve a complete molecular response was found to be influenced by the presence of somatic mutations in addition to *JAK2* (*CALR* status was unknown at the time of the study).

In particular, patients with *TET2* mutations showed a smaller decrease in *JAK2* allele burden with treatment and a lower rate of complete molecular response [42]. PEG-IFNα-2a may also be effective in treating *CALR*-positive ET. Complete long-term hematologic remission was documented in two patients with *CALR*-positive ET. At the time of the report, these patients had been in remission for 18 months and over 5 years, respectively, after discontinuation IFNα [43].

Anagrelide, a drug that inhibits maturation of megakaryocytes into platelets, has also been used as a cytoreductive treatment in patients with ET who cannot tolerate or are resistant to HU. This drug has been associated with a greater incidence of thrombotic events and a higher incidence of transformation to acute leukemia compared with HU in one study [44], but with no adverse effects relative to HU in another study [45].

Although the Janus kinase inhibitors including ruxolitinib have been used to treat PV and PMF, there are few studies documenting Janus kinase inhibitor treatment in ET. In one study, ruxolitinib decreased platelet and leukocyte counts, reduced spleen size, and improved disease-related symptoms in patients with ET [46].

## Conclusion

While morphological criteria for the diagnosis of ET have changed little, our understanding of the molecular underpinnings of this disease have progressed greatly in recent years. The updated 2016 WHO morphological diagnostic criteria remain similar to the 2008 classification, with emphasis on the distinction of ET from pre-PMF based on morphological and laboratory values [3].

In contrast, the molecular diagnostic WHO criteria now incorporate mutation status of *CALR* and *MPL*, in addition to the previously known driver mutations in *JAK2*. Clinical features including age at presentation, cell counts, propensity for thrombosis, and risk of progression to more aggressive disease are influenced by molecular features, including which gene is mutated, the structure of the mutated protein, and in some cases by the mutant allele burden. Furthermore, nondriver mutations and the order in which they are acquired relative to driver mutations also have clinical correlates.

With greater understanding of these molecular features, the future holds great potential for expanding targeted therapy for ET and other MPNs beyond Janus kinase inhibitors.

## References

1. Thiel J, Kvasnicka HM, Orazi A, et al. Essential thrombocythaemia. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al., editors. WHO classification of tumours of haematopoietic and lymphoid tissues. 4th ed. Lyon: IARC; 2008. p. 48–50.

2. Epstein E, Goedel A. Hamorrhagischethrombo-zythamiebeivascularerschrumpfmilz (hemorrhagic thrombocythemia with a vascular, sclerotic spleen). Virchows Arch A Pathol Anat Histopathol. 1934;293:233–48.
3. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–405. doi:10.1182/blood-2016-03-643544.
4. Srour SA, Devesa SS, Morton LM, et al. Incidence and patient survival of myeloproliferative neoplasms and myelodysplastic/myeloproliferative neoplasms in the united states, 2001–12. Br J Haematol. 2016;174(3):382–96. doi:10.1111/bjh.14061.
5. Mesa RA, Miller CB, Thyne M, et al. Differences in treatment goals and perception of symptom burden between patients with myeloproliferative neoplasms (MPNs) and hematologists/oncologists in the United States: findings from the MPN landmark survey. Cancer. 2016. doi:10.1002/cncr.30325.
6. Thiele J, Kvasnicka HM, Mullauer L, et al. Essential thrombocythemia versus early primary myelofibrosis: a multicenter study to validate the WHO classification. Blood. 2011;117(21):5710–8. doi:10.1182/blood-2010-07-293761.
7. Barbui T, Thiele J, Passamonti F, et al. Survival and disease progression in essential thrombocythemia are significantly influenced by accurate morphologic diagnosis: an international study. J Clin Oncol. 2011;29(23):3179–84. doi:10.1200/JCO.2010.34.5298.
8. Gisslinger H, Jeryczynski G, Gisslinger B, et al. Clinical impact of bone marrow morphology for the diagnosis of essential thrombocythemia: comparison between the BCSH and the WHO criteria. Leukemia. 2016;30(5):1126–32. doi:10.1038/leu.2015.360.
9. Gianelli U, Vener C, Bossi A, et al. The European consensus on grading of bone marrow fibrosis allows a better prognostication of patients with primary myelofibrosis. Mod Pathol Off J US Can Acad Pathol Inc. 2012;25(9):1193–202. doi:10.1038/modpathol.2012.87.
10. Chachoua I, Pecquet C, El-Khoury M, et al. Thrombopoietin receptor activation by myeloproliferative neoplasm associated calreticulin mutants. Blood. 2016;127:1325–35. doi:10.1182/blood-2015-11-681932.
11. Baxter EJ, Scott LM, Campbell PJ, et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Lancet. 2005;365(9464):1054–61. doi:10.1016/S0140-6736(05)71142-9.
12. James C, Ugo V, Le Couedic J-P, et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. Nature. 2005;434(7037):1144–8. doi:10.1038/nature03546.
13. Kralovics R, Passamonti F, Buser AS, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med. 2005;352(17):1779–90. doi:10.1056/NEJMoa051113.
14. Levine RL, Wadleigh M, Cools J, et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. Cancer Cell. 2005;4:387–97. doi:10.1016/j.ccr.2005.03.023.
15. Barbui T, Thiele J, Vannucchi AM, et al. Rationale for revision and proposed changes of the WHO diagnostic criteria for polycythemia vera, essential thrombocythemia and primary myelofibrosis. Blood Cancer J. 2015;5(8):e337. doi:10.1038/bcj.2015.64.
16. Scott LM, Scott MA, Campbell PJ, et al. Progenitors homozygous for the V617F mutation occur in most patients with polycythemia vera, but not essential thrombocythemia. Blood. 2006;108:2435–7. doi:10.1182/blood-2006-04-018259.
17. Passamonti F, Rumi E, Daniela P, et al. Relation between JAK2 (V617F) mutation status, granulocyte activation, and constitutive mobilization of CD34+ cells into peripheral blood in myeloproliferative disorders. Blood. 2006;107:3676–82. doi:10.1182/blood-2005-09-3826.
18. Hussein K, Bock O, Theophile K, von Neuhoff N, et al. JAK2(V617F) allele burden discriminates essential thrombocythemia from a subset of prefibrotic-stage primary myelofibrosis. Exp Hematol. 2009;37(10):1186–1193.e7. doi:10.1016/j.exphem.2009.07.005.
19. Antonioli E, Guglielmelli P, Poli G, et al. Influence of JAK2V617F allele burden on phenotype in essential thrombocythemia. Haematologica. 2008;93(1):41–8. doi:10.3324/haematol.11653.

20. Tefferi A, Strand JJ, Lasho TL, et al. Bone marrow JAK2V617F allele burden and clinical correlates in polycythemia vera. Leukemia. 2007;21(9):2074–5.
21. Vannucchi AM, Antonioli E, Guglielmelli P, et al. Clinical profile of homozygous JAK2 617V>F mutation in patients with polycythemia vera or essential thrombocythemia. Blood. 2007;110(3):840–6. doi:10.1182/blood-2006-12-064287.
22. Nangalia J, Massie CE, Baxter EJ, et al. Somatic CALR mutations in myeloproliferative neoplasms with nonmutated JAK2. NEJM. 2013;369(25):2391–405. doi:10.1056/NEJMoa1312542.
23. Klampfl T, Gisslinger H, Harutyunyan AS, et al. Somatic mutations of calreticulin in myeloproliferative neoplasms. N Engl J Med. 2013;369(25):2379–90. doi:10.1056/NEJMoa1311347.
24. Pietra D, Rumi E, Ferretti VV, Di Buduo CA, et al. Differential clinical effects of different mutations subtypes in CALR-mutant myeloproliferative neoplasms. Leukemia. 2016;30(2):431–8. doi:10.1038/leu.2015.277.
25. Palandri F, Latagliata R, Polverelli N, et al. Mutations and long-term outcome of 217 young patients with essential thrombocythemia or early primary myelofibrosis. Leukemia. 2015;29(6):1344–9. doi:10.1038/leu.2015.87.
26. Rotunno G, Pacilli A, Artusi V, et al. Epidemiology and clinical relevance of mutations in post-polycythemia vera and postessential thrombocythemia myelofibrosis: a study on 359 patients of the AGIMM group. Am J Hematol. 2016;91(7):681–6. doi:10.1002/ajh.24377.
27. Milosevic Feenstra JD, Nivarthi H, Gisslinger H, et al. Whole-exome sequencing identifies novel MPL and JAK2 mutations in triple-negative myeloproliferative neoplasms. Blood. 2016;127(3):325–32. doi:10.1182/blood-2015-07-661835.
28. ST O, Simonds EF, Jones C, et al. Novel mutations in the inhibitory adaptor protein LNK drive JAK-STAT signaling in patients with myeloproliferative neoplasms. Blood. 2010;116:988–92. doi:10.1182/blood-2010-02-270108.
29. Lundberg P, Karow A, Nienhold R, et al. Clonal evolution and clinical correlates of somatic mutations in myeloproliferative neoplasms. Blood. 2014;123(14):2220–8. doi:10.1182/blood-2013-11-537167.
30. Tefferi A, Pardanani A, Lim KH, et al. TET2 mutations and their clinical correlates in polycythemia vera, essential thrombocythemia and myelofibrosis. Leukemia. 2009;23(5):905–11. doi:10.1038/leu.2009.47.
31. Delic S, Rose D, Kern W, et al. Application of an NGS-based 28-gene panel in myeloproliferative neoplasms reveals distinct mutation patterns in essential thrombocythaemia, primary myelofibrosis and polycythaemia vera. Br J Haematol. 2016. doi:10.1111/bjh.14269.
32. Landgren O, Goldin L, Kristinsson S, et al. Increased risks of polycythemia vera, essential thrombocythemia, and myelofibrosis among 24,577 first-degree relatives of 11,039 patients with myeloproliferative neoplasms in Sweden. Blood. 2008;112:2199–204. doi:10.1182/blood-2008-03-143602.
33. Olcaydu D, Harutyunyan A, Jager R, et al. A common JAK2 haplotype confers susceptibility to myeloproliferative neoplasms. Nat Genet. 2009;41:450–4. doi:10.1038/ng.341.
34. Pardanani A, Fridley BL, Lasho TL, et al. Host genetic variation contributes to phenotypic diversity in myeloproliferative disorders. Blood. 2008;111:2785–9. doi:10.1182/blood-2007-06-095703.
35. Hinds DA, Barnholt KE, Mesa RA, et al. Germ line variants predispose to both JAK2 V617F clonal hematopoiesis and myeloproliferative neoplasms. Blood. 2016;128:1121–8. doi:10.1182/blood-2015-06-652941.
36. Tapper W, Jones AV, Kralovics R, et al. Genetic variation at MECOM, TERT, JAK2 and HBS1L-MYB predisposes to myeloproliferative neoplasms. Nat Commun. 2015;6:6691. doi:10.1038/ncomms7691.
37. Barbui T, Vannucchi AM, Buxhofer-Ausch V, et al. Practice-relevant revision of IPSET-thrombosis based on 1019 patients with WHO-defined essential thrombocythemia. Blood Cancer J. 2015;5:e369. doi:10.1038/bcj.2015.94.

38. Haider M, Gangat N, Lasho T, et al. Validation of the revised international prognostic score of thrombosis for essential thrombocythemia (IPSET-thrombosis) in 585 mayo clinic patients. Am J Hematol. 2016;91(4):390–4. doi:10.1002/ajh.24293.
39. Tefferi A, Guglielmelli P, Larson DR, et al. Long-term survival and blast transformation in molecularly annotated essential thrombocythemia, polycythemia vera, and myelofibrosis. Blood. 2014;124:2507–13. doi:10.1182/blood-2014-05-579136.
40. Cerquozzi S, Tefferi A. Blast transformation and fibrotic progression in polycythemia vera and essential thrombocythemia: a literature review of incidence and risk factors. Blood Cancer J. 2015;5:e366. doi:10.1038/bcj.2015.95.
41. Tefferi A, Barbui T. Polycythemia vera and essential thrombocythemia: 2015 update on diagnosis, risk-stratification and management. Am J Hematol. 2015;90(2):162–73. doi:10.1002/ajh.23895.
42. Quintás-Cardama A, Abdel-Wahab O, Manshouri T, et al. Molecular analysis of patients with polycythemia vera or essential thrombocythemia receiving pegylated interferon α-2a. Blood. 2013;122:893–901. doi:10.1182/blood-2012-07-44201.
43. Cassinat B, Verger E, Kiladjian JJ. Interferon alfa therapy in CALR-mutated essential thrombocythemia. N Engl J Med. 2014;371(2):188–9. doi:10.1056/NEJMc1401255.
44. Landolfi R, Marchioli R, Kutti J, et al. Efficacy and safety of low-dose aspirin in polycythemia vera. N Engl J Med. 2004;350(2):114–24. doi:10.1056/NEJMoa035572.
45. Gisslinger H, Gotic M, Holowiecki J, et al. Anagrelide compared with hydroxyurea in WHO-classified essential thrombocythemia: the ANAHYDRET Study, a randomized controlled trial. Blood. 2013;121(10):1720–8. doi:10.1182/blood-2012-07-443770.
46. Verstovsek S, Passamonti F, Rambaldi A, et al. Long-term results from a phase II open-label study of ruxolitinib in patients with essential thrombocythemia refractory to or intolerant of hydroxyurea. Blood. 2014;124:184.
47. Tefferi A, Wassie EA, Lasho TL, et al. Calreticulin mutations and long-term survival in essential thrombocythemia. Leukemia. 2014;(12):2300–3. doi:10.1038/leu.2014.148.
48. Jones AV, Cross NC. Inherited predisposition to myeloproliferative neoplasms. Ther Adv Hematol. 2013;4:237–53.

## Recommended Reading

Milosevic JD, Kralovics R. Genetic and epigenetic alterations of myeloproliferative disorders. Int J Hematol. 2013;97(2):183–97. doi:10.1007/s12185-012-1235-2.
Reilly JT. Pathogenetic insight and prognostic information from standard and molecular cytogenetic studies in the BCR-ABL-negative myeloproliferative neoplasms (MPNs). Leukemia. 2008;22:1818–27. doi:10.1038/leu.2008.218.
Rumi E, Pietra D, Ferretti V, et al. JAK2 or CALR mutations status defines subtypes of essential thrombocythemia with substantially different clinical course and outcomes. Blood. 2014;123(10):1544–51. doi:10.1182/blood-2013-11-539098.
Tefferi A, Barbui T. Personalized management of essential thrombocythemia—application of recent evidence to clinical practice. Leukemia. 2013;27:1617–20. doi:10.1038/leu.2013.99.

# Chapter 8
# Primary Myelofibrosis

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## Introduction

Primary myelofibrosis (PMF) is previously called chronic idiopathic myelofibrosis in the 2001 WHO classification and also formerly known as agnogenic myeloid metaplasia and myelofibrosis/sclerosis with myeloid metaplasia. Along with polycythemia vera (PV) and essential thrombocythemia (ET), it is one of the three classic Philadelphia chromosome (*BCR-ABL1*) negative myeloproliferative neoplasms (Ph-MPNs) which share a common central theme in pathogenesis which is persistent activation of *JAK/STAT* signaling pathway.

## Epidemiology

PMF is the least frequent among the three Ph-MPNs. The incidence of PMF in the United States is approximately 0.2/100,000. It occurs mainly in middle aged and elderly patients. Both sexes are nearly equally affected. The median age at presentation is 67 years old. Median survival is estimated to be 6 years. Children are rarely affected. The causes of death include leukemic transformation, progressive cachexia, vascular events, and infections.

The pathogenesis of PMF is not entirely clear. In the cases with activating mutations in *JAK2, MPL,* and *CALR*, persistent activation of *JAK/STAT* signaling pathway is considered the driving event. In a minority of cases, the pathogenesis has been linked to exposure to benzene or ionizing radiation. A very high incidence of PMF has been noted in patients given thorium-based radiographic contrast material

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and in individuals exposed to atomic bombs at Hiroshima. Familial PMF (FPMF) or Inherited forms of PMF have been reported in both adults as autosomal dominance and in children as an autosomal recessive disorder.

## Clinical Presentation

Clinical presentations usually correlate with the stage of histopathological changes in the bone marrow. In the prefibrotic or early stage, the marrow is hypercellular, with absent or only slight fibrosis. In this stage, there is usually peripheral blood thrombocytosis; extramedullary hematopoiesis (EMH) is minimal if any. Patients may present with bleeding or thrombosis, and because the platelet count is often markedly elevated, the clinical picture may overlap with that of ET. Studies have shown that the incidence of arterial and venous thrombotic events in PMF is approximately the same as that seen in ET, which is significantly lower than that seen in PV. Portal vein thrombosis is a recognized complication of PMF which sometimes may occur preceding the clinical onset of the disease.

At the fibrotic stage, the bone marrow is markedly fibrotic leading to ineffective marrow hematopoiesis. Anemia can occur, and some patients can become transfusion dependent. EMH is common and may occur in any organ. Prominent hepatosplenomegaly, the hallmark of PMF, is seen in approximately 90% of patients. Palpable hepatomegaly is present in 40 to 70% of patients. Portal hypertension may develop as a result of increased splanchnic flow due to splenomegaly and/or intrahepatic obstruction associated with extramedullary hematopoiesis.

The production of cytokines during the disease progression causes profound constitutional symptoms such as severe fatigue, weight loss, and signs of a hypermetabolic state including low-grade fever, bone pain, and night sweats. Secondary gout or renal stones due to chronic overproduction of uric acid may also occur.

Approximately 15–30% of patients are asymptomatic with a diagnostic workup trigged by an abnormal peripheral blood cell count (e.g., anemia, leukocytosis, or marked thrombocytosis), or an incidental finding of splenomegaly.

## Morphology and Diagnosis

### *Morphology*

#### Peripheral Blood

The most striking finding on the peripheral blood (PB) smear in the prefibrotic stage is usually marked thrombocytosis, resembling ET. Modest anemia and mild leukocytosis, especially neutrophilia with a left shift, may also be seen. Myeloblasts, nucleated RBCs, and teardrop-shaped RBCs are only rarely observed.

The classic findings of leukoerythroblastosis with numerous teardrop-shaped RBCs and large, abnormal platelets are evident in the fibrotic stage. This is largely due to the abnormal release of immature and abnormal cells from sites of EMH. In this stage, lower platelet counts, leukopenia, or anemia may occur as a result of ineffective hematopoiesis secondary to myelofibrosis. Mild leukocytosis can occur. Circulating megakaryocyte nuclei and fragments are frequently observed. Blasts can be seen up to 5–9% of the WBCs. In patients with history of established diagnosis of PMF, the presence of 9–19% blasts in the PB indicates an acceleration of the disease.

### Bone Marrow

In the prefibrotic stage PMF or pre-PMF, the bone marrow (BM) is hypercellular and shows increased proliferation of both granulocyte lineage and megakaryocyte lineage and a decrease in erythropoiesis with left shift. There may be a mild left shift in granulopoiesis, but band and segmented neutrophils comprise the majority of granulocytes. There is no increase in myeloblasts. The megakaryocytes are markedly increased cluster and have a pleomorphic and bizarre appearance. They vary in size and often show abnormal nuclear/cytoplasmic ratios and hyperchromatic nuclei with disorganized lobation.

Since at this stage of PMF, there is minimal myelofibrosis, but instead, there is significant proliferation of megakaryocytes in the marrow and thrombocytosis in the peripheral blood. As such, pre-PMF is difficult to be distinguished from ET. Nonetheless, distinction between the two entities is critical because PMF is a dismal disease and is managed differently compared with ET.

In the fibrotic stage, as fibrosis of the marrow becomes more obvious, marrow cellularity gradually decreases. The marrows are progressively replaced by fibrotic tissues and fat. Dilated marrow sinuses may be prominent, which may contain megakaryocytes and immature hematopoietic cells. Atypical megakaryocytes are often the predominant cells in the marrow, and they are usually present in tight clusters. New bone formation and osteosclerosis may occur.

Morphologically, the fibrotic stage of PMF cannot be distinguished from secondary myelofibrosis (post-PV or post-ET).

### New Diagnostic Criteria of PMF

With the discovery of recently identified mutations, the 2016 WHO diagnostic guidelines for PMF have incorporated more molecular markers into diagnostic criteria. The separate diagnostic criteria for prefibrotic/early primary myelofibrosis or pre-PMF and overt PMF were established and are listed below in Tables 8.1 and 8.2 [1].

**Table 8.1** WHO criteria for pre-PMF

| WHO pre-PMF criteria |
|---|
| Major criteria |
| 1. Megakaryocytic proliferation and atypia, without reticulin fibrosis > grade 1*, accompanied by increased age-adjusted BM cellularity, granulocytic proliferation, and often decreased erythropoiesis |
| 2. Not meeting the WHO criteria for BCR-ABL1+ CML, PV, ET, myelodysplastic syndromes, or other myeloid neoplasms |
| 3. The presence of *JAK2*, *CALR*, or *MPL* mutation or the absence of these mutations, the presence of another clonal marker, or the absence of minor reactive BM reticulin fibrosis |
| Minor criteria |
| The presence of at least 1 of the following, confirmed in 2 consecutive determinations: |
| (a) Anemia not attribute to a comorbid condition |
| (b) Leukocytosis $\geq 11 \times 10^9$/L |
| (c) Palpable splenomegaly |
| (d) LDH increased to above upper normal limit of institutional reference range |
| Diagnosis of pre-PMF requires meeting all 3 major criteria, and at least 1 minor criterion |

From Arber et al [1]
*Reticulin fibrosis grade 1: Loose network of reticulin with many intersections, especially in perivascular area

**Table 8.2** WHO criteria for overt PMF

| WHO overt PMF criteria |
|---|
| Major criteria |
| 1. The presence of megakaryocytic proliferation and atypia, accompanied by either reticulin and/or collagen fibrosis grades 2 or 3 |
| 2. Not meeting WHO criteria for ET, PV, BCR-ABL1+ CML, myelodysplastic syndromes, or other myeloid neoplasms |
| 3. The presence of *JAK2*, *CALR*, or *MPL* mutation or the absence of these mutations, the presence of another clonal marker, or the absence of reactive myelofibrosis |
| Minor criteria |
| The presence of at least 1 of the following, confirmed in 2 consecutive determinations: |
| (a) Anemia not attribute to a comorbid condition |
| (b) Leukocytosis $\geq 11 \times 10^9$/L |
| (c) Palpable splenomegaly |
| (d) LDH increased to above upper normal limit of institutional reference range |
| (e) Leukoerythroblastosis |
| Diagnosis of overt PMF requires meeting all 3 major criteria, and at least 1 minor criterion |

From Arber et al. [1]

## Cytogenetics and Molecular Genetics

### *Cytogenetics*

No cytogenetic abnormalities are specific for PMF. Clonal abnormalities are reported in 30% to 50% of patients with PMF at the time of diagnosis, and the frequency gradually increases over time. More than half of patients with PMF have

normal karyotype. Normal karyotype is associated with higher hemoglobin level, WBC count, and platelet count, and is a good prognostic factor.

The presence of either del(13)(q12–22) or der(6)t(1;6)(q21–23;p21.3) is strongly suggestive but not diagnostic of PMF. The most common abnormalities include del(13q), del(20q), trisomy 8, trisomy 9, and abnormalities of chromosome 1q. Deletions affecting the long arms of chromosomes 7 and 5 occur as well but may be associated with prior cytotoxic therapy used to treat the myeloproliferative process. When present as the sole abnormality, del(20q) and del(13q) are considered prognostically favorable abnormalities. Del(20q) is associated with lower leukocyte count and thrombocytopenia, whereas del(13q) is associated with thrombocytosis. Del(13q) is correlated with *CALR* mutations.

Unfavorable karyotype includes complex karyotype, monosomal karyotype, sole, or 2 abnormalities that include trisomy 8, −7/7q-, isochromosome 17q [i(17q)], inv.(3), −5/5q-, del(12p), or 11q23 rearrangement. One study showed that monosomal karyotype and inv.(3)/i(17q) were associated with a greater than 80% 2 year mortality in patients with PMF (Table 8.3).

## *Molecular Genetics*

The most common mutation in PMF is *JAK2* V617F, followed by mutations in *CALR* and *MPL*. The mutations in these three genes are called "driver mutations" because they are activating mutations causing activation of JAK/STAT signaling pathway in the absence of ligands. Some patients with PMF are found to have no driver mutations, which are called "triple negative" cases.

Base on the presence of driver mutations, PMF can be divided into four molecular subtypes (Table 8.4).

**Table 8.3** summarizes the common cytogenetic abnormalities and their prognosis impact in PMF

| | |
|---|---|
| Good prognosis | Normal karyotype, 20q-, 13q-, +9 |
| Poor prognosis | Complex karyotype, monosomal karyotype, +8, −7/7q-, i(17q), inv.(3), −5/5q-, 12p-, or 11q23 |

**Table 8.4** Molecular subtypes of PMF

| Genes involved | Frequency | Most common mutations | Prognosis |
|---|---|---|---|
| *JAK2* | 50–60% | V617F | Poor |
| *CALR* | 20–35% | Type 1 (52-bp deletion) | Good |
| | | Type 2 (5-bp insertion) | Poor |
| *MPL* | 5–8% | W515 K/L | Not predictive |
| Triple negative | 8–10% | | Poor |

## JAK2 *Mutations*

### Pathogenesis

*JAK2* (Janus kinase 2) gene encodes a tyrosine kinase located in the cytoplasm which plays a critical role in signal transduction in various processes such as cell growth, development, differentiation, or histone modifications. It involves in regulation of both innate immunity and adaptive immunity. It achieves its function via its association with type-I cytokine receptors such as receptors for growth hormone, prolactin, leptin, erythropoietin, thrombopoietin; or type-II cytokine receptors including receptors of IFN-alpha, IFN-beta, IFN-gamma, and multiple interleukins. Activation of the receptors leads to JAK2 autophosphorylation, which creates docking sites for recruitment of STAT (Signal Transducer and Activator of Transcription) proteins. STAT proteins are subsequently phosphorylated and form homodimer or heterodimers which translocate to the nucleus to activate gene transcription.

*JAK2* gene is located at chromosome 9p24.1, consisting of 25 exons. The JAK2 protein comprises an N-terminal FERM (band 4.1, ezrin, radixin, moesin) domain, a SRC homology 2 (SH2)-like domain, a Janus homology 2 (JH2) pseudokinase domain, and Janus homology 1 (JH1) active tyrosine kinase domain. N-terminal domain is associated with binding to cytokine receptors. Precise role of SH2-like domain is unclear. JH1 is activated via transphosphorylation of tandem tyrosines in activation loop. JH2 has autoinhibitory activity and regulates activity of JH1.

The most common mutation of *JAK2* in PMF is a point mutation, substitution of G to T, at nucleotide 1849, resulting in the change of amino acid from valine to phenylalanine at codon 617 in exon 14 in the JH2 pseudokinase domain. *JAK2* V617F is a gain-of-function mutation, capable of activating STAT-mediated transcription in the absence of ligands. Because JH2 has autoinhibitory activity and regulates activity of JH1, mutation in JH2 domain such as V617F disrupts the inhibitory interaction and causes persistent activation of JH1. In addition, V617F impairs JH2 catalytic activity, which further enhances JH1 activity. Therefore, V617F appears to achieve hyperactivity through a gain-of-function steric mechanism on JH1 and a loss-of-function catalytic mechanism on JH2. This acquired somatic mutation occurs at the level of the hematopoietic stem cell, giving rise to lineage-specific cells that are hypersensitive to cytokine stimulation.

*JAK2* V617F has also been shown to activate ERK/MAPK and PI-3/AKT pathways in the absence of cytokine stimulation.

The second common mutation of *JAK2* is exon 12 mutation, which is usually not seen in PMF.

## Clinical Significance

*JAK2* V617F is found in 50–60% of PMF. The presence of this mutation is associated with older age, higher white blood cell count, hemoglobin level, and platelet count. *JAK2* V617F homozygous patients are more symptomatic. Patients tend to have large splenomegaly and significantly higher risk of cardiovascular events. In PMF, the rate of major thrombotic event is around 2% patient year, and *JAK2* mutation emerged as an independent risk factor for these events.

Regarding prognosis, *JAK2* V617F was found to be associated with leukemic transformation and poorer survival [2].

In terms of allele burden, studies have shown that patients with PMF with a low mutant allele burden appear to have more aggressive disease and inferior survival [3].

## Detection Methods

Because *JAK2* V617F is the most frequent mutation in PMF, and it has prognostic impact, it is critical to choose a sensitive assay for establishing diagnosis, phenotypic association, prognosis, and follow-up for minimal residual disease. In fact, testing of *JAK2* V617F is now recommended in the initial workup of all suspected Ph-MPNs.

The current assays either specifically target the c.1849G > T point mutation or target the region of exon 14 encompassing the c.1849G > T mutation. Allele-specific PCR is an assay that specifically targets the mutation. It uses mutation-specific primers or probes to amplify the region mutated. Mutation scanning assays that target the region of exon 14 encompassing the c.1849G > T mutation include direct sequencing and high-resolution DNA melting curve analysis.

*JAK2* V617F allele burden is designated as the ratio between mutated and total alleles in the patient's specimen. It needs to be determined through quantitative assays. Monitoring *JAK2* V617F allele burden before and after therapy is not yet as routine as that for *BCR-ABL* in the management of CML; however, as more and more effective therapies for PMF emerge, this will be increasingly desired. It will be of particular interest for detection of minimal residual disease, especially after allogeneic stem cell transplantation (ASCT).

Specificity and sensitivity of the assays are essential for both qualitative and quantitative assays. For specificity, the assays must demonstrate a clearly defined background level such that *JAK2* V617F negative and positive cases can be readily distinguished.

According to the UK guidelines for the detection of *JAK2* V617F and other relevant mutations, the sensitivity of quantitative assays must be high enough to be able to identify a *JAK2* V617F mutant allele with a burden as low as 1–3% [4]. This threshold has been shown to be pathogenetically relevant and carry clinical significance.

The most common quantitative test is allele-specific real-time PCR. To quantify the mutant allele burden, a granulocyte enrichment step is needed and at least 20 ng of genomic DNA, equivalent to 3030 diploid genomes is required [4]. Sanger sequencing is not recommended as a quantitative assay because it only has a sensitivity of 10–20%. Other assays that possess a sensitivity of 3–5%, such as pyrosequencing or allele-specific PCR followed by agarose gel electrophoresis may fail to identify a small number of patients who carry a low-level mutant allele.

Targeted next-generation sequencing (NGS) panels such as the TruSight Myeloid Sequencing Panel (Illumina, Inc.) have been proven successful in detection of mutations in exon 14 and exon 12 of *JAK2,* exon 9 of *CALR*, exon 10 of *MPL*, and other genes associated with myeloid malignancies. This technique allows for broad multigene coverage in a single assay and, at the same time, provides estimation of mutant allele burden. However, sensitivity of the assay can be an issue. Approximately 1000-fold coverage is needed for a given base to achieve a 99% confidence of detecting more than 5% heterozygous mutant alleles. As more and more sensitive NGS assays are being developed and validated for clinical application, NGS is likely to replace conventional PCR for detection of mutations and quantification of mutant allele burdens in *JAK2* and many other genes.

## CALR *Mutations*

### Pathogenesis

The *CALR* gene is located on chromosome 19p13.2 with 9 exons. *CALR* encodes calreticulin protein which is composed of 417 amino acids. Calreticulin consists of three main domains. N-terminus is lectin-binding domain, which plays a role in chaperone activity and Zn2+ binding; the middle domain is a proline-rich P domain that contains high-affinity, low-capacity binding sites for Ca2+; the C-terminus is a domain that contains multiple calcium-binding sites.

Calreticulin is a multifunctional calcium binding protein chaperone mostly localized both within and outside of the endoplasmic reticulum. It has several diverse functions, including regulation of intracellular calcium homeostasis, steroid-mediated gene regulations, and chaperone activity, thus, playing an important role in cellular proliferation, differentiation, apoptosis, and immunogenic cell death.

*CALR* mutations are observed in hematopoietic stem and progenitor cells and are mutually exclusive with mutations in *JAK2* and *MPL* genes.

More than 50 different *CALR* mutations have been reported. Clinically relevant *CALR* gene mutations are limited to exon 9. Almost all mutations are somatic insertion/deletions (indels), resulting in one base pair reading frame shift. All mutant calreticulin proteins share a novel amino acid sequence at the C-terminus. These mutations result in the replacement of the negatively charged C-terminal amino acids by positively charged amino acids such as arginine and methionine [5]. These alterations cause loss of most of the C-terminal acidic domain and the KDEL

endoplasmic reticulum retention motif. Ca2 + −binding function of the mutant protein may be destroyed, and the protein may have an altered subcellular localization.

How these changes result in *JAK-STAT* pathway activation is currently being investigated. In a recent study, through extensive mutagenesis-based structure-function experiments and biochemical assays, a detailed mechanism was proposed to explain the mutant *CALR*-mediated oncogenic transformation. Specifically, this study shows that mutant *CALR* is sufficient to initiate an ET-like phenotype in vivo, and that mutant CALR proteins are involved in activation of *JAK/STAT* signaling pathway through direct physical interaction with MPL via their positively charged C-terminus. This study solidifies *JAK–STAT* activation as the central pathway driving oncogenic transformation in MPNs and also provides a biological basis as to why *JAK2, MPL,* and *CALR* mutations are mutually exclusive in patients with MPNs [6].

## Clinical Significance

*CALR* mutations are detected in 20–35% of patients with PMF, among whom 88% have nonmutated *JAK2* or *MPL*. So far, more than 50 types of *CALR* mutations have been identified. The most common mutation is type-1 mutation (45–53%) which is a 52-bp deletion (c.1092_1143del), causing frameshift (p.L367 fs*46). The second most common mutation is type-2 mutation (32–41%) which is a 5-bp insertion (c.1154_1155insTTGTC), also causing frameshift (p.K385 fs*47).

The majority of *CALR* mutations that are neither type 1 nor type 2 are operationally classified into "type 1-like" and "type 2-like" mutants, based on their structural similarities to type-1 and type-2 mutations [7].

In patients with PMF, *CALR* mutations are associated with younger age, higher platelet count, lower International Prognostic Scoring System (IPSS)-plus score, and lower incidence of anemia, and leukocytosis compared with other molecular subtypes (i.e., *JAK2* mutated, *MPL* mutated, and "triple negative"). *CALR*-mutated patients are also less likely to be transfusion dependent [8]. These patients have a lower risk of thrombosis compared with patients with mutated *JAK2*. Spliceosome mutations are infrequent in *CALR*-mutated PMF.

Survival of patients with PMF with *CALR* mutations is longer than those with *JAK2* or *MPL* mutations and triple-negative cases [8]. The favorable impact of *CALR* mutations on survival appears to be independent of the current prognostic scoring systems. In terms of both long-term survival and leukemia transformation, outcomes appear to be best for *CALR*-mutated patients and worst for "triple-negative" patients. Among 617 subjects with PMF in a European study, the median survival times were 17.7 years for *CALR*-mutated patients, 9.2 years for *JAK2* mutated, 9.1 years for *MPL* mutated, and 3.2 years for "triple-negative" patients [9].

Type-1 and type-2 *CALR* mutations demonstrate significantly different phenotypes and clinical impact. Patients with type 2 *CALR* mutations had significantly

higher dynamic IPSS plus (DIPSS-pus) scores, leukocyte counts, and circulating blasts than those with type-1 *CALR* mutations. In a study of 358 patients with PMF, survival was found to be significantly longer in those with type-1 *CALR* mutations compared with those with either *JAK2* or type-2 *CALR* mutations [10].

The difference between type-1 and type-2 phenotypes can be explained by their structural dissimilarity. Although all *CALR* mutations generate a novel C terminus with loss of the KDEL signal and impaired calcium binding, type-1 mutations eliminate nearly all the negatively charged amino acids, whereas type-2 mutations retain approximately one-half of these. In a study on the relationship between mutation subtypes and biological and clinical features of PMF, patients with type-1 mutation, but not those with type 2, were shown to have abnormal cytosolic calcium signals in cultured megakaryocytes [7].

Similar to *JAK2*- or *MPL*-mutated cases, *CALR*-mutated PMF cases respond to JAK inhibitors. However, a phase-III trial (COMFORT-II) demonstrated that *CALR* mutation status did not influence treatment outcomes of ruxolitinib. In this trial, ruxolitinib was shown to associate with reductions in splenomegaly in *CALR*-mutated patients; however, the three-year follow-up study showed that the relative reduction in the risk of death in *CALR*-mutated patients was comparable with that of the overall population on ruxolitinib [11].

Therapeutically, the altered peptide sequence at the C-terminal domain of the mutated *CALR* offers opportunity for immunological targeting because it represents a tumor-specific epitope. In addition, novel therapeutic inhibitors can be developed to target the physical interaction between mutant *CALR* and *MPL* in mutant *CALR*-mediated MPNs.

Morphologically, *CALR*-mutated MPNs have a higher frequency of megakaryocytic aberrancies compared with *CALR*-wild type cases. The megakaryocytes of *CALR*-mutated PMF are more hyperchromatic compared with *CALR*-wild type cases [12].

## Detection Methods

The identification of somatic *CALR* mutations can be used to confirm the diagnosis of a myeloproliferative disorder in Philadelphia chromosome negative, *JAK2* and *MPL* wild-type patients with thrombocytosis.

Somatic mutations in *CALR* gene were first discovered via whole-exome sequencing in patients with ET and PMF lacking *JAK2* and *MPL* mutations [5]. Subsequently, targeted sequencing of *CALR* gene was performed in cohorts of patients with MPNs.

Currently, allele-specific PCR amplifying exon 9 followed by fragment sizing analysis by capillary electrophoresis is the mainstay technique for qualitative and quantitative testing of *CALR* mutations. The type of mutation is determined by comparing the size of the mutant allele PCR product with the size of the wild-type allele PCR product.

High-resolution DNA melting curve analysis can be used as a screening method to detect possible mutations. Confirmation studies such as fragment size analysis or sequencing are needed eventually to determine mutation type.

New technologies such as digital PCR can be used to detect *CALR* mutations and determine the mutant allele burdens. Compared with the commonly used fluorescent PCR product analysis, digital PCR is more precise, reproducible, and accurate with a very high sensitivity. It can be used for minimal residual disease monitoring.

Detection of large indels such as the 52-bp deletion in type 1 *CALR* mutation is currently a challenging issue for most NGS technologies; therefore, NGS is not recommended as a first-line detection method.

Because all pathogenic *CALR* mutations result in an identical C-terminal protein, immunohistochemistry can be used to identify these mutant proteins. One study showed that using a commercially available mouse monoclonal antibody (clone CAL2), *CALR* mutations could be detected on the bone marrow trephine with specificity of 100%, sensitivity of 82–91%, positive predictive value 100%, and negative predictive value 90–95% [13].

## *MPL Mutations*

### Pathogenesis

*MPL* encodes thrombopoietin receptor which belongs to a cytokine receptor superfamily. It is located on chromosome 1p34 and includes 12 exons. Thrombopoietin binds to the extracellular domain as a growth factor and causes phosphorylation and activation of JAK2 which in turn leads to MPL phosphorylation and activation of downstream signaling pathways such as STAT and ERK.

*MPL* is an important regulator of megakaryopoiesis. Studies have shown that expression level of *MPL* is important for the development and progression of ET and PMF, both of which involve proliferation of megakaryocytes.

### Clinical Significance

*MPL* mutations are found in 5–8% of PMF. The most common site of mutation is codon 515 in exon 10. The most common mutation is W515 K/L (tryptophan being substituted with either lysine or leucine). The W515 residue forms part of the key amphipathic K/RWQFP motif just after the transmembrane domain that plays an important role in maintaining the receptor within its inactive state in the absence of ligand. W515 K/L mutation causes spontaneous activation of *JAK/STAT* signaling that leads to cytokine-independent proliferation of hematopoietic cells. In one study, bone marrow transplantation in mice with W515 L mutation resulted in myeloproliferative disorder with marked thrombocytosis, splenomegaly, splenic infarction,

and myelofibrosis. *MPL* W515 L-mutated cells were demonstrated to associate with cell proliferation in patients with MF due to thrombopoietin hypersensitivity compared with nonmutated *MPL*.

Less common mutations include W515R and W515A.

Clinically, *MPL* mutations demonstrated inconsistent phenotypes in PMF. Some studies showed that *MPL*-mutated patients were older, showing higher platelet and serum erythropoietin levels, lower hemoglobin, and bone marrow cellularity, and they had significant risk for thrombotic complications and higher tendency to become transfusion dependent compared with *JAK2*V617F-positive patients. Other studies showed that the presence of mutant *MPL* was associated with older age and constitutional symptoms but not associated with platelet count, hemoglobin level, or prognostic scores. Nonetheless, *MPL* mutations are not found to have clear association with the prevalence of splenomegaly, abnormal cytogenetics, myelofibrotic transformation, overall survival, or leukemia-free survival [14].

## *Detection Methods*

Because the majority of *MPL* mutations are point mutations, the detection methods for *JAK2* mutations can be used to identify *MPL* mutations.

### Triple Negative PMF

About 8–10% of patients with PMF are found to have no mutations in *JAK2, MPL*, and *CALR* genes. These cases are called triple-negative cases, reminiscent of triple-negative breast cancers.

Triple-negative PMF cases show very poor prognosis compared with all other molecular subtypes [9]. One study reported median survival of only 2.5 years.

A recent study has demonstrated that triple-negative cases of PMF do not represent a homogenous disease entity. In this study, whole-exome sequencing and Sanger sequencing of the entire exons of *MPL* and *JAK2* gene*s* were performed on paired tumor and control samples from triple-negative patients with PMF. In two patients, mutations in *MPL* (*MPL*-S204P), *CBL, TET2, ASXL1*, and *SRSF2* were identified. In one patient with PMF, a germline mutation of *MPL* (V285E) was identified. The identified *MPL* mutations were shown to be gain-of-function mutations [15].

## *Other Mutations*

Mutations in other genes other than the aforementioned driver mutations occur in a substantial number of patients. These mutations often coexist with one another and with the driver mutations. They might cooperate with the driver mutations in the pathogenesis of PMF.

Specifically, mutations in this regard include those relevant to epigenetic (e.g., *ASXL1, TET2, EZH2, IDH1, IDH2, DNMT3A*), RNA splicing (e.g., *SRSF2, U2AF1, SF3B1*), or transcriptional regulations (*TP53, IKZF1, NF-E2, CUX1*).

Several of these mutations have been shown to have detrimental prognostic impact in PMF, which include *ASXL1, SRSF2, EZH2,* and *IDH1/2*. One study found that an increased number of detrimental mutations were associated with poorer outcome. Specifically, patients with 0 vs 1 vs 2 mutations showed significantly different overall survival (median 12.3 years vs 7 years vs 2.6 years, respectively) and leukemia-free survival. The prognostic significance was independent of IPSS and DIPSS-plus systems [16].

### ASXL1 Mutations

*ASXL1* (Additional Sex Combs Like 1) gene is located at chromosome 20q11.1. It encodes a protein associated with the polycomb group of proteins. It is involved in transcriptional regulation mediated by ligand-bound nuclear hormone receptors, such as retinoic acid receptors and peroxisome proliferator-activated receptor gamma.

*ASXL1* mutations may contribute to the pathogenesis of MPNs through several pathways. *ASXL1* mutations cause increased expression of HoxA9 and HoxA10 proteins; *ASXL1* depletion results in loss of recruitment of *EZH2* to its target loci; *ASXL1* mutations also interact with *NRAS/KRAS* mutations.

One study has shown that mutations in *ASXL1* are more frequent in PMF (20%) compared with in PV (7%) and ET (4%).

*ASXL1* mutations have been shown to associate with leukocytosis, circulating blasts, anemia, splenomegaly, and constitutional symptoms. *ASXL1* mutations are more likely to occur in the presence of normal karyotype [2].

In one study of 879 patients with PMF, *ASXL1, SRSF2, EZH2,* and *IDH1/2* mutations were found to associate with risk for premature death or leukemic transformation. However, only *ASXL1* mutations retained prognostic significance independent of the DIPSS-plus model [2].

Experts have proposed to use *CALR* and *ASXL1* mutation status as an independent prognostic marker to classify patients with PMF. It was demonstrated that patients with *CALR* + *ASXL1*− genotype had the longest survival, and those with *CALR* − *ASXL1*+ genotype had the shortest survival, whereas *CALR* + *ASXL1*+ and *CALR* − *ASXL1*− patients had similar survival. *CALR* − *ASXL1*+ PMF are, therefore, considered "high molecular risk" diseases [17].

### EZH2 Mutations

*EZH2* (Enhancer of Zeste Homolog 2) gene is located at chromosome 7q36.1. This gene encodes an enzyme that is a member of the polycomb group. Polycomb repressive complex (PRC) is a protein complex that has a global role in epigenetic

transcriptional repressors. Two main families of PRC, PRC1 and PRC2, are identified in mammals. The catalytic core of PRC2 is composed of EZH1, EZH2, etc. EZH catalyzes the trimethylation of lysine 27 of histone H3 leading to maintaining transcriptional repression state of target gene.

*EZH2* mutations are found in 6–13% of patients with PMF. In a cohort of 879 patients with PMF, *EZH2* mutations have been found associated with leukocytosis, ≥1% circulating blasts, shortened survival, but the data did not remained significant in the context of IPSS [2].

### SRSF2 Mutations

Serine/arginine-rich splicing factor 2 (SRSF2) is a gene that codes for one of the several serine/arginine-rich splicing factors. SRSF2 is a member of the spliceosome and is involved in mRNA processing.

*SRSF2* mutations are present in 14% of patients with PMF. *SRSF2* mutations are associated with older age, leukocytosis, ≥1% circulating blasts, constitutional symptoms, anemia, and transfusion need [2].

### IDH1/2 Mutations

*IDH1/2* (Isocitrate Dehydrogenase 1 and 2) genes encode isocitrate dehydrogenases that catalyze the oxidative decarboxylation of isocitrate to 2-oxoglutarate. IDH1 localizes to cytoplasm and peroxisomes, whereas IDH2 localizes to mitochondria. Both are NADP-dependent enzymes. *IDH1/2* mutations change enzymatic activity of IDH1 and IDH2 and alter methylation of DNA.

*IDH1* or *IDH2* mutations are seen in 4% of patients PMF, which are mutually exclusive. In a study of 879 patients with PMF, *IDH1* or *IDH2* mutations have been shown to associate with significantly increased risk of leukemia transformation [2]. *IDH* mutations have been reported to cluster with *SRSF2* mutations in patients with PMF and to potentially collaborate with *JAK2* V617F in leukemogenesis.

### TET2 Mutations

*TET2* (Ten-eleven-translocation 2) gene is located at chromosome 4q24. It encodes a methylcytosine dioxygenase that catalyzes the conversion of 5-methylcytosine to 5-hydroxymethylcytosine. This enzyme plays an important role in active DNA demethylation. This function is shared by two other TET proteins, TET1 and TET3. This protein has been shown to have an important role in myelopoiesis.

*TET2* mutations are present in 17% of patients with PMF. Mutation types include insertions, deletions, nonsense, and missense mutations. *TET2* mutations can coexist with *JAK2* mutations and *MPL* mutations and might be associated with higher *JAK2* V617F allele burdens.

*TET2* mutations can either be somatic or germline mutations. Some study demonstrated that *TET2* mutations are associated with advanced age.

### DNMT3A Mutations

*DNMT3A* (DNA Methyltransferase 3A) encodes a DNA methyltransferase which plays a role in de novo methylation. It belongs to a family of DNA methyltransferases including DNMT1, DNMT3A, and DNMT3B. The DNMT3A protein localizes to the cytoplasm and nucleus.

*DNMT3A* mutations are present in 4–7% of patients with PMF, some of which coexist with other mutations such as mutations in *JAK2, TET2,* and *ASXL1*. The majority of *DNMT3A* mutations are heterozygous missense mutations that occur at residue R882 in the methyltransferase domain near the carboxyl terminus of the DNMT3A protein.

## Prognosis and Molecular Targeted Therapy

### *Molecular Prognostication of PMF*

In PMF patient care, sometimes it is challenging to make therapeutic decisions as to whether or when the patients need therapy. Currently, such decisions rely upon prognostic scoring systems developed by the International Working Group for Myeloproliferative neoplasm Research and Treatment (IWG-MRT), including IPSS (applicable at the time of diagnosis), the dynamic IPSS (DIPSS) (applicable at any time during the disease course), and DIPSS-plus [18].

Both IPSS and DIPSS use five adverse factors, including age > 65 years, hemoglobin <10 g/dl, leukocyte count >25 $\times 10^9$/l, ≥1% circulating blasts, and the presence of constitutional symptoms, to distinguish among low, intermediate-1, intermediate-2, and high-risk patients. These patients have respective median survivals of 11.3, 7.9, 4.0, and 2.3 years, per IPSS, or not reached, 14.2, 4.0, and 1.5 years, per DIPSS.

DIPSS-plus is modified DIPSS which incorporated three additional DIPSS-independent risk factors: platelet count <100 $\times 10^9$/L, red cell transfusion need and unfavorable karyotype; median survival for the low, intermediate-1, intermediate-2, and high-risk categories were 15.4, 6.5, 2.9, and 1.3 years in one study [18].

To improve upon these prognostic models by incorporating recently described molecular markers, independent cohorts of patients with PMF were performed in Europe and US. These studies demonstrated that five mutations (*ASXL1, EZH2, SRSF2, IDH1,* and *IDH2*) were associated with shorter leukemia-free survival and overall survival; these mutations were, therefore, considered "detrimental" for disease outcome. Accordingly, experts proposed to classify patients with PMF possess-

ing at least one detrimental mutation as "high molecular risk (HMR)" and those with no such mutation as "low molecular risk (LMR)". HMR and LMR are determined to be independent prognostic factors for risk evaluation via multivariable analysis. However, when the five mutations were evaluated individually in the context of DIPSS-plus, only *ASXL1* mutations retained prognostic significance [2, 16].

The HMR and LMR groups were further evaluated for gene expression patterns, and each group was demonstrated to have unique gene expression profiles [2].

When integrated with DIPSS-plus, significant differences in survival between the HMR and LMR groups were observed in low and intermediate-1 risk disease categories despite not apparent in DIPSS plus high-risk disease category [16, 17, 19].

## *Molecular Targeted Therapies*

Treatment of PMF is similar to secondary myelofibrosis (MF) (post-ET or post-PV) in that all MF, whether primary or secondary, are associated with persistent activation of *JAK2/STAT* pathway and may potentially benefit from JAK inhibitors.

One study showed that ruxolitinib, the first JAK inhibitor approved for treating MF, improved survival independent of the mutation profile in patients with MF and reduced the risk of death in patients harboring detrimental mutations compared with the best available therapy provided by the trial.

Although JAK inhibition clearly reduces the symptomatic burden of MF due to its ability to block inflammatory cytokine activities, it does not control the stem cell-derived clonal myeloproliferation that drives the disease; therefore, it is neither curative nor effective in reducing the risk of leukemic transformation or results in complete remissions. The discontinuation rate is high due to severe side effects.

Chronic inhibition of JAK2 leads to drug resistance as demonstrated by the persistence of MPN cells. JAK2 inhibitor persistence is associated with reactivation of *JAK/STAT* signaling pathway and with heterodimerization between activated JAK2 and JAK1 or TYK2, consistent with activation of JAK2 in trans by other JAK kinases. Discontinuation of JAK inhibitors generally leads to symptom return within a short period of time. JAK2 inhibitor withdrawal is associated with resensitization to JAK2 kinase inhibitors and with reversible changes in JAK2 expression [20].

Strategies to improve JAK inhibition therapy are being explored. Improving targeting of JAK2 such as degrading JAK2 may lead to increased therapeutic efficacy. In addition, JAK inhibitors can be combined with other novel therapies to overcome JAK inhibitor resistance or target additional mechanisms of pathogenesis. Patient selection and proper dosing of JAK inhibitors are also important factors for effective treatment.

The only possible curative therapy is allogeneic stem cell transplant (ASCT) which, unfortunately, offers durable remissions in only one-third of patients. The plausibility of combining JAK inhibitors with ASCT is being investigated.

There is an urgent need for disease-modifying drugs. Numerous targeted therapies are being investigated, which include modulators of epigenetic regulation,

pathways that work downstream from *JAK/STAT* (i.e., PI3K/AKT/mTOR), heat shock protein 90, hedgehog signaling, profibrotic factors, abnormal megakaryocytes, and telomerase.

## JAK *Inhibitors*

*Ruxolitinib* is a JAK1/JAK2 inhibitor. It is the first JAK inhibitor approved in the United States, Canada, and Europe for treatment of intermediate- and high-risk myelofibrosis, including both patients with PMF and secondary MF. The approval was based on two randomized phase 3 clinical trials, COMFORT-I and COMFORT-II, comparing ruxolitinib with either placebo or the best available therapy (BAT) [21, 22]. In both trials, the patients on ruxolitinib showed significant reduction in spleen size and improvement of disease-related symptoms. At three-year follow-up interval of both COMFORT-I and COMFORT-II, patients who received ruxolitinib had prolonged survival compared with patients who received placebo or BAT [23].

A follow-up analysis of the trials revealed that responses to ruxolitinib were observed in both *JAK2*-mutated patients and *JAK2* wild-type patients. Another follow-up study demonstrated that ruxolitinib improved survival independent of mutation profile and reduced the risk of death in patients harboring a set of prognostically detrimental mutations *(ASXL1, EZH2, SRSF2*, or *IDH1/2)* vs the best available therapy [24]. Moreover, one study revealed that in patients treated with ruxolitinib, harboring >3 mutations was inversely correlated with spleen response and time to treatment discontinuation.

*Newer JAK inhibitors* are under investigation to improve selectivity for *JAK2* V617F and overcome the resistance to JAK inhibitors. Momelotinib, pacritinib, and fedratinib are new JAK inhibitors, which, like ruxolitinib, are type I inhibitors; NVPCHZ868 is a novel type-II inhibitor. Type-I inhibitors compete with ATP for the drug-binding pocket whereas JAK2 is in its active conformation. Type-II inhibitors bind the inactive kinase, completely suppressing *JAK–STAT* signaling in type-I JAK inhibitor-persistent cells.

*Momelotinib* (CYT387) is a JAK1/JAK2 inhibitor that showed a favorable effect on anemia compared with ruxolitinib. A phase-II trial of momelotinib in MF reported reductions in splenomegaly and symptoms. 70% of transfusion-dependent patients achieving more than 2 years of transfusion-free period. Momelotinib is currently undergoing phase III trials being compared with ruxolitinib or BAT (NCT01969838, NCT02101268).

*Pacritinib* (SB1518) is selective for JAK2 over other JAKs but has activity against FLT3. It demonstrated favorable safety outcomes in phase I/II trials including patients with thrombocytopenia. Thirty-one percent achieved reduction of 35% or greater in spleen volume at 24 weeks and 48% patients showed reduction in symptoms. Phase-III studies are being conducted to identify its efficacy in MF compared with BAT (NCT01773187 and NCT02055781).

*Fedratinib* (SAR302503) is a potent selective JAK2 inhibitor, which is generally well tolerated. Different from other JAK2 inhibitors, fedratinib was found to decrease the *JAK2* V617F allele burden by approximately 60% during therapy and reduce the marrow fibrosis scores. Despite its effectiveness in spleen size reduction and symptom improvement, its association to Wernicke encephalopathy led to its discontinuation.

*NVP-CHZ868* has been shown to cause significant reductions in *JAK2* mutant allele burden in murine models of PV and MF.

## *Histone Deacetylase Inhibitors*

Histone deacetylases (HDACs) downregulate transcription through changing DNA conformation. They remove acetyl groups from lysine residues on histone tails, inducing an inactive or closed conformation that results in restricting access of transcription factors to DNA. Apart from regulating histone modification, HDACs also regulate the post-translational acetylation status of many nonhistone proteins, including transcription factors, chaperones, and signaling molecules, resulting in changes in protein stability, protein–protein interactions, and protein-DNA interactions. To date, 18 different mammalian HDACs have been identified and divided into four classes based on their sequence similarity to yeast counterparts. HDACs from the classical family are dependent on Zn2+ for deacetylase activity and constitute classes I, II, and IV. Class III HDACs consist of seven sirtuins, which require the NAD + cofactor for activity.

HDAC inhibitors demonstrate pleiotrophic activities through modulating the acetylation status of histones as well as other nonhistone proteins. Although the mechanisms of their anticancer effect are not fully understood, the predominant responses include induction of tumor cell death, inhibition of proliferation, and modulation of tumor immunogenicity.

Three HDAC inhibitors have been approved by FDA for the treatment of hematologic malignancies including cutaneous T-cell lymphoma and multiple myeloma, and several others are in clinical development for a wide array of cancers.

HDAC inhibitors are found to act synergistically with other anticancer drugs as evidenced by compelling cell line and animal models. HDAC inhibitor in combination with JAK2 inhibitor for the treatment of PMF is being investigated in several clinical trials including a phase-Ib European study and a phase-I/II US study (PRIME).

*Panobinostat* (Farydak) is the first HDAC inhibitor approved to treat multiple myeloma. It is a potent pan-histone deacetylase inhibitor that enhances acetylation of histones H3, H4, and heat shock protein. In a phase-II study, panobinostat was used as a monotherapy to treat 35 patients with intermediate/high risk MF. 69% of patients experienced a 25% or more reduction in spleen size. A phase-I dose-escalation study of single-agent panobinostat administered at lower doses was conducted in 18 patients with MF. Prolonged administration of panobinostat in 5

patients resulted in elimination of leukoerythroblastic blood features, improvement in anemia, resolution of splenomegaly, and reduction in MF symptoms, and in 2 cases, improvement in bone marrow histopathological features and regression of marrow fibrosis were observed.

Panobinostat is currently being used in combination with ruxolitinib to treat patients with myelofibrosis in a phase-I/II trial (i.e., PRIME trial).

*Pracinostat* is a pan-HDAC inhibitor that has preferential selectivity for classes 1 and 2 HDACs. In a phase-II study of 22 patients with intermediate- and high-risk MF, pracinostat monotherapy resulted in reduction in splenomegaly in 27% of patients.

*Givinostat* is an inhibitor of class 1 and 2 HDACs. In a phase-II trial in patients with *JAK2* V617F+ MPNs, 38% of patients with PMF showed reduction in splenomegaly.

## *DNA Methyltransferase Inhibitors*

DNA methyltransferases catalyze the methylation of CpG islands within promoter sites of DNA, thereby downregulating transcription by blocking access to transcription factor complexes.

Hypermethylation of genes that negatively regulate the hyperactive *JAK/STAT* signaling pathway is identified in patients with MF. Several reports have suggested clinical activity of hypomethylating agents in the management of patients with MF.

5-azacitidine (Vidaza) and 5-aza-2′-deoxycitidine (Decitabine) are approved for treatment of MDS. In a phase II study in MF, Vidaza was given to newly diagnosed, relapsed, or refractory intermediate- or high-risk patients with MF. Reponses (based on IWG-MRT) were observed in 8 (24%) patients with median response time of 5 months. Seven (21%) patients, with or without *JAK2* V617F mutations, experienced clinical improvements. Unfortunately, the majority of patients eventually lost response.

In another trial, Decitabine was used to treat DIPSS-plus high-risk PMF. 82% (9 out of 11 patients) of patients benefited from this therapy. The median response duration was 9 months, and the median overall survival was 32 months in responders vs 16.3 months in nonresponders.

## **mTOR** *Inhibitors*

PI3K/AKT/mTOR pathway is one of the downstream components of the *JAK/STAT* signaling pathway. This pathway plays an important role in cell growth and proliferation in many malignancies, including MF.

BEZ235 inhibits PI3K kinase and mTOR kinase in the PI3K/AKT/mTOR kinase signaling pathway, which may result in tumor cell apoptosis and growth inhibition

in PI3K/mTOR-overexpressing tumor cells. Co-treatment of PMF cells with BEZ235 and the JAK2 inhibitor fedratinib enhances JAK2 inhibitor-mediated loss of survival, and BEZ235 also induces apoptosis in JAK2 inhibitor-resistant MPN cells.

Everolimus, an mTOR inhibitor, was used to as a monotherapy to treat intermediate- or high-risk patients with MF in a phase-I/II study. 23% of patients responded to therapy based on IWG-MRT criteria. 69% of patients had complete systemic symptom resolution. 44% of patients had reduction of splenomegaly by greater than 30%. . However, clinical responses were not associated with reduced *JAK2* V617F mutant allele burden, circulating CD34 + cells, or cytokine levels.

## *Telomerase Inhibitors*

Telomeres are repetitive DNA sequences (TTAGGG) at the end of chromosomes that protect coding DNA from genetic damage as well as cells from replicative senescence. Telomerase is a holoenzyme that is made up of human telomerase reverse transcriptase, an RNA template, and specialized proteins. It takes part in telomere synthesis and length maintenance in rapidly dividing cells such as cancer cells.

Imetelstat (GRN163L) is a 13-mer lipid-conjugated oligonucleotide that targets the RNA template of human telomerase reverse transcriptase. It inhibits telomerase activity and cell proliferation. This drug has been investigated as a promising agent to treat solid tumors and some hematologic malignancies.

Imetelstat was shown to have the potential to reverse bone marrow fibrosis. In one study, imetelstat was used to treat primary or secondary patients with MF ($N = 33$) with intermediate-2 or high-risk (DIPSS-Plus) disease. 48% of patients had received prior JAK inhibitor therapy. Complete remission (CR) or partial remission (PR) was achieved in 21% (7/33) of patients; median response duration was 18 months for CR and 10 months for PR, respectively. Among the four patients with CR, all had bone marrow fibrosis reversal, and 3 of the 4 patients demonstrated molecular response. Response rates were 27% among patients with a *JAK2* mutation versus 0% among those without a *JAK2* mutation, and 32% among patients without an *ASXL1* mutation versus 0% among those with an *ASXL1* mutation. Unfortunately, imetelstat was found to cause myelosuppresion [25].

## Hsp90 *Inhibitors*

Hsp90 (Heat-shock protein 90) is involved in activation of *JAK/STAT* signaling pathway through its function as an ATP-dependent dimeric molecular chaperone. This chaperone folds and stabilizes its client proteins, including JAK2 and STAT5, into their active conformations.

Hsp90 inhibitors bind to the N-terminal ATP-binding domain of Hsp90 and inhibit its chaperone function, which has been shown to induce proteasome-mediated degradation of client proteins in MPN cells.

Hsp90 inhibitors such as AUY922 have been shown to deplete JAK2 and induce apoptosis in MPN cells. In addition, they are also known to disrupt chaperone association of JAK2 with Hsp90 and induce degradation of JAK2. Therefore, they can be used in combination with JAK2 inhibitors to increase JAK2 targeting efficacy.

In one study, Hsp90 inhibitor was used in combination with ruxolitinib to treat patients with MF. The combination therapy reduced total and phosphorylated-JAK2 and achieved more potent inhibition of downstream signaling than ruxolitinib monotherapy. Compared with ruxolitinib alone, the combination therapy improved blood counts, spleen weights, and reduced bone marrow fibrosis.

## *Hedgehog Inhibitors*

The Hedgehog (Hh) proteins comprise a group of secreted proteins that regulate cell growth, differentiation, and survival. In adults, the Hh pathway is mainly quiescent, with the exception of roles in tissue maintenance and repair, and its inappropriate reactivation has been linked to several disparate human cancers. Moreover, Hh pathway is implicated in the development of fibrosis in the biliary and pulmonary system. The Hh signaling pathway has been shown to play a role in normal hematopoiesis and in the tumorigenesis of hematologic malignancies.

Saridegib (IPI-926) is an Hh inhibitor that inhibits signaling via a membrane protein called smoothened. In a phase-II study, 14 patients were treated with Saridegib orally for a median duration of 5 months. Twelve patients had slight reductions in spleen size (less than 50% from baseline), but symptoms did not improve consistently. Reductions in mRNA and protein levels of GLI1 (a key Hedgehog pathway target), JAK2V617F allele burden, degree of fibrosis, or cytokine levels were observed in some patients but were not significant when evaluated for the cohort. Unfortunately, all patients discontinued therapy by 7.5 months because of lack or loss of response or adverse effects. Two patients developed acute leukemia. These results, therefore, did not support Saridegib as an effective monotherapy for MF.

Hh inhibitor might be used in combination with JAK inhibitor to achieve additive or synergic therapeutic effect. In a mouse model of MF, combined inhibition of the *Hh* and *JAK* pathways showed promising results. The combination therapy caused reduction in *JAK2* mutant allele burden, bone marrow fibrosis, white blood cell, and platelet counts.

### *Allogeneic Stem Cell Transplantation*

ASCT has a definitive role in the treatment of patients with MF and is the only modality with proven curative potential. ASCT is an established treatment capable of eradication of the disease process and normalization of bone marrow findings. It can reverse bone marrow reticulin and collagen fibrosis and produces durable disease-free survival. However, this particular treatment is associated with a high rate of mortality and morbidity; therefore, it is only recommended for those with high DIPSS-plus score or high molecular risk.

In patients with MF, ASCT has conventionally been performed with busulfan/cyclophosphamide-based myeloablative chemotherapy regimens. Recently improved regimen such as ASCT with busulfan/fludarabine conditioning results in a dramatic decrease in JAK2 activity, splenomegaly, and bone marrow fibrosis.

The impact of treatment with JAK1/2 inhibitors on ASCT, in particular, timing of ASCT, is not clear and remains an essential area of research. Recently, a retrospective multicenter study was performed to analyze the outcomes of 100 consecutive patients who underwent ASCT for MF with prior exposure to JAK1/2 inhibitors. It was found that patients who experienced clinical improvement with JAK1/2 inhibitor therapy before ASCT had favorable outcomes, and prior exposure to JAK1/2 inhibitors did not adversely affect post-transplantation outcomes. Experts, therefore, suggest that JAK1/2 inhibitor therapy should be continued near to the start of conditioning therapy.

## Familial Primary Myelofibrosis

Familial PMF (FPMF) or inherited forms of PMF have been reported in both adults and children. The discussion here will be focused on adults. Familial PMF (FPMF) appears to be the member of the familial MPN, which are defined when in the same pedigree at least two relatives have an MPN as PV, ET, or PMF[26]. The prevalence of familial cases within MPN is at least 7.6% with an equal distribution of cases of PV, ET, and PMF. The clinical presentation at diagnosis of familial MPNs can be indistinguishable from sporadic disease. The presence of multilineage proliferation in probands or in the same pedigree is suggestive of familial MPN. Patients with familial MPN develop the same type of complications (thrombosis and hemorrhage) and disease evolution (post-PV myelofibrosis, post-ET myelofibrosis, and leukemia) observed in patients with sporadic MPN. The 10-year survival is 30% for those with FPMF.

In FPMF, the detection rate of *JAK2* V617F mutations is 75–90% [26]. The *JAK2* mutations are acquired and occur as secondary genetic events. As both mutations of the *JAK2* gene have been reported in the same pedigree, a genetic predisposition to the acquisition of the *JAK2* mutations is supposed to be inherited. The inheritance pattern of FMPN is consistent with an autosomal dominant trait with decreased

penetrance. A study done by the French Group of FMPN on 93 families and 227 patients including 14 FPMF revealed that a high JAK2 V617F allele burden (>50%) was not correlated with more frequent transformation to acute leukemia. The correlation of JAK2 V617F with thrombotic event is less clear [27]. In FMPN, TET2 mutations occur in both JAK2 V617F-positive and JAK2 V617F-negative cases; they are commonly acquired at the time of leukemic transformation [27].

## Prefibrotic Primary Myelofibrosis vs. Essential Thrombocythemia

PMF and ET are two distinct MPN entities by the new 2016 WHO classification. Prefibrotic PMF or pre-PMF is the early stage of PMF. Distinguish pre-PMF from true ET is very important because the pre-PMF has significantly higher leukemic transformation rate, higher rate of progression to overt myelofibrosis, and low survival rate than true ET [28]. However, their overlapping morphology, molecular markers, and clinical features make the distinction extremely difficult.

Clinically, pre-PMF and ET have similar age and gender distribution. However, some differences in clinical data have been observed between the two. Higher leukocyte counts, higher LDH, and lower hemoglobin level are seen in pre-PMF when compared with ET. The incidence of palpable splenomegaly and circulating CD34 cell counts is higher in pre-PMF than in ET.

Morphologically, BM of patient with ET shows predominantly large to giant, mature megakaryocytes with hyperlobulated nuclei and a random distribution or loose clustering within BM space without significant dysplasia. There is usually no or very rarely, less than 5%, grade-1 reticulin fibrosis. In pre-PMF, increased age-matched BM cellularity, increased megakaryocytes with atypical histotopography (endosteal translocation, dense clusters), and distinctive nuclear features (hypolobulation, clumsy-cloud-like, maturation defects), granulocytic proliferation, and reduced erythropoiesis are seen. Reticulin fibers are normal or minor (grade 0/1). Due to the lack or minimal marrow fibrosis and the presence of megakaryocytic hyperplasia in both conditions, the distinction of ET and pre-PMF morphologically is extremely challenged. Despite the morphological distinctions listed above, according to several investigations by different groups, the consensus among morphological diagnosis in distinguish ET from pre-PMF ranges from 53 to 88% [29]. Since 2005, more than 20 somatic mutations have been found in PMN. However, none of them have proven to be specific for a specific MPN and no specific molecular markers can help differentiate ET from pre-PMF [30].

Distinguish ET from pre-PMF is not trivial. Recognizing specific BM histology patterns, adequate clinical information and laboratory results together with mutational studies are necessary to accurately differentiate the two.

## Conclusions

PMF is one of the chromosome-negative myeloproliferative neoplasms derived from clonal stem cell proliferation. Compared with ET and PV, the prognosis of PMF is worse, and it is, therefore, critical to distinguish it from the other two diseases. The central theme of pathogenesis in PMF is persistent activation of *JAK/STAT* signaling pathway through driver mutations, i.e., mutations in *JAK2, CALR,* and *MPL* genes. Mutations in five genes, *ASXL1, EZH2, SRSF2, IDH1,* and *IDH2*, are recognized as detrimental mutations because they are associated with shorter leukemia-free survival and overall survival; accordingly, patients with PMF with at least one of these mutation are classified as having "high molecular risk." Currently molecular profiling of PMF is recommended in clinical management to refine prognostic models and stratify therapeutic decisions. So far, there is no effective treatment for PMF except ASCT, which is not mortality free. The currently approved drugs including ruxolitinib can only provide palliative solutions. There is an urgent need for disease-modifying drugs. Numerous targeted therapies are being investigated, and some of them have demonstrated promising results.

## References

1. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;27(20):2391–405.
2. Vannucchi AM, Lasho TL, Guglielmelli P, et al. Mutations and prognosis in primary myelofibrosis. Leukemia. 2013;27(9):1861–9.
3. Tefferi A, Lasho TL, Huang J, et al. Low JAK2V617F allele burden in primary myelofibrosis, compared to either a higher allele burden or unmutated status, is associated with inferior overall and leukemia-free survival. Leukemia. 2008;22(4):756–61.
4. Bench AJ, White HE, Foroni L, et al. Molecular diagnosis of the myeloproliferative neoplasms: UK guidelines for the detection of JAK2 V617F and other relevant mutations. Br J Haematol. 2013;160(1):25–34.
5. Klampfl T, Gisslinger H, Harutyunyan AS, et al. Somatic mutations of calreticulin in myeloproliferative neoplasms. N Engl J Med. 2013;369(25):2379–90.
6. Stanley RF, Steidl U. Molecular mechanism of mutant CALR-mediated transformation. Cancer Discov. 2016;6(4):344–6.
7. Pietra D, Rumi E, Ferretti VV, et al. Differential clinical effects of different mutation subtypes in CALR-mutant myeloproliferative neoplasms. Leukemia. 2016;30(2):431–8.
8. Tefferi A, Lasho TL, Finke CM, et al. CALR vs JAK2 vs MPL-mutated or triple-negative myelofibrosis: clinical, cytogenetic and molecular comparisons. Leukemia. 2014;28(7):1472–7.
9. Rumi E, Pietra D, Pascutto C, et al. Clinical effect of driver mutations of JAK2, CALR, or MPL in primary myelofibrosis. Blood. 2014;124(7):1062–9.
10. Tefferi A, Lasho TL, Finke C, et al. Type 1 vs type 2 calreticulin mutations in primary myelofibrosis: differences in phenotype and prognostic impact. Leukemia. 2014;28(7):1568–70.
11. Guglielmelli P, Rotunno G, Bogani C, et al. Ruxolitinib is an effective treatment for CALR-positive patients with myelofibrosis. Br J Haematol, 2016;173:938–49.
12. Loghavi S, Bueso-Ramos CE, Kanagal-Shamanna R, et al. Myeloproliferative neoplasms with calreticulin mutations exhibit distinctive morphologic features. Am J Clin Pathol. 2016;145(3):418–27.

13. Andrici J, Farzin M, Clarkson A, et al. Mutation specific immunohistochemistry is highly specific for the presence of calreticulin mutations in myeloproliferative neoplasms. Pathology. 2016;48(4):319–24.
14. Pardanani A, Guglielmelli P, Lasho TL, et al. Primary myelofibrosis with or without mutant MPL: comparison of survival and clinical features involving 603 patients. Leukemia. 2011;25(12):1834–9.
15. Milosevic Feenstra JD, Nivarthi H, Gisslinger H, et al. Whole-exome sequencing identifies novel MPL and JAK2 mutations in triple-negative myeloproliferative neoplasms. Blood. 2016;127(3):325–32.
16. Guglielmelli P, Lasho TL, Rotunno G, et al. The number of prognostically detrimental mutations and prognosis in primary myelofibrosis: an international study of 797 patients. Leukemia. 2014;28(9):1804–10.
17. Tefferi A, Guglielmelli P, Lasho TL, et al. CALR and ASXL1 mutations-based molecular prognostication in primary myelofibrosis: an international study of 570 patients. Leukemia. 2014;28(7):1494–500.
18. Gangat N, Caramazza D, Vaidya R, et al. DIPSS plus: a refined dynamic international prognostic scoring system for primary myelofibrosis that incorporates prognostic information from karyotype, platelet count, and transfusion status. J Clin Oncol. 2011;29(4):392–7.
19. Tefferi A. Myeloproliferative neoplasms: a decade of discoveries and treatment advances. Am J Hematol. 2016;91(1):50–8.
20. Koppikar P, Bhagwat N, Kilpivaara O, et al. Heterodimeric JAK-STAT activation as a mechanism of persistence to JAK2 inhibitor therapy. Nature. 2012;489(7414):155–9.
21. Verstovsek S, Mesa RA, Gotlib J, et al. A double-blind, placebo-controlled trial of ruxolitinib for myelofibrosis. N Engl J Med. 2012;366(9):799–807.
22. Harrison C, Kiladjian JJ, Al-Ali HK, et al. JAK inhibition with ruxolitinib versus best available therapy for myelofibrosis. N Engl J Med. 2012;366(9):787–98.
23. Vannucchi AM, Kantarjian HM, Kiladjian JJ, et al. A pooled analysis of overall survival in COMFORT-I and COMFORT-II, 2 randomized phase III trials of ruxolitinib for the treatment of myelofibrosis. Haematologica. 2015;100(9):1139–45.
24. Guglielmelli P, Biamonte F, Rotunno G, et al. Impact of mutational status on outcomes in myelofibrosis patients treated with ruxolitinib in the COMFORT-II study. Blood. 2014;123(14):2157–60.
25. Tefferi A, Lasho TL, Begna KH, et al. A pilot study of the telomerase inhibitor imetelstat for myelofibrosis. N Engl J Med. 2015;373(10):908–19.
26. Rumi E. Familial chronic myeloproliferative disorders: the state of the art. Hematol Oncol. 2008;26(3):131–8.
27. Malak S, Labopin M, Saint-Martin C, Bellanne-Chantelot C, Najman A. French Group of Familial Myeloproliferative Disorders. Long term follow up of 93 families with myeloproliferative neoplasms: life expectancy and implications of JAK2V617F in the occurrence of complications. Blood Cells Mol Dis. 2012;49(3–4):170–6.
28. Barbui T, Thiele J, Vannucchi AM, Tefferi A. Myeloproliferative neoplasms: morphology and clinical practice. Am J Hematol. 2016;91(4):430–3.
29. Barbui T, Thiele J, Vannucchi AM, Tefferi A. Rationale for revision and proposed changes of the WHO diagnostic criteria for polycythemia vera, essential thrombocythemia and primary myelofibrosis. Blood Cancer J. 2015;5:e337. doi:10.1038/bcj.2015.64.
30. Barbui T, Thiele J, Vannucchi AM, Tefferi A. Problems and pitfalls regarding WHO-defined diagnosis of early/prefibrotic primary myelofibrosis versus essential thrombocythemia. Leukemia. 2013;27(10):1953–8.

# Chapter 9
# Mastocytosis

**David Czuchlewski and Tracy I. George**

## Introduction

Mastocytosis represents a heterogeneous group of disorders ranging from urticaria pigmentosa in children that typically spontaneously resolves to aggressive malignancies such as mast cell leukemia in adults with short survival and poor prognosis. These different subtypes share in common a proliferation of neoplastic mast cells and activating mutations of *KIT* (particularly D816V), resulting in activation of *KIT* and downstream signaling pathways. In this chapter, we will review the molecular pathology of mastocytosis involving *KIT* mutations and other genetic mutations, including the therapeutic and prognostic importance of these mutations.

### *Epidemiology*

The incidence of mastocytosis is unknown, but it is recognized as a rare disease. Cutaneous forms of mastocytosis are more common than the systemic forms of the disease. Cutaneous mastocytosis (CM) is primarily a disease of childhood with onset during the first year of life; a second peak is seen in older adults aged 50–60 years where skin disease occurs as a manifestation of systemic disease [1]. The advanced forms of mast cell disease, including mast cell leukemia (MCL), aggressive systemic mastocytosis (ASM), and systemic mastocytosis with an associated hematologic neoplasm (SM-AHN) represent a minority of the adults with systemic mastocytosis (SM). Most adults with SM have indolent disease. For example, fewer than 100 cases of MCL have been reported in the literature to date [2].

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## *Etiology*

Mast cells are of hematopoietic origin and arise from a bone marrow-derived CD34-positive and KIT-positive pluripotent progenitor, and human mast cells differentiate under the influence of stem cell factor, which binds to the KIT tyrosine kinase receptor [3]. Recurrent activating somatic mutations in *KIT* have been detected in neoplastic mast cells of SM and to a lesser extent, CM [4]. Rare familial cases of mastocytosis are discussed later in the chapter.

## *Clinical Features and Classification*

Two large categories of mastocytosis are described, cutaneous disease (skin only) and systemic disease, which includes at least one extracutaneous organ. Typically, systemic disease involves the bone marrow and spleen, but can also involve lymph nodes, liver, and skin, among other organs. SM is further divided into indolent and advanced forms of disease based on a mix of clinical and pathological findings. The WHO classification of mastocytosis includes the major categories of mastocytosis (Table 9.1) [5], but does not include myelomastocytic leukemia or well-differentiated mastocytosis. Diagnostic criteria for SM and mastocytosis variants are listed in Tables 9.2 and 9.3, respectively [6].

CM is subclassified on clinical features into maculopapular cutaneous mastocytosis (MPCM), which includes urticaria pigmentosa, diffuse cutaneous mastocytosis (DCM), and cutaneous mastocytoma (Table 9.4) [1]. The flushing, urticaria, pruritus and dermatographism associated with CM are very common symptoms and the result of local release of mast cell mediators. The vast majority of patients with CM includes children with the characteristic red or brown flat or raised skin lesions of MCPM. Whealing and reddening of the lesions when rubbing or stroking of the skin lesions is typical (Darier's sign). In adults, the presence of MCPM is highly suggestive of SM, most commonly indolent SM [8] and careful staging for systemic disease is recommended. The skin lesions in adult are typically distributed over the thigh and trunk, whereas in children these polymorphous lesions are found over the trunk, head, and extremities. DCM manifests as generalized erythema accompanied by thickened skin; initially, this might present with blisters. Cutaneous mastocytoma is usually a solitary brown or yellow lesion and these lesions do not typically progress past childhood; multiple lesions of mastocytoma are also described. In the past, a telangiectatic variant of CM was described as telangiectasia macularis eruptiva perstans (TMEP); this has been eliminated from the classification [1]. In the authors' experience, lesions of TMEP often represent skin manifestations of indolent SM (ISM).

**Table 9.1** WHO 2016 classification of mastocytosis [5]

| |
|---|
| Cutaneous mastocytosis |
| Systemic mastocytosis |
| Indolent systemic mastocytosis |
| Smoldering systemic mastocytosis |
| Systemic mastocytosis with an associated hematologic neoplasm |
| Aggressive systemic mastocytosis |
| Mast cell leukemia |
| Mast cell sarcoma |

**Table 9.2** Criteria for systemic mastocytosis [6]

| |
|---|
| 1 major and 1 minor *OR* 3 minor criteria |
| *Major* |
| Multifocal dense aggregates of mast cells in extracutaneous organ(s) |
| *Minor* |
| > 25% atypical or immature mast cells in aspirate smears or sections |
| Activating point mutation at codon 816 in *KIT* |
| CD25 expression in mast cells, with or without CD2 |
| Serum total tryptase level >20 ng/mL (unless an associated myeloid neoplasm is present which would invalidate this criterion) |

An aggregate is defined as 15 or more mast cells in clusters, in bone marrow and/or another extracutaneous organ.
In smears, more than 25% of total mast cells should be atypical or immature. In biopsy sections of the bone marrow or other extracutaneous organs, more than 25% of mast cells are spindle shaped or show atypical morphological characteristics.
Mast cells in peripheral blood, bone marrow, or other extracutaneous organ express CD25, +/− CD2, in addition to normal mastocytosis markers (tryptase, CD117, etc.)
An associated non-mast cell hematologic neoplasm may also secrete tryptase which is why this minor criterion cannot be used when an associated myeloid neoplasm is present.

Mediator release symptoms caused by mast cell degranulation result in severe hypotension, syncope, headache, anaphylaxis, flushing, pruritus, urticaria, and gastrointestinal symptoms including diarrhea, abdominal pain, nausea, and vomiting. Malabsorption with weight loss can also occur in advanced forms of mastocytosis. Consumption of alcohol, certain foods, stress, or other triggers can precipitate symptoms. Bone involvement can lead to bone pain, osteoporosis, and pathologic fractures. Bone marrow involvement leads to cytopenias. Spleen involvement leads to splenomegaly with hypersplenism and liver involvement leads to hepatomegaly with impaired liver function, ascites and/or portal hypertension. Clinicopathologic findings have led to the development of "B" and "C" findings used as criteria in the diagnosis of smoldering SM and ASM, respectively (Tables 9.5 and 9.6) [7].

**Table 9.3** Diagnostic criteria for mastocytosis variants [1, 7]

| |
|---|
| Cutaneous mastocytosis |
| Does not meet criteria for systemic mastocytosis[a] |
| Typical skin lesions of mastocytosis associated with Darier's sign (major) |
| Increased numbers of mast cells in skin biopsy of lesion (minor) |
| Activating *KIT* mutation in skin biopsy of lesion (minor) |
| Indolent systemic mastocytosis |
| Meets systemic mastocytosis criteria[a] |
| No B- or C-findings |
| No associated hematologic neoplasm |
| Smoldering systemic mastocytosis |
| Meets systemic mastocytosis criteria[a] |
| No C-findings |
| No associated hematologic neoplasm |
| 2 or more B-findings[b] |
| Systemic mastocytosis with an associated hematologic neoplasm |
| Meets systemic mastocytosis criteria[a] |
| Meets criteria for an associated clonal non-mast cell associated neoplasm per the WHO classification |
| Aggressive systemic mastocytosis |
| Meets systemic mastocytosis criteria[a] |
| 1 or more C-findings[c] |
| No evidence of mast cell leukemia |
| Mast cell leukemia |
| 20% or more mast cells on aspirate smears |
| Mast cell sarcoma |
| Does not meet criteria for systemic mastocytosis[a] |
| Unifocal lesion with destructive growth pattern and high grade cytology |

Cutaneous mastocytosis in children particularly may be diagnosed by the absence of systemic mastocytosis and the presence of the major criterion above; skin biopsy is not always necessary. Adults with cutaneous mastocytosis should be staged to evaluate for systemic disease. In indolent systemic mastocytosis, skin lesions are often present. Isolated bone marrow mastocytosis is a type of indolent disease that lacks skin lesions. Indolent systemic mastocytosis typically has a low mast cell burden in the bone marrow, in contrast to aggressive systemic mastocytosis and mast cell leukemia. Mast cell leukemia tends to have a diffuse pattern of bone marrow infiltration, often by atypical and/or immature mast cells; this variant of mastocytosis often lacks skin lesions

[a]See Table 9.2

[b]See Table 9.5

[c]See Table 9.6

**Table 9.4** Cutaneous mastocytosis classification [1]

| |
|---|
| Maculopapular cutaneous mastocytosis |
| Monomorphic variant |
| Polymorphic variant |
| Diffuse cutaneous mastocytosis |
| Cutaneous mastocytoma |

The monomorphic variant of maculopapular cutaneous mastocytosis has small maculopapular lesions and is more typically seen in adult patients, whereas the polymorphic variant is more typical in pediatric patients and which resolves around puberty

**Table 9.5** "B" findings [7]

| |
|---|
| Bone marrow biopsy with >30% mast cells and/or serum tryptase >20 ng/mL |
| Signs of dysplasia or proliferation in non-mast cell lineage insufficient for a diagnosis of an associated hematologic neoplasm, with normal or slightly abnormal blood counts |
| Hepatomegaly without impaired liver function, and/or palpable splenomegaly without hypersplenism, and/or lymphadenopathy |

The diagnosis of smoldering systemic mastocytosis meets criteria for systemic mastocytosis, with 2 more of the above "B" findings, but no "C" findings

**Table 9.6** "C" findings [7]

| |
|---|
| Cytopenias without an associated hematologic malignancy (ANC $<1.0 \times 10^9$/L, Hb < 10 g/dL, platelets $<100 \times 10^9$/L) |
| Palpable hepatomegaly with impaired liver function, ascites and/or portal hypertension |
| Bone involvement with large osteolytic lesions and/or pathologic fractures |
| Palpable splenomegaly with hypersplenism |
| Malabsorption with weight loss due to gastrointestinal mast cell lesions |

The diagnosis of aggressive systemic mastocytosis meets criteria for systemic mastocytosis with one or more "C" findings, with no evidence of mast cell leukemia (≥20% mast cells on aspirate smears)

## Morphology and Immunophenotype

### *Cytology*

The cytology of mast cells as visualized in air-dried preparation stained with Romanowsky-type stains is summarized in Table 9.7. Well-differentiated mast cells are small with a round nucleus and a cytoplasm containing tightly packed and uniform metachromatic granules. Nucleoli are absent or indistinct. Well-differentiated mast cells are found in normal bone marrow, mast cell hyperplasia, and a variant of

**Table 9.7** Cytology of mast cells

| Mast cell type | Size/shape | Nucleus | Nuclear Chromatin | Cytoplasm | Nuclear to cytoplasmic ratio | Associated disorders |
|---|---|---|---|---|---|---|
| Well-differentiated | Small-medium, round or oval | Central, round or oval | Condensed | Well granulated | Low | Normal, mast cell hyperplasia, well-differentiated mastocytosis |
| Atypical Type I (Spindle shaped) | Elongated cytoplasmic extensions (spindle shaped) | Central or eccentric, oval | Condensed | Hypogranular, focal granule accumulation without degranulation | Variable | Systemic mastocytosis |
| Atypical Type II (Promastocyte) | Variable | Bi- or poly-lobed | Fine or condensed | Hypogranular without degranulation | Variable | Mast cell leukemia, myelomastocytic leukemia |
| Metachromatic[a] Blast | Medium-large, round or oval | Prominent nucleoli | Fine | Few metachromatic granules | High | Mast cell leukemia, myelomastocytic leukemia |

[a]Metachromatic: a cell that characteristically takes on a color different from that of the dye with which it is stained

mastocytosis known as well-differentiated mastocytosis, which is not currently recognized in the WHO 2016 classification. Atypical type I or spindle shaped mast cells have elongated cytoplasmic projections which are often hypogranular and oval nuclei. Increased atypical type I mast cells are seen in many different variants of systemic mastocytosis (SM) (Fig. 9.1). Promastocytes or atypical type II mast cells contain bilobed, multilobated or indented nuclei with abundant hypogranular cytoplasm; nuclear chromatin may be immature or condensed. Metachromatic blasts are immature mast cells with blastic chromatin, high nuclear-to-cytoplasmic ratios, prominent nucleoli, and a few large metachromatic granules; immunophenotyping is necessary to confirm a mast cell lineage in these cells. Promastocytes and metachromatic blasts are more typically found in MCL and myelomastocytic leukemia [9–11].

## *Histology*

Histologic examination of tissues involved by mastocytosis is necessary for the diagnosis of disease with the exception of typical MPCM (Table 9.4) [1]. Hematoxylin and eosin (H&E) stained sections and immunohistochemistry or cytochemical stains should be used to confirm the presence of mast cells and aberrant antigen expression, if present. On H&E stained sections, mast cells often have abundant clear or lightly eosinophilic cytoplasm with oval to round to indented nuclei and they can be admixed with fibroblasts, eosinophils, small lymphocytes, and plasma cells (Fig. 9.2). The histologic findings of mast cells can mimic other neoplasms or non-neoplastic conditions and is described in further detail below.

In CM, mast cells infiltrate the dermis with aggregates of mast cells filling the papillary dermis and extending into the reticular dermis in MPCM, demonstrating a band-like dermal pattern in diffuse cutaneous mastocytosis and a single lesion with large aggregates in the dermis and sometimes subcutis in cutaneous mastocytoma. The subtle infiltration by mast cells is best visualized by immunohistochemistry.

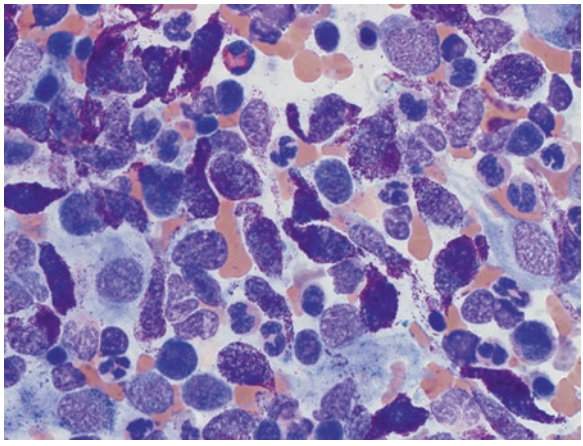

**Fig. 9.1** Mast cell leukemia, cytology. Numerous spindle shaped mast cells are present in this bone marrow aspirate smear. The cytoplasm is less granular than normal tissue mast cells and nuclei are oval shaped. Admixed hematopoiesis is present including neutrophilic and erythroid precursors. Wright Giemsa stain, 60×

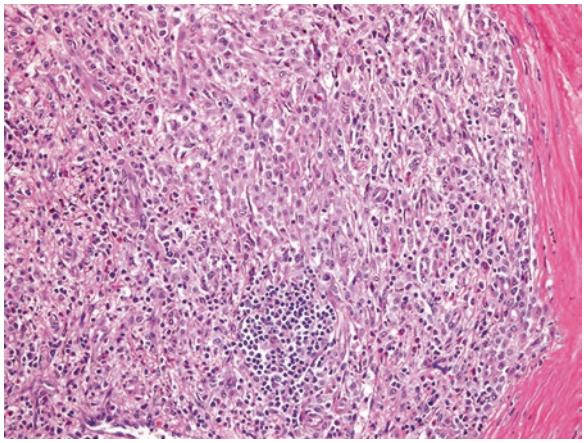

**Fig. 9.2** Mast cell leukemia, histology. Sheets of neoplastic mast cells have replaced the spleen leaving only small islands of lymphocytes. The mast cells have abundant lightly eosinophilic cytoplasm and are admixed with eosinophils. Spleen, Hematoxylin & eosin stain, 20×

Cytologic atypia is not usually a feature of CM. However, in mast cell sarcoma, cytologic atypia is marked such that the neoplastic cells may not resemble normal mast cells and must be identified via immunohistochemistry. Rarely, mast cell sarcoma may transform into mast cell leukemia.

Bone marrow infiltration by mast cells in SM may be interstitial, focal and dense, diffuse and dense patterns, or present as mixed patterns [12]. While most cases of SM contain focal dense aggregates with accompanying reticulin and collagen fibrosis and osteosclerosis, early indolent disease may show an entirely interstitial pattern that has also been termed "occult" SM [13]. A detailed description of mast cell disease in other organs is beyond the scope of this review, but is well described elsewhere [14]. It should also be noted that in SM-AHN, features of both SM and the associated hematologic neoplasm are present, where the latter are typically myeloid neoplasms including myeloproliferative neoplasm (MPN), myelodysplastic syndrome (MDS), MDS/MPN, and acute myeloid leukemia.

## *Cytochemistry and Immunophenotype*

Mast cells will be highlighted by cytochemical stains (Giemsa, toluidine blue, naphthol-ASD-chloroacetate esterase), but these stains are less specific than immunophenotypic studies such as tryptase, which is regarded as the most specific marker for mast cells [15]. One important exception is in the gastrointestinal tract where tryptase expression in neoplastic mast cells is highly variable with only a subset of mast cells being positive [16]. Immunophenotyping of mast cells may be performed by immunohistochemistry or flow cytometry (Figs. 9.3 and 9.4). Despite the low percentage of mast cells found in bone marrow aspirate specimens due to fibrotic-rich mast cell aggregates not aspirating well, flow cytometry is quite sensitive for

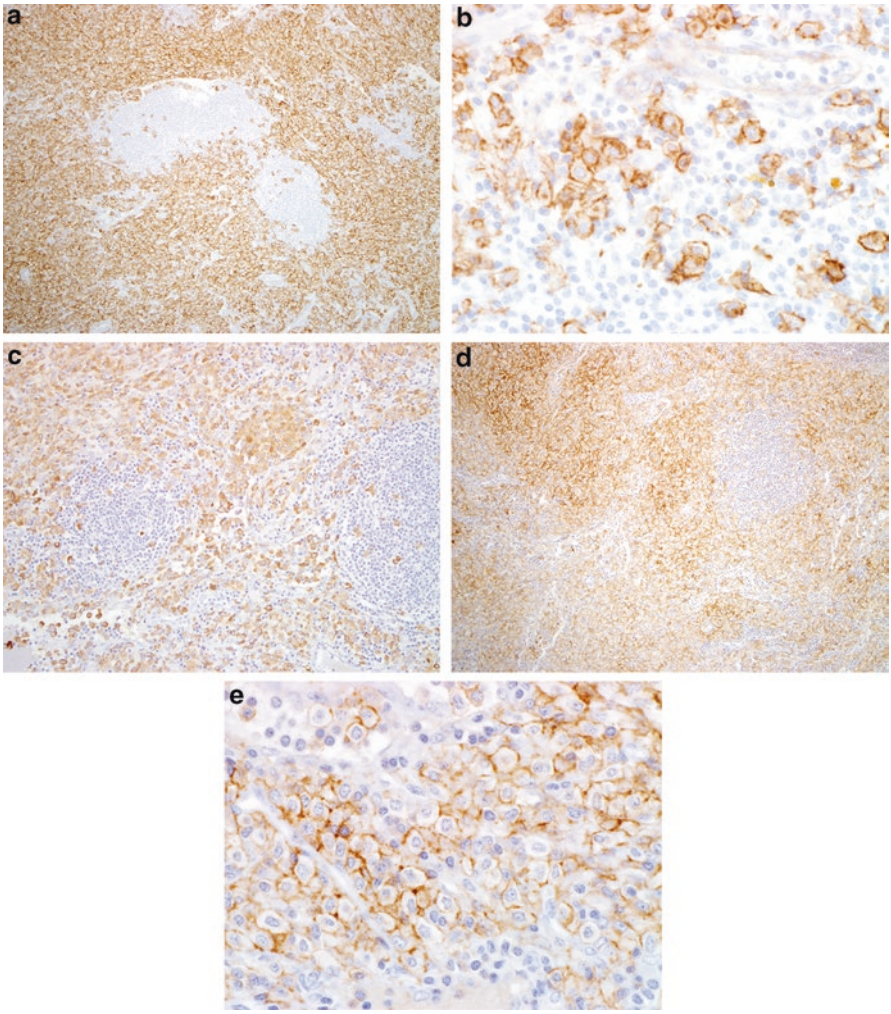


**Fig. 9.3** Mast cell leukemia, immunohistochemistry. (**a**) Sheets of CD117-positive mast cells have largely replaced this lymph node in this patient with mast cell leukemia, where splenic involvement is shown in Fig. 9.2. CD117, 20×. (**b**) On higher power, the CD117 staining is shown to be membranous with occasional perinuclear staining. CD117, 60×. (**c**) Tryptase staining of the same lymph node shows a similar pattern of involvement with cytoplasmic staining. Tryptase, 20×. (**d**) Immunohistochemistry with antibody to CD25 highlights more variable intensity of staining. CD25, 20×. (**e**) A membranous staining pattern is also seen with CD25 immunostain. CD25, 60×

detecting mast cells when a selective gating strategy is performed [17]. Mast cells express CD9, CD11c, CD29, CD33, CD43, CD44, CD45, CD49d, CD49e, CD51, CD54, CD68, CD71, CD117, FceRI [18] and neoplastic mast cells may express CD25, CD2, CD35, CD59, CD63, CD69, high light scatter and autofluorescence

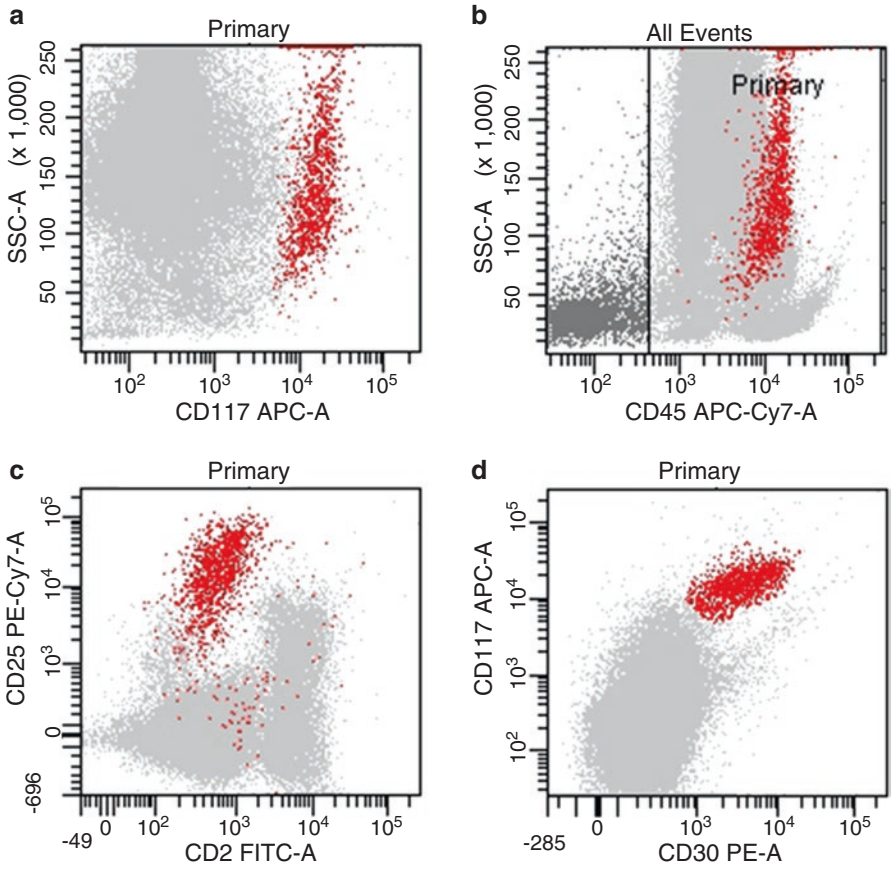


**Fig. 9.4** Advanced systemic mastocytosis, flow cytometry. (**a**) Flow cytometry of bone marrow aspirate from a patient with advanced systemic mastocytosis shows very bright CD117 with an increased amount of side scatter. The mast cells are a lot brighter in intensity than other CD117-positive bone marrow cells, such as myeloblasts. (**b**) Neoplastic mast cells also show bright CD45 expression. (**c**) Coexpression of CD25 is present in neoplastic mast cells without CD2 coexpression. (**d**) Coexpression of CD30 is also present in CD117-bright mast cells

[17]. Flow cytometry is more sensitive in detecting CD25 and CD2 expression on mast cells. In routine practice, immunohistochemistry for tryptase, CD117, and CD25 are frequently used to detect neoplastic mast cell infiltrates, with CD30 staining described recently [19]. One important exception to this rule is SM with well-differentiated mast cells (or well-differentiated mastocytosis) where mast cells do not coexpress CD25 or CD2, lack exon 17 *KIT* mutations, but are often responsive to imatinib [20]. One pattern of bone marrow infiltration by mast cells described with tryptase staining is the tryptase-positive compact round cell infiltrate of the bone marrow (TROCI-BM) [21]. This pattern of staining is rare, but can be seen in a specific set of myeloid neoplasms including SM.

## Genetic Findings

The *KIT* gene (Fig. 9.5), located at 4q12, encodes a transmembrane receptor tyrosine kinase protein that serves as the receptor for stem cell factor. It is expressed on melanocytes, germ cells, and pacemaker cells within the gastrointestinal tract [22]. Upon ligand binding, the KIT protein dimerizes and becomes transphosphorylated, allowing the transmission of prosurvival and migration signaling through subsequent downstream mediators. Structurally, the 21 exons of the protein comprise an extracellular domain with five immunoglobulin-like subunits, a hydrophobic transmembrane domain, and an intracellular domain that includes an autoinhibitory juxtramembrane domain (JMD) as well as the kinase domain. The latter is separated into two regions with an intervening hinge domain [23].

Activating mutations of *KIT* are seen not only in mastocytosis, but also in other neoplasms such as acute myeloid leukemia, gastrointestinal stromal tumor, germ cells tumors, and melanoma. The exact mechanism by which the *KIT* mutation effects protein activation may vary, depending on the location of the mutation within the protein structure. Even for the most common codon D816V mutation, however, the structural consequences are not completely clear. Some evidence suggests that changes in the activation loop of the protein (codons 810–835) may have a positional effect on the autoinhibitory JMD, resulting in increased protein kinase activity [24]. The change, which might also promote protein dimerization, is similar to the structural effect of common changes in *EGFR* and *BRAF* [24]. *KIT* mutations have also been shown to alter the cellular localization of the protein and the precise substrate specificity for downstream signaling purposes [25, 26].

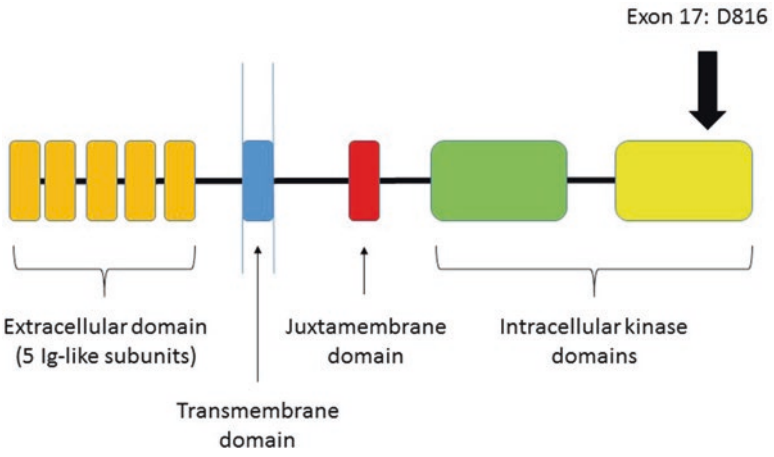


**Fig. 9.5** *KIT* gene. A schematic representation of the *KIT* gene highlighting the most common site of the activating *KIT* mutation in mastocytosis (*arrow*)

## *Familial Mastocytosis*

Germline mutations in *KIT* have been described in association with familial mastocytosis (Table 9.8), but other patients have not had any known *KIT* mutations [37]. All subtypes of CM have been reported in association with familial mastocytosis [27, 30, 31, 38–40]. An autosomal dominant pattern of inheritance with incomplete penetrance is the most common inheritance pattern [41, 42]. Interestingly, one study of well-differentiated mastocytosis has described a familial association in 13 (39%) patients including D816V (3), K509I (3) and wildtype *KIT* (7) with all patients presenting with CM, except for the three patients with K509I who had SM including MCL (2) and ISM (1) [20].

## *Cutaneous Mastocytosis*

The distribution of *KIT* mutations is dramatically different in pediatric CM. These cases are much less likely to harbor the D816V mutation, which is seen in approximately 1/3 of such cases. Instead, alternative *KIT* mutations are often observed, especially in codon 8 (e.g., p.Asp419del, ~17%), codon 9 (e.g., pAla502_Tyr503dup, ~6%) and codon 11 [23, 34]. Some 25% of these cases appear to be *KIT* wild-type [23, 43]. An interesting wrinkle in the molecular analysis of pediatric CM is the presence of germline *KIT* mutations in rare cases of familial mastocytosis as discussed above [35].

**Table 9.8** KIT mutations in familial mastocytosis

| Exon | Mutation | Mastocytosis | Other tumors | Reference |
|---|---|---|---|---|
| 8 | del D419 | DCM | GIST | [27] |
| 9 | K509I | SM, MCPM | | [28, 29] |
| | S451C | DCM | | [30] |
| 10 | A533D | DCM | | [31] |
| | M541 L | CM | | |
| 11 | V559A | MCPM | GIST | [32] |
| 13 | R634W | MCPM | | [33] |
| 17 | D816V | MCPM | | [34] |
| | N822I | MCPM | | [35] |
| 18 | S849I | CM, MCAS | | [36] |
| | M835 K | CM, MCAS | | [36] |

*CM* cutaneous mastocytosis, *DCM* diffuse cutaneous mastocytosis, *GIST* gastrointestinal stromal tumor, *MCAS* mast cell activation syndrome, *MCPM* maculopapular cutaneous mastocytosis, *SM* systemic mastocytosis

### *Indolent Mastocytosis*

*KIT* mutations in mastocytosis display heterogeneity that mirrors the clinical and morphological complexity of this disease entity. In indolent SM, the vast majority of cases (~95%) show the D816V mutation when appropriately sensitive assay techniques are utilized, as described below [44]. Other *KIT* mutations more rarely seen in cases of adult SM include D816Y, D816F, D816H, and D816I, D820G, and V559I.

### *Advanced Mastocytosis*

Advanced mastocytosis includes MCL, ASM, and SM-AHN. The prevalence of *KIT* D816V mutations appears to be somewhat lower in MCL and mast cell sarcoma [2, 45].

Interestingly, in many cases of SM-AHN, *KIT* mutation is detected in both the neoplastic mast cells and the non-mast cell lineage neoplastic cells, supporting in some cases the involvement of a common progenitor cell and/or divergent cellular maturation of the neoplastic clone [46]. The clonal identity between mast cell and non-mast cell neoplastic cells is more common in cases with chronic myelomonocytic leukemia as the non-mast cell lineage disease than in cases occurring with myeloproliferative neoplasms or AML [46].

With the advent of next generation sequencing, molecular analysis of mastocytosis has expanded beyond the *KIT* gene to include a number of other genes that are known to be recurrently mutated in myeloid neoplasms. Briefly, these include:

- *TET2*, mutated in ~20–30% of cases of mastocytosis [47, 48]. Some early data suggested an association between *TET2* mutation and aggressive disease [49], but this was not substantiated in later studies [48, 50].
- *ASXL1*, mutated in ~12–21% of cases of mastocytosis, independently associated with inferior overall survival in multiple cohorts [48, 50].
- Additional recurrently mutated genes include *SRSF2, SF3B1, U2AF1, NRAS, KRAS, CBL, DNMT3A, ETV6, EZH2, JAK2,* and *SETBP1* [23, 48].

Of note, non-*KIT* mutations tend to be seen more frequently in aggressive forms of SM and in SM-AHN, while being less common in indolent SM (14% of cases) [48]. In particular, SM-AHN is a multimutated myeloid neoplasm with mutations in *TET2*, *SRSF2,* or *ASXL1* preceding *KIT* D816V [51]. It is possible that these additional mutations underlie the more aggressive clinical behavior of these cases. In fact, the number of additional non-*KIT* mutations correlates with the degree of survival decrement [48]. Such observations underscore the opportunities for prognostic and therapeutic insight afforded by our increased understanding of mast cell disease at the molecular level.

### *Techniques*

In discussing the molecular detection of *KIT* mutations, it is important to grasp the impact of assay methodology on the detection rate, and therefore the reported incidence, of *KIT* mutations. Neoplastic mast cells tend to be distributed in the bone marrow in many cases in a patchy fashion, and the yield of neoplastic cells on aspirate or clot sections can be variable. Thus, when using relatively insensitive detection techniques such as Sanger sequencing on unfractionated bone marrow cells, a substantial fraction of cases of SM may appear to lack the *KIT* mutation. However, the detection rate substantially increases when using assays of higher analytic sensitivity such as allele-specific quantitative PCR, nested PCR, digital PCR, microdissection and/or cell sorting techniques [52–54]. Of note, recent data suggest that even next generation sequencing at a high depth of coverage may be insufficient for *KIT* mutation detection in some SM cases [55].

## Prognosis and Therapy

CM has a benign clinical course in the vast majority of patients with spontaneous regression of lesions around the time of puberty. In one long-term follow-up study of children with CM, 10 of 15 children had complete resolution of their symptoms and disease 20 years later [56]. Thus, therapeutic approaches for CM, but also SM, targets the treatment of mediator related symptoms [57, 58]. This includes the use of histamine receptor antagonists, both HR1 and HR2 antagonists. Proton pump inhibitors and the mast cell stabilizer cromolyn sodium are also used. Short courses of glucocorticosteroids may be used for anaphylaxis and mediator-related symptoms that do not respond to histamine receptor antagonists and mast cell stabilizers. Other treatments for CM include a topical calcineurin inhibitor [59], narrowband ultraviolet B phototherapy [60, 61], and miltefosine [62]. Masatinib, a multikinase KIT inhibitor that targets wild type KIT, has shown improvement in symptoms in patients with indolent SM and CM associated with significant quality-of-life issues [63].

Patients with SM may have marked osteoporosis or osteopenia for which bisphosphonates are used [64]. For those patients with severe allergic symptoms, allergen immunotherapy may be helpful and an epinephrine pen for emergency use [65, 66]. For patients with progressive smoldering SM, cladribine has been recommended [67].

When advanced SM is present, cytoreductive agents and targeted therapies have been used including cladribine, interferon alpha, cytarabin, fludarabine, hydroxyurea, and tyrosine kinase inhibitors [68]. It is also important to note that the *KIT* status also informs the choice of therapy. The common D816 mutations are insensitive to imatinib [69], while the alternative mutations affecting the JMD and the extracellular domain predict clinical responsiveness to this agent. Recently, alternative kinase inhibitors such as midostaurin and dasatinib have shown promise

in some cases of D816-mutated and nonmutated SM [70, 71]. Hematopoietic stem cell transplantation is recommended for young patients with a suitable donor [72], and patients with SM-AHN should have treatment directed toward the ANM part of the disease as if no SM was there and vice versa [64]. Newer treatment strategies targeting the D816V *KIT* mutation and downstream signaling pathways are currently in development (mTOR blockers, PI3 kinase blockers), as well as immunotherapy aimed at CD30-positive advanced systemic mastocytosis [73].

## Conclusion

Mastocytosis is a clinically heterogeneous disorder united by a common thread of mutations in *KIT* resulting in constitutive expression of KIT protein and downstream signaling pathways. Advances in molecular medicine targeting KIT and related pathways have led to new effective therapies. With additional genetic mutations recently described, especially in advanced mastocytosis, our understanding of this neoplasm is still evolving.

## References

1. Hartmann K, Escribano L, Grattan C, Brockow K, Carter MC, Alvarez-Twose I, et al. Cutaneous manifestations in patients with mastocytosis: consensus report of the European competence network on mastocytosis; the American Academy of Allergy, Asthma & Immunology; and the European Academy of Allergology and Clinical Immunology. J Allergy Clin Immunol. 2016;137(1):35–45.
2. Georgin-Lavialle S, Lhermitte L, Dubreuil P, Chandesris MO, Hermine O, Damaj G. Mast cell leukemia. Blood. 2013;121(8):1285–95.
3. Valent P, Spanblochl E, Sperr WR, Sillaber C, Zsebo KM, Agis H, et al. Induction of differentiation of human mast cells from bone marrow and peripheral blood mononuclear cells by recombinant human stem cell factor (SCF)/kit ligand (KL) in long term culture. Blood. 1992;80(9):2237–45.
4. Sotlar K, Escribano L, Landt O, Mohrle S, Herrero S, Torrelo A, et al. One-step detection of c-kit point mutations using peptide nucleic acid-mediated polymerase chain reaction clamping and hybridization probes. Am J Pathol. 2003;162(3):737–46.
5. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–405.
6. Horny H-P, Akin C, Arber DA, Peterson L, Tefferi A, Metcalfe DD, et al. Mastocytosis. In: World Health Organization classification of tumors of haematopoietic and lymphoid tissues. 5th ed. Lyon: IARC Press; 2016.
7. Horny H-P, Metcalfe DD, Bennett JM, Bain BJ, Akin C, Escribano L, et al. Mastocytosis. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW, editors. WHO classification of tumours of haematopoietic and lymphoid tissues. 4th ed. Lyon: IARC Press; 2008. p. 54–63.
8. Berezowska S, Flaig MJ, Rueff F, Walz C, Haferlach T, Krokowski M, et al. Adult-onset mastocytosis in the skin is highly suggestive of systemic mastocytosis. Mod Pathol. 2014;27(1):19–29.
9. Valent P, Samorapoompichi P, Sperr WR, Horny HP, Lechner K. Myelomastocytic leukemia: myeloid neoplasm characterized by partial differentiation of mast cell-lineage cells. Hematol J. 2002;3(2):90–4.

10. Valent P, Horny HP, Escribano L, Longley BJ, Li CY, Schwartz LB, et al. Diagnostic criteria and classification of mastocytosis: a consensus proposal. Leuk Res. 2001;25(7):603–25.
11. Arredondo A, Gotlib J, Shier L, Medeiros B, Wong K, Cherry A, et al. Myelomastocytic leukemia versus mast cell leukemia versus systemic mastocytosis associated with acute myeloid leukemia: a diagnostic challenge. Am J Hematol. 2010;85(8):600–6.
12. Krokowski M, Sotlar K, Krauth M-T, Fodinger M, Valent P, Horny HP. Delineation of patterns of bone marrow mast cell infiltration in systemic mastocytosis: value of CD25, correlation with subvariations of the disease and separation from mast cell hyperplasia. Am J Clin Pathol. 2005;124(4):560–8.
13. Reichard KK, Chen D, Pardanani A, McClure RF, Howard MT, Kurtin PJ, et al. Morphologically occult systemic mastocytosis in bone marrow: clinicopathologic features and an algorithmic approach to diagnosis. Am J Clin Pathol. 2015;144(3):493–502.
14. George TI, Sotlar K, Valent P, Horny H-P. Mastocytosis. In: Jaffe ES, Arber DA, Harris NL, Quintanilla-Martinez L, editors. Hematopathology. 2nd ed. Philadelphia: Elsevier; 2017. p. 911–30.
15. Horny H-P, Sillaber C, Menke D, Kaiserling E, Wehrmann M, Stehberger B, et al. Diagnostic value of immunostaining for tryptase in patients with mastocytosis. Am J Surg Pathol. 1998;22(9):1132–40.
16. Doyle LA, Sepehr GJ, Hamilton MJ, Akin C, Castells MC, Hornick JL. A clinicopathology study of 24 cases of systemic mastocytosis involving the gastrointestinal tract and assessment of mucosal mast cell density in irritable bowel syndrome and asymptomatic patients. Am J Surg Pathol. 2014;38(6):832–43.
17. Escribano L, Diaz-Agustin B, Lopez A, Nunez Lopez R, Garcia-Montero A, Almeida J, et al. Immunophenotypic analysis of mast cells in mastocytosis: when and how to do it. Proposals of the Spanish network on mastocytosis (REMA). Cytometry B Clin Cytom. 2004;58(1):1–8.
18. Escribano L, Orfao A, Diaz-Agustin B, Villarrubia J, Cervero C, Lopez A, et al. Indolent systemic mast cell disease in adults: immunophenotypic characterization of bone marrow mast cells and its diagnostic implications. Blood. 1998;91(8):2731–6.
19. Morgado JM, Perbellini O, Johnson RC, Teodósio C, Matito A, Álvarez-Twose I, et al. CD30 expression by bone marrow mast cells from different diagnostic variants of systemic mastocytosis. Histopathology. 2013;63(6):780–7.
20. Alvarez-Twose I, Jara-Acevedo M, Morgado JM, Garcia-Montero A, Sanchez-Munoz L, Teodosio C, et al. Clinical, immunophenotypic, and molecular characteristics of well-differentiated systemic mastocytosis. J Allergy Clin Immunol. 2016;137(1):168–78.
21. Horny HP, Sotlar K, Stellmacher F, Krokowski M, Agis H, Schwartz LB, et al. The tryptase positive compact round cell infiltrate of the bone marrow (TROCI-BM): a novel histopathological finding requiring the application of lineage specific markers. J Clin Pathol. 2006;59(3):298–302.
22. Metcalfe DD. Mast cells and mastocytosis. Blood. 2008;112(4):946–56.
23. Bibi S, Langenfeld F, Jeanningros S, Brenet F, Soucie E, Hermine O, et al. Molecular defects in mastocytosis. Immunol Allergy Clin N Am. 2012;34(2):239–62.
24. Laine E, Chauvot de Beauchene I, Perahia D, Auclair C, Tchertanov L. Mutation D816V alters the internal structure and dynamics of c-KIT receptor cytoplasmic region: implications for dimerization and activation mechanisms. PLoS Comput Biol. 2011;7(6):e1002068.
25. Xiang Z, Kreisel F, Cain J, Colson A, Tomasson MH. Neoplasia driven by mutant c-KIT is mediated by intracellular, not plasma membrane, receptor signaling. Mol Cell Biol. 2007;27(1):267–82.
26. Sun J, Pedersen M, Rönnstrand L. The D816V mutation of c-kit circumvents a requirement for Src family kinases in c-kit signal transduction. J Biol Chem. 2009;284(17):11039–47.
27. Hartmann K, Wardelmann E, Ma Y, Merkelbach–Bruse S, Preussner LM, Woolery C, et al. Novel germline mutation of KIT associated with familial gastrointestinal stromal tumors and mastocytosis. Gastroenterology. 2005;129(3):1042–6.
28. Zhang LY, Smith ML, Schultheis B, Fitzgibbon J, Lister TA, Melo JV, et al. A novel K509I mutation of KIT identified in familial mastocytosis—in vitro and in vivo responsiveness to imatinib therapy. Leuk Res. 2006;30(4):373–8.
29. De Melo Campos P, Machado-Neto JA, Scopim-Ribeiro R, Visconte V, Tabarroki A, Duarte AS, et al. Familial systemic mastocytosis with germline KIT K509I mutation is sensitive to treatment with imatinib, dastainib and PKC412. Leuk Res. 2014;38(10):1245–51.
30. Wang HJ, Lin ZM, Zhang J, Yin JH, Yang Y. A new germline mutation in KIT associated with diffuse cutaneous mastocytosis in a Chinese family. Clin Exp Dermatol. 2014;39(2):146–9.

31. Tang X, Boxer M, Drummond A, Ogston P, Hodgins M, Burden AD. A germline mutation in KIT in familial diffuse cutaneous mastocytosis. J Med Genet. 2004;41(6):e88.
32. Beghini A, Tibiletti M, Roversi G, Chiaravalli A, Serio G, Capella C, et al. Germline mutation in the juxtamembrane domain of the KIT gene in a family with gastrointestinal stromal tumors and urticaria pigmentosa. Cancer. 2001;92(3):657–62.
33. Pollard WL, Beachkofsky TM, Kobayashi TT. Novel R634W c-kit mutation identified in familial mastocytosis. Pediatr Dermatol. 2015;32(2):267–70.
34. Bodemer C, Hermine O, Palmerini F, Yang Y, Grandpeix-Guyodo C, Leventhal PS, et al. Pediatric mastocytosis is a clonal disease associated with $D^{816}V$ and other activating c-KIT mutations. J Invest Dermatol. 2010;130(3):804–15.
35. Wasag B, Niedoszytko M, Piskorz A, Lange M, Renke J, Jassem E, et al. Novel, activating KIT-N822I mutation in familial cutaneous mastocytosis. Exp Hematol. 2011;39(8):859–65.
36. Wohrl S, Moritz KB, Bracher A, Fischer G, Stingl G, Loewe R. A c-kit mutation in exon 18 in familial mastocytosis. J Invest Dermatol. 2013;133(3):839–41.
37. Fett N, Teng J, Longley BJ. Familial urticaria pigmentosa: report of a family and review of the role of KIT mutations. Am J Dermatopathol. 2013;35(1):113–6.
38. Sato-Matsumura KC, Matsumura T, Koizumi H, Sato H, Nagashima K, Ohkawara A. Analysis of c-kit exon 11 and exon 17 of urticaria pigmentosa that occurred in monozygotic twin sisters. Br J Dermatol. 1999;140(6):1130–2.
39. de la Sotta P, Romero WA, Kramer D, Cardenas C, Gonzalez S. Cutaneous mastocytosis in twins: multiple mastocytomas and urticaria pigmentosa in two pairs of monozygotic twins. Pediatr Dermatol. 2011;28(5):585–7.
40. Chang A, Tung RC, Schlesinger T, Bergfeld WF, Dijkstra J, Kahn TA. Familial cutaneous mastocytosis. Pediatr Dermatol. 2001;18(4):271–6.
41. Fowler JF Jr, Parsley WM, Cotter PG. Familial urticaria pigmentosa. Arch Dermatol. 1986;122(1):80–1.
42. Clark DP, Buescher L, Havey A. Familial urticaria pigmentosa. Arch Intern Med. 1990;150(8):1742–4.
43. Meni C, Bruneau J, Georgin-Lavialle S, Le Sache de Peufeilhoux L, Damaj G, Hadj-Rabia S, et al. Paediatric mastocytosis: a systematic review of 1747 cases. Br J Dermatol. 2015;172(3):642–51.
44. Kristensen T, Vestergaard H, Møller MB. Improved detection of the KIT D816V mutation in patients with systemic mastocytosis using a quantitative and highly sensitive real-time qPCR assay. J Mol Diagn. 2011;13(2):180–8.
45. Georgin-Lavialle S, Aguilar C, Guieze R, Lhermitte L, Bruneau J, Fraitag S, et al. Mast cell sarcoma: a rare and aggressive entity--report of two cases and review of the literature. J Clin Oncol. 2013;31(6):e90–7.
46. Sotlar K, Colak S, Bache A, Berezowska S, Krokowski M, Bültmann B, et al. Variable presence of KITD816V in clonal haematological non-mast cell lineage diseases associated with systemic mastocytosis (SM-AHNMD). J Pathol. 2010;220(5):586–95.
47. Tefferi A, Levine RL, Lim KH, Abdel-Wahab O, Lasho TL, Patel J, et al. Frequent TET2 mutations in systemic mastocytosis: clinical, KITD816V and FIP1L1-PDGFRA correlates. Leukemia. 2009;23(5):900–4.
48. Pardanani A, Lasho T, Elala Y, Wassie E, Finke C, Reichard KK, et al. Next-generation sequencing in systemic mastocytosis: derivation of a mutation-augmented clinical prognostic model for survival. Am J Hematol. 2016;91(9):888–93.
49. Soucie E, Hanssens K, Mercher T, Georgin-Lavialle S, Damaj G, Livideanu C, et al. In aggressive forms of mastocytosis, TET2 loss cooperates with c-KITD816V to transform mast cells. Blood. 2012;120(24):4846–9.
50. Damaj G, Joris M, Chandesris O, Hanssens K, Soucie E, Canioni D, et al. ASXL1 but not TET2 mutations adversely impact overall survival of patients suffering systemic mastocytosis with associated clonal hematologic non-mast-cell diseases. PLoS One. 2014;9(1):e85362.
51. Jawhar M, Schwaab J, Schnittger S, Sotlar K, Horny HP, Metzgeroth G, et al. Molecular profiling of myeloid progenitor cells in multi-mutated advanced systemic mastocytosis identifies KIT D186V as a distinct and late event. Leukemia. 2015;29(5):1115–22.
52. Akin C. Molecular diagnosis of mast cell disorders: a paper from the 2005 William Beaumont Hospital symposium on molecular pathology. J Mol Diagn. 2006;8(4):412–9.

53. Jara-Acevedo M, Teodosio C, Sanchez-Muñoz L, Álvarez-Twose I, Mayado A, Caldas C, et al. Detection of the KIT D816V mutation in peripheral blood of systemic mastocytosis: diagnostic implications. Mod Pathol. 2015;28(8):1138–49.
54. Sotlar K. C-kit mutational analysis in paraffin material. Methods Mol Biol. 2013;999:59–78.
55. Kristensen T, Broesby-Olsen S, Vestergaard H, Bindslev-Jensen C, Møller MB. Targeted ultradeep next-generation sequencing as a method for KIT D816V mutation analysis in mastocytosis. Eur J Haematol. 2016;96(4):381–8.
56. Uzzaman A, Maric I, Noel P, Kettelhut BV, Metcalfe DD, Carter MC. Pediatric-onset mastocytosis: a long term clinical follow-up and correlation with bone marrow histopathology. Pediatr Blood Cancer. 2009;53(4):629–34.
57. Castells M, Metcalfe DD, Escribano L. Diagnosis and treatment of cutaneous mastocytosis in children: practical recommendations. Am J Clin Dermatol. 2011;12(4):259–70.
58. Valent P. Diagnosis and management of mastocytosis: an emerging challenge in applied hematology. Hematology Am Soc Hematol Educ Program. 2015;2015:98–105.
59. Correia O, Duarte AF, Quirino P, Azevedo R, Delgado L. Cutaneous mastocytosis: two pediatric cases treated with topical pimecrolimus. Dermatol Online J. 2010;16(5):8.
60. Brazzelli V, Grasso V, Manna G, et al. Indolent systemic mastocytosis treated with narrow-band UVB phototherapy: study of five cases. J Eur Acad Dermatol Venereol. 2012;26(4):465–9.
61. Prignano F, Troiano M, Lotti T. Cutaneous mastocytosis: successful treatment with narrow-band ultraviolet B phototherapy. Clin Exp Dermatol. 2010;35(8):914–5.
62. Hartmann K, Siebenhaar F, Belloni B, Brockow K, Eben R, Hartmann B, et al. Effects of topical treatment with the raft modulator miltefosine and clobetasol in cutaneous mastocytosis: a randomized, double-blind, placebo-controlled trial. Br J Dermatol. 2010;162(1):185–90.
63. Paul C, Sans B, Suarez F, Casassus P, Barete S, Lanternier F, et al. Masitinib for the treatment of systemic and cutaneous mastocytosis with handicap: a phase 2a study. Am J Hematol. 2010;85(12):921–5.
64. Valent P, Akin C, Escribano L, Fodinger M, Hartmann K, Brockow K, et al. Standards and standardization in mastocytosis: consensus statements on diagnostics, treatment recommendations and response criteria. Eur J Clin Investig. 2007;37(6):435–53.
65. Gonzalez de Olano D, Alvarez-Twose I, Esteban-Lopez MI, Sanchez-Munoz L, de Durana MD, Vega A, et al. Safety and effectiveness of immunotherapy in patients with indolent systemic mastocytosis presenting with Hymenoptera venom anaphylaxis. J Allergy Clin Immunol. 2008;121(2):519–26.
66. Niedoxzytko M, de Monchy J, van Doormaal JJ, Jassem E, Oude Elberink JN. Mastocytosis and insect venom allergy: diagnosis, safety and efficacy of venom immunotherapy. Allergy. 2009; 64(9):1237–45.
67. Kluin-Nelemans HC, Oldhoff JM, Van Doormaal JJ, Van 't Wout JW, Verhoef G, Gerrits WB, et al. Cladribine therapy for systemic mastocytosis. Blood. 2003;102(13):4270–6.
68. Arock M, Valent P. Pathogenesis, classification and treatment of mastocytosis: state of the art in 2010 and future perspectives. Expert Rev Hematol. 2010;3(4):497–516.
69. Ma Y, Zeng S, Metcalfe DD, Akin C, Dimitrijevic S, Butterfield JH, et al. The c-KIT mutation causing human mastocytosis is resistant to STI571 and other KIT kinase inhibitors; kinases with enzymatic site mutations show different inhibitor sensitivity profiles than wild-type kinases and those with regulatory-type mutations. Blood. 2002;99(5):1741–4.
70. Verstovsek S, Tefferi A, Cortes J, O'Brien S, Garcia-Manero G, Pardanani A, et al. Phase II study of dasatinib in Philadelphia chromosome-negative acute and chronic myeloid diseases, including systemic mastocytosis. Clin Cancer Res. 2008;14(12):3906–15.
71. Gotlib J, Kluin-Nelemans HC, George TI, Akin C, Sotlar K, Hermine O, et al. Efficacy and safety of midostaurin in advanced systemic mastocytosis. N Engl J Med. 2016;374(26):2530–41.
72. Ustun C, Gotlib J, Popat U, Artz A, Scott B, Litzaow M, et al. Consensus opinion on allogeneic hematopoietic cell transplantation in advanced systemic mastocytosis. Biol Blood Marrow Transplant. 2016;22(8):1348–56.
73. Ustun C, Arock M, Kulin-Nelemans HC, Reiter A, Sperr WR, George T, et al. Advanced systemic mastocytosis: from molecular and genetic progress to clinical practice. Haematologica. 2016;101(10):1133–43.

# Chapter 10
# Chronic Myeloproliferative Neoplasm, Rare Types

**Jerald Z. Gong and Guldeep K. Uppal**

## Chronic Neutrophilic Leukemia

### *Introduction*

Chronic neutrophilic leukemia (CNL) is a rare type of myeloproliferative neoplasm (MPN) characterized by sustained leukocytosis ($\geq 25 \times 10^9$/L) with neoplastic proliferation of neutrophilic granulocytes in blood and bone marrow. Since first described in 1920 by Tuohy, approximately 200 cases of CNL have been reported to date [1, 2]. The literature includes many case reports and a handful of small case series. The diagnostic criteria for CNL have only been defined more recently, and it is unclear from the literature how many are true cases of CNL. It is likely that less than 40% of reported cases meet the current WHO diagnostic criteria [3, 4].

In 2013, Maxon et al. reported high frequency of oncogenic mutations in colony stimulating factor 3 receptor (*CSF3R*) in CNL [5]. These findings were supported by another study that reported 100% frequency of *CSF3R* mutation in 12 patients with WHO-defined CNL [6]. *CSF3R* mutations have been incorporated in the diagnostic criteria in the 2016 WHO classification of CNL [7]. Table 10.1 lists the updated diagnostic criteria.

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**Table 10.1** Diagnostic criteria for chronic neutrophilic leukemia [7]

| | |
|---|---|
| 1 | Peripheral blood WBC ≥ 25 × $10^9$/L<br>Segmented neutrophils plus band forms ≥80% of WBC<br>Neutrophil precursors (promyelocytes, myelocytes, and Metamyelocytes) <10% of WBC<br>Myeloblasts rarely observed<br>Monocytes <1 × $10^9$/L<br>No dysgranulopoiesis |
| 2 | Hypercellular bone marrow<br>Neutrophil granulocytes increased in percentage and number<br>Neutrophil maturation appears normal<br>Myeloblasts <5% of nucleated cells |
| 3 | Not meeting WHO criteria for *BCR-ABL1*-positive CML, PV, ET, or PMF |
| 4 | No rearrangement of *PDGFRα, PDGFRβ, FGFR1,* or *PCM1-JAK2* |
| 5 | Presence of *CSF3R* T618I or other activating *CSF3R* mutation |
| or | In the absence of *CSF3R* mutation, persistent neutrophilia (at least 3 months), splenomegaly, and no identifiable cause of reactive neutrophilia including absence of a plasma cell neoplasm or, if present, demonstration of clonality of myeloid cells by cytogenetic or molecular studies |

## *Clinical Features*

With rare exceptions, CNL is primarily a disease of elderly with most patients presenting in their 60s. A review of 33 published cases of CNL reported a median age of 62.5 years and male to female ratio of 2:1 [8]. Many patients are asymptomatic at the time of diagnosis. In other patients, fatigue is the most common symptom [9]. A small number of patients presents with weight loss, night sweats, bone pain, gout, or pruritus [10]. The most common and consistent finding on physical examination is splenomegaly. Hepatomegaly can be seen, but lymphadenopathy is uncommon [10–12]. Some cases in the literature have reported increased incidence of hemorrhagic diathesis and mucosal bleeding [10, 11]. The bleeding tendency could result from thrombocytopenia and platelet dysfunction or from vascular wall infiltration by the neoplastic leukocytes [13, 14].

## *Morphology*

Peripheral blood shows leukocytosis with neutrophilia. The white blood cell (WBC) count is moderately elevated with an average of 50 × $10^9$/L. The diagnostic leukocyte count threshold in the 2008 WHO classification is ≥25 × $10^9$/L. [2] In contrast to chronic myeloid leukemia (CML), leukocytosis in CNL consists of a proliferation of primarily mature neutrophilic granulocytes (Fig. 10.1). Segmented neutrophils and bands comprise >80% of the total WBCs [2]. Neutrophils frequently show toxic granulations and Döhle bodies, which suggest an activated state [15]. Features of dysplasia are usually absent. There are no monocytosis, basophilia, or

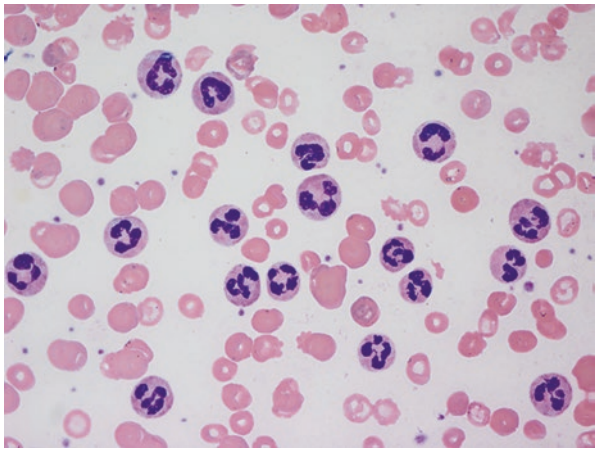

**Fig. 10.1** Peripheral blood smear from a patient with CNL showing leukocytosis with neutrophilia without left shift (Wright-Giemsa stain, 500×)

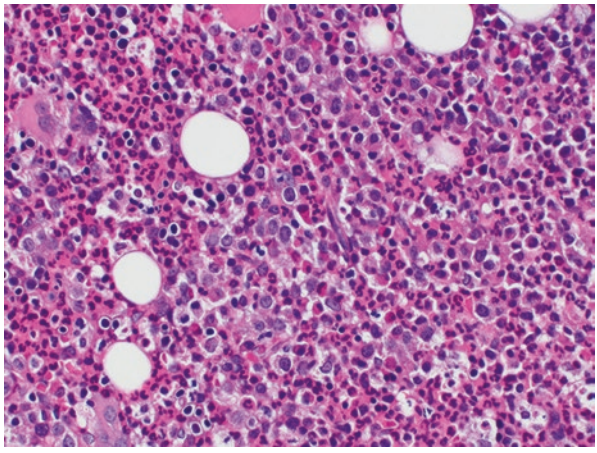

**Fig. 10.2** Bone marrow trephine biopsy from a patient with CNL showing hypercellularity with marked myeloid hyperplasia. The erythropoiesis and megakaryocytes are normal in number and maturation (Hematoxylin & Eosin, 400×)

eosinophilia. Intermediate and early myeloid precursors and nucleated red blood cells are rare. In particular, circulating myeloblasts are almost never seen. Platelet count is usually normal except in late disease stages when mild thrombocytopenia can be seen with increasing splenomegaly and progression of disease.

The bone marrow is hypercellular with marked myeloid hyperplasia. Myeloid to erythroid ratio is often >20:1. There is an increase in myelocytes, metamyelocytes, and bands, but blasts or promyelocytes are not increased typically (Fig. 10.2). Erythroid precursors are relatively reduced and show normal maturation. In general, megakaryocytes are normal in number and morphology. Some cases may show mild megakaryocytic hyperplasia [2]. Significant dyspoiesis is not seen; if present, should prompt one to rule out atypical chronic myeloid leukemia (aCML). Reticulin fibrosis is not observed.

Rare cases of CNL have been reported to harbor clonal plasma cells in bone marrow. Bone marrow should be carefully examined for plasma cells, and appropriate

immunohistochemical stains should be performed while diagnosing CNL [16, 17]. In cases where clonal plasma cells are detected, the diagnosis of CNL should be supported by molecular or cytogenetic studies to prove clonality [2].

Splenomegaly is a consistent finding in CNL. Infiltration of spleen by CNL primarily involves red pulp cords and sinuses. The white pulp is relatively spared from leukemic infiltration. The red pulp is expanded and filled with segmented neutrophils and precursors. Rarely megakaryocytes and erythroid precursors can be seen along with neutrophils [11]. Similar to spleen, involvement of liver is seen as infiltration of the sinuses and portal areas by neutrophils [5, 6]. Lymph node involvement has been reported only rarely [12, 18].

## *Cytogenetics and Molecular Findings*

Many case reports and a few small series have supported the clonal nature of CNL based on X-inactivation partners and karyotypic abnormalities [19]. However, in majority of the cases, cytogenetic studies showed normal karyotype [2, 3]. One review series reported cytogenetic abnormalities in 37% cases of CNL [3]. The reported abnormalities included trisomy 8, trisomy 21, deletion 11q, and deletion 20q [20–22]. The most frequent cytogenetic abnormality was deletion 20q. 20q deletions are not specific for CNL and have been reported in other myeloproliferative neoplasms (MPNs). It is possible that the reported cytogenetic abnormalities are secondary events in pathogenesis and represent cytogenetic evolution [23].

The major breakthrough in the pathogenesis of CNL came in 2013 when mutations in the gene coding for colony stimulating factor 3 receptor (*CSF3R*) were reported in 16 of 27 patients (59%) with CNL or *BCR-ABL1*-negative atypical CML. The reported mutation frequencies were 89% in CNL cases (8/9) and 40% in aCML cases (8/18) [5]. These findings were supported by another study that reported 100% frequency of *CSF3R* mutation in 12 patients with WHO-defined CNL [6].

The *CSF3R* gene maps on chromosome 1p34.3 and encodes the transmembrane receptor for granulocyte colony stimulating factor (G-CSF; CSF3). It is known to play an important role in proliferation and differentiation of granulocytes. Two types of mutations are found in *CSF3R*: the majority occurs in the extracellular domain (membrane proximal point mutations) and a small number occurs in the cytoplasmic portion of the receptor (nonsense or frameshift mutations) leading to truncation of the cytoplasmic domain. The most common membrane proximal mutations include T618I and T615A. These mutations result in ligand-independent activation of *CSF3R* that initiates downstream signaling through JAK2 [5]. The point mutation is usually present in isolation or can be seen along with compound frameshift or nonsense mutations. *CSF3R* mutations have not been reported in patients with reactive neutrophilia [5, 6]. *CSF3R* T618I or other activating mutations in *CSF3R* are part of the diagnostic criteria in revised 2016 WHO classification of hematolymphoid neoplasms [7].

Since the initial discovery of the *JAK2* V617F mutation, a few cases of CNL with this mutation have been published [24, 25]. Despite being rare, when detected,

*JAK2* V617F mutation indicates the clonal nature of the disease. Other mutations that have been reported to occur in CNL include *SETBP1*, calreticulin (*CALR*), and *ASXL1* mutations [26–28]. In one study, *SETBP1* mutations were seen along with *CSF3R* T618I mutations in 33% patients [26]. A recent study has reported *SETBP1* and *ASXL1* mutations in 14 cases of CNL with mutated *CSF3R*. Eight cases (57%) showed *SETBP1* mutation whereas five (38%) cases showed *ASXL1* and/or *SETBP1* mutations. The presence of coexisting *SETBP1* and *CSF3R* mutations may indicate a worse prognosis [26].

## *Clinical Course and Disease Progression*

The clinical course of the disease is variable. The survival time ranges from 6 months to more than 20 years [9, 22]. Unlike CML, there are no established criteria for progression. The disease progression is characterized by progressive neutrophilia, worsening splenomegaly, resistance to previously effective therapy, anemia, and thrombocytopenia. Transformation to acute myeloid leukemia has been reported in 10–15% of cases [22]. A review of 40 cases of CNL reported median overall survival of 23.5 months and median time to progression to acute myeloid leukemia (AML) of 21 months [28]. The most frequently reported causes of death were intracranial hemorrhage, progressive disease, and treatment-related toxicity from chemotherapy or transplantation [21]. A recent study evaluated role of various factors for prognostication including age, LDH levels, splenomegaly, hemoglobin level, thrombocytopenia, total bilirubin levels, *SETBP1* mutation, *ASXL1* mutation, and "T618I versus other *CSF3R* mutation" in a group of 14 cases of *CSF3R*-mutated CNL. On a multivariate analysis, only *ASXL1* mutation and thrombocytopenia were found to be independently predictive of short survival. The median survival in this group was 23.2 months [27]. A trend of short survival has been reported in patients with coexisting *CSF3R* and *SETBP1* mutations [5]. A case of CNL has been reported with coexistent *CSF3R* and *SETBP1* mutations that showed in vitro lack of response to JAK inhibitor [24]. A study reported transformation of two *SETBP1*-mutated cases of CNL to acute myeloid leukemia. The same study also reported evolution of CNL to chronic myelomonocytic leukemia (CMML) in patients with the presence of *ASXL1* mutation and lack of *SETBP1* mutation [27]. These co-operative mutations likely play an important role in disease transformation.

## *Conclusion*

CNL is a rare myeloprolife*βα*rative neoplasm that is characterized by persistent neutrophilic leukocytosis in peripheral blood and bone marrow and by frequent hepatosplenomegaly. Oncogenic mutations in *CSF3R* appear to be specific driver events in CNL. The role of additional subclonal mutations such as *CALR, JAK2,*

*SETP1,* and *ASXL1* is being evaluated. *SETBP1* and *ASXL1* are emerging as new prognostic indicators while awaiting more conclusive studies. Before testing for *CSF3R* mutations, one should always keep in mind that CNL is a very rare myeloid neoplasm. Although molecular testing of *CSF3R* mutations will quickly become available in diagnostic laboratories, other common causes of neutrophilia must be ruled out before considering the confirmative molecular test.

## Chronic Eosinophilic Leukemia, Not Otherwise Specified

### *Introduction*

Chronic eosinophilic leukemia, not otherwise specified (CEL-NOS) is defined by 2008 WHO classification as clonal eosinophil disorder with persistent increase of eosinophils in blood, bone marrow, and peripheral tissues. The diagnosis requires a blood eosinophil count of $>1.5 \times 10^9$/L plus at least one of the following two criteria: increase of blasts in blood and/or bone marrow (>2% in blood, >5% in marrow) and evidence of clonality in eosinophils. Cases with greater than 20% blasts in blood or bone marrow are excluded from CEL and are diagnosed as acute myeloid leukemia. Evidence of clonality is demonstrated by cytogenetics or pathological mutations [29]. There is no major update in the 2016 WHO classification on CEL [7]. Table 10.2 lists the 2008 WHO diagnostic criteria of CEL.

CEL is a rare disease. There are no epidemiological reports on the incidence. CEL mainly occurs in adult male with a peak incidence of fourth decade. Clinical symptoms are associated with eosinophil-mediated organ damage, such as cardiomyopathy, pneumonitis, dermatitis, neuropathy, and gastrointestinal (GI) inflammation. The patients typically present with nonspecific constitutional symptoms including fever, malaise, cough, angioedema, pruritus, muscle pain, and diarrhea. A small subset of patients presents with symptoms of cardiac damage with restrictive cardiomyopathy and congestive heart failure, and valve damage. Dislodge of cardiac thrombi may result in emboli of end organs. CNS and peripheral neuropathies are also frequent. Extensive bone marrow infiltration of eosinophils often results in

**Table 10.2** Diagnostic criteria of chronic eosinophilic leukemia, not otherwise specified [29]

| | |
|---|---|
| 1 | Eosinophil count $\geq 1.5 \times 10^9$/L |
| 2 | No Ph chromosome or *BCR-ABL1* or other myeloproliferative neoplasms (PV, ET, PMF) or MDS/MPN (CMML or aCML) |
| 3 | No rearrangement of *PDGFRα, PDGFRβ, FGFR1, or PCM1-JAK2* |
| 4 | No inv.(16)(p13q22) or t(16;16)(p13;q22) or other feature of diagnostic of AML |
| 5 | Blast count in peripheral blood or bone marrow <20% |
| 6 | There is a clonal cytogenetic or molecular genetic abnormality, or blasts >2% in peripheral blood or >5% in bone marrow |

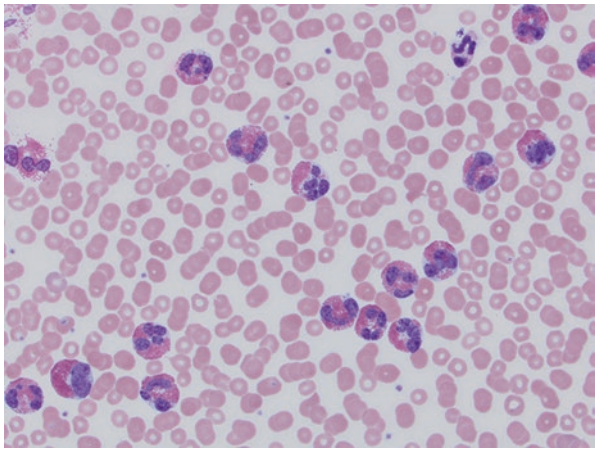

**Fig. 10.3** Peripheral blood smear from a patient with CEL showing numerous eosinophils. The eosinophils are mature in morphology and show no cytological atypia (Wright-Giemsa stain, 500×) (Courtesy of Dr. Zenggang Pan)

anemia and thrombocytopenia. Liver and spleen involvement is present in 30–50% patients. Some patients may be asymptomatic, and the diagnoses are made by incidental finding [29, 30].

## *Morphology*

Peripheral blood eosinophilia is a consistent finding, usually greater than $1.5 \times 10^9$/L. The eosinophils are predominantly segmented forms, and immature eosinophils are infrequent. Dysplasia such as hypersegmented nuclei, hypogranular cytoplasm, cytoplasmic vacuoles, and enlarged nuclear size are often present in eosinophils (Fig. 10.3). However, these features are not specific for leukemic eosinophils as similar morphology can present in reactive eosinophilia. Neutrophilia may be present but dysplastic neutrophils are absent. Monocytosis is usually absent.

The bone marrow is hypercellular, and the dominant feature is eosinophilic infiltrate. Maturation of eosinophils is usually orderly although left shift is not uncommon. Increase of blasts may be seen, and their presence supports the diagnosis of CEL. If blasts are greater than 20%, then a diagnosis of acute myeloid leukemia with associated eosinophilia should be entertained. Neutrophilic granulocytes, erythrocytes, and megakaryocytes are usually normal in morphology and maturation (Fig. 10.4).

Cases having similar clinical presentation of CEL but lack clonal cytogenetic changes or increase of blasts are classified as idiopathic hypereosinophilic syndrome (HES). The 2008 WHO classification defines HES as persistent eosinophilia $>1.5 \times 10^9$/L for greater than 6 months with no demonstrable clonal cytogenetic changes or increase of blasts. In addition, the diagnosis requires demonstration of organ damage such as cardiomyopathy, pulmonary infiltrate, and renal disease [29]. It is now widely accepted that HES represents a heterogeneous group that includes

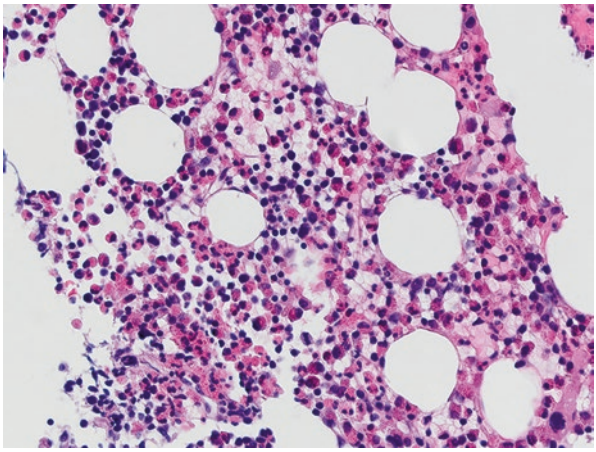

**Fig. 10.4** Bone marrow trephine biopsy from a patient with CEL showing hypercellularity with increase of eosinophils and precursors (Hematoxylin & Eosin, 400×) (Courtesy of Dr. Zenggang Pan)

patients of CEL and reactive eosinophilia. The CEL patients have mutations or cryptic chromosome abnormalities that are not detected by the currently available technology. The remaining patients have sustained eosinophilia caused by reactive conditions that are cytokine- driven due to hyperproliferation of T cells or other cytokine producing cells.

In some HES patients, increase of CD4+ T cells and clonal T-cell gene rearrangement can be demonstrated in bone marrow and/or blood. These cases are known as "lymphoid variant of HES" (L-HES). In order to make a diagnosis of L-HES, the cases must meet the diagnostic criteria of HES plus additional evidence of increased CD3-CD4+ T cells. Clonal T-cell receptor gamma gene rearrangement can be detected in the majority, if not all, patients. Expansion of CD3-CD4+ T cells (also known as Th2 T cells) results in overproduction of interleukin 5 (IL-5) which in turn drives proliferation of eosinophils. Clinical manifestations related to IL-5 include atopic skin disorders (up to 80%), diarrhea and abdominal pain (~20%), eosinophilic pneumonia (~20%), and rheumatologic arthralgia, arthritis, and synovitis (~30%). The CD3-CD4+ Th2 T cells may have variant expression of other T cell markers such as CD7-, CD25+, and TCRαβ-. Clonal TCR gamma gene rearrangement can be detected in up to 75% of patients. The majority of the patients have no evidence of overt T-cell lymphoma. In rare patients, concurrent T-cell lymphoma is present suggesting that the abnormal T cells are lymphoma cells [31, 32].

## *Cytogenetics and Molecular Findings*

Various chromosome abnormalities have been described in CEL and in other eosinophil-associated neoplasms. Cases with recurrent translocations involving tyrosine kinase genes *PDGFRα*, *PDGFRβ*, *FGFR1*, and *PCM1-JAK2* are categorized separately by WHO classification to emphasize the potential therapeutic

options of tyrosine kinase inhibitors for these patients. These cases are excluded from CEL. Chronic myeloid leukemia with *BCR-ABL1*, often accompanied by eosinophilia, can occasionally mimic CEL. The presence of *BCR-ABL1* excludes CEL [29].

After the aforementioned entities are excluded, the neoplastic nature of CEL must be proved by the presence of clonality or increase of blasts. Clonality can be demonstrated by chromosome abnormalities, pathogenic mutations, or by an alternative method such as X chromosome inactivation (Humara). One must aware that eosinophilia is not uncommon in other bone marrow neoplasms, in which the eosinophils can be either a part of neoplastic components (e.g., acute myeloid leukemia with inversion 16; systemic mastocytosis) or a reactive response to neoplastic stimuli (e.g., certain peripheral T-cell lymphomas). In these situations, the diagnoses are based on their primary malignancies. Due to these reasons, the presence of another neoplastic process must be first ruled out before a diagnosis of CEL can be made.

The majority of CEL is diagnosed based on the presence of clonal cytogenetic alteration. The chromosomal changes range from single karyotype abnormality to complex karyotypes. These changes encompass a wide variety with no specific recurrent chromosome types. Myelodysplastic syndrome (MDS)-associated chromosome changes such as trisomy 8 and deletion 5q are also seen as isolated abnormalities in CEL [33, 34]. Isochromosome 17p, most frequently found in accelerated and blast phases of chronic myeloid leukemia, has been reported as sole abnormality in CEL [35, 36]. Approximately 25% CEL have a complex karyotype [37].

Large-scale sequencing has been recently applied to CEL and HES, and recurrent mutations were found in both categories. Pathological mutations were detected in up to 50% of CEL and 30% of HES. The most frequent mutations were found in *ASXL1* (43%), *TET2* (36%), *EZH2* (29%), and genes involved in DNA methylation and chromatin modification. Other frequently mutated genes included *SETBP1*, *CBL*, *NOTCH1*, and spliceosome genes [37]. Contradictory results were reported on *KIT* mutations in CEL and HES. Some studies reported high frequency of *KIT* mutations, whereas others found no *KIT* mutations [37, 38]. It is possible that the cases with eosinophilia and *KIT* D816V mutations are not true CEL but rather systemic mastocytosis with eosinophilia. Rare cases with *JAK2* mutations were reported in CEL/HES. Likewise, it is uncertain whether these cases are CEL or other MPN with increased eosinophils [39, 40]. *NRAS* mutations were reported in rare cases [41].

Methylation studies have identified considerable differences in methylation patterns between HES and reactive eosinophilia. HES patients have shown frequent hypomethylation signature, whereas patients with reactive eosinophilia are constitutively hypermethylated. These methylation alterations were seen in a 128 relevant gene signatures, with the highest numbers of methylation abnormalities seen in *Mir886*, *GSTM5*, *TNXB*, *ZADH2*, *LGR6*, *HLA-C*, *HLA-DRB1*, *S100A13*, and *HIVEP3*. These genes involve various functional pathways in tumorigenesis such as cancer, cell death and survival, hematologic diseases, and inflammatory response [42].

In female patients, clonality can be assessed by X-inactivation analysis of human androgen receptor gene (HUMARA). This assay detects polymorphisms of trinucleotide repeats adjacent to differential methylation sites in human androgen receptor genes in X chromosome. Due to high levels of polymorphism, amplification of this region using methylation-specific polymerase chain reaction (PCR) can determine tumor of paternal or maternal origin (monoclonal) or both (polyclonal). There are several limitations in this method. (1) The method can be only used in females. (2) The method is less informative in hematologic malignancies as compared with solid tumor due to highly skewed X-inactivation in hematologic cells. (3) The result is affected by reactive cell components within the tumor. (4) The assay requires normal control sample from the same patient. Due to these limitations, HUMARA assay has lost favor in assessing clonality in hematologic malignancies [43].

### *Prognosis and Therapy*

CEL is a clinically aggressive disease with poor prognosis. A recent study showed median survival of 22 months and acute transformation developed in half of the patients within 3 years. Median survival from acute transformation to death was 2 months [44]. Hydroxyurea, combination chemotherapy, and stem cell transplantation are used to treat aggressive CEL with variable outcomes. Rare patients that have *KIT* M541 L mutations have shown good response to Imatinib [45]. Corticosteroid is effective in control of organ damage and is the first-line treatment for HES. The asymptomatic patients are usually managed by "watch and wait" approach with close follow-up [46].

## Conclusions

Although CEL and HES are rare clinical syndromes, it is important to distinguish them from reactive eosinophilia due to their severe clinical consequences. Recent studies have shown that recurrent oncogenic mutations are present in a subset of CEL and HES. Higher frequencies of mutations are found in genes involving DNA methylation and chromatin modification. The finding of similar pathological mutations in CEL and HES suggests that a subset of HES is more closely resemble CEL. These patients are older in age, more likely to have abnormal eosinophil morphology, and have shorter overall survival. On the other hand, the HES mutation-negative patients are younger in age and are more frequently associated with symptoms of eosinophil activation such as GI, pulmonary, skin, and rheumatoid manifestations. These findings suggest that the subset of mutation-positive HES may represent clonal neoplastic process similar to CEL. Due to the limited targets in most of the currently performed next generation sequencing panels, mutations involving targets outside of the panels are not investigated. Large-scale sequencing

such as whole-exon sequencing may help us to further understand the pathogenesis and to better classify CEL and HES. It is anticipated that as the new pathological mutations been discovered in HES, some patients who are currently been classified as HES will be reclassified as CEL.

## Bibliography


1. Tuohy E. A case of splenomegaly with polymorphonuclear neutrophil hperleukocytosis. Am J Med Sci. 1920;160:18–25.
2. Bain BJ, Brunning RD, Vardiman JW, Thiele J. Chronic neutrophilic leukaemia. In: Swerdlow SH, Campo E, Harris NL, editors. WHO classification of tumors of haematopoietic and lymphoid tissues. Lyon: IARC Press; 2008. p. 38–9.
3. Reilly JT. Chronic neutrophilic leukaemia: a distinct clinical entity? Br J Haematol. 2002;116(1):10–8.
4. Uppal G, Gong J. Chronic neutrophilic leukaemia. J Clin Pathol. 2015;68(9):680–4.
5. Maxson JE, Gotlib J, Pollyea DA, Fleischman AG, Agarwal A, Eide CA, et al. Oncogenic CSF3R mutations in chronic neutrophilic leukemia and atypical CML. N Engl J Med. 2013;368(19):1781–90.
6. Pardanani A, Lasho TL, Laborde RR, Elliott M, Hanson CA, Knudson RA, et al. CSF3R T618I is a highly prevalent and specific mutation in chronic neutrophilic leukemia. Leukemia. 2013;27(9):1870–3.
7. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–405.
8. Elliott MA. Chronic neutrophilic leukemia and chronic myelomonocytic leukemia: WHO defined. Best Pract Res Clin Haematol. 2006;19(3):571–93.
9. Hasle H, Olesen G, Kerndrup G, Philip P, Jacobsen N. Chronic neutrophil leukaemia in adolescence and young adulthood. Br J Haematol. 1996;94(4):628–30.
10. You W, Weisbrot IM. Chronic neutrophilic leukemia. Report of two cases and review of the literature. Am J Clin Pathol. 1979;72(2):233–42.
11. Zittoun R, Rea D, Ngoc LH, Ramond S. Chronic neutrophilic leukemia. A study of four cases. Ann Hematol. 1994;68(2):55–60.
12. Nakamine H, Hirano K, Tsujimoto M, Nishino E, Takenaka T, Maeda J, et al. Lymph node involvement in chronic neutrophilic leukemia. An immunohistochemical study. Virchows Arch A Pathol Anat Histopathol. 1988;412(3):241–5.
13. Hossfeld DK, Lokhorst HW, Garbrecht M. Neutrophilic leukemia accompanied by hemorrhagic diathesis: report of two cases. Blut. 1987;54(2):109–13.
14. Noguchi T, Ikeda K, Yamamoto K, Ashiba A, Yoshida J, Munemasa M, et al. Severe bleeding tendency caused by leukemic infiltration and destruction of vascular walls in chronic neutrophilic leukemia. Int J Hematol. 2001;74(4):437–41.
15. Ohtsuki T, Katsura Y, Mizukami H, Matsu-ura Y, Kimura F, Ohnishi M, et al. Elevated neutrophil function in chronic neutrophilic leukemia. Am J Hematol. 1992;41(1):50–6.
16. Dincol G, Nalcaci M, Dogan O, Aktan M, Kucukkaya R, Agan M, et al. Coexistence of chronic neutrophilic leukemia with multiple myeloma. Leuk Lymphoma. 2002;43(3):649–51.
17. Cehreli C, Undar B, Akkoc N, Onvural B, Altungoz O. Coexistence of chronic neutrophilic leukemia with light chain myeloma. Acta Haematol. 1994;91(1):32–4.
18. Feremans W, Marcelis L, Ardichvili D. Chronic neutrophilic leukaemia with enlarged lymph nodes and lysozyme deficiency. J Clin Pathol. 1983;36(3):324–8.
19. Bohm J, Kock S, Schaefer HE, Fisch P. Evidence of clonality in chronic neutrophilic leukaemia. J Clin Pathol. 2003;56(4):292–5.

20. Orazi A, Cattoretti G, Sozzi GA. Case of chronic neutrophilic leukemia with trisomy 8. Acta Haematol. 1989;81(3):148–51.
21. Terre C, Garcia I, Bastie JN, Mayeur D, Decombe L, Gruyer P, et al. A case of chronic neutrophilic leukemia with deletion (11)(q23). Cancer Genet Cytogenet. 1999;110(1):70–1.
22. Matano S, Nakamura S, Kobayashi K, Yoshida T, Matsuda T, Sugimoto T. Deletion of the long arm of chromosome 20 in a patient with chronic neutrophilic leukemia: cytogenetic findings in chronic neutrophilic leukemia. Am J Hematol. 1997;54(1):72–5.
23. Elliott MA. Chronic neutrophilic leukemia: a contemporary review. Curr Hematol Rep. 2004;3(3):210–7.
24. Kako S, Kanda Y, Sato T, Goyama S, Noda N, Shoda E, et al. Early relapse of JAK2 V617F-positive chronic neutrophilic leukemia with central nervous system infiltration after unrelated bone marrow transplantation. Am J Hematol. 2007;82(5):386–90.
25. Gajendra S, Gupta R, Chandgothia M, Kumar L, Gupta R, Chavan SM. Chronic neutrophilic leukemia with V617F JAK2 mutation. Indian J Hematol Blood Transfus. 2014;30(2):139–42.
26. Lasho TL, Mims A, Elliott MA, Finke C, Pardanani A, Tefferi A. Chronic neutrophilic leukemia with concurrent CSF3R and SETBP1 mutations: single colony clonality studies, in vitro sensitivity to JAK inhibitors and lack of treatment response to ruxolitinib. Leukemia. 2014;28(6):1363–5.
27. Lasho TL, Elliott MA, Pardanani A, Tefferi A. CALR mutation studies in chronic neutrophilic leukemia. Am J Hematol. 2014;89(4):450.
28. Elliott MA, Pardanani A, Hanson CA, Lasho TL, Finke CM, Belachew AA, et al. ASXL1 mutations are frequent and prognostically detrimental in CSF3R-mutated chronic neutrophilic leukemia. Am J Hematol. 2015;90(7):653–6.
29. Bain BJ, Gilliland DG, Wardiman JW, Horny H-P. Chronic eosinophilic leukemia, not otherwise specified. In: Swerdlow SH, Jaffe ES, Harris NL, editors. WHO. 4th ed. Lyon: IARC; 2008. p. 51–3.
30. Bain BJ. Eosinophilia and chronic eosinophilic leukemia, including myeloid/lymphoid neoplasms with eosinophilia and abnormalities of PDGFRA, PDGFRB, and FGFR1. In: Jaffe ES, Harris NL, Vardiman JW, Campo E, Arber DA, editors. Hematopathology. Philadelphia: Saunders/Elsevier; 2011. p. 785–6.
31. Means-Markwell M, Burgess T, deKeratry D, O'Neil K, Mascola J, Fleisher T, et al. Eosinophilia with aberrant T cells and elevated serum levels of interleukin-2 and interleukin-15. N Engl J Med. 2000;342(21):1568–71.
32. Lefevre G, Copin MC, Staumont-Salle D, Avenel-Audran M, Aubert H, Taieb A, et al. The lymphoid variant of hypereosinophilic syndrome: study of 21 patients with CD3-CD4+ aberrant T-cell phenotype. Medicine (Baltimore). 2014;93(17):255–66.
33. Ma SK, Kwong YL, Shek TW, Wan TS, Chow EY, Chan JC, et al. The role of trisomy 8 in the pathogenesis of chronic eosinophilic leukemia. Hum Pathol. 1999;30(7):864–8.
34. Maubach PA, Bauchinger M, Emmerich B, Rastetter J. Trisomy 7 and 8 in Ph-negative chronic eosinophilic leukemia. Cancer Genet Cytogenet. 1985;17(2):159–64.
35. Bain BJ. Eosinophilic leukaemias and the idiopathic hypereosinophilic syndrome. Br J Haematol. 1996;95(1):2–9.
36. Parreira L, Tavares de Castro J, Hibbin JA, Marsh JC, Marcus RE, Babapulle VB, et al. Chromosome and cell culture studies in eosinophilic leukaemia. Br J Haematol. 1986;62(4):659–69.
37. Wang SA, Tam W, Tsai AG, Arber DA, Hasserjian RP, Geyer JT, et al. Targeted next-generation sequencing identifies a subset of idiopathic hypereosinophilic syndrome with features similar to chronic eosinophilic leukemia, not otherwise specified. Mod Pathol. 2016;Aug;29(8):854–64.
38. Iurlo A, Gianelli U, Beghini A, Spinelli O, Orofino N, Lazzaroni F, et al. Identification of kit(M541L) somatic mutation in chronic eosinophilic leukemia, not otherwise specified and its implication in low-dose imatinib response. Oncotarget. 2014;5(13):4665–70.
39. Helbig G, Stella-Holowiecka B, Majewski M, Lewandowska M, Holowiecki J. Interferon alpha induces a good molecular response in a patient with chronic eosinophilic leukemia (CEL) carrying the JAK2V617F point mutation. Haematologica. 2007;92(11):e118–9.

40. Jones AV, Kreil S, Zoi K, Waghorn K, Curtis C, Zhang L, et al. Widespread occurrence of the JAK2 V617F mutation in chronic myeloproliferative disorders. Blood. 2005;106(6):2162–8.
41. Dahabreh IJ, Giannouli S, Zoi C, Zoi K, Loukopoulos D, Voulgarelis M. Hypereosinophilic syndrome: another face of janus? Leuk Res. 2008;32(9):1483–5.
42. Andersen CL, Nielsen HM, Kristensen LS, Sogaard A, Vikesa J, Jonson L, et al. Whole-exome sequencing and genome-wide methylation analyses identify novel disease associated mutations and methylation patterns in idiopathic hypereosinophilic syndrome. Oncotarget. 2015;6(38):40588–97.
43. Kopp P, Jaggi R, Tobler A, Borisch B, Oestreicher M, Sabacan L, et al. Clonal X-inactivation analysis of human tumours using the human androgen receptor gene (HUMARA) polymorphism: a non-radioactive and semiquantitative strategy applicable to fresh and archival tissue. Mol Cell Probes. 1997;11(3):217–28.
44. Helbig G, Soja A, Bartkowska-Chrobok A, Kyrcz-Krzemien S. Chronic eosinophilic leukemia-not otherwise specified has a poor prognosis with unresponsiveness to conventional treatment and high risk of acute transformation. Am J Hematol. 2012;87(6):643–5.
45. Iurlo A, Fracchiolla NS, Ferla V, Cassin R, Gottardi E, Beghini A, et al. Successful treatment with imatinib in a patient with chronic eosinophilic leukemia not otherwise specified. J Clin Oncol. 2014;32(10):e37–9.
46. Tefferi A, Gotlib J, Pardanani A. Hypereosinophilic syndrome and clonal eosinophilia: point-of-care diagnostic algorithm and treatment update. Mayo Clin Proc. 2010;85(2):158–64.

# Chapter 11
# Atypical Chronic Myeloid Leukemia, *BCR/ABL1* Negative

**Katherine Boothe Levinson and Adam Bagg**

## Introduction

Atypical chronic myeloid leukemia (aCML) is a neoplasm of hematopoietic stem cells characterized by overlapping myelodysplastic and myeloproliferative features at the time of diagnosis, and is hence classified under the myelodysplastic/myeloproliferative (MDS/MPN) disease category. The hallmark of this uncommon disorder is an overabundance of dysplastic mature granulocytic cells and their immature precursors, found in both the peripheral blood and bone marrow. To date, the specific causative molecular mechanisms underlying this enigmatic entity remain elusive, and there is no single genetic feature that defines the disease. Initially, studies investigating the biological underpinnings of aCML were limited by both technology and a lack of consensus criteria for diagnosing the disorder. The formalized diagnostic requirements for aCML, originally described in the 2001 World Health Organization (WHO) classification and later refined in 2008 and 2016, set the stage for an explosion of SNP array and next generation sequencing research in recent years. These efforts have identified numerous novel, recurrent somatic mutations seen in association with aCML as well as numerous other myeloid malignancies [1]. While none of these mutations is specific to aCML, alterations in certain genes, particularly *SETBP1* and *ETNK1*, appear to occur more frequently in aCML than in other myelodysplastic syndromes (MDS), myeloproliferative neoplasms (MPN),

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and MDS/MPN. These findings are of critical importance due to their potential diagnostic, prognostic, and therapeutic implications for patients with aCML, which, at present, has few effective treatment options and a dismal overall prognosis.

## *Epidemiology*

Atypical CML is one of the rarest myeloid neoplasms. For many years, it was postulated that there were only one to two cases of aCML (by definition t(9;22)*/BCR-ABL1*-negative) for every 100 cases of "typical" CML (by definition t(9;22)*/BCR-ABL1*-positive) [2]. A recent review of United States cancer registry data collected between 2001 and 2012 confirmed that aCML is indeed quite rare, with an incidence of 0.1 cases per one million person-years [3]. Atypical CML is somewhat more common in males, with an approximate male-to-female ratio of 1.5:1. Although its occurrence has been documented in patients of many ages, including rare cases in the pediatric population [4, 5], aCML is most commonly diagnosed in the sixth or seventh decades of life. Cases of aCML have been observed in individuals from many different racial/ethnic backgrounds, although recent data suggest that the disease is much more common in patients who identify as white than in patients who identify as Hispanic, black, or Asian/Pacific Islander [3].

## *Clinical Features*

There is a paucity of published information regarding the clinical presentation of aCML. Based on the limited data available, the most common signs and symptoms of aCML are B-symptoms (fevers, night sweats, weight loss), occurring in 38% of patients [6], and hepato- or splenomegaly, sometimes with associated early satiety and abdominal pain, occurring in 44–75% of patients [2, 6, 7]. Patients with aCML can present with variable abnormalities in hemoglobin and platelet counts. Atypical CML case series have reported median hemoglobin concentrations ranging from 9.4 to 11.7 g/dL and median platelet counts ranging from 87 to 319 × $10^9$ cells/L at the time of diagnosis [2, 5–9]. If cytopenias are severe enough, aCML patients may present with related clinical findings, including dyspnea on exertion and fatigue from anemia, as well as bleeding and bruising from thrombocytopenia. While aCML patients by definition produce an excessive number of leukocytes, they appear to have a predisposition to develop recurrent infections, presumably a consequence of the cells' qualitative dysfunction. A single case series showed that by the time patients were diagnosed with aCML, 30% had experienced infection, 38% had experienced hemorrhage, and 65% had developed a transfusion requirement [2].

## Morphology and Immunophenotype

The diagnosis of aCML relies on a combination of morphologic features seen in the peripheral blood and bone marrow, which have been outlined by the WHO (see Table 11.1) [10, 11]. A summary of the findings that help distinguish aCML from chronic myelomonocytic leukemia (CMML) and chronic neutrophilic leukemia (CNL), two entities commonly in the differential diagnosis of aCML, is presented in Table 11.2.

### *Peripheral Blood Morphology*

Examination of the peripheral blood smear can reveal many features required for the diagnosis of aCML. An essential peripheral blood finding is the presence of a persistent granulocytic leukocytosis in excess of $13 \times 10^9$ cells/L [12]. The median white blood cell count reported in aCML case series varies widely, ranging from 23.7 to $152 \times 10^9$ cells/L [2, 5–9, 12]. The leukocytosis of aCML is left-shifted, with immature precursors, including metamyelocytes, myelocytes, and promyelocytes, accounting for at least 10% of the total white blood cell differential count (Fig. 11.1). Dysgranulopoiesis should be present, although no numeric cutoff for percentage of dysplastic cells or rigorous dysplasia grading system have been formally established. Features of granulocytic dysplasia may include unusually small or large cell

**Table 11.1** Summary of the 2016 WHO diagnostic criteria for atypical chronic myeloid leukemia

| |
|---|
| Peripheral blood: |
| Neutrophilic leukocytosis >$13 \times 10^9$ cells/L |
| Myeloid precursors (promyelocytes, myelocytes, metamyelocytes) ≥10% of leukocytes |
| Dysgranulopoiesis |
| Monocytes <10% of leukocytes with no or minimal absolute monocytosis |
| Basophils usually <2% of leukocytes with no or minimal absolute basophilia |
| Blasts <20% of leukocytes |
| Bone marrow: |
| Hypercellular with granulocytic expansion |
| Dysgranulopoiesis with or without erythroid and megakaryocytic dysplasia |
| Blasts <20% of nucleated cells |
| Genetic: |
| No Philadelphia chromosome or *BCR-ABL1* fusion gene |
| No rearrangement of *PDGFRA, PDGFRB,* or *FGFR1* |
| No *PCM1-JAK2* fusion gene |

Data from Swerdlow et al. 2008 and Arber et al. 2016 [10, 11]

**Table 11.2** Comparative summary of 2016 WHO morphologic criteria for atypical chronic myeloid leukemia (aCML), chronic neutrophilic leukemia (CNL), and chronic myelomonocytic leukemia (CMML) in the peripheral blood (PB) and bone marrow (BM)

| | aCML | CNL | CMML |
|---|---|---|---|
| WBC | >13 × $10^9$ cells/L | ≥25 × $10^9$ cells/L | NA |
| % Immature granulocytes PB | >10% | <10% | NA |
| % Monocytes PB | <10% | NA | ≥10% |
| Absolute monocytes PB | Normal to mildly increased | <1 × $10^9$ cells/L | ≥1 × $10^9$ cells/L |
| % Blasts PB | <20% | Rarely observed | <20% |
| % Blasts BM | <20% | <5% | <20% |
| Granulocytic dysplasia | + | − | ± |
| Other hematopoietic dysplasia | ± | − | ± |

Adapted from Arber et al. (2016) [10]

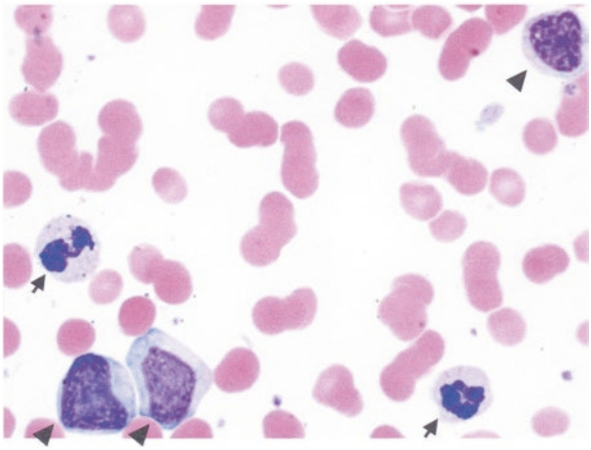

**Fig. 11.1** High-power view of atypical CML peripheral blood smear with dysplastic neutrophils (*arrows*) and immature precursors (*arrowheads*). Wright-Giemsa, 100×

size, exaggerated chromatin clumping, abnormalities in nuclear segmentation (including hypolobation, pseudo Pelger-Huet cell formation, and irregular hypersegmentation), and abnormalities in granulation (including hypogranularity, agranularity, or enlarged, pseudo Chediak-Higashi granules) (Fig. 11.2) [11]. An accurate diagnosis of aCML hinges on the absence of certain additional findings. Basophils and monocytes should account for <2% and <10% of peripheral white blood cells, respectively. Blasts typically account for <5% of peripheral blood leukocytes and should never exceed 20% of the total.

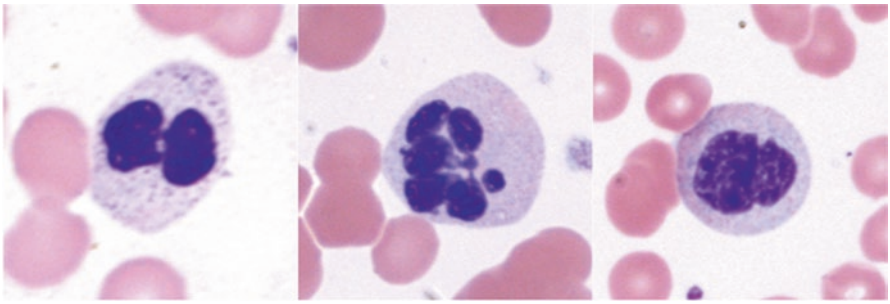

**Fig. 11.2** High power view of dysplastic peripheral blood neutrophils in atypical CML. Pseudo Pelger Huet morphology (*left panel*); nuclear hyperlobation and cytoplasmic hypogranulation (*central panel*); and nuclear hypolobation and cytoplasmic hypogranulation (*right panel*). Wright-Giemsa, 100×

## *Bone Marrow Morphology*

An aCML bone marrow biopsy should be hypercellular, with mature granulocytic cells and precursors comprising the majority of the cellularity (Fig. 11.3). Typically, the myeloid to erythroid ratio is in excess of 10:1 [11]. The overall number of megakaryocytes can be decreased, normal, or increased [11]. Similar to the peripheral blood, dysplasia should be present in the granulocytic lineage (Fig. 11.4). Median frequencies of erythroid dysplasia seen in aCML have varied substantially, ranging from 12 to 91% [2, 5, 7, 8]. Dysmegakaryopoiesis is slightly more common, with median reported frequencies ranging from 44 to 90% [2, 5, 7, 8]. The dysplastic megakaryocytes seen in aCML may have features typical of MDS, such as small cell size, abnormal nuclear lobation, or nuclear hypolobation; features typical of MPN, such as large cell size, nuclear hypersegmentation, and clustering; or features that fall somewhere in between (Fig. 11.5) [7]. A single, recent study found that among 61 patients with aCML, 54% had MDS-like megakaryocytes, 26% had MPN-like megakaryocytes, and 8% had mixed MDS/MPN-like megakaryocytes [7]. Reticulin fibrosis is uncommon in aCML, but may be seen in occasional cases, with a frequency of 18% reported in one case series [2]. The bone marrow blast count is usually <5% [2, 5, 7] and must be <20%. In addition, blasts should not form visible sheets or clusters [11].

## *Immunophenotype and Cytochemistry*

There are no specific data regarding the immunophenotype of aCML. However, immunophenotyping of peripheral blood and bone marrow for CD14, CD68R, and/or CD163 may facilitate monocyte quantification in cases where both aCML and CMML are in the differential diagnosis [11]. It should be noted that immunophenotyping,

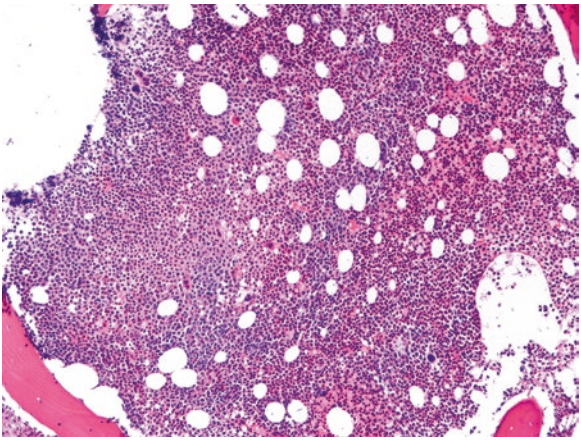

**Fig. 11.3** Low power view of a bone marrow core biopsy of atypical CML. The biopsy is notable for hypercellularity and a markedly increased myeloid to erythroid ratio. Hematoxylin and eosin 10×

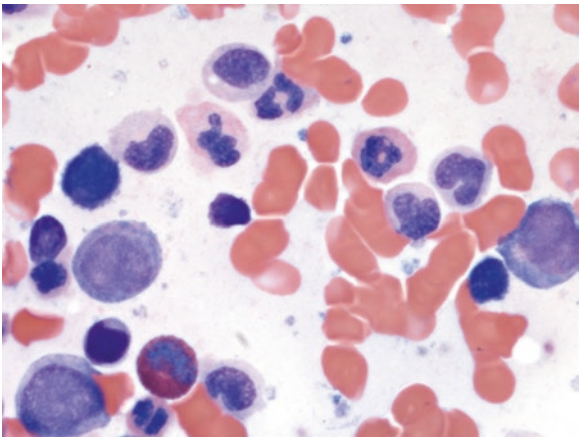

**Fig. 11.4** High-power view of atypical CML bone marrow aspirate smear with dysplastic granulocytic cells. Wright-Giemsa, 100×

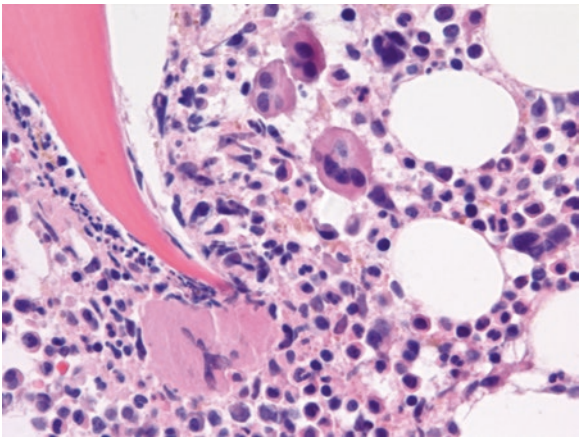

**Fig. 11.5** High power view of bone marrow core biopsy of atypical CML showing abnormal megakaryocytes with both myelodysplastic and myeloproliferative features. Hematoxylin and eosin 40×

even in this limited capacity, is not without challenges. Flow cytometric and immunohistochemical evaluation of monocytes may be impacted by alterations of antigen expression, including CD14, in immature and neoplastic populations [13, 14]. While such aberrancies may be helpful in the qualitative identification of abnormal monocytic populations, they can also limit precise enumeration by both flow cytometry and immunohistochemistry. In addition, it has been shown that staining of bone marrow core biopsies for CD68R and CD163 could not reliably distinguish between aCML and CMML in one small study [15]. For these reasons, cytochemical staining for nonspecific esterase is still advocated in addition to flow cytometry and immunohistochemistry as the most reliable means of identifying neoplastic monocytic populations [11, 13, 16].

There are other immunohistochemical findings in bone marrow core biopsies that can serve as a useful alternative to counting monocytes when attempting to distinguish aCML from CMML. For example, immunohistochemical staining for CD123 can be used to identify plasmacytoid dendritic cell nodules on bone marrow core biopsies, which are a specific (although somewhat insensitive) finding that favors a diagnosis of CMML [15].

## Cytogenetics and Molecular Findings

### *Cytogenetics*

There is no single cytogenetic feature that can confirm or establish a diagnosis of aCML. Although the Philadelphia chromosome is by definition absent in all cases of aCML, other nonspecific karyotypic abnormalities are quite common, having been reported in 20–88% of patients carrying the diagnosis [2, 5–8]. Studies have shown that there are some recurrent cytogenetic findings in aCML patients, many of which are also observed in MDS and CMML [11]. The most common aberrations in aCML, observed across six case series, are trisomy 8 and deletions of the long arm of chromosome 20 [2, 5–8, 12]. The next most common cytogenetic abnormalities noted in those studies were alterations in chromosome 7 (either monosomy or deletion of the long arm) and the presence of isochromosome 17q. Additional abnormalities of chromosomes 5, 11, 12, 13, 14, 17, 19, 21, and X were identified at lower frequencies. While complex karyotypes and multiple-anomaly karyotypes are relatively frequent findings in aCML, balanced translocations are quite rare.

### *Molecular*

Scientific understanding of aCML's molecular underpinnings remains in its infancy. Until recently, aCML's only defining molecular features were the absence of key genetic findings diagnostic of other similar neoplasms, specifically the *BCR-ABL1* fusion seen in CML and the *PDGFRA*, *PDGFRB*, *FGFR1*, and, most recently,

*PCM1-JAK2* fusions seen in myeloid and lymphoid neoplasms associated with eosinophilia [10]. Over the past several years, aCML has transitioned from a disease defined by the mutations it lacks into a disease with a few signature molecular alterations. Recent genetic sequencing studies have revealed that two genes, *SETBP1* and *ETNK1*, are recurrently mutated in aCML. Because mutations in these genes are considered relatively specific for aCML, they will be the primary focus of this section. There are, however, multiple additional, nonspecific genetic alterations seen in aCML which recur across the spectrum of myeloid neoplasms; these will be summarized here and discussed elsewhere. The relative frequencies of the various mutations seen in aCML and other closely related disorders are summarized in Table 11.3.

**Table 11.3** Frequencies of gene mutations as seen in atypical chronic myeloid leukemia (aCML) and other morphologically similar diagnostic entities, including chronic myelomonocytic leukemia (CMML), myelodysplastic/myeloproliferative neoplasm – unclassifiable (MDS/MPN-U), and chronic neutrophilic leukemia (CNL)

| | aCML | CMML | MDS/MPN-U | CNL |
|---|---|---|---|---|
| *SETBP1* | 24–33% | 4–15% | 10% | 14–55%[a] |
| *ETNK1* | 9% | 3–14% | 0% | 0% |
| *CSF3R* | <10%[b] | 0–1% | 0% | 43–100% |
| Cell Signaling | | | | |
| *N/KRAS* | 0–40% | 4–57% | 10–14% | NR |
| *CBL* | 7–12% | 10–21% | >10% | 0% |
| *JAK2* | 0–7% | 0–13% | 0–19% | 0% |
| *FLT3* | 0–7% | 0–3% | 3% | NR |
| *CALR* | 0–4% | 3% | 0% | 0–8% |
| *MPL* | 0–2% | <1% | 0% | 0% |
| Transcription Regulation | | | | |
| *CEBPA* | 5–12% | 4–20% | 0–4% | NR |
| *RUNX1* | 2% | 9–37% | 14% | NR |
| RNA Splicing | | | | |
| *SRSF2* | 40% | 36–51% | NR | 21% |
| *U2AF1* | 13% | 5–15% | NR | NR |
| Epigenetic Regulation | | | | |
| *ASXL1* | 20–66% | 27–49% | NR | 57% |
| *TET2* | 25–41% | 36–61% | 29–30% | 29% |
| *EZH2* | 13–20% | 6–13% | 10% | NR |
| *IDH1/2* | 0–5% | 1–10% | 0–10% | NR |

*NR* not reported

Sequencing methodologies and extent of gene analyzed were heterogeneous across different studies. The categories denoted with a "/" (*N/KRAS* and *IDH1/2*) each feature two closely related genes which were sometimes reported individually and sometimes reported as an aggregate

[a]Percentages are from small studies and may not reflect the true mutational frequency in the designated patient population

[b]Average percentage across majority of available studies, although initial research reported mutational frequencies of up to 44%

## SETBP1

The gene SET binding protein 1 (*SETBP1)* is located on chromosome 18q21.1, encodes a protein of the same name which contains 1596 amino acids (NCBI reference sequence NP_056374.2), and is predominantly located in the nucleus [17]. The structure of the SETBP1 protein has not been fully elucidated, but its known structural components are depicted in Fig. 11.6. Although its biological function is still under investigation, SETBP1 has been proposed to influence cell proliferation by inhibiting the known tumor suppressor phosphatase 2A (PP2A) via interactions with its substrate SET [17–20], by regulating the expression of cell differentiation homeobox genes, homeobox A9 (*Hoxa9)* and homeobox A10 (*Hoxa10)* [21], and, possibly, by modulating Ski/Ski homodimer and/or Ski/SnoN heterodimer formation via its SKI homologous domain [17].

*SETBP1* became relevant to aCML when multiple publications showed evidence of recurrent *SETBP1* mutations in up to 33% of patients with the disease [22–25]. Mutations in *SETBP1* were also identified in patients with CMML and myelodysplastic/myeloproliferative neoplasm, unclassifiable (MDS/MPN-U), but they occurred at lower rates than in aCML, at frequencies of 4–15% [23–29] and 10% [25], respectively. A handful of *SETBP1* mutations were also identified in 1 of 4 [25], 2 of 14 [23], 5 of 13 [30], 5 of 9 [31], and 4 of 12 [29] patients with CNL, although the small number of cases examined in these studies makes it difficult to know the true mutation frequency in CNL. While the initial study found no evidence of *SETBP1* mutations in hundreds of other hematologic and nonhematologic malignancies [25], subsequent studies showed that they can be seen with some frequency in secondary AML (2–17%) and less commonly in cases of MPN (0–3%), MDS (2–4%), and primary AML (0–< 1%) [24, 26, 28, 32].

The initial study reporting *SETBP1* alterations in aCML patients showed that the *SETBP1* mutations were all heterozygous missense mutations occurring almost exclusively in the protein's SKI-homologous region [25]. Six of the mutations seen in the

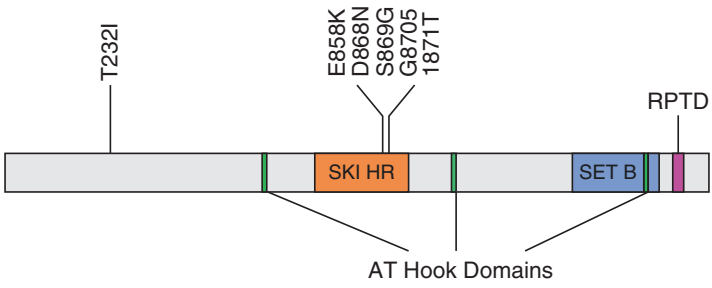


**Fig. 11.6** Schematic representation of SETBP1 protein. The primary protein isoform consists of 1596 amino acids and contains three AT hook domains (*green*), a SKI homologous domain (SKI HR, *orange*), SET binding domain (SET B, *blue*), and repeat domain (RPTD, *pink*). The mutations specifically associated with aCML are listed, and those confirmed to be somatic are in bold (Adapted from Piazza 2012 [33])

aCML patients were confirmed to be somatically acquired by tandem analysis of constitutive DNA [25]. The mutational hotspot included 16 amino acid residues in positions 858–874 [24, 25]. Comparative RNA analysis between *SETBP1* wild type and *SETBP1* mutated aCML cases revealed 14 differentially expressed genes belonging to the group transcriptionally controlled by TGF-β, a finding which was highly statistically significant [25]. Because of the known associations between SKI and TGF-β signaling [33], these findings suggested one potential mechanism by which mutations in *SETBP1*'s SKI homologous domain could induce oncogenesis in aCML. It was also noted that a portion of the *SETBP1* mutational hotspot (amino acids 868–873) encodes a virtually perfect binding site for β-TrCP1, the substrate recognition subunit of the protein-degrading E3 ubiquitin ligase [25]. Additional experiments not only confirmed that the mutated versions of SETBP1 seen in aCML are resistant to degradation, but also demonstrated that cells expressing mutant *SETBP1* have significantly lower levels of PP2A activity and significantly increased proliferation rates compared to wild type controls [25], suggesting a second mechanism by which mutations in *SETBP1* could induce oncogenesis in aCML.

There are only limited data connecting *SETBP1* mutations to clinical features, cytogenetic findings, and other mutations specifically in aCML. The seminal study of *SETBP1* mutations in aCML revealed that the only clinical variable significantly associated with *SETBP1* mutations was a higher white blood cell count at diagnosis, with a median of $81 \times 10^9$ cells/L in the *SETBP1* mutated group compared to a median of $38.5 \times 10^9$ cells/L in those with wild type *SETBP1* [25]. One subsequent study found that aCML patients with *SETBP1* mutations had significantly higher hemoglobin concentrations than those with wild type *SETBP1* [23], a finding which was not seen in the initial study [25]. There is currently no aCML-specific information regarding associations between *SETBP1* mutations and cytogenetic alterations, although studies of other myeloid neoplasms have shown significant correlations between *SETBP1* mutations and several cytogenetic anomalies that are frequently seen in aCML, including i(17)(q10), monosomy 7, and del(7q) [24, 28] . Several studies of patients with aCML have noted preliminary associations between *SETBP1* mutations and alterations in other genes that are commonly mutated across the broad spectrum of myeloid neoplasms. The only statistically significant association observed has been between *SETBP1* mutations and *SRSF2* mutations [23]. Multiple other genes have been reportedly mutated in tandem with *SETBP1* in aCML, including *CBL* [24, 25], *ASXL1* [22, 24, 25], *EZH2* [25], *N/KRAS* [22, 25], *TET2* [25], *ETNK1* [22], and *CSF3R* [31]. These findings, however, were either not statistically significant or were only anecdotal. Additional, larger studies will be needed to firmly establish these associations and to expand our understanding of how *SETBP1* mutations interact with other concurrent molecular alterations to produce the unique aCML disease phenotype.

Although they can be found in multiple different myeloid neoplasms, and while they are not evident in the majority of aCMLs, the current data suggest that *SETBP1* mutations are one of the most promising molecular markers in the diagnosis and pathogenesis of aCML. Preliminary research has suggested multiple potential mechanisms by which mutations in *SETBP1* can induce neoplastic transformation

in hematopoietic cells. More investigation is needed to further our understanding of the precise molecular mechanisms by which *SETBP1* mutations contribute specifically to the development of aCML.

## ETNK1

The gene ethanolamine kinase 1 (*ETNK1*, also known as *EKI1*) is located on chromosome 12p12.1 and encodes a 452 amino acid protein called ETNK1 [34]. Little is known about the structure of the protein, which is depicted in Fig. 11.7. ETNK1 is responsible for facilitating the ATP-dependent phosphorylation of ethanolamine (Etn) to produce phosphoethanolamine (P-Etn). The conversion of Etn to P-Etn is the first step in a biochemical chain of events known as the CDP-ethanolamine pathway (also known as the Kennedy pathway), which ultimately results in the production of phosphatidylethanolamine (PE) [35]. PE is the second most abundant phospholipid in mammalian cells, and is involved in many essential cellular processes, including cell division and membrane protein orientation [35]. These functions are, of course, quite nonspecific. Although mechanisms linking them to neoplasia could be postulated, further research is needed to link PE and ETNK1 to oncogenesis at the molecular level.

The only study examining *ETNK1* mutations in aCML performed whole exome and transcriptome sequencing on 15 patients with aCML, and identified two somatic, heterozygous missense mutations in *ETNK1* that altered two adjacent amino acid residues, H243Y and N244S [22]. In that same study, targeted sequencing of numerous additional clonal hematologic disorders revealed *ETNK1* mutations in 4 of 53 additional cases of aCML, bringing the overall *ETNK1* mutation frequency in aCML to 9% (6 of 68 cases). Although *ETNK1* mutations were also identified in 2 of 77 (3%) cases of CMML, they were not seen in MDS/MPN-U ($n = 10$), CNL ($n = 1$), other hematologic neoplasms, solid tumors, cancer cell lines, or healthy controls [22]. The additional six mutations were all heterozygous N244S mutations, bringing the overall mutation counts to one H243Y variant and seven N244S variants [22]. Review of the ETNK1 structure revealed that the mutations were clustering in the region encoding the protein's highly conserved kinase domain [22]. Follow up experiments on seven aCML primary samples indicated that *ETNK1*

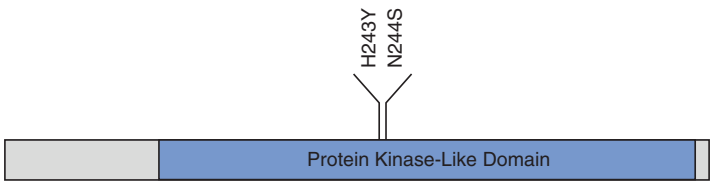


**Fig. 11.7** Schematic representation of ETNK1 protein. The primary protein isoform consists of 452 amino acids, with a protein kinase-like domain spanning amino acids 100–444 (*blue*). The mutations specifically associated with aCML are listed (Adapted from Gambacorti-Passerini 2015 and Lasho 2015 [30, 42])

mutations significantly lowered intracellular levels of P-Etn relative to wild type controls, and that transduction of myeloid cell lines with wild type and mutant forms of *ETNK1* produced similar results [22]. These findings suggest that mutations in *ETNK1* interfere with the function of the ETNK1 enzyme, but until more research is performed, it remains unknown how diminished ETNK1 activity contributes to oncogenesis.

At present, there are no data available regarding the clinical characteristics and cytogenetic findings associated with *ETNK1* mutations in aCML or in any other hematologic neoplasm. Very limited data suggest that concurrent *ETNK1* and *SETBP1* mutations may be seen in aCML [22], although how these mutations may interact and contribute to oncogenesis has not been explored. Another important consideration is that, although the breakthrough study suggested that *ETNK1* mutations were highly specific for aCML, an additional study reported *ETNK1* mutations in 4 of 29 (14%) cases of CMML, which would make the mutational frequency in CMML higher than that reported in aCML [36]. In addition, *ETNK1* mutations were seen in 5 of 82 (6%) cases of systemic mastocytosis (SM) and in 1 of 137 (<1%) cases of "idiopathic hypereosinophilia" [36]. All of these findings suggest that alterations in *ETNK1* may not be as unique to aCML as previously thought. Larger-scale studies are needed to clarify the relative incidences of *ETNK1* mutations in aCML, CMML, SM, and "idiopathic hypereosinophilia" so that we may better understand their utility in classifying these related disorders.

## CSF3R

*CSF3R* (granulocyte colony-stimulating factor 3 receptor, also known as *GCSFR*) encodes the trans-membrane cell surface receptor for granulocyte-colony stimulating factor (G-CSF, also known as CSF3), which has long been known to promote the proliferation and differentiation of granulocytic cells via its interaction with CSF3R [37]. Given the essential role that *CSF3R* plays in granulocytic cell growth and maturation, it is not surprising that *CSF3R* mutations would be seen in neoplasms characterized by aberrations in the myeloid lineage, including aCML and CNL. Although initially reported in 40–44% of patients with aCML [31, 38], *CSF3R* mutations are now considered rare in aCML, occurring in fewer than 10% of cases [7, 22, 23, 29]. The *CSF3R* mutations seen in aCML generally fall into one of two main categories: either missense mutations occurring predominantly in the membrane proximal domain or truncating mutations occurring in the cytoplasmic domain [38]. Occasionally, both mutation types occur simultaneously [38]. Follow-up experiments have demonstrated that both mutation subtypes can induce cell line transformation in vitro [38]. Although *CSF3R* mutations are occasionally identified in aCML, they are substantially more common in CNL, occurring in 43–4100% of cases [23, 30, 31, 38, 29]. Consequently, the 2016 WHO classification of myeloid neoplasms lists *CSF3R* mutations among the diagnostic criteria for CNL and notes that the detection of *CSF3R* mutations in potential cases of aCML should prompt efforts to exclude an alternative diagnosis of CNL or other myeloid neoplasm [10].

### Other Molecular Findings in aCML

Many of the other mutations seen in aCML are not disease specific, but rather, are seen across a wide spectrum of myeloid neoplasms, including CMML, MDS/MPN-U, and CNL. These mutations, summarized in Table 11.3, occur in genes involved with cell signaling, such as *N/KRAS* [1, 7, 8, 22, 25, 39–41], *CBL* [1, 23–25, 41–43], *JAK2* [1, 7, 23, 29–31, 39, 41, 42, 44], *FLT3* [1, 7, 39, 45], *CALR* [7, 23, 30, 46], and *MPL* [7, 23], transcription regulation, such as *CEBPA* [1, 7, 25] and *RUNX1* [1, 25, 41], RNA-splicing, such as *SRSF2* [1, 23] and *U2AF1* [1, 22], and epigenetic regulation, such as *ASXL1* [1, 22–25, 30, 41], *TET2* [1, 23–25, 41, 42], *EZH2* [1, 22, 25, 41, 47], and *IDH1/2* [1, 7, 25, 41]. The mechanisms by which these various genes promote cellular proliferation and oncogenesis are quite diverse and beyond the scope of this chapter. Because none of these mutated genes is particularly unique to aCML, finding alterations in them is of little value diagnostically. However, mutations in these genes should not be overlooked, as they may have prognostic and therapeutic implications for aCML patients, which will be discussed in the next section. In addition, observations regarding which mutations tend to co-occur and which ones are mutually exclusive will hopefully shed light on the molecular pathogenesis of aCML and other myeloid neoplasms.

## Therapy and Prognosis

Presently, aCML has a fairly dismal prognosis, with a median survival ranging from 12.4 to 36 months across several case series [2, 5–8]. The most robust risk factor associated with shorter overall survival in aCML is leukocytosis in excess of $50 \times 10^9$ cells/L, documented in two separate case series [2, 7]. A few other features, including older age (>65 years) [2], female sex [2], and higher numbers of circulating immature precursors [7], have also been associated with shorter overall survival, but these were noted only in single case series.

The only molecular marker that has been associated with prognosis specifically in aCML is the presence of mutated *SETBP1,* however, the studies examining this relationship have shown inconclusive results [24, 25]. While one study showed that *SETBP1* mutations were significantly associated with worse prognosis in aCML, with a median survival of 22 months compared to 77 months in nonmutated patients (median follow up not specified) [25], a second study found that aCML patients with *SETBP1* mutations had an overall survival of 32.9 months, compared to 15.6 months in nonmutated aCML patients (median follow up 17.1 months) [24]; importantly, the latter finding did not reach statistical significance. The fact that there have been significant associations between *SETBP1* mutations and other adverse prognostic factors such as increased white blood cell count [25], supports the notion that *SETBP1* mutations are markers of poor prognosis in aCML, although more studies are needed to confirm this finding.

In addition to having a poor overall prognosis, patients with aCML have a substantial risk of transforming into acute leukemia, with an absolute progression risk of 13–40% [2, 6, 7] and a median time to leukemic transformation of 11.2–18 months [2, 7]. Many clinical and laboratory parameters have been associated with the risk of transformation in aCML, including transfusional requirement, palpable hepatosplenomegaly, higher white blood cell count, higher percentage circulating myeloid precursors, percentage monocytes of 3–8% with an absolute monocyte count $<1 \times 10^9$ cells/L, >5% bone marrow blasts, marked dyserythropoiesis, and karyotypic changes [2, 7].

There is no current gold standard for managing patients with aCML. Many different treatment modalities are utilized in aCML, including hydroxyurea, immunomodulators, hypomethylating agents, histone deacetylase inhibitors, traditional chemotherapy, and hematopoietic stem cell transplantation (HSCT) [2, 5–7]. While there are rare case series demonstrating these therapies can be beneficial in aCML [6, 48], the data regarding the efficacy of these treatments and their impact on patient survival are quite limited. The most evidence-based therapeutic strategy in aCML is HSCT, and even this approach is only supported by a few small studies, which followed an aggregate total of 18 patients over median follow up periods of 22–97.6 months [49–51]. Across these three studies, two patients relapsed, six patients died, nine developed acute graft versus host disease, and 15 developed chronic graft versus host disease. These aggregate data seem to suggest that HSCT is a viable treatment option for aCML, but the morbidity and mortality associated with the procedure highlights a need for additional therapeutic options.

The advent of molecular diagnostics in aCML has introduced the possibility of targeted, lower-toxicity treatment options for patients with certain disease-associated mutations. At present, most of the data regarding these treatments are limited to single case reports. For example, one group showed that transformed cells expressing an oncogenic *SPTBN1-FLT3* fusion protein derived from an aCML patient exhibited dose-dependent growth inhibition by three different FLT3 inhibitors [45]. Another study showed that an aCML patient with a heterozygous *NRAS* mutation had improvements in cell counts, normalization of liver and kidney function tests, and increased energy levels after receiving off-label trametinib, an FDA-approved MEK1/2 inhibitor [52]. There is a somewhat more substantial body of evidence supporting mutation-targeted treatment in aCML patients harboring *CSF3R* mutations. Although *CSF3R* mutations are not as prevalent in aCML as initially posited, studies have suggested that drugs targeting downstream effectors of CSF3R may be helpful therapeutic options for patients expressing mutant forms of the protein. In vitro experiments with *CSF3R*-mutated patient samples, cell lines, and murine bone marrow cells have demonstrated that cells with membrane proximal mutations like T618I are sensitive to JAK inhibitors and that cells with cytoplasmic tail truncating mutations like S783fs are sensitive to the SRC (and ABL1) inhibitor dasatinib [38]. There are multiple case reports showing that patients and mice with *CSF3R* T618I-mutated neoplasms respond to ruxolitinib therapy [4, 38, 53, 54]. The magnitude of response to treatment has varied from case to case, ranging from isolated reduction in white blood cell count [4] to complete normalization of peripheral blood counts with fewer

circulating granulocytes, improved neutrophil granulation, improvements in bone marrow morphology, reduction in splenic volume, and improvement in symptom scores [53]. Interestingly, a single aCML patient with concurrent *CSF3R* T618I and *SETBP1* G870S mutations did not respond to ruxolitinib therapy, suggesting that *SETBP1* mutations may modify *CSF3R* T618I mutations' responsiveness to therapy by an as-of-yet unknown mechanism [55].

In addition to guiding therapeutic decision making in aCML, *CSF3R* mutations may also have a role in monitoring therapeutic efficacy. *CSF3R* T618I mutation levels were found to correlate with the presence of disease in two aCML HSCT patients, suggesting this molecular marker may be useful in monitoring disease burden post-transplantation [56]. Although a single case report showed that clinical responses to ruxolitinib therapy are not necessarily accompanied by reductions in *CSF3R* T618I allele frequency [53], it will be worthwhile to further assess the utility of *CSF3R* allele frequency as a marker for response to targeted therapies. While these reports offer hope for the future of therapeutics in aCML, it is important to note that these findings are only anecdotal. Many more studies will be required to optimize therapeutic approaches and significantly alter patient prognosis.

The lack of consensus regarding the optimal management of aCML patients is multifactorial, and generating informative clinical trials will be challenging for several reasons. First, correctly identifying cases of aCML can be difficult, as the current diagnostic criteria were only recently established, and even these criteria may be open to subjective interpretation. In addition, aCML is a rare disease, making it hard to generate large clinical trials comparing efficacies of different treatment modalities. Finally, there are no formally accepted guidelines for evaluating response to treatment or for assessing disease progression in aCML, which hampers accurate assessment of therapeutic efficacy in research studies. Although such metrics are established in MDS [57] and subtypes of MPN [58], they are suboptimal for use in MDS/MPN because those criteria do not account for the simultaneous myelodysplastic and myeloproliferative features seen in this unique disease category. These important issues were discussed in a recent commentary where the authors proposed sets of criteria specifically designed to measure treatment response and disease progression in patients with MDS/MPN [59]. These recommendations are an excellent step toward standardizing aCML research, but they will require further refinement and thorough validation before they can be adopted in clinical practice.

## Conclusion

Atypical CML is an uncommon myeloid neoplasm with overlapping myelodysplastic/myeloproliferative features and a poor prognosis. Although aCML is currently best defined by morphologic criteria, advances in the field of genetics have identified *SETBP1* and *ETNK1* as relatively disease-specific molecular markers. Many other mutations have also been reported in aCML. Although these mutations are not unique to aCML and are therefore of limited use diagnostically, they may carry

increasing significance as the arsenal of targeted therapeutic options expands. At present, more research is needed to further our understanding of the molecular mechanisms driving the development of aCML and to identify treatment regimens supported by robust, well-designed clinical trials.

## References


1. Zoi K, Cross NCP. Molecular pathogenesis of atypical CML, CMML and MDS/MPN-unclassifiable. Int J Hematol. 2015;101:229–42. doi:10.1007/s12185-014-1670-3.
2. Breccia M, Biondo F, Latagliata R, Carmosino I, Mandelli F, Alimena G. Identification of risk factors in atypical chronic myeloid leukemia. Haematologica. 2006;91:1566–8.
3. Srour SA, Devesa SS, Morton LM, Check DP, Curtis RE, Linet MS, Dores GM. Incidence and patient survival of myeloproliferative neoplasms and myelodysplastic/myeloproliferative neoplasms in the United States, 2001-12. Br J Haematol. 2016;177:331. doi:10.1111/bjh.14061.
4. Freedman JL, Desai AV, Bailey LC, Aplenc R, Burnworth B, Zehentner BK, Teachey DT, Wertheim G. Atypical chronic myeloid leukemia in two pediatric patients: atypical CML in pediatric patients. Pediatr Blood Cancer. 2016;63:156–9. doi:10.1002/pbc.25694.
5. Hernandez JM, del Canizo MC, Cuneo A, Garcia JL, Gutierrez NC, Gonzalez M, Castoldi G, San Miguel JF. Clinical, hematological and cytogenetic characteristics of atypical chronic myeloid leukemia. Ann Oncol. 2000;11:441–4.
6. Kurzrock R, Bueso-Ramos CE, Kantarjian H, Freireich E, Tucker SL, Siciliano M, Pilat S, Talpaz M. BCR rearrangement–negative chronic myelogenous leukemia revisited. J Clin Oncol. 2001;19:2915–26.
7. Wang SA, Hasserjian RP, Fox PS, Rogers HJ, Geyer JT, Chabot-Richards D, Weinzierl E, Hatem J, Jaso J, Kanagal-Shamanna R, Stingo FC, Patel KP, Mehrotra M, Bueso-Ramos C, Young KH, Dinardo CD, Verstovsek S, Tiu RV, Bagg A, Hsi ED, Arber DA, Foucar K, Luthra R, Orazi A. Atypical chronic myeloid leukemia is clinically distinct from unclassifiable myelodysplastic/myeloproliferative neoplasms. Blood. 2014;123:2645–51.
8. Martiat P, Michaux JL, Rodhain J. Philadelphia-negative (Ph-) chronic myeloid leukemia (CML): comparison with Ph+ CML and chronic myelomonocytic leukemia. The Groupe Francais de Cytogenetique Hematologique. Blood. 1991;78:205–11.
9. Shepherd PCA, Ganesan TS, Galton DAG. Haematological classification of the chronic myeloid leukaemias. Baillières Clin Haematol. 1987;1:887–906.
10. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, Bloomfield CD, Cazzola M, Vardiman JW. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127:2391–405. doi:10.1182/blood-2016-03-643544.
11. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Theile J, Vardiman JW. WHO classification of tumours of haematopoietic and lymphoid tissues. 4th ed. Lyon: International Agency for Research on Cancer; 2008.
12. Bennett J, Catovsky D, Daniel M, Flandrin G, Galton D, Gralnick H, Sultan C, Cox C. The chronic myeloid leukaemias: guidelines for distinguishing chronic granulocytic, atypical chronic myeloid, and chronic myelomonocytic leukaemia. Proposals by the French-American-British cooperative Leukaemia group. Br J Haematol. 1994;87:746–54.
13. Dunphy CH, Orton SO, Mantell J. Relative contributions of enzyme cytochemistry and flow cytometric immunophenotyping to the evaluation of acute myeloid leukemias with a monocytic component and of flow cytometric immunophenotyping to the evaluation of absolute monocytoses. Am J Clin Pathol. 2004;122:865–74. doi:10.1309/BH588HVG6UHN2RF2.
14. Shen Q, Ouyang J, Tang G, Jabbour EJ, Garcia-Manero G, Routbort M, Konoplev S, Bueso-Ramos C, Medeiros LJ, Jorgensen JL, Wang SA. Flow cytometry immunophenotypic findings in chronic myelomonocytic leukemia and its utility in monitoring treatment response. Eur J Haematol. 2015;95:168–76. doi:10.1111/ejh.12477.

15. Orazi A, Chiu R, O'Malley DP, Czader M, Allen SL, An C, Vance GH. Chronic myelomonocytic leukemia: the role of bone marrow biopsy immunohistology. Mod Pathol. 2006;19:1536–45.
16. Patnaik MM, Parikh SA, Hanson CA, Tefferi A. Chronic myelomonocytic leukaemia: a concise clinical and pathophysiological review. Br J Haematol. 2014;165:273–86. doi:10.1111/bjh.12756.
17. Minakuchi M, Kakazu N, Gorrin-Rivas MJ, Abe T, Copeland TD, Ueda K, Adachi Y. Identification and characterization of SEB, a novel protein that binds to the acute undifferentiated leukemia-associated protein SET. Eur J Biochem. 2001;268:1340–51.
18. Cristobal I, Blanco FJ, Garcia-Orti L, Marcotegui N, Vicente C, Rifon J, Novo FJ, Bandres E, Calasanz MJ, Bernabeu C, Odero MD. SETBP1 overexpression is a novel leukemogenic mechanism that predicts adverse outcome in elderly patients with acute myeloid leukemia. Blood. 2010;115:615–25. doi:10.1182/blood-2009-06-227363.
19. Li M, Makkinje A, Damuni Z. The myeloid leukemia-associated protein SET is a potent inhibitor of protein phosphatase 2A. J Biol Chem. 1996;271:11059–62.
20. Grech G, Baldacchino S, Saliba C, Grixti MP, Gauci R, Petroni V, Fenech AG, Scerri C. Deregulation of the protein phosphatase 2A, PP2A in cancer: complexity and therapeutic options. Tumor Biol. 2016;37:11691. doi:10.1007/s13277-016-5145-4.
21. Oakley K, Han Y, Vishwakarma BA, Chu S, Bhatia R, Gudmundsson KO, Keller J, Chen X, Vasko V, Jenkins NA, Copeland NG, Du Y. Setbp1 promotes the self-renewal of murine myeloid progenitors via activation of Hoxa9 and Hoxa10. Blood. 2012;119:6099–108. doi:10.1182/blood-2011-10-388710.
22. Gambacorti-Passerini CB, Donadoni C, Parmiani A, Pirola A, Redaelli S, Signore G, Piazza V, Malcovati L, Fontana D, Spinelli R, Magistroni V, Gaipa G, Peronaci M, Morotti A, Panuzzo C, Saglio G, Usala E, Kim D-W, Rea D, Zervakis K, Viniou N, Symeonidis A, Becker H, Boultwood J, Campiotti L, Carrabba M, Elli E, Bignell GR, Papaemmanuil E, Campbell PJ, Cazzola M, Piazza R. Recurrent ETNK1 mutations in atypical chronic myeloid leukemia. Blood. 2015;125:499–503. doi:10.1182/blood-2014-06-579466.
23. Meggendorfer M, Haferlach T, Alpermann T, Jeromin S, Haferlach C, Kern W, Schnittger S. Specific molecular mutation patterns delineate chronic neutrophilic leukemia, atypical chronic myeloid leukemia, and chronic myelomonocytic leukemia. Haematologica. 2014;99:e244–6. doi:10.3324/haematol.2014.113159.
24. Meggendorfer M, Bacher U, Alpermann T, Haferlach C, Kern W, Gambacorti-Passerini C, Haferlach T, Schnittger S. SETBP1 mutations occur in 9% of MDS/MPN and in 4% of MPN cases and are strongly associated with atypical CML, monosomy 7, isochromosome i(17)(q10), ASXL1 and CBL mutations. Leukemia. 2013;27:1852–60. doi:10.1038/leu.2013.133.
25. Piazza R, Valletta S, Winkelmann N, Redaelli S, Spinelli R, Pirola A, Antolini L, Mologni L, Donadoni C, Papaemmanuil E, Schnittger S, Kim D-W, Boultwood J, Rossi F, Gaipa G, De Martini GP, di Celle PF, Jang HG, Fantin V, Bignell GR, Magistroni V, Haferlach T, Pogliani EM, Campbell PJ, Chase AJ, Tapper WJ, Cross NCP, Gambacorti-Passerini C. Recurrent SETBP1 mutations in atypical chronic myeloid leukemia. Nat Genet. 2012;45:18–24. doi:10.1038/ng.2495.
26. Damm F, Itzykson R, Kosmider O, Droin N, Renneville A, Chesnais V, Gelsi-Boyer V, de Botton S, Vey N, Preudhomme C, Clavert A, Delabesse E, Park S, Birnbaum D, Fontenay M, Bernard OA, Solary E. SETBP1 mutations in 658 patients with myelodysplastic syndromes, chronic myelomonocytic leukemia and secondary acute myeloid leukemias. Leukemia. 2013;27:1401–3. doi:10.1038/leu.2013.35.
27. Laborde RR, Patnaik MM, Lasho TL, Finke CM, Hanson CA, Knudson RA, Ketterling RP, Pardanani A, Tefferi A. SETBP1 mutations in 415 patients with primary myelofibrosis or chronic myelomonocytic leukemia: independent prognostic impact in CMML. Leukemia. 2013;27:2100–2. doi:10.1038/leu.2013.97.
28. Makishima H, Yoshida K, Nguyen N, Przychodzen B, Sanada M, Okuno Y, Ng KP, Gudmundsson KO, Vishwakarma BA, Jerez A, Gomez-Segui I, Takahashi M, Shiraishi Y, Nagata Y, Guinta K, Mori H, Sekeres MA, Chiba K, Tanaka H, Muramatsu H, Sakaguchi H, Paquette RL, McDevitt MA, Kojima S, Sauntharajah Y, Miyano S, Shih L-Y, Du Y, Ogawa S, Maciejewski JP. Somatic SETBP1 mutations in myeloid malignancies. Nat Genet. 2013;45:942–6. doi:10.1038/ng.2696.

29. Pardanani A, Lasho TL, Laborde RR, Elliott M, Hanson CA, Knudson RA, Ketterling RP, Maxson JE, Tyner JW, Tefferi A. CSF3R T618I is a highly prevalent and specific mutation in chronic neutrophilic leukemia. Leukemia. 2013;27:1870–3. doi:10.1038/leu.2013.122.
30. Elliott MA, Pardanani A, Hanson CA, Lasho TL, Finke CM, Belachew AA, Tefferi A. *ASXL1* mutations are frequent and prognostically detrimental in *CSF3R* -mutated chronic neutrophilic leukemia: Asxl1 mutations are frequent and prognostically detrimental in CSF3R-mutated CNL. Am J Hematol. 2015;90:653–6. doi:10.1002/ajh.24031.
31. Gotlib J, Maxson JE, George TI, Tyner JW. The new genetics of chronic neutrophilic leukemia and atypical CML: implications for diagnosis and treatment. Blood. 2013;122:1707–11. doi:10.1182/blood-2013-05-500959.
32. Thol F, Suchanek KJ, Koenecke C, Stadler M, Platzbecker U, Thiede C, Schroeder T, Kobbe G, Kade S, Löffeld P, Banihosseini S, Bug G, Ottmann O, Hofmann W-K, Krauter J, Kröger N, Ganser A, Heuser M. SETBP1 mutation analysis in 944 patients with MDS and AML. Leukemia. 2013;27:2072–5. doi:10.1038/leu.2013.145.
33. Luo K, Stroschein SL, Wang W, Chen D, Martens E, Zhou S, Zhou Q. The ski oncoprotein interacts with the Smad proteins to repress TGFbeta signaling. Genes Dev. 1999;13:2196–206.
34. Lykidis A, Wang J, Karim MA, Jackowski S. Overexpression of a mammalian ethanolamine-specific kinase accelerates the CDP-ethanolamine pathway. J Biol Chem. 2001;276:2174–9. doi:10.1074/jbc.M008794200.
35. Calzada E, Onguka O, Claypool SM. Phosphatidylethanolamine metabolism in health and disease. Int Rev Cell Mol Biol. Elsevier. 2016;321:29–88.
36. Lasho TL, Finke CM, Zblewski D, Patnaik M, Ketterling RP, Chen D, Hanson CA, Tefferi A, Pardanani A. Novel recurrent mutations in ethanolamine kinase 1 (ETNK1) gene in systemic mastocytosis with eosinophilia and chronic myelomonocytic leukemia. Blood Cancer J. 2015;5:e275. doi:10.1038/bcj.2014.94.
37. Liongue C, Ward AC. Granulocyte colony-stimulating factor receptor mutations in myeloid malignancy. Front Oncol. 2014;4:93. doi:10.3389/fonc.2014.00093.
38. Maxson JE, Gotlib J, Pollyea DA, Fleischman AG, Agarwal A, Eide CA, Bottomly D, Wilmot B, McWeeney SK, Tognon CE, Pond JB, Collins RH, Goueli B, ST O, Deininger MW, Chang BH, Loriaux MM, Druker BJ, Tyner JW. Oncogenic *CSF3R* mutations in chronic neutrophilic leukemia and atypical CML. N Engl J Med. 2013;368:1781–90. doi:10.1056/NEJMoa1214514.
39. Tyner JW, Loriaux MM, Erickson H, Eide CA, Deininger J, MacPartlin M, Willis SG, Lange T, Druker BJ, Kovacsovics T, Maziarz R, Gattermann N, Deininger MW. High-throughput mutational screen of the tyrosine kinome in chronic myelomonocytic leukemia. Leukemia. 2009;23:406–9. doi:10.1038/leu.2008.187.
40. Hirsch-Ginsberg C, LeMaistre AC, Kantarjian H, Talpaz M, Cork A, Freireich EJ, Trujillo JM, Lee M-S, Stass SA. RAS mutations are rare events in Philadelphia chromosome-negative/bcr gene rearrangement-negative chronic myelogenous leukemia, but are prevalent in chronic myelomonocytic leukemia. Blood. 1990;76:1214–9.
41. Muramatsu H, Makishima H, Maciejewski JP. Chronic myelomonocytic leukemia and atypical chronic myeloid leukemia: novel pathogenetic lesions. Semin Oncol. 2012;39:67–73. doi:10.1053/j.seminoncol.2011.11.004.
42. Ernst T, Chase A, Hidalgo-Curtis C, Zoi K, Zoi C, Hochhaus A, Reiter A, Vainchenker W, Grand F, Cross NCP. Frequent inactivating mutations of TET2 and CBL are associated with acquired uniparental disomy in atypical chronic myeloid leukemia and related disorders. [abstract]. Blood. 2009;114:3258.
43. Grand FH, Hidalgo-Curtis CE, Ernst T, Zoi K, Zoi C, McGuire C, Kreil S, Jones A, Score J, Metzgeroth G, Oscier D, Hall A, Brandts C, Serve H, Reiter A, Chase AJ, Cross NCP. Frequent CBL mutations associated with 11q acquired uniparental disomy in myeloproliferative neoplasms. Blood. 2009;113:6182–92. doi:10.1182/blood-2008-12-194548.
44. Fend F, Horn T, Koch I, Vela T, Orazi A. Atypical chronic myeloid leukemia as defined in the WHO classification is a JAK2 V617F negative neoplasm. Leuk Res. 2008;32:1931–5. doi:10.1016/j.leukres.2008.04.024.

45. Grand FH, Iqbal S, Zhang L, Russell NH, Chase A, Cross NCP. A constitutively active SPTBN1-FLT3 fusion in atypical chronic myeloid leukemia is sensitive to tyrosine kinase inhibitors and immunotherapy. Exp Hematol. 2007;35:1723–7. doi:10.1016/j.exphem.2007.07.002.
46. Nangalia J, Massie CE, Baxter EJ, Nice FL, Gundem G, Wedge DC, Avezov E, Li J, Kollmann K, Kent DG, Aziz A, Godfrey AL, Hinton J, Martincorena I, Van Loo P, Jones AV, Guglielmelli P, Tarpey P, Harding HP, Fitzpatrick JD, Goudie CT, Ortmann CA, Loughran SJ, Raine K, Jones DR, Butler AP, Teague JW, O'Meara S, McLaren S, Bianchi M, Silber Y, Dimitropoulou D, Bloxham D, Mudie L, Maddison M, Robinson B, Keohane C, Maclean C, Hill K, Orchard K, Tauro S, M-Q D, Greaves M, Bowen D, Huntly BJP, Harrison CN, Cross NCP, Ron D, Vannucchi AM, Papaemmanuil E, Campbell PJ, Green AR. Somatic *CALR* mutations in Myeloproliferative neoplasms with Nonmutated *JAK2*. N Engl J Med. 2013;369:2391–405. doi:10.1056/NEJMoa1312542.
47. Ernst T, Chase AJ, Score J, Hidalgo-Curtis CE, Bryant C, Jones AV, Waghorn K, Zoi K, Ross FM, Reiter A, Hochhaus A, Drexler HG, Duncombe A, Cervantes F, Oscier D, Boultwood J, Grand FH, Cross NCP. Inactivating mutations of the histone methyltransferase gene EZH2 in myeloid disorders. Nat Genet. 2010;42:722–6. doi:10.1038/ng.621.
48. Jabbour E, Kantarjian H, Cortes J, Thomas D, Garcia-Manero G, Ferrajoli A, Faderl S, Richie MA, Beran M, Giles F, Verstovsek S. PEG-IFN-α-2b therapy in BCR-ABL–negative myeloproliferative disorders: final result of a phase 2 study. Cancer. 2007;110:2012–8. doi:10.1002/cncr.23018.
49. Koldehoff M, Beelen DW, Trenschel R, Steckel NK, Peceny R, Ditschkowski M, Ottinger H, Elmaagacli AH. Outcome of hematopoietic stem cell transplantation in patients with atypical chronic myeloid leukemia. Bone Marrow Transplant. 2004;34:1047–50. doi:10.1038/sj.bmt.1704686.
50. Lim S-N, Lee J-H, Lee J-H, Kim D-Y, Kim SD, Kang Y-A, Lee Y-S, Lee K-H. Allogeneic hematopoietic cell transplantation in adult patients with myelodysplastic/myeloproliferative neoplasms. Blood Res. 2013;48:178. doi:10.5045/br.2013.48.3.178.
51. Mittal P, Saliba RM, Giralt SA, Shahjahan M, Cohen AI, Karandish S, Onida F, Beran M, Champlin RE, de Lima M. Allogeneic transplantation: a therapeutic option for myelofibrosis, chronic myelomonocytic leukemia and Philadelphia-negative/BCR-ABL-negative chronic myelogenous leukemia. Bone Marrow Transplant. 2004;33:1005–9. doi:10.1038/sj.bmt.1704472.
52. Khanna V, Pierce ST, Dao K-HT, Tognon CE, Hunt DE, Junio B, Tyner JW, Druker BJ. Durable disease control with MEK inhibition in a patient with NRAS-mutated atypical chronic myeloid leukemia. Cureus. 2015;7:e414. doi:10.7759/cureus.414.
53. Dao K-HT, Solti MB, Maxson JE, Winton EF, Press RD, Druker BJ, Tyner JW. Significant clinical response to JAK1/2 inhibition in a patient with CSF3R-T618I-positive atypical chronic myeloid leukemia. Leuk Res Rep. 2014;3:67–9. doi:10.1016/j.lrr.2014.07.002.
54. Fleischman AG, Maxson JE, Luty SB, Agarwal A, Royer LR, Abel ML, MacManiman JD, Loriaux MM, Druker BJ, Tyner JW. The CSF3R T618I mutation causes a lethal neutrophilic neoplasia in mice that is responsive to therapeutic JAK inhibition. Blood. 2013;122:3628–31. doi:10.1182/blood-2013-06-509976.
55. Ammatuna E, Eefting M, van Lom K, Kavelaars FF, Valk PJM, Touw IP. Atypical chronic myeloid leukemia with concomitant CSF3R T618I and SETBP1 mutations unresponsive to the JAK inhibitor ruxolitinib. Ann Hematol. 2015;94:879–80. doi:10.1007/s00277-014-2272-0.
56. Langabeer SE, McCarron SL, Haslam K, O'Donovan MT, Conneally E. The CSF3R T618I mutation as a disease-specific marker of atypical CML post allo-SCT. Bone Marrow Transplant. 2014;49:843–4. doi:10.1038/bmt.2014.35.
57. Cheson BD, Greenberg PL, Bennett JM, Lowenberg B, Wijermans PW, Nimer SD, Pinto A, Beran M, de Witte TM, Stone RM, Mittelman M, Sanz GF, Gore SD, Schiffer CA, Kantarjian H. Clinical application and proposal for modification of the international Working group (IWG) response criteria in myelodysplasia. Blood. 2006;108:419–25. doi:10.1182/blood-2005-10-4149.
58. Tefferi A, Cervantes F, Mesa R, Passamonti F, Verstovsek S, Vannucchi AM, Gotlib J, Dupriez B, Pardanani A, Harrison C, Hoffman R, Gisslinger H, Kröger N, Thiele J, Barbui T, Barosi

G. Revised response criteria for myelofibrosis: international Working group-Myeloproliferative neoplasms research and treatment (IWG-MRT) and European LeukemiaNet (ELN) consensus report. Blood. 2013;122:1395–8. doi:10.1182/blood-2013-03-488098.

59. Savona MR, Malcovati L, Komrokji R, Tiu RV, Mughal TI, Orazi A, Kiladjian J-J, Padron E, Solary E, Tibes R, Itzykson R, Cazzola M, Mesa R, Maciejewski J, Fenaux P, Garcia-Manero G, Gerds A, Sanz G, Niemeyer CM, Cervantes F, Germing U, Cross NCP, List AF, on behalf of the MDS/MPN International Working Group. An international consortium proposal of uniform response criteria for myelodysplastic/myeloproliferative neoplasms (MDS/MPN) in adults. Blood. 2015;125:1857–1865. doi: 10.1182/blood-2014-10-607341.

# Chapter 12
# Chronic Myelomonocytic Leukemia: Clinical and Pathologic Features

**Michael Gentry and Eric D. Hsi**

## Introduction

Chronic myelomonocytic leukemia (CMML) is a myeloid neoplasm characterized by a persistent absolute monocytosis, often with a background of dysplastic morphologic features. The diagnosis encompasses a heterogeneous group of cases with considerable variability in morphologic dysplasia, cytopenias, leukocytosis, and the presence or absence of organomegaly. As such, the diagnostic category has features of a myelodysplastic syndrome as well as a myeloproliferative neoplasm. It was originally categorized as a form of myelodysplastic syndrome (MDS) in the early French-American-British (FAB) classification [1]. But in later editions of the World Health Organization (WHO) classification of tumours of haematopoietic and lymphoid tissues, it was placed within the newly created category of "Myelodysplastic/Myeloproliferative Neoplasms" [2].

## Clinical

The diagnosis of CMML requires a persistent peripheral blood (PB) monocytosis ($\geq 1 \times 10^9$ cells /L and $\geq 10\%$ of the white blood cell differential count as defined in the 2016 WHO classification of hematopoietic neoplasms) with <20% blasts in the peripheral blood and bone marrow (BM) and the absence of a *BCR-ABL1* rearrangement [3]. There should be morphologic dysplasia; however, if dysplasia is minimal

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**Table 12.1** Diagnostic criteria for CMML [2, 3]

| |
|---|
| Persistent peripheral blood monocytosis ≥1 × $10^9$/L & ≥10% of WBC |
| Absence of *BCR-ABL* rearrangement |
| No rearrangement of *PDGFRA, PDGFRB,* or *FGFR1* and no *PCM1-JAK2* fusion[a] |
| <20% blasts in peripheral blood and bone marrow[b] |
| Dysplasia in one or more myeloid lineages; |
| if dysplasia is minimal/absent then need: |
| Acquired clonal cytogenetic or molecular genetic present in hematopoietic cells or |
| Monocytosis has persisted ≥3 months and all other causes of monocytosis are excluded |
| CMML-0: <2% blasts in PB and <5% blasts in BM[c]; |
| CMML-1: 2–4% blasts in PB and/or 5–9% blasts in the BM[c]; |
| CMML-2: 5–19% blasts in PB, 10–19% blasts in the BM, and/or presence of any Auer rods[c] |

[a]Should be excluded in cases with eosinophilia
[b]Blasts include myeloblasts, monoblasts, and promonocytes
[c]The PB or BM blast count that results in the highest category should be used

or absent, there should be an acquired clonal cytogenetic or molecular genetic abnormality or monocytosis lasting ≥3 months with exclusion of other causes of monocytosis [2]. Additionally, in cases with eosinophilia, rearrangements involving *PDGFRA, PDGFRB*, and *FGFR1*, as well as the *PCM1-JAK2* fusion, should be excluded [3]. If eosinophils are >1.5 × $10^9$ cells/L and there is no rearrangement with the aforementioned genes, the diagnosis is CMML with eosinophilia (see Table 12.1).

There is some evidence to support dividing CMML into "dysplastic" (<13 × $10^9$ WBC/L) and "proliferative" (≥13 × $10^9$ WBC/L) categories based on distinctive molecular and clinical features of each subset [4–10]. In addition, CMML is categorized by the percentage of blasts[1] present in the peripheral blood and bone marrow as CMML-0: <2% blasts in PB and <5% blasts in BM; CMML-1: 2–4% blasts in

[1] The blast count in either the PB or BM that results in the highest CMML category should be used.

PB and/or 5–9% blasts in the BM; and CMML-2: 5–19% blasts in PB, 10–19% blasts in the BM, and/or presence of any Auer rods [3].

In two large epidemiological studies in Europe and the United States, the incidence of CMML was 3–4.1/1,000,000 person-years [11, 12]. The median age at diagnosis was 76 years with a male predominance [12]. The overall survival at 5 years was 18% [11]. Patients have a spectrum of MDS to MPN-like presentations. The majority have elevated WBC counts, although some have normal or decreased counts. Symptoms include fatigue, weight loss, fever, night sweats, infections, and bleeding. Splenomegaly or hepatomegaly may occur, especially with the myeloproliferative subgroup. Typically, patients present with <5% circulating blasts and <10% BM blasts, corresponding to CMML-0/1 [2].

## Morphology and Immunophenotype

In the blood (see Fig. 12.1), a monocytosis of $\geq 1 \times 10^9$cells/L and ≥10% of total WBCs is a requirement, in distinction from chronic myeloid leukemia, *BCR-ABL1* positive, which may have an absolute monocytosis, but it is typically <10% of all WBCs [2, 3]. The monocytes of CMML can have abnormal morphology with atypical granulation and nuclear lobation, or immature chromatin that is somewhat denser than that of promonocytes or monoblasts; overall however, the monocytes are usually mature and morphologically unremarkable [2]. Monoblasts are large with abundant gray-to-blue cytoplasm, possible pseudopod formation, and round nuclei with delicate chromatin and prominent nucleoli. Promonocytes also have abundant gray or blue cytoplasm and nuclei with finely reticulated chromatin, but the nuclei have delicate folds with or without a small nucleolus (see Fig. 12.2) [2]. Cytopenias are often present and there may be neutrophilia [2]. There is usually dysgranulopoiesis, which may manifest as nuclear hypolobation, abnormal nuclear lobation, or hypogranular granulocytes [2].

Of note, cases of MPN can be associated with monocytosis or can develop monocytosis during the course of the disease. In these rare situations, a previously documented history of MPN excludes CMML. Additionally, the presence of MPN features in the bone marrow and/or of MPN associated mutations (*JAK2*, *CALR* or *MPL*) tend to support MPN with monocytosis rather than CMML.

The bone marrow (see Fig. 12.1) is usually hypercellular, but it can be normocellular or hypocellular [2]. A granulocytic proliferation is often the most prominent finding and may obscure the monocytic proliferation [2]. Most cases have dysgranulopoiesis and dysmegakaryopoiesis, and many have dyserythropoiesis [2]. Reticulin fibrosis occurs in up to 30% of cases and 20% of cases have nodules of clonally related, neoplastic plasmacytoid dendritic cells [2]. In cases with splenomegaly, the red pulp is typically infiltrated by leukemic cells [2].

Phenotypically, the leukemic cells typically express the myeloid associated antigens CD33 and CD13 [2]. Monocytic antigens such as CD14, CD68, and CD64 are variably expressed [2]. Oftentimes, there is an aberrant immunophenotype on

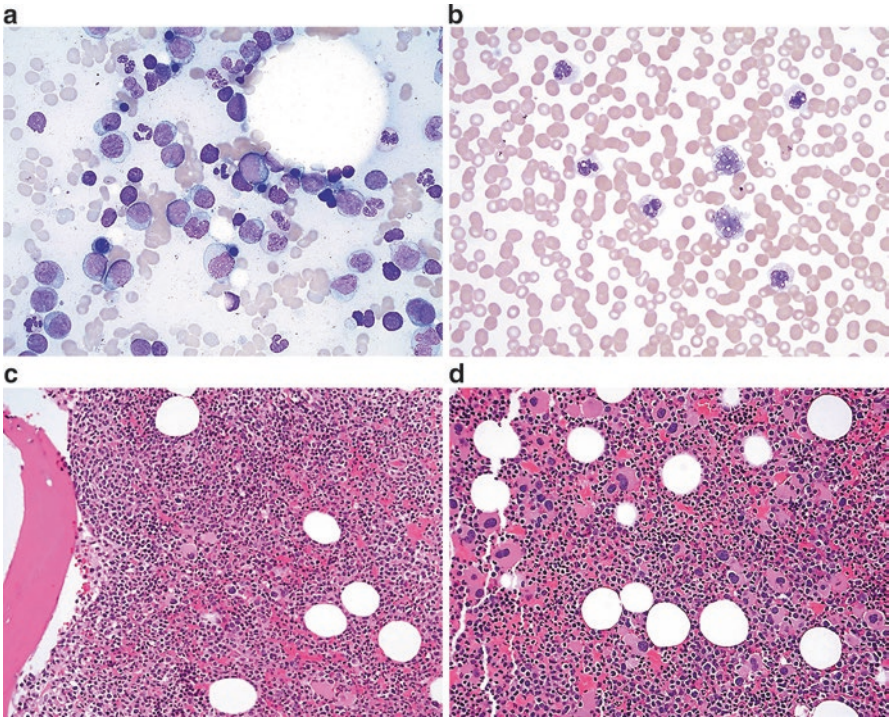


**Fig. 12.1** (**a**) Bone marrow aspirate of CMML-2 (Wright stain, 500× original magnification). (**b**) Peripheral blood of CMML (Wright stain, 500× original magnification). (**c**) Bone marrow core biopsy (hematoxylin and eosin, 200× original magnification) with (**d**) numerous dysplastic megakaryocytes (hematoxylin and eosin, 400× original magnification) (Courtesy of Dr. H. Joyce Rogers, Pathology and Laboratory Medicine Institute, Cleveland Clinic, Cleveland, OH)

monocytes with ≥2 immunophenotypic aberrancies such as expression of CD56 or CD2, decreased expression of CD14 (possibly indicative of immaturity), and/or decreased expression of HLA-DR, CD13, CD15, CD64, and CD36 [2]. Monoblasts and promonocytes are typically negative for CD34. The monocytes are positive for lysozyme, nonspecific esterase, and are negative for naphthol-ASD-chloroacetate esterase [2].

## Cytogenetics and Molecular

Approximately 30% of CMML cases have cytogenetic abnormalities [13, 14]. Among the most common findings are +8, −Y, del(20q), +21, der(3q), and chromosome 7 abnormalities including −7 and del(7q) [14, 15]. Karyotypes having multiple abnormalities are also common [13–15]. Isolated abnormalities of chromosome 5 (−5/del(5q)) are relatively rare [13–15]. Single nucleotide polymorphism (SNP)

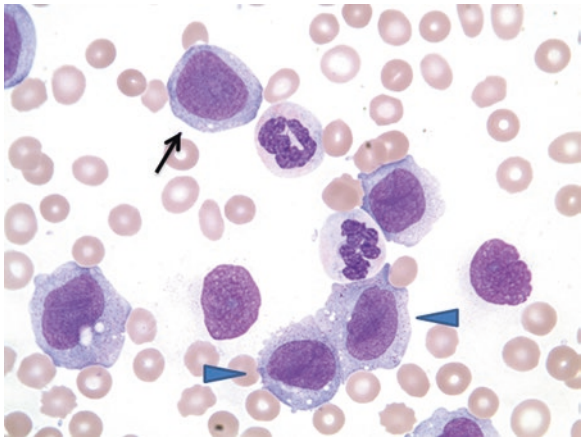

**Fig. 12.2** Peripheral blood of CMML highlighting a monoblast (*arrow*) and promonocytes (*arrowhead*) (Wright stain, 1000× original magnification)

arrays have shown increased numbers of chromosomal alterations than appreciated by karyotype analysis alone including frequent copy neutral loss of heterozygosity (LOH) [16, 17].

Over 90% of CMML cases have identifiable gene mutations [18, 19] (see Table 12.2). Molecular genetic or cytogenetic abnormalities can be used as evidence of clonality in cases without significant morphologic dysplasia, especially when involving gene mutations commonly associated with CMML. Many of these genetic mutations can be broadly divided into 3 pathways: epigenetic regulation/histone modification, spliceosome machinery, and cell signaling/transcription factors.

It is important to bear in mind that many of the mutations found in CMML (such as *TET2, ASXL1, SRSF2, CBL*) can also be found in isolation in hematologically normal appearing patients or patients with cytopenia(s) who do not otherwise meet criteria for a myelodysplastic syndrome (MDS) or CMML [20, 21]. Thus, the presence of one of these mutations should be carefully considered in the context of the duration of the monocytosis, exclusion of other reactive causes of monocytosis, and of the presence of other clinical data such as cytopenias or splenomegaly that might support CMML.

In CMML patients, the most commonly identified mutations in genes encoding proteins involved in epigenetic regulation/histone modification involve *TET2, DNMT3A, IDH2, ASXL1, EZH2,* and *UTX* (see Table 12.2) [19, 22, 23]. Of these, *TET2* and *ASXL1* are the most frequent and most important. *TET2* catalyzes the hydroxylation of methylated DNA and as a result, somatic mutations in *TET2* are believed to lead to epigenetic dysregulation [24] . *TET2* mutations are found in 46–58% of CMML cases [19, 22] and *TET2* deletions have been detected in 7% CMML cases, with a higher incidence of cryptic deletions noted than in acute myeloid leukemia (AML) or MDS [25]. Mutations of *IDH1, IDH2* and *TET2* tend to be mutually exclusive since they are functionally redundant [22, 26] . Mutated *IDH1/IDH2* results in abnormal production of the metabolite 2-hydroxyglutarate(2-HG) [24], which inhibits multiple enzymes including *TET2,* leading to hypermethylation [26]. *ASXL1* is thought to affect histone modification through effects on the

**Table 12.2** Mutational profile of CMML [19, 22]

| Gene | No. of tested Samples | Number mutated | % range | overall % |
|---|---|---|---|---|
| *ASXL1* | 487 | 207 | 40–46.9 | 42.5 |
| *TET2* | 437 | 231 | 45.7–58 | 52.9 |
| *SRSF2* | 395 | 194 | 46–53.1 | 49.1 |
| *RUNX1* | 438 | 64 | 14.3–15 | 14.6 |
| *NRAS* | 438 | 50 | 11–12 | 11.4 |
| *CBL* | 439 | 52 | 10–14.3 | 11.8 |
| *JAK2* | 438 | 28 | 4–8 | 6.4 |
| *KRAS* | 263 | 20 | 8 | 7.6 |
| *ZRSF2* | 364 | 24 | 5.1–8 | 6.6 |
| *IDH2* | 404 | 21 | 4.5–6 | 5.2 |
| *SF3B1* | 395 | 23 | 5.7–6 | 5.8 |
| *U2AF1* | 395 | 25 | 5–8 | 6.3 |
| *EZH2* | 348 | 10 | 1.1–5 | 2.9 |
| *FLT3* | 439 | 9 | 0.57–3 | 2.1 |
| *DNMT3A* | 402 | 14 | 2–5.1 | 3.5 |
| *CEBPA* | 175 | 11 | 6.3 | 6.3 |
| *SETBP1* | 370 | 45 | 6.2–18.9 | 12.2 |
| *PTPN11* | 175 | 8 | 4.5 | 4.5 |
| *SH2B3* | 175 | 8 | 4.5 | 4.5 |
| *TP53* | 377 | 11 | 1–5.1 | 2.9 |
| *BCOR*[a] | 54 | 4 | 7.4 | 7.4 |
| *STAG2*[a] | 88 | 9 | 10.2 | 10.2 |
| *IDH1* | 404 | 4 | <1–1.7 | 1 |

These data taken from separate studies [40, 42]

polycomb group repressive complex proteins (PRC1/2) [27]. The most common mutations associated with *ASXL1* are c.1934dupG;p.Gly646TrpfsX12 and 1900_1922_ del [28]. *ASXL1* mutations are associated with a higher WBC count, lower hemoglobin, extramedullary disease and an abnormal karyotype [15, 22]. *EZH2* is a component of the PRC2 complex [29] and *UTX* is a lysine specific demethylase with effects on histone H3K27 [23].

The most commonly identified spliceosome mutations involve the following genes: *SF3B1, SRSF2, U2AF1*, and *ZRSR2* [19, 22]. Mutations within this category affect the machinery involved in pre-mRNA splicing [30, 31] and tend to be exclusive of each other in cases of CMML [22, 30, 32]. Of these genes, mutations of *SRSF2* are by and far the most common. Mutations of *SRSF2* tend to result in alterations at the 95th amino acid residue, normally occupied by proline and are associated with a normal karyotype [15, 30] . Mutations of *SF3B1* have an association with der(3q) and, as with cases of MDS, tend to be associated with ring sideroblasts [15, 30]. Recurrent *SF3B1* mutations include K700E, H662Q, and K666N [30, 32]. *U2AF1* encodes a small nuclear RNA auxiliary factor. Mutations in this gene (most commonly S34F, Q157, [30] are associated with a normal karyotype, but can also

be seen with a monosomal karyotype (defined as having loss of two chromosomes or loss of one chromosome plus another structural alteration) [15].

There are several recurring mutations in genes involved in signaling/tyrosine kinase pathways including *JAK2*, *RAS* (*KRAS* + *NRAS*), *CBL*, *PTPN11*, and *BRAF* [22, 33–35] . The *RAS* gene family is composed of multiple isoforms, including *KRAS* and *NRAS*, which have GTPase activity and are involved in cell signaling pathways [36]. *BRAF* is a kinase intimately involved with *RAS* signaling and is an important activator of the *MEK/ERK* pathway [37]. *JAK2* is a tyrosine kinase involved with cell signaling and proliferation via the *STAT* pathway [38]. *JAK2* mutated CMML tends to share some morphologic features with JAK2+ myeloproliferative neoplasms such as mild/moderate reticulin fibrosis, erythroid and megakaryocytic hyperplasia, occasional megakaryocytic clustering and atypia, and dilated sinusoids [39]. Overall, these morphologic features appear to be less developed than in a pure MPN. Additional factors that would lend support to a diagnosis of JAK2+ CMML would include a lack of a history of MPN or lack of cell counts consistent with an MPN, and finding morphologic features of dysplasia. *CBL* regulates receptor tyrosine kinase activity by ubiquitination [34]. It is associated with *TET2* mutations and monosomy 7 and tends to associate with wild type *JAK2* and *KRAS/NRAS* [34].

Other reported common mutations found in CMML are of *RUNX1, SETBP1, STAG2*, and *BCOR* [19, 22, 33, 40–43]. The cohesin complex is a multimer composed of four subunits, including *STAG2*, thought to be involved in cohesion of sister chromatids during cell division, postreplicative DNA repair, and regulation of gene expression [40]. Among myeloid neoplasms, *STAG2* mutations are often found with other mutations such as *TET2, ASXL1*, and *EZH2* [40]. *RUNX1* encodes the alpha subunit of the core-binding factor and is essential for hematopoiesis/differentiation and helps regulate expression of GCSF and MPO [44]. *SETBP1* is a binding partner for *SET* protein, a protein which has downstream effects on transcription and nucleosome assembly [45, 46]. *SETBP1* mutations have an association with mutations of *ASXL1* or spliceosome machinery and often occur with a normal karyotype [47].

From a molecular perspective, the myelodysplastic type of CMML(MD-CMML) is associated with mutations of spliceosome proteins such as SRSF2, SF3B1, ZRSR2 and U2AF35 and epigenetic regulators of DNA methylation such as TET2 and IDH1/2 [10]. The myeloproliferative type of CMML (MP-CMML) is associated with mutations of ASXL1 and signal pathway mutations such as CBL, FLT3, JAK2 and KRAS/NRAS, in addition to the mutations involving spliceosome machinery and regulators of DNA methylation [8, 10, 39]. Even in cases originally diagnosed as MD-CMML, the identification of signal pathway mutations (RAS) at the time of diagnosis or during the disease course has been associated with progression to MP-CMML [8]. Investigation of the mutational hierarchy of CMML [18] indicates that mutations affecting epigenetic regulators (such as TET2 and ASXL1) and spliceosome mutations are often associated with early neoplastic clones whereas signal pathway mutations tend to be later mutational hits.

## Prognosis and Therapy

Although there have been several large studies that have examined various clinical and pathologic markers for prognostic utility regarding CMML, there is no universally accepted prognostic model for CMML. Factors that have been found to have some prognostic significance at one time or another in multivariate analysis include increased age, high WBC count, increased bone marrow blasts, cytogenetic risk stratification, circulating immature myeloid cells, thrombocytopenia, anemia, and the presence of frameshift or nonsense *ASXL1* mutations [6, 22, 28, 47–50].

Three prognostic models with external validation include the CPSS (CMML-specific prognostic scoring system), the GFM (Group Francophone des Myélodysplasies), and the Mayo Model (see Table 12.3) [22, 28, 49]. The CPSS model identified 4 factors for stratifying overall survival (OS) and (acute) leukemia free survival (LFS) risk: French-American-British (FAB) classification, WHO classification, the CMML-specific cytogenetic risk stratification, and blood transfusion dependency. The CMML-specific cytogenetic risk stratification considered a normal karyotype and –Y as low risk; complex cytogenetics (≥3 chromosomal abnormalities), chromosome 7 abnormalities, and trisomy 8 as poor risk; and other chromosomal abnormalities as intermediate risk [14]. All positive risk factors were assigned a value of 1, except for high risk cytogenetics, which is assigned a value of 2. A total score was obtained from the sum of the individual scores and is placed into 1 of 4 categories: Low risk, Intermediate-1, Intermediate-2, and high risk. The GFM model used five risk factors for OS and LFS: age > 65 years, WBC $>15 \times 10^9$/L, anemia, platelets $<100 \times 10^9$/L, and presence of a nonsense or frameshift *ASXL1* mutation. Positive risk factors were assigned a value of 1, 2, or 3 and summed for a total score that was placed in one of three categories: low, intermediate, and high risk. The Mayo Model identified four significant prognostic variables for OS and LFS: absolute monocyte count ($>10 \times 10^9$/L), circulating immature mononuclear cells (defined as any of myeloblasts, promyelocytes, myelocytes, metamyelocytes), anemia (<10 g/dL), and thrombocytopenia ($<100 \times 10^9$/L). Three prognostic categories were created from this: low risk (0 risk factors), intermediate risk (1 risk factor), and high risk (≥2 risk factors).

Although mutations of various genes (*RUNX1, TET2, NRAS, CBL, SETBP1, SRSF2*) have been associated with differences in overall survival (OS) or leukemia-free survival (LFS), the data are inconsistent and further validations are necessary before drawing conclusions [19, 22, 30, 33, 34, 41, 43, 47, 51, 52].

Cytogenetic risk stratifications have also yielded variable results with regard to prognosis, but some common themes have emerged. These common themes include complex karyotypes are associated with a worse prognosis and a normal karyotype or –Y is associated with a better OS [14, 15]. When applied to multiple prognostic models in multivariate analysis, the Mayo-French cytogenetic model retained independent prognostic significance [15]. This model effectively predicted leukemic transformation and stratifies cases into one of three risk groups—high risk: complex karyotype or monosomal (defined as having at least one autosomal monosomy and

**Table 12.3** Comparison of three CMML prognostic models with external validation [22, 28, 49]

| Prognostic model | Risk factors | Prognostic score | Low risk category survival (months) | Intermediate risk category survival (months) | | High risk category survival (months) | Risk of acute leukemic transformation |
|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | | |
| CPSS | • CMML FAB type (WBC <13 × $10^9$=0 pt; >13 × $10^9$ = 1 pt)<br>• CMML WHO type (CMML-1= 0 pt, CMML-2=1 pt)<br>• CMML-specific cytogenetics (low risk=0, intermediated = 1, high=2)<br>• RBC transfusion dependence (1 pt) | Low risk: 0 pt<br>Intermediate risk 1: 1 pt<br>Intermediate risk 2: 2–3 pts<br>High risk: 4–5 pts | 72 | 31 | 13 | 5 | % AML transformed at 5 years: 13, 29, 60 and 73%, respectively |
| GFM | • Age >65 years (2 pt)<br>• WBC >15 × $10^9$/L (3 pt)<br>• Anemia (2 pt) females Hb<10 g/dL males Hb<11 g/dL<br>• Platelets <100 × $10^9$/L (2 pt)<br>• ASXL1 mutation (nonsense or frameshift) (2 pt) | Low risk: 0–4 pts<br>Intermediate risk: 5–7 pts<br>High risk: 8–12 pts | Not reached | 385 | | 14.4 | AML free survival: 56.0, 274, 9.2 months, respectively |

(continued)

**Table 12.3** (continued)

| Prognostic model | Risk factors | Prognostic score | Low risk category survival (months) | Intermediate risk category survival (months) | | High risk category survival (months) | Risk of acute leukemic transformation |
|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | | |
| Mayo Model | • Absolute monocyte count >10 × $10^9$/L<br>• Circulating immature mononuclear cells (myeloblasts, promyelocytes, myelocytes, metamyelocytes)<br>• Hemoglobin <10 g/dL<br>• Platelet count <100 × $10^9$/L | Low risk: 0 risk factor<br>Intermediate risk: 1 risk factor<br>High risk: ≥2 risk factors | 32 | 18.5 | | 10 | Relative risk for AML transformation: 4.9 for high risk; 2.6 for intermediate risk |

one more structural abnormality or having at least two autosomal monosomies) karyotype; low risk: normal karyotype, −Y, order(3q); or intermediate risk: all others. Another study [53] examining the prognostic impact of cytogenetic abnormalities acquired during the course of CMML disease showed they were associated with an overall decrease in LFS by multivariate analysis. Acquisition of a complex karyotype was associated with leukemia progression, but del(20q) was associated with stable disease [53].

Therapy for CMML has largely been drawn from treatments for myelodysplastic syndrome and myeloproliferative neoplasms. Supportive care including erythropoietic stimulating agents and transfusions are typically utilized for significant anemia [54]. Hypomethylating agents, including 5-azacitidine and decitabine, have been approved by the United States Food and Drug Administration for treatment of CMML. In several studies, these two drugs collectively have shown overall response rates ranging from 25–69% and median OS from 12–37 months [55–61]. Proliferative phase CMML is typically treated with hydroxyurea. In a randomized control trial comparing hydroxyurea with etoposide, hydroxyurea was associated with better treatment response (60% versus 36%) and OS (20 months versus 9 months) than etoposide [62]. Even so, treatment outcomes with hypomethylating agents and hydroxyurea are still relatively poor and there is a strong need for more effective therapies. Allogeneic hematopoietic stem cell transplant (HSCT) is the only known cure; however, due to the advanced age and/or comorbidities often associated with CMML patients, this option is often not available [63].

Although *TET2* mutations have been associated with response to hypomethylating agents in MDS patients, there are no consistent molecular mutation predictors of response to hypomethylating agents in CMML patients [58, 64–66], and analysis of predictive models of methylation patterns have so far yielded mixed results [66]. Numerous investigational therapies such as the *JAK2* inhibitor ruxolitinib and *RAS* pathway inhibitors (farnesyltransferase inhibitors) have been evaluated in patients with CMML with variable but limited responses [67–69].

## Conclusion

CMML is a myeloid neoplasm with overlapping features of a myelodysplastic and myeloproliferative neoplasm. The diagnosis requires a combination of a persistent absolute monocytosis ($>1 \times 10^9$ cells/L) and either a background of morphologic dysplasia, clonal cytogenetic/molecular genetic abnormalities or persistence of monocytosis for ≥3 months with exclusion of other causes of monocytosis. There are no disease-defining cytogenetic or molecular genetic abnormalities. However, the presence of a cytogenetic or molecular abnormality may help to make a diagnosis of CMML in the appropriate clinical context. The independent prognostic and therapeutic value of molecular mutations is currently limited. Yet, as our knowledge of the mutational landscape is expanded and refined, and newer therapies become

available, our understanding of the molecular basis of CMML may yield additional insight into the treatment potential of CMML.

## References


1. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, et al. Proposals for the classification of the myelodysplastic syndromes. Br J Haematol. 1982;51:189–99.
2. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW. WHO classification of tumours of haematopoietic and lymphoid tissue. Lyon: IARC Press; 2008. p. 439.
3. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127:2391–405.
4. Schuler E, Schroeder M, Neukirchen J, Strupp C, Xicoy B, Kündgen A, et al. Refined medullary blast and white blood cell count based classification of chronic myelomonocytic leukemias. Leuk Res. 2014;38:1413–9.
5. Nösslinger T, Reisner R, Grüner H, Tüchler H, Nowotny H, Pittermann E, et al. Dysplastic versus proliferative CMML--a retrospective analysis of 91 patients from a single institution. Leuk Res. 2001;25:741–7.
6. Onida F, Kantarjian HM, Smith TL, Ball G, Keating MJ, Estey EH, et al. Prognostic factors and scoring systems in chronic myelomonocytic leukemia : a retrospective analysis of 213 patients. Blood. 2010;99:840–9.
7. Voglová J, Chrobák L, Neuwirtová R, Malasková V, Straka L. Myelodysplastic and myeloproliferative type of chronic myelomonocytic leukemia — distinct subgroups or two stages of the same disease? Leuk Res. 2001;25:493–9.
8. Ricci C, Fermo E, Corti S, Molteni M, Faricciotti A, Cortelezzi A, et al. RAS mutations contribute to evolution of chronic myelomonocytic leukemia to the proliferative variant. Clinical Cancer Res Off J Am Assoc Cancer Res. 2010;16:2246–56.
9. Gelsi-Boyer V, Cervera N, Bertucci F, Brecqueville M, Finetti P, Murati A, et al. Molecular similarity between myelodysplastic form of chronic myelomonocytic leukemia and refractory anemia with ring sideroblasts. Haematologica. 2013;98:576–83.
10. Cervera N, Itzykson R, Coppin E, Prebet T, Murati A, Legall S, et al. Gene mutations differently impact the prognosis of the myelodysplastic and myeloproliferative classes of chronic myelomonocytic leukemia. Am J Hematol. 2014;89:604–9.
11. Visser O, Trama A, Maynadié M, Stiller C, Marcos-Gragera R, De Angelis R, et al. Incidence, survival and prevalence of myeloid malignancies in Europe. Eur J cancer Oxford England 1990. 2012;48:3257–66.
12. Srour SA, Devesa SS, Morton LM, Check DP, Curtis RE, Linet MS, et al. Incidence and patient survival of myeloproliferative neoplasms and myelodysplastic/myeloproliferative neoplasms in the United States, 2001-12. Br J Haematol. 2016;174:382.
13. Tang G, Zhang L, Fu B, Hu J, Lu X, Hu S, et al. Cytogenetic risk stratification of 417 patients with chronic myelomonocytic leukemia from a single institution. Am J Hematol. 2014;89:813–8.
14. Such E, Cervera J, Costa D, Solé F, Vallespí T, Luño E, et al. Cytogenetic risk stratification in chronic myelomonocytic leukemia. Haematologica. 2011;96:375–83.
15. Wassie EA, Itzykson R, Lasho TL, Kosmider O, Finke CM, Hanson CA, et al. Molecular and prognostic correlates of cytogenetic abnormalities in chronic myelomonocytic leukemia: a Mayo Clinic-French consortium study. Am J Hematol. 2014;89:1111–5.
16. Dunbar AJ, Gondek LP, O'Keefe CL, Makishima H, Rataul MS, Szpurka H, et al. 250K single nucleotide polymorphism array karyotyping identifies acquired uniparental disomy and

homozygous mutations, including novel missense substitutions of c-Cbl, in myeloid malignancies. Cancer Res. 2008;68:10349–57.
17. Gondek LP, Tiu R, O 'keefe CL, Sekeres MA, Theil KS, Maciejewski JP. Chromosomal lesions and uniparental disomy detected by SNP arrays in MDS, MDS/MPD, and MDS-derived AML. Blood. 2008;111:1534–42.
18. Itzykson R, Kosmider O, Renneville A, Morabito M, Preudhomme C, Berthon C, et al. Clonal architecture of chronic myelomonocytic leukemias. Blood. 2013;121:2186–98.
19. Patnaik MM, Lasho TL, Vijayvargiya P, Finke CM, Hanson CA, Ketterling RP, et al. Prognostic interaction between ASXL1 and TET2 mutations in chronic myelomonocytic leukemia. Blood Cancer J. 2016;6:e385.
20. Mason CC, Khorashad JS, Tantravahi SK, Kelley TW, Zabriskie MS, Yan D, et al. Age-related mutations and chronic myelomonocytic leukemia. Leukemia. 2016;30:906–13.
21. Steensma DP, Bejar R, Jaiswal S, Lindsley RC, Sekeres MA, Hasserjian RP, et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. Blood. 2015;126:1546–58.
22. Itzykson R, Kosmider O, Renneville A, Gelsi-Boyer V, Meggendorfer M, Morabito M, et al. Prognostic score including gene mutations in chronic myelomonocytic leukemia. J Clin Oncol Off J Am Soc Clin Oncol. 2013;31:2428–36.
23. Jankowska AAM, Makishima H, Tiu RRV, Szpurka H, Huang Y, Traina F, et al. Mutational spectrum analysis of chronic myelomonocytic leukemia includes genes associated with epigenetic regulation: UTX, EZH2, and DNMT3A. Blood. 2011;118:3932–41.
24. Abdel-Wahab O, Levine RL. Mutations in epigenetic modifiers in the pathogenesis and therapy of acute myeloid leukemia. Blood. 2013;121:3563–72.
25. Bacher U, Weissmann S, Kohlmann A, Schindela S, Alpermann T, Schnittger S, et al. TET2 deletions are a recurrent but rare phenomenon in myeloid malignancies and are frequently accompanied by TET2 mutations on the remaining allele. Br J Haematol. 2012;156:67–75.
26. Figueroa ME, Abdel-Wahab O, Lu C, Ward PS, Patel J, Shih A, et al. Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. Cancer Cell. 2010;18:553–67.
27. Abdel-Wahab O, Adli M, LaFave LM, Gao J, Hricik T, Shih AH, et al. ASXL1 mutations promote myeloid transformation through loss of PRC2-mediated gene repression. Cancer Cell. 2012;22:180–93.
28. Patnaik MM, Padron E, LaBorde RR, Lasho TL, Finke CM, Hanson CA, et al. Mayo prognostic model for WHO-defined chronic myelomonocytic leukemia: ASXL1 and spliceosome component mutations and outcomes. Leukemia. 2013;27:1504–10.
29. Chung YR, Schatoff E, Abdel-Wahab O. Epigenetic alterations in hematopoietic malignancies. Int J Hematol. 2012;96:413–27.
30. Patnaik MM, Lasho TL, Finke CM, Hanson CA, Hodnefield JM, Knudson RA, et al. Spliceosome mutations involving SRSF2, SF3B1, and U2AF35 in chronic myelomonocytic leukemia: prevalence, clinical correlates, and prognostic relevance. Am J Hematol. 2013;88:201–6.
31. Yoshida K, Sanada M, Shiraishi Y, Nowak D, Nagata Y, Yamamoto R, et al. Frequent pathway mutations of splicing machinery in myelodysplasia. Nature. 2011;478:64–9.
32. Kar SA, Jankowska A, Makishima H, Visconte V, Jerez A, Sugimoto Y, et al. Spliceosomal gene mutations are frequent events in the diverse mutational spectrum of chronic myelomonocytic leukemia but largely absent in juvenile myelomonocytic leukemia. Haematologica. 2013;98:107–13.
33. Kohlmann A, Grossmann V, Klein H-U, Schindela S, Weiss T, Kazak B, et al. Next-generation sequencing technology reveals a characteristic pattern of molecular mutations in 72.8% of chronic myelomonocytic leukemia by detecting frequent alterations in TET2, CBL, RAS, and RUNX1. J Clin Oncol. 2010;28:3858–65.
34. Schnittger S, Bacher U, Alpermann T, Reiter A, Ulke M, Dicker F, Eder C, Kohlmann A, Grossmann V, Kowarsch A, Kern W, Haferlach C, Haferlach T. Use of CBL exon 8 and 9

mutations in diagnosis of myeloproliferative neoplasms and myelodysplastic/myeloproliferative disorders: an analysis of 636 cases. Haematologica. 2012;97:1890.
35. Zhang L, Singh RR, Patel KP, Stingo F, Routbort M, You MJ, et al. BRAF kinase domain mutations are present in a subset of chronic myelomonocytic leukemia with wild-type RAS. Am J Hematol. 2014;89:499–504.
36. Bos JL. Ras oncogenes in human cancer: a review. Cancer Res. 1989;49(17):4682–9.
37. Roring M, Herr R, Fiala GJ, Heilmann K, Braun S, Eisenhardt AE, et al. Distinct requirement for an intact dimer interface in wild-type, V600E and kinase-dead B-Raf signalling. EMBO J. 2012;31(11):2629–47.
38. Aaronson DS, Horvath CMA. Road map for those who don't know JAK-STAT. Science New York NY. 2002;296(5573):1653–5.
39. Pich A, Riera L, Sismondi F, Godio L, Bonino LD, Marmont F, et al. JAK2V617F activating mutation is associated with the myeloproliferative type of chronic myelomonocytic leukaemia. J Clin Pathol. 2009;62:798–801.
40. Kon A, Shih L-Y, Minamino M, Sanada M, Shiraishi Y, Nagata Y, et al. Recurrent mutations in multiple components of the cohesin complex in myeloid neoplasms. Nat Genet. 2013;45:1232–7.
41. Laborde RR, Patnaik MM, Lasho TL, Finke CM, Hanson CA, Knudson RA, et al. SETBP1 mutations in 415 patients with primary myelofibrosis or chronic myelomonocytic leukemia: independent prognostic impact in CMML. Leukemia. 2013;27:2100–2.
42. Damm F, Chesnais V, Nagata Y, Yoshida K, Scourzic L, Okuno Y, et al. BCOR and BCORL1 mutations in myelodysplastic syndromes and related disorders. Blood. 2013;122:3169–77.
43. Kuo M-C, Liang D-C, Huang C-F, Shih Y-S, J-H W, Lin T-L, et al. RUNX1 mutations are frequent in chronic myelomonocytic leukemia and mutations at the C-terminal region might predict acute myeloid leukemia transformation. Leukemia. 2009;23:1426–31.
44. Speck NA, Gilliland DG. Core-binding factors in haematopoiesis and leukaemia. Nat Rev Cancer. 2002;2:502–13.
45. Kutney SN, Hong R, Macfarlan T, Chakravarti DA. Signaling role of histone-binding proteins and INHAT subunits pp32 and set/TAF-Ibeta in integrating chromatin hypoacetylation and transcriptional repression. J Biol Chem. 2004;279:30850–5.
46. Seo SB, McNamara P, Heo S, Turner A, Lane WS, Chakravarti D. Regulation of histone acetylation and transcription by INHAT, a human cellular complex containing the set oncoprotein. Cell. 2001;104:119–30.
47. Patnaik MM, Itzykson R, Lasho TL, Kosmider O, Finke CM, Hanson CA, et al. ASXL1 and SETBP1 mutations and their prognostic contribution in chronic myelomonocytic leukemia: a two-center study of 466 patients. Leukemia. 2014;28:2206–12.
48. Kantarjian H, O'Brien S, Ravandi F, Cortes J, Shan J, Bennett JM, et al. Proposal for a new risk model in myelodysplastic syndrome that accounts for events not considered in the original international prognostic scoring system. Cancer. 2008;113:1351–61.
49. Such E, Germing U, Malcovati L, Cervera J, Kuendgen A, Della Porta MG, et al. Development and validation of a prognostic scoring system for patients with chronic myelomonocytic leukemia. Blood. 2013;121(15):3005.
50. Greenberg PL, Tuechler H, Schanz J, Sanz G, Garcia-Manero G, Solé F, et al. Revised international prognostic scoring system for myelodysplastic syndromes. Blood. 2012;120:2454–65.
51. Padron E, Garcia-Manero G, Patnaik MM, Itzykson R, Lasho T, Nazha A, et al. An international data set for CMML validates prognostic scoring systems and demonstrates a need for novel prognostication strategies. Blood cancer journal. 2015;5:e333.
52. Meggendorfer M, Roller A, Haferlach T, Eder C, Dicker F, Grossmann V, et al. SRSF2 mutations in 275 cases with chronic myelomonocytic leukemia (CMML). Blood. 2012;120:3080–8.
53. Tang G, Fu B, Hu S, Lu X, Tang Z, Li S, et al. Prognostic impact of acquisition of cytogenetic abnormalities during the course of chronic myelomonocytic leukemia. Am J Hematol. 2015;90:882–7.

54. Patnaik MM, Tefferi A. Chronic myelomonocytic leukemia: 2016 update on diagnosis, risk stratification, and management. Am J Hematol. 2016;91:631–42.
55. Ades L, Sekeres MA, Wolfromm A, Teichman ML, Tiu RV, Itzykson R, et al. Predictive factors of response and survival among chronic myelomonocytic leukemia patients treated with azacitidine. Leuk Res. 2013;37(6):609–13.
56. Fianchi L, Criscuolo M, Breccia M, Maurillo L, Salvi F, Musto P, et al. High rate of remissions in chronic myelomonocytic leukemia treated with 5-azacytidine: results of an Italian retrospective study. Leuk Lymphoma. 2013;54(3):658–61.
57. Costa R, Abdulhaq H, Haq B, Shadduck RK, Latsko J, Zenati M, et al. Activity of azacitidine in chronic myelomonocytic leukemia. Cancer. 2011;117(12):2690–6.
58. Braun T, Itzykson R, Renneville A, de Renzis B, Dreyfus F, Laribi K, et al. Molecular predictors of response to decitabine in advanced chronic myelomonocytic leukemia: a phase 2 trial. Blood. 2011;118(14):3824–31.
59. Aribi A, Borthakur G, Ravandi F, Shan J, Davisson J, Cortes J, et al. Activity of decitabine, a hypomethylating agent, in chronic myelomonocytic leukemia. Cancer. 2007;109(4):713–7.
60. Thorpe M, Montalvao A, Pierdomenico F, Moita F, Almeida A. Treatment of chronic myelomonocytic leukemia with 5-Azacitidine: a case series and literature review. Leuk Res. 2012;36(8):1071–3.
61. Wijermans PW, Rüter B, Baer MR, Slack JL, Saba HI, Lübbert M. Efficacy of decitabine in the treatment of patients with chronic myelomonocytic leukemia (CMML). Leuk Res. 2008;32:587–91.
62. Wattel E, Guerci A, Hecquet B, Economopoulos T, Copplestone A, Mahé B, et al. A randomized trial of hydroxyurea versus VP16 in adult chronic myelomonocytic leukemia. Groupe Français des Myélodysplasies and European CMML group. Blood. 1996;88:2480–7.
63. Cheng H, Kirtani VG, Gergis U. Current status of allogeneic HST for chronic myelomonocytic leukemia. Bone Marrow Transplant. 2012;47:535–41.
64. Patnaik MM, Wassie EA, Padron E, Onida F, Itzykson R, Lasho TL, et al. Chronic myelomonocytic leukemia in younger patients: molecular and cytogenetic predictors of survival and treatment outcome. Blood Cancer J. 2015;5:e280.
65. Bejar R, Lord A, Stevenson K, Bar-Natan M, Pérez-Ladaga A, Zaneveld J, et al. TET2 mutations predict response to hypomethylating agents in myelodysplastic syndrome patients. Blood. 2014;124:2705–12.
66. Meldi K, Qin T, Buchi F, Droin N, Sotzen J, Micol J-B, et al. Specific molecular signatures predict decitabine response in chronic myelomonocytic leukemia. J Clin Invest. 2015;125:1857–72.
67. Padron E, Dezern A, Andrade-Campos M, Vaddi K, Scherle P, Zhang Q, et al. A multi-institution phase 1 trial of ruxolitinib in patients with chronic myelomonocytic leukemia (CMML). Clin Cancer Res. 2016;22:3746–54.
68. Feldman EJ, Cortes J, DeAngelo DJ, Holyoake T, Simonsson B, O'Brien SG, et al. On the use of lonafarnib in myelodysplastic syndrome and chronic myelomonocytic leukemia. Leukemia. 2008;22:1707–11.
69. Fenaux P, Raza A, Mufti GJ, Aul C, Germing U, Kantarjian H, et al. A multicenter phase 2 study of the farnesyltransferase inhibitor tipifarnib in intermediate- to high-risk myelodysplastic syndrome. Blood. 2007;109:4158–63.

# Chapter 13
# Juvenile Myelomonocytic Leukemia

**Joanna Wiszniewska and Choladda V. Curry**

## Introduction

Juvenile myelomonocytic leukemia (JMML) is a rare myelodysplastic/myeloproliferative neoplasm of early childhood that results from abnormal activation of the RAS/MAPK pathway in the hematopoietic stem cells. Involvement of RAS/MAPK pathway gene mutations in the pathogenesis of JMML have been recognized over the two past decades and resulted in the inclusion of specific molecular findings in the diagnostic criteria for JMML in the recently published 2016 revisions to the WHO classification of hematopoietic malignancies [1]. The diagnostic findings, in addition to absolute monocytosis, blast percentage below 20%, splenomegaly, and absence of *BCR/ABL1* rearrangement, now also include identification of mutations in *PTPN11*, *KRAS*, *NRAS*, *NF1* or *CBL* genes. In patients who show clinical and hematologic features of JMML but lack genetic aberration in one of these genes, additional criteria must be fulfilled such as presence of monosomy 7, elevated hemoglobin F levels, or evidence of RAS/MAPK pathway activation by GM-CSF colony stimulating assay or STAT5 phosphorylation assay [1].

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### *Epidemiology*

JMML accounts for 2–3% of all childhood hematologic malignancies and has an estimated incidence of 1.2 per million per year. It is a disorder of infancy and early childhood with median age at diagnosis of 1.8 years and most cases presenting before age of 5 years. Males are affected more frequently than females with M:F ratio of 2-3:1 [2].

### *Clinical Presentation*

Children with JMML commonly present with nonspecific symptoms of pallor, fever, skin rash, bleeding, and infections that make a diagnosis challenging. Splenomegaly and lymphadenopathy are noted at diagnosis in majority of the patients. In addition, local leukemic infiltration may affect other sites and organs giving rise to a variety of site specific symptoms. Laboratory evaluations often reveal elevated levels of hemoglobin F, lactic dehydrogenase, and serum concentrations of polyclonal immunoglobulins [3].

## Morphology and Immunophenotyping

A peripheral blood cell count at diagnosis shows leukocytosis, thrombocytopenia, and often anemia. Two thirds of patients present with a WBC below $50 \times 10^9$/L; a minority of patients may present with a WBC above $100 \times 10^9$/L. A WBC $< 10 \times 10^9$/L is uncommon and it is usually seen in association with monosomy 7. The leukocytosis constitutes of neutrophilic granulocytes with left shift and monocytosis including immature and dysplastic monocytes [3] (Fig. 13.1). An absolute monocyte count of more than $1 \times 10^9$/L is a defining abnormality required for a diagnosis of JMML [1, 3]. In contrast to *BCR/ABL1* positive chronic myeloid leukemia, basophilia is not a prominent feature of JMML. The percentage of blasts in the peripheral blood typically is less than 5%. Nucleated red blood cells are also often seen.

Morphologic evaluation of the bone marrow will show normal or increased cellularity for age with granulocytic hyperplasia and typically a reduced number of megakaryocytes (Fig. 13.2). Dysplastic features are often minimal. If monosomy 7 is present, an erythroid hyperplasia, eosinophilia, and higher blast count may be observed. A monocytosis, which will be present in the bone marrow, may be less pronounced, or obvious, in comparison to the peripheral blood [3]. No specific immunophenotypic features of blasts, granulocytes or monocytes are diagnostic for JMML; however, abnormal expression of CD56 and CD16 on monocytes may be seen, as in chronic myelomonocytic leukemia (CMML), adult counterpart to JMML.

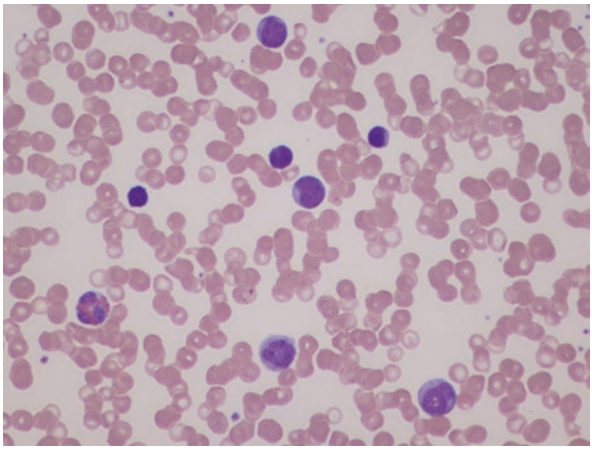

**Fig. 13.1** Juvenile myelomonocytic leukemia. Peripheral blood smear shows monocytosis with atypical monocytes

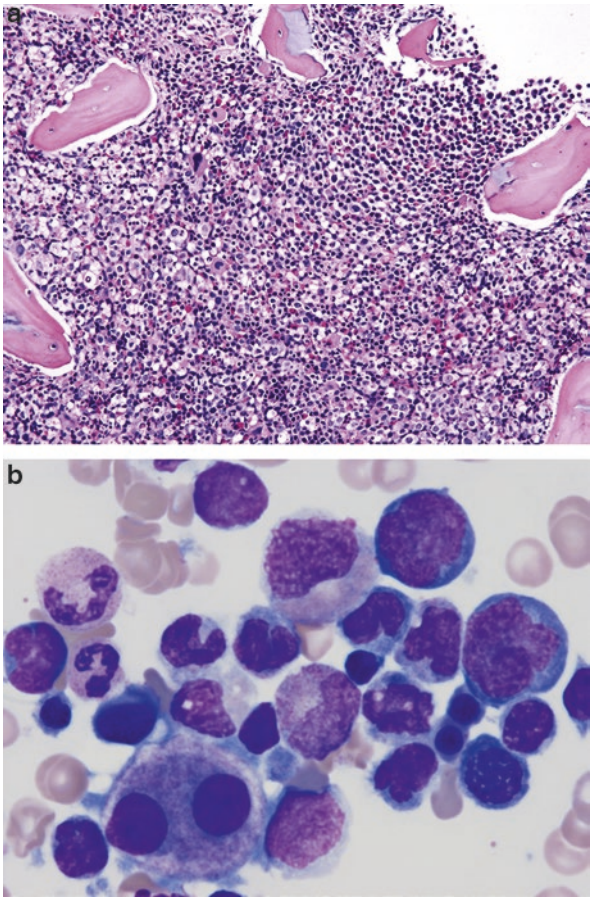


**Fig. 13.2** Juvenile myelomonocytic leukemia. (**a**) Bone marrow biopsy shows a hypercellular bone marrow with mild granulocytic hyperplasia and slightly decreased number of megakaryocytes. (**b**) Aspirate smear shows an increased monocytic component. A dysplastic megakaryocyte showing binucleation with widely separate nuclear lobes is also depicted (left lower)

## Molecular and Cytogenetic Findings

Dysregulation of the RAS/MAPK signaling pathway leads to uncontrollable proliferation of monocytic and granulocytic cell lineages and rapid progression of the disease in untreated JMML patients. The hallmark selective hypersensitivity of myeloid progenitor cells to GM-CSF in in vitro colony stimulation assays is thought to reflect excessive activation of the RAS/MAPK pathway [4]. This complex pathway is an essential signaling cascade that controls cell proliferation, differentiation, and survival. It is therefore heavily implicated in oncogenesis with approximately 30% of tumors shown to harbor mutations in one of the *RAS* genes. Signaling is activated upon stimulation by numerous cytokines, hormones, and growth factors [5]. RAS proteins are small GTPases that operate as molecular switches, cycling between active GTP-bound and inactive GDP-bound conformations, and activate downstream effectors through sequential substrate phosphorylation (Fig. 13.3). Studies of inherited cancer predisposition syndromes were pivotal in discerning involvement of specific molecular pathways in the development of leukemias and tumorigenesis in general. Mendelian autosomal dominant disorders with overlapping clinical features named comprehensively as RASopathies are caused by germline mutations in genes encoding RAS/MAPK pathway proteins. RASopathies include Noonan syndrome, Cardiofaciocutaneous syndrome, Costello syndrome, Legius syndrome, LEOPARD syndrome and CBL or SHOC2 associated Noonan-like syndromes. Patients diagnosed with these syndromes have an increased risk of

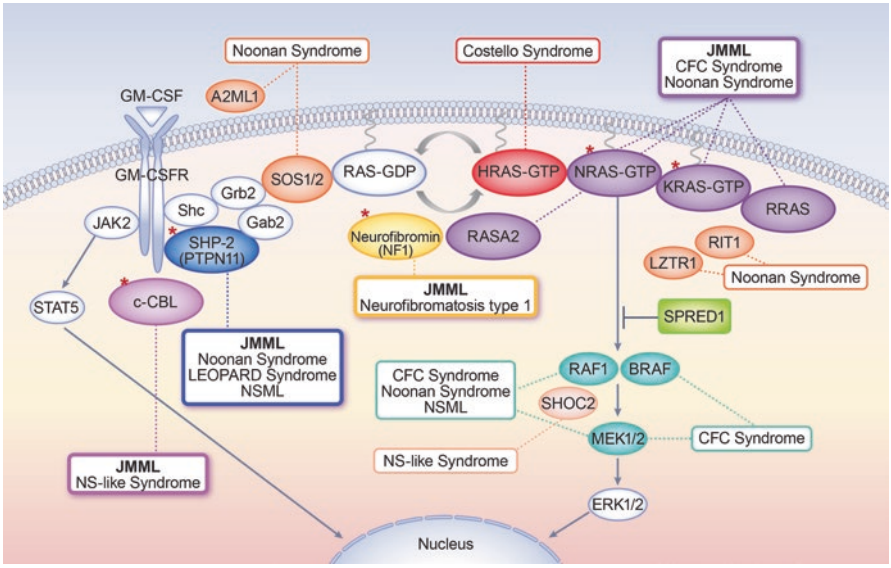


**Fig. 13.3** RAS/MAPK pathway and genetic abnormalities in juvenile myelomonocytic leukemia (JMML) and inherited RASopathies. *CFC* cardiofaciocutaneous syndrome, *NS-like* Noonan-like syndrome, *NSML* Noonan syndrome with multiple lentigines, *canonical JMML genes. Modified form Chang et al. [15] and Aoki et al. [35]

developing a variety of cancers including acute leukemias and myeloproliferative disorders. Multiple cases of JMML/transient myeloproliferative disorder have been reported in Noonan syndrome and Noonan-like syndrome with germline mutations in *PTPN11* and *CBL* respectively, although the exact risk is not known [6, 7]. Neurofibromatosis type 1 (NF1) which is caused by mutations in the negative modulator of RAS function, neurofibromin, is also well known to increase the risk of developing JMML with some studies indicating a 350-fold increase compared with patients without NF1 [3]. Emerging data from genetic profiling and whole exome sequencing of JMML patients indicate that in addition to canonical driver mutations in RAS/MAPK pathway, activating mutations affecting JAK-STAT, PI3K, mTORC2 pathways, and alterations in epigenetic modifiers play a role in the oncogenic process in JMML [8–10]. JMML is a genetically heterogeneous disease with 85–90% of patients harboring molecular alterations in five of the RAS pathway genes [3, 9, 11–14]. Table 13.1 summarizes the contribution of the most commonly involved (canonical) RAS pathway genes. Recent studies have shown that somatic mutations in *PTPN11*, *NRAS*, *KRAS*, *CBL*, and *NF1* are not mutually exclusive as it was previously thought, but can coexist in 11–17% of patients, with *PTPN11* and *NF1* aberrations co-occurring most frequently [8, 9]. In the below section, the five canonical RAS-MAPK genes involved in JMML are discussed. Other genetic alterations and chromosomal aberrations are also reviewed.

## *NF1*

Neurofibromatosis type 1 (NF1) was one the first syndromes recognized to be associated with JMML [3, 15]. NF1 is a relatively common autosomal dominant disorder with an incidence of approximately 1 in 3000 individuals, and it is characterized by the presence of cafe-au-lait spots, Lisch nodules in the eye, and fibromatous tumors of the skin. Individuals with NF1 are at increased risk of developing benign and malignant tumors derived from neural crest cells, most commonly optic gliomas, pheochromocytomas, and nerve sheet tumors. This group of patients is also at high risk (200–350 folds) of acquiring myeloid disorders and monosomy 7 syndrome. Interestingly, the risk for developing leukemias is limited only to the pediatric NF1 population [3, 16]. Retrospective analyses of NF1 cases has shown that JMML is typically diagnosed at a later age than in sporadic JMML cases (after age of 5 years) and presents with higher platelet and blast counts [3]. NF1 is caused by loss of function mutations in neurofibromin gene located on chromosome 17q11. There is a vast allelic heterogeneity (>500 pathogenic variants) with nonsense, missense, and splicing mutations, as well as large deletions and rare duplications reported in NF1 families and sporadic cases. Therefore, a comprehensive molecular diagnostic approach may be required, including sequencing techniques and other methods such as Multiplex Ligation-dependent Probe Amplification (MLPA) or chromosomal microarrays for detection of large deletions/duplications. Neurofibromin acts as a tumor suppressor by binding its GTPase-activation domain to Ras and accelerating the conversion of Ras-GTP to Ras-GDP [14]. Somatic loss of neurofibromin function,

**Table 13.1** Contribution of canonical genetic alterations to JMML

| Gene | Protein | Function | Mutation type | Germline/somatic | Percent of mutations in JMML | Associated inherited syndrome |
|---|---|---|---|---|---|---|
| *PTPN11* | SHP2 | protein tyrosine phosphatase | Gain of function | Somatic and germline | ~35% somatic<br>~10% germline | NS |
| *KRAS* | KRAS | GTPase | Gain of function | Somatic | <10% | NS, CFC |
| *NRAS* | NRAS | GTPase | Gain of function | Somatic | ~15–20% | NS |
| *NF1* | Neurofibromin 1 | negative regulator of RAS/MAPK pathway | Loss of function, LOH | Germline | ~12% | NF1 |
| *CBL* | CBL | E3 ubiquitin ligase | Loss of function, LOH | Germline and somatic | ~10–15% | NS-like |

*NS* Noonan syndrome, *CFC* cardiofaciocutaneous syndrome, *NF1* neurofibromatosis type 1, *NS-like* Noonan syndrome like, *LOH* loss of heterozygosity

predominantly through acquired uniparental isodisomy and resulting in duplication of a constitutional mutation, leads to elevated levels of Ras-GTP and constitutive activation of Ras/MAPK pathway in hematopoietic cells [15]. None of the *NF1* alleles appears to be specific to leukemia [16]. Recent genome-wide studies have shown that a high percentage (approximately 25%) of NF1 patients with JMML have a second mutation in a canonical RAS pathway gene. Additionally, these patients commonly have additional genetic alterations impairing the PCR2 complex, which is involved in epigenetic transcription regulation [8].

## *PTPN11*

The *PTPN11* gene located on chromosome 12q24.13 encodes for SHP-2 (also referred to as Tyrosine-protein phosphatase nonreceptor type 11), a widely expressed cytoplasmic phosphatase that acts as a positive modulator of RAS signal transduction pathways. SHP-2 is composed of two tandemly arranged amino-terminal SH2 domains (N-SH2 and C-SH2), a single catalytic (PTP) domain, and a C-terminal tail containing residues that undergo reversible phosphorylation. In the inactive state, the N-SH2 domain interacts with the PTP domain blocking the catalytic site. After binding to the ligand, the N-SH2 domain undergoes a conformational change that makes the catalytic site available to substrate, thereby activating the phosphatase [17]. Missense gain-of-function mutations in the *PTPN11* gene that result in locking of the SHP-2 in the open, constitutively active conformation were first described in ~50% of patients with Noonan syndrome (NS) [18]. This genetically heterogenous autosomal dominant disorder is characterized by facial dysmorphism, short stature, and heart defects. A small percentage of NS patients with *PTPN11* mutations develop JMML, an observation which led to consideration of *PTPN11* involvement in the development of hematopoietic malignancies. Indeed, further analyses of de novo JMML cases revealed the presence of somatic *PTPN11* missense mutations in ~35% of cases [11] and occasionally in acute myeloid leukemia and myelodysplastic syndromes [19]. The distribution of affected residues rarely overlaps in inherited and somatically acquired *PTPN11* mutations, with leukemia-associated variants infrequently occurring in Noonan syndrome patients. Mutations detected in sporadic JMML are typically located in exons 3, 4, and 13 (residues D61, A72, E76, G503), whereas Noonan syndrome causing mutations are more widely spread among all exons [11, 19, 20]. Functional studies comparing activation of RAS/MAPK pathway by germline mutations occurring commonly in NS patients without and with JMML, and acquired mutations occurring in JMML, showed a significant difference in strength of activation with the most potent effect exerted by the latter ones. These analyses indicate that the impact of the leukemia-associated mutations may be incompatible with life if they occur in germline, whereas milder mutations associated with NS may predispose these individuals to hematologic malignancies. There is however a mutation spectrum overlap between the Noonan syndrome patients who developed JMML and those who did not [20]. A fraction of NS patients

develop a JMML-like myeloproliferating disorder early in infancy, which may regress without treatment or follow an aggressive clinical course similar to JMML. It has been proposed that some NS-associated mutations may have milder effect on myeloid precursor proliferation and result in a transient JMML-like disorder somewhat akin to Down syndrome-related transient abnormal myelopoiesis [20, 21].

## *CBL*

C-CBL (CASITAS B-lineage lymphoma protooncogene) is a ubiquitously expressed ubiquitin-protein ligase (E3) that tags active protein-tyrosine kinase receptors for degradation and therefore functions as negative regulator of RAS signal transduction. In addition, CBL acts as an adaptor protein in tyrosine phosphorylation-dependent signaling, interacting with many molecules including Grb2, which in turn prevents binding of CBL to SOS1 [13, 22]. CBL consists of an N-terminal tyrosine kinase-binding domain, short linker region, and a zinc-binding RING-finger domain that mediates the E3 ubiquitin ligase activity. Homozygous *CBL* mutations are detected in about 10–15% of JMML cases. Missense, splicing, and small in frame deletion mutations affect predominantly the linker region or RING-finger domain (exons 8 and 9) leading to impairment of ubiquitin ligase activity. The most commonly affected residue is Y371 in the linker region of CBL [13, 15]. Functional studies of the mutated protein indicate that specific disruption of the E3 ligase activity might leave intact the adaptor functions resulting in the relative imbalance of the CBL effects on signal transduction [12, 13]. Cells from these patients exhibit GM-CSF hypersensitivity and STAT5 hyperphosphorylation equivalent to other RAS pathway mutations [13]. Similar to *NF1*, *CBL* functions as a tumor suppressor gene with the initial constitutional mutation inherited in an autosomal dominant manner or de novo germline event and a second somatic event occurring in hematopoietic stem cells. Somatic alteration typically leads to loss of heterozygosity through acquired uniparental disomy involving chromosome 11q where the *CBL* gene resides [8, 12, 13]. Patients with heterozygous germline *CBL* mutations display variable phenotype with many clinical features overlapping with Noonan syndrome (Noonan Syndrome-like phenotype) [12, 23]. They commonly present with dysmorphic features, developmental delay, cryptorchidism, heart defects, and predisposition to JMML. Leukemia often spontaneously resolves in these patients, although it may also have an aggressive clinical course in some cases [2].

## *RAS*

*NRAS*, *KRAS* and *HRAS* genes are the homologs of the rodent sarcoma virus genes and encode related Ras proteins that belong to the family of small GTPase activating proteins (GAPs). GAP proteins bind guanine and negatively regulate signal

transduction by accelerating hydrolysis of GTP to GDP. In JMML, mutations in *KRAS* and *NRAS* are present in about 20–25% of cases and almost exclusively affect codons 12, 13, and 61 of the RAS genes [14, 24]. These activating mutations cause Ras to accumulate in the active GTP-bound state by impairing intrinsic GTPase activity and conferring resistance to GTPase activating proteins [25]. *KRAS* and *NRAS* activating mutations represent somatic events in almost all JMML cases. Germline alterations are a rare cause of Noonan and Cardiofaciocutaneous syndromes, but similarly to *PTPN11*, they affect different codon spectrum and have different activation strength compared to leukemogenic somatic events [5]. Interestingly, few JMML nonsyndromic patients with mosaicism for codon 12 *KRAS* and *NRAS* oncogenic mutations have been described in the literature [26, 27]. Activating mutations in *RRAS*, another member of RAS subfamily, and loss of function mutations in *RASA2* encoding Ras GTPase activating protein have been recently described as very rare causes of Noonan syndrome [28, 29]. Alterations in these genes were also detected in nonsyndromic cases of JMML, some of which lacked canonical driver mutations [8, 9, 28].

## *Other Genetic and Epigenetic Alterations*

The development of high throughput sequencing methods has allowed for the systematic evaluation of the genomic landscape of many tumor types. Whole exome sequencing studies of JMML have identified low frequency mutations in many additional genes, many of which were previously detected in other myeloproliferative/myelodysplastic disorders and other tumor types. Somatic mutations in JMML occur at a low rate with about 0.38 events/Mb/case versus 0.61 events/Mb/case on average in other childhood cancers [8]. These secondary genetic alterations target small number of interlacing networks affecting RAS regulators, transcription factors such as *GATA2* and *RUNX1*, spliceosome complex, methylation modifiers, and exerting activation of additional signal transduction pathways including JAK-STAT [8, 9]. Recurrent *SETBP1* protooncogene mutations were one of the first secondary genetic hits described in JMML. They are thought to contribute to the disease progression rather than initiation as they are primarily found in subpopulations of leukemic clones [10]. *SETBP1* mutations are present at diagnosis at a very low allelic frequency in up to 30% of patients and have been shown to be associated with poor clinical outcome [9]. Mutations in several additional RAS pathway regulators have been described in JMML including GTPase *RAC2*. Interestingly, functional analyses showed that gain of function of RAC2 leads to activation of PI3K and mTORC2 pathways, but does not have significant effect of RAF-MEK-ERK pathway itself [8]. These findings have potential implications for use in targeted therapies in JMML in the future. Components of epigenetic regulators are frequently mutated in the subset of pediatric cancers, and recent studies also have highlighted the role of secondary epigenetic aberrations in the development and prognosis of JMML. Mutations targeting multiple genes leading to impairment of PRC2 complex function were

detected in 15–30% of sporadic JMML cases and a high percentage of NF1 patients [8, 9]. The PRC2 complex is involved in transcriptional repression of genes via Histone H3 methylation. *ASXL1*, also mutated in some sporadic JMML cases, inhibits PRC2 recruitment leading to global hypermethylation [8, 9]. Hypermethylation of CpG islands in *BMP4*, *CALCA*, *CDKN2B*, and *RARB* has been associated with older age, and elevated hemoglobin F at diagnosis, and poor prognosis suggesting that a high-methylation phenotype characterizes an aggressive biologic variant of JMML [30]**.** Further studies are needed to evaluate the potential benefits of DNA-hypomethylating agents in treatment regimens for patients exhibiting aberrant methylation patterns.

### *Chromosomal Aberrations*

In the majority of JMML cases, chromosome analysis reveals a normal karyotype. Monosomy 7 is the most commonly observed abnormality present in about 25% of bone marrows and an additional 4% of cases harbor 7q deletion [3]. Patients with chromosome 7 abnormality tend to present with lower white blood cell count with higher percentage of monocytes, red blood cell macrocytosis, and normal to moderately elevated hemoglobin F levels compared to those with normal karyotype [3]. Other chromosomal abnormalities including numerical abnormalities, inversions, translocation and complex rearrangements involving range of chromosomes, co-occurring with monosomy 7 in some cases, were reported in rare patients [3, 31].

## Prognosis and Therapy

In the vast majority of cases, JMML has an aggressive course with a fatal outcome if not treated with hematopoietic stem cell transplantation. HSCT is the only curative treatment for JMML, although post-transplant relapse rate is high reaching approximately 50% [32]. Currently, clinical risk assessment includes age at diagnosis, platelet count and hemoglobin F levels as main prognostic factors. Studies indicate that age > 2 years at diagnosis, platelet count $\leq 33 \times 10^9$/L, and hemoglobin F $\geq$ 15% are predictors of short survival [2] . It is recognized that genetic heterogeneity is an important variable in the natural history of JMML, and genetic subgrouping has become a foundation for treatment indications. Hematopoietic stem cell transplantation is recommended for all children with JMML associated with somatic *PTPN11* and *KRAS* mutations as well as for neurofibromatosis type 1 patients, and the majority of cases associated with somatic *NRAS* mutations. A "Watch and wait" strategy and/or mild chemotherapy is recommended for syndromic *CBL, PTPN11,* and *NRAS* associated JMML as the disease often regresses spontaneously in these cases [2]. JMML associated with somatic *NRAS* mutations appears to display the most diverse outcomes. Although the majority follows an aggressive progression of

the disease, genotype–phenotype correlation studies indicate that some patients with somatic *NRAS* mutations may have resolution of the neoplasm without treatment [33, 34].

Genome wide analyses have shown that secondary genetic alterations accumulate only in a limited number of JMML cases—predominantly in those with neurofibromatosis type 1 and nonsyndromic cases. Importantly, the number of these events rather than specific type of secondary alterations is associated with poorer outcomes [8, 9]. JMML with double mutations in the RAS pathway also display a much more aggressive disease with rapid evolution to AML with myelodysplasia-related changes and significantly lower overall survival rates [8]. In the foreseeable future, routine genome wide mutation analysis of juvenile myelomonocytic leukemia will likely assist in risk stratification and treatment decisions.

## Conclusion

Juvenile myelomonocytic leukemia is a clinically and genetically heterogenous disease. Recent advances in our understanding of the genetic landscape of JMML have facilitated its diagnostic classification and provided a foundation for further studies of disease mechanisms and development of targeted treatments for this disease.

## References

1. Arber DA, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–405.
2. Locatelli F, Niemeyer CM. How I treat juvenile myelomonocytic leukemia. Blood. 2015;125(7):1083–90.
3. Niemeyer CM, et al. Chronic myelomonocytic leukemia in childhood: a retrospective analysis of 110 cases. European working group on Myelodysplastic syndromes in childhood (EWOG-MDS). Blood. 1997;89(10):3534–43.
4. Emanuel PD, et al. Selective hypersensitivity to granulocyte-macrophage colony-stimulating factor by juvenile chronic myeloid leukemia hematopoietic progenitors. Blood. 1991;77(5):925–9.
5. Schubbert S, Shannon K, Bollag G. Hyperactive Ras in developmental disorders and cancer. Nat Rev Cancer. 2007;7(4):295–308.
6. Kratz CP, et al. Cancer in Noonan, Costello, cardiofaciocutaneous and LEOPARD syndromes. Am J Med Genet C Semin Med Genet. 2011;157C(2):83–9.
7. Aoki Y, Matsubara Y. Ras/MAPK syndromes and childhood hemato-oncological diseases. Int J Hematol. 2013;97(1):30–6.
8. Caye A, et al. Juvenile myelomonocytic leukemia displays mutations in components of the RAS pathway and the PRC2 network. Nat Genet. 2015;47(11):1334–40.
9. Stieglitz E, et al. The genomic landscape of juvenile myelomonocytic leukemia. Nat Genet. 2015;47(11):1326–33.
10. Sakaguchi H, et al. Exome sequencing identifies secondary mutations of SETBP1 and JAK3 in juvenile myelomonocytic leukemia. Nat Genet. 2013;45(8):937–41.

11. Tartaglia M, et al. Somatic mutations in PTPN11 in juvenile myelomonocytic leukemia, myelodysplastic syndromes and acute myeloid leukemia. Nat Genet. 2003;34(2):148–50.
12. Niemeyer CM, et al. Germline CBL mutations cause developmental abnormalities and predispose to juvenile myelomonocytic leukemia. Nat Genet. 2010;42(9):794–800.
13. Loh ML, et al. Mutations in CBL occur frequently in juvenile myelomonocytic leukemia. Blood. 2009;114(9):1859–63.
14. Flotho C, et al. RAS mutations and clonality analysis in children with juvenile myelomonocytic leukemia (JMML). Leukemia. 1999;13(1):32–7.
15. Chang TY, Dvorak CC, Loh ML. Bedside to bench in juvenile myelomonocytic leukemia: insights into leukemogenesis from a rare pediatric leukemia. Blood. 2014;124(16):2487–97.
16. Side L, et al. Homozygous inactivation of the NF1 gene in bone marrow cells from children with neurofibromatosis type 1 and malignant myeloid disorders. N Engl J Med. 1997;336(24):1713–20.
17. Tartaglia M, et al. SHP-2 and myeloid malignancies. Curr Opin Hematol. 2004;11(1):44–50.
18. Tartaglia M, et al. Mutations in PTPN11, encoding the protein tyrosine phosphatase SHP-2, cause Noonan syndrome. Nat Genet. 2001;29(4):465–8.
19. Loh ML, et al. Mutations in PTPN11 implicate the SHP-2 phosphatase in leukemogenesis. Blood. 2004;103(6):2325–31.
20. Tartaglia M, et al. Diversity and functional consequences of germline and somatic PTPN11 mutations in human disease. Am J Hum Genet. 2006;78(2):279–90.
21. Kratz CP, et al. The mutational spectrum of PTPN11 in juvenile myelomonocytic leukemia and Noonan syndrome/myeloproliferative disease. Blood. 2005;106(6):2183–5.
22. Thien CB, Walker F, Langdon WY. RING finger mutations that abolish c-Cbl-directed polyubiquitination and downregulation of the EGF receptor are insufficient for cell transformation. Mol Cell. 2001;7(2):355–65.
23. Martinelli S, et al. Heterozygous germline mutations in the CBL tumor-suppressor gene cause a Noonan syndrome-like phenotype. Am J Hum Genet. 2010;87(2):250–7.
24. Kalra R, et al. Genetic analysis is consistent with the hypothesis that NF1 limits myeloid cell growth through p21ras. Blood. 1994;84(10):3435–9.
25. Zenker M, et al. Expansion of the genotypic and phenotypic spectrum in patients with KRAS germline mutations. J Med Genet. 2007;44(2):131–5.
26. Kato M, et al. Aggressive transformation of juvenile myelomonocytic leukemia associated with duplication of oncogenic KRAS due to acquired uniparental disomy. J Pediatr. 2013;162(6):1285–8. 1288 e1
27. Doisaki S, et al. Somatic mosaicism for oncogenic NRAS mutations in juvenile myelomonocytic leukemia. Blood. 2012;120(7):1485–8.
28. Flex E, et al. Activating mutations in RRAS underlie a phenotype within the RASopathy spectrum and contribute to leukaemogenesis. Hum Mol Genet. 2014;23(16):4315–27.
29. Chen PC, et al. Next-generation sequencing identifies rare variants associated with Noonan syndrome. Proc Natl Acad Sci U S A. 2014;111(31):11473–8.
30. Olk-Batz C, et al. Aberrant DNA methylation characterizes juvenile myelomonocytic leukemia with poor outcome. Blood. 2011;117(18):4871–80.
31. Yoshida N, et al. Correlation of clinical features with the mutational status of GM-CSF signaling pathway-related genes in juvenile myelomonocytic leukemia. Pediatr Res. 2009;65(3):334–40.
32. Locatelli F, et al. Hematopoietic stem cell transplantation (HSCT) in children with juvenile myelomonocytic leukemia (JMML): results of the EWOG-MDS/EBMT trial. Blood. 2005;105(1):410–9.
33. Matsuda K, et al. Spontaneous improvement of hematologic abnormalities in patients having juvenile myelomonocytic leukemia with specific RAS mutations. Blood. 2007;109(12):5477–80.
34. Flotho C, et al. Genotype-phenotype correlation in cases of juvenile myelomonocytic leukemia with clonal RAS mutations. Blood. 2008;111(2):966–7; author reply 967-8.
35. Aoki, et al. Recent advances in RASopathies. J Hum Genet. 2016;61:33–9.

# Chapter 14
# Down Syndrome-Associated Hematologic Disorders and Leukemia

**Amy M. Coffey, Brian Y. Merritt, and Cholladda V. Curry**

## Transient Abnormal Myelopoiesis

### *Introduction*

Transient abnormal myelopoiesis (TAM), also referred to as transient myeloproliferative disorder (TMD) or transient leukemia (TL), is characterized by increased circulating myeloid blasts that have an acquired GATA-binding protein 1 (*GATA1*) mutation in children with Down syndrome [2].

### *Epidemiology*

TAM occurs in around 20–30% of children with Down syndrome. Approximately 10–15% of neonates with Down syndrome have blasts >10%, and clinical and hematological features of TAM, referred to as clinical TAM, whereas another

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10–15% have acquired *GATA1* mutations with a low number of blasts (<10%) and no clinical or hematological features suggestive of TAM, the so-called silent TAM [2, 3]. Clinical TAM most commonly manifests during the early neonatal period. The median age at presentation is 3–7 days; most cases will manifest by 2 months but can be diagnosed up to 6 months old [4, 5]. TAM has been reported in partial trisomy 21 and trisomy 21 mosaicism [6, 7].

## *Clinical Features*

Clinical features of TAM vary; typical presenting features include hepatomegaly, splenomegaly, pericardial/pleural effusions, and skin rash [2, 3, 8]. Down syndrome children who have silent TAM may be diagnosed on review of peripheral blood smear and complete blood count (CBC) but otherwise appear well clinically; on the other hand, patients may be very sick with disseminated leukemic infiltration with massive hepatosplenomegaly, effusions, coagulopathy, and multi-organ failure [8]. Characteristic hematologic features of TAM include leukocytosis, anemia, and thrombocytopenia. Leukocytosis in TAM shows increased circulating blasts, neutrophilia, monocytosis, basophilia, and increased myelocytes. Other laboratory features are abnormal liver function tests and abnormal coagulation [2, 3, 8].

## *Morphology and Immunophenotyping*

Many cases of TAM are morphologically and immunophenotypically indistinguishable from acute megakaryoblastic leukemia. Blasts in the peripheral blood and bone marrow show typical features of megakaryoblasts, which include a modest amount of agranular basophilic cytoplasm with cytoplasmic blebs, irregular nuclear contours, and a nuclear chromatin pattern that may be more condensed than traditional myeloblasts with infrequent nucleoli (Fig. 14.1). The blast percentage in the peripheral blood may be disproportionately higher than in the bone marrow, reflecting probable megakaryopoiesis in the liver [4, 9]. If a bone marrow biopsy is performed, multilineage dysplasia may be seen, most often with involvement of megakaryocytic and erythroid cell lines. Cases with prominent dysplasia may more closely resemble a conventional myelodysplastic syndrome. Marrow fibrosis is variable. Peripheral basophilia may be present. Liver biopsies performed for liver dysfunction may show necrosis and/or fibrosis, which is usually accompanied by atypical myeloid progenitors, including megakaryoblasts, within sinusoids. Examination of the placenta may show marked expansion of the chorionic plate and stem vessels by the circulating myeloid cells (Fig. 14.2) and/or features of fetal thrombotic vasculopathy.

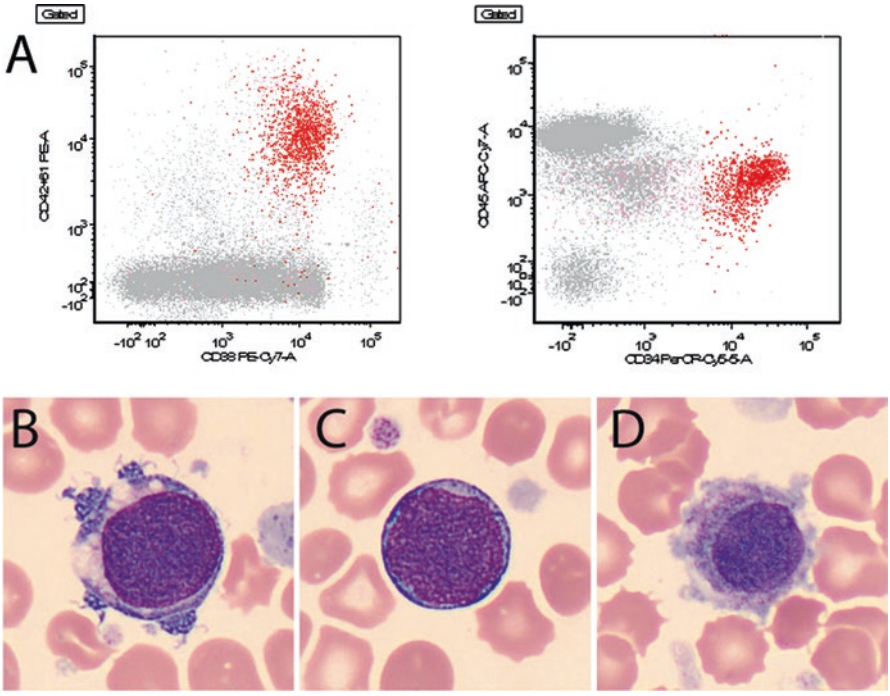


**Fig. 14.1** Peripheral blood findings in TAM in a 2-week old. (**a**) Flow cytometry *dot plots* show a 5% population of cells expressing megakaryocytic markers CD42 + CD61 corresponding to megakaryoblasts; the blasts are also positive for CD34, CD117, CD45 (dim), CD13, CD33, CD11b, CD38, and CD71. (**b**–**d**) Peripheral blood smears show leukocytosis with circulating megakaryoblasts (**b**–**c**, Wright-Giemsa 1000×) and other abnormal megakaryocytic precursors (**d**, Wright-Giemsa 1000×). Cytoplasmic pseudopod formation is a characteristic feature of megakaryoblastic differentiation (**b**), but a more constant feature is the presence of deeply basophilic agranular cytoplasm with features otherwise typical for myeloblasts (**c**)

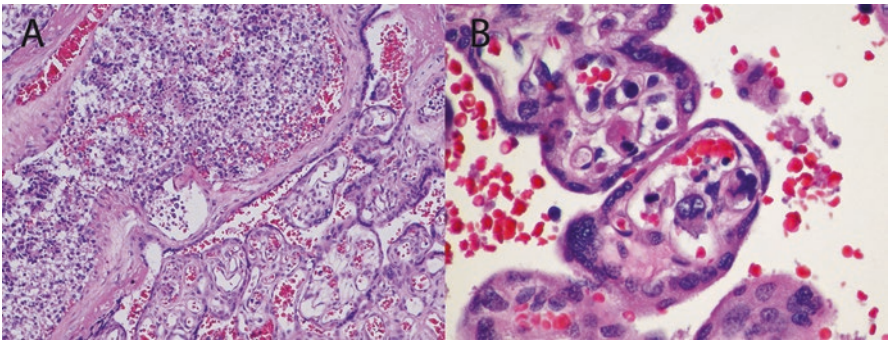


**Fig. 14.2** Placenta findings in TAM. Stem vessels are expanded by an atypical myeloid infiltrate (**a**), and atypical megakaryocytic precursors are visualized within the fetal vasculature of chorionic villi (**b**) (case provided by Edwina Popek, DO, Baylor Collge of Medicine/Texas Children's Hospital, Pavilion for Women)

### *Immunophenotyping of Blasts by Flow Cytometry*

In the majority of cases, blasts are positive for CD34, CD56, CD117, CD13, CD33, CD7, CD4 dim, CD41, CD42, CD36, CD61, CD71, TPO-R, and IL-3R [5]. Myeloperoxidase is negative. HLA-DR expression is variable. Overall, the immunophenotype of blasts is variable and there is no reliable morphologic or immunophenotypic profile to allow distinguishing between blasts from patients with TAM (with *GATA1* mutation) and those without *GATA1* mutation [2, 3]. Subtle immunophenotypic differences between TAM and Down syndrome-associated acute myeloid leukemia have been described; CD34, CD56, and CD41 are less frequently expressed in Down syndrome-associated acute myeloid leukemia (see discussion below) [5].

### *Cytogenetics and Molecular Findings*

Clonal cytogenetic abnormalities are generally absent in TAM. There are isolated reports of oligoclonal abnormalities preceding progression to Down syndrome-associated acute myeloid leukemia (DS-ML) [10]. Detection of karyotypic abnormalities in TAM may indicate clonal evolution with progression to DS-ML [11].

The molecular events involved in the development from TAM to DS-ML have been recently reviewed [2]. Briefly, it is best described as a three-step model in fetal liver-derived hematopoietic stem or progenitor cells involving (1) trisomy 21, (2) an acquired *GATA1* mutation, and (3) at least one additional oncogenic mutation (Fig. 14.3). Of note, step 3 will be discussed in the DS-ML section below.

Constitutional trisomy 21, the first in the three-step model, leads to altered myeloid progenitor self-renewal, altered lineage development, and increased clonogenicity of megakaryocyte precursors (Fig. 14.3) [12–14]. Trisomy 21 leads to increased megakaryocyte-erythroid progenitors (MEP) and increased size and characteristics of immunophenotypic characteristics of the hematopoietic stem cell (HSC) compartment; HSC and multipotent myeloid progenitors in trisomy 21 fetal liver proliferate more and have increased erythroid-megakaryocyte output and gene expression (increased HSC with MK/E bias) [12]. Although megakaryocytes (MK) are increased, their differentiation is impaired, leading to thrombocytopenia in both the fetal and neonatal periods, suggesting that dysmegakaryopoiesis occurs in Trisomy 21 [2, 12]. Increased expression of various genes on chromosome 21, particularly *ERG* and *DYRK1a*, may be responsible for the abnormal megakaryopoiesis seen in Down syndrome [2]. On a larger scale, trisomy 21 causes genome-wide alterations in gene expression, directly or indirectly affecting multiple genes on most chromosomes [2, 15].

Acquired somatic mutations in GATA-binding protein 1 (*GATA1*), the second of the previously mentioned three-step model, serve as the "second hit" in the development of abnormal myeloid proliferations and are a defining feature of both TAM

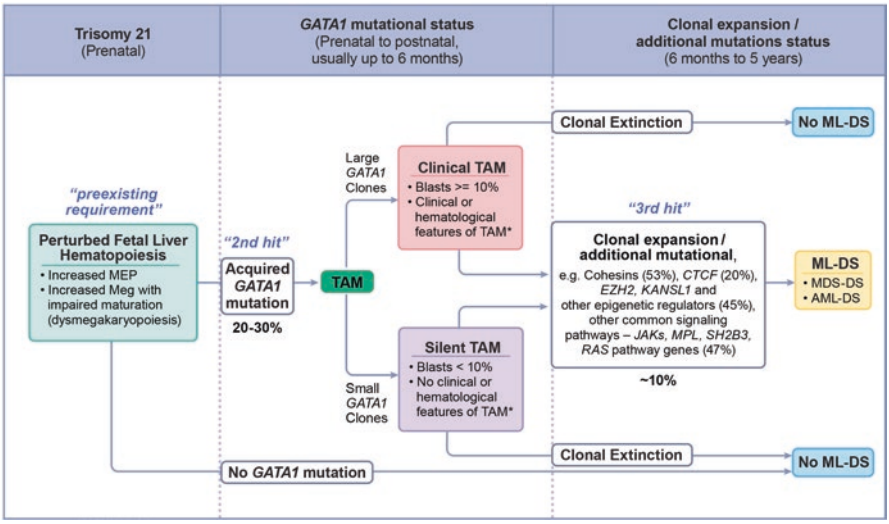


**Fig. 14.3** Natural history with stepwise progression of TAM to DS-ML. The pathogenesis begins in utero with perturbed fetal liver hematopoiesis due to constitutional trisomy 21. Acquired *GATA1* mutations occur in a subset of infants with trisomy 21, either prenatally or shortly after birth, and serve as the "second hit" leading to either clinical or silent TAM. The "third hit" comes with the accumulation of additional genetic events leading to clonal expansion and promotion of leukemogenesis (Adapted from Fig. 1 of Ref. [2])

and DS-ML (Fig. 14.3). It was previously thought that *GATA1* aberrations occur in 4–10% of neonates with Down syndrome [16–18]; however, with the relatively recent discovery of silent TAM, acquired *GATA1* mutations are present at higher frequency, occurring in 25–30% of all neonates with Down syndrome and preceding all cases of DS-ML [2, 3]. The affected site of acquired *GATA1* mutations is in exon 2, or less commonly exon 3, or at the intronic boundary of exons 1 and 2. Insertions, deletions, and duplications, overall accounting for three-quarters of all mutations, are more common than point mutations. The *GATA1* gene, located at Xp11.23, encodes a zinc finger transcription factor required for normal megakaryopoiesis and erythropoiesis. The mutated gene encodes a truncated protein devoid of its amino terminus that is thought to impair *GATA1*-mediated regulation of other transcription factors such as *GATA2*, *MYB*, *MYC,* and IKAROS family zinc finger 1 (*IKZF1*); this in turn is thought to block megakaryocyte differentiation [7].

The unique combination of constitutional trisomy 21 and *GATA1* mutations is responsible for the development of TAM [7]. In fact, *GATA1* mutations in the absence of trisomy 21 cause anemia and neutropenia without leukemogenesis [19]. Additional transforming events (the third step of the three-step model), however, are required for progression to acute leukemia (discussed in section on DS-ML) (Fig. 14.3). Aside from *GATA1*, *JAK3* mutations have uncommonly been reported (1 in 15 cases in one series) [20].

### *Prognosis and Therapy*

Spontaneous remission occurs in the majority of cases, usually within the first 3–6 months of life. The overall disease resolution rate is 85–90%, with a mortality rate of up to 20% [8, 21, 22]. Notable complications as a result of hyperviscosity and/or megakaryocyte-derived cytokines include liver fibrosis/liver failure, heart and respiratory failure, and disseminated intravascular coagulation. Low-dose cytarabine may be administered in such cases. High-risk features include failure of spontaneous remission, markedly elevated white blood cell count greater than 100 × 10^9/L, hepatic or renal dysfunction, hepatic fibrosis, prematurity, coagulopathy, massive organomegaly causing respiratory compromise, and hydrops [12, 21]. Progression to myeloid leukemia occurs in 20–30% of cases and usually occurs between 2 and 4 years of age.

Molecular markers are not currently utilized to monitor for disease progression to DS-ML. Transcriptional differences between TAM and DS-ML have been demonstrated in a single study that may have diagnostic utility. *CDKN2C* and *PRAME* transcripts were shown to be increased in DS-ML, while *MYCN* transcripts were increased in TAM [23]. An immunohistochemical study targeting the PRAME protein failed to discriminate between cases of TAM and DS-ML [24]. For the time being, the distinction between TAM and DS-ML relies predominantly on the integration of clinical parameters, hematologic features, pathologic, cytogenetic, and molecular findings (Table 14.1).

### *Conclusion*

TAM (both clinical and silent TAM) occurs in up to a third of infants with Down syndrome. *GATA1* alterations play a key role in the pathogenesis. Spontaneous remission is seen in the majority of cases, but subsequent development of DS-ML may occur. Children with TAM require close follow-up to monitor for a possible development of DS-ML in a subset of these patients. Consensus clinical guidelines with regard to the detection and monitoring of acquired *GATA1* mutation by molecular methods have not yet been established.

## Myeloid Leukemia Associated with Down Syndrome

### *Introduction*

There is a 150-to-500-fold increased risk of developing acute myeloid leukemia (AML) in children with Down syndrome compared to the general population, with an overall incidence of 0.5–2% in this population [16, 25]. Approximately 70% of

**Table 14.1** Clinical, pathologic, and molecular features of TAM vs. DS-ML

| Parameter | TAM | DS-ML |
|---|---|---|
| *Clinical* | | |
| Age | 3–7 days, up to 6 months | 1–2 years, up to 4 years |
| Organomegaly, organ dysfunction, etc.[a] | Variable | Variable |
| *Hematologic* | | |
| Leukocytosis | Variable | Variable |
| Blast percentage | Variable, often high | Variable |
| Anemia | Usually present | Usually present |
| Thrombocytopenia | Usually present | Usually present |
| *Morphology* | | |
| Blasts with megakaryoblastic differentiation | Present | Present |
| Erythroid and megakaryocytic dysplasia | Usually present | Usually present |
| Reticulin fibrosis | Variable | Usually present |
| *Immunophenotype* | Coexpression of CD34, CD56, CD41, CD61, and CD36 is common, along with other myeloid markers. Aberrant CD4 and CD7 expression may be seen | Similar to TAM, with less frequent expression of CD34, CD56 and CD41 |
| *Karyotypic abnormalities* | Generally absent | +8, +11, +21, −7, dup(1q), del(6q), del(7p), dup(7q), del(16q), der(3q), low hyperdiploidy, pseudodiploidy |
| *Molecular aberrations*[b] | | |
| Acquired GATA1 mutation | Present | Present |
| Additional driver genetic events | Generally absent or not sufficient for leukemogenesis | Generally required for leukemogenesis |

*PB* peripheral blood, *BM* bone marrow
[a]Clinical manifestations may include but are not limited to hepatomegaly, splenomegaly, effusions, skin rash, coagulopathy, multi-organ failure
[b]Refer to Fig. 14.3

AML in Down syndrome manifests as acute megakaryoblastic leukemia. Myeloid leukemia associated with Down syndrome (DS-ML) is recognized as a distinct entity by the 2016 revision of the World Health Organization classification based on its distinct clinical and molecular features in comparison to other types of AML [26]. As a note on terminology, older texts separate Down syndrome-associated AML from Down syndrome-associated myelodysplastic syndrome (MDS) because some children with Down syndrome present with cytopenias, dyspoiesis, and fewer

than 20% blasts, thus meeting the criteria for MDS. By the current WHO classification schema, the term "myeloid leukemia associated with Down syndrome" encompasses both AML and MDS since they represent a continuum of the same biologic process, and all cases fulfilling the criteria for MDS will inevitably evolve into AML [5, 26].

## *Epidemiology*

DS-ML develops 1–3 years following TAM, with a mean age of 1–1.8 years at diagnosis, but can occur up to 4 years old. Previously, it was thought that DS-ML may develop without prior TAM [26, 27]; however, recent data suggested all DS-ML preceded by TAM, either clinically or silently, the so-called clinical TAM or silent TAM, respectively (refer to the TAM section, above). It is therefore most likely that patients who develop DS-ML without a prior history of TAM are those who have silent TAM which was not diagnosed earlier. Previous studies based on retrospective data suggest that 20–30% of neonates with TAM (in general imply clinical TAM) will subsequently develop DS-ML. In consideration of the recent discovery of silent TAM, which suggests a much higher frequency of *GATA1* mutations at birth, and the estimated population-based frequency of DS-ML, which is approximately 0.5–2% before 4 years of age, the risk of progression is probably closer to 5–10% [2, 7, 16, 28]. Progression to DS-ML may immediately follow clinical TAM but more commonly occurs following a period of clinical remission.

## *Clinical Features*

DS-ML is most often characterized by an indolent clinical course with initial myelodysplasia and progressive pancytopenia, particularly thrombocytopenia and leukopenia, with low circulating blast counts for many months followed by the eventual development of overt AML [2].

## *Morphology and Immunophenotyping*

Morphology in the peripheral blood and bone marrow may be indistinguishable from TAM (Fig. 14.4). Blasts have megakaryoblastic features. Erythroblastic differentiation may also occur but is rare (Fig. 14.5). Megakaryocytic/erythroid dysplasia is often present. Erythroid precursors may show megaloblastic change in addition to dysplastic forms. Dysgranulopoiesis may also be present. Reticulin

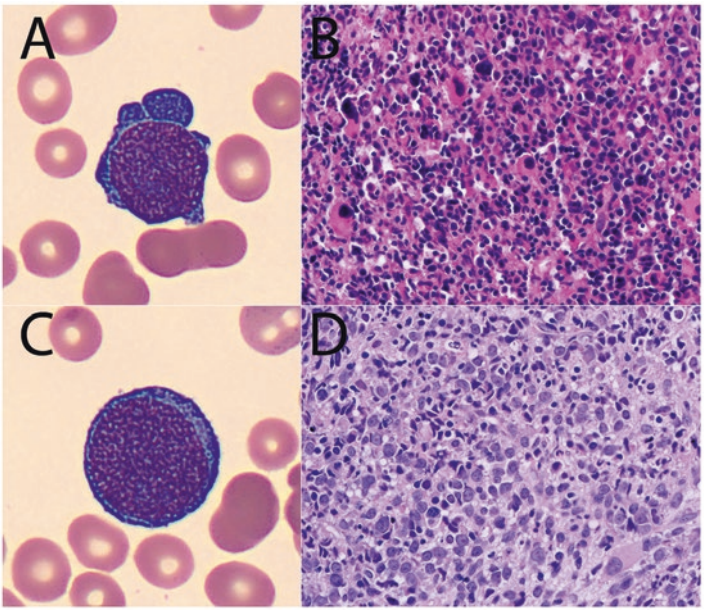


**Fig. 14.4** Bone marrow findings in myeloid leukemia associated with Down syndrome, with features resembling myelodysplastic syndrome (**a**, **b**) and features of acute megakaryoblastic leukemia (**c**, **d**). Cases behaving as myelodysplastic syndrome show uni- or multilineage dyspoiesis including megakaryocytic dysplasia in a background of reticulin fibrosis. (**a**) Blasts show features of megakaryoblastic differentiation with deep basophilic cytoplasm and cytoplasmic blebs. In panel **b**, megakaryocytes are increased in number and show abnormal nuclear forms including small, hypolobated forms and nuclear hyperchromasia. In this case, there is no increase in blasts (**a** Wright-Giemsa 1000×, B H&E 500×). Cases behaving as acute megakaryoblastic leukemia show more than 20% marrow involvement by leukemic blasts showing megakaryoblastic differentiation. (**d**) Bone marrow core biopsy shows large aggregates of blasts, in a background of dyspoiesis with reticulin fibrosis (**c** Wright-Giemsa 1000×, D H&E 500×)

fibrosis in the marrow is variable but often present; therefore, it is essential to obtain a bone marrow trephine core biopsy for a more accurate assessment of blast involvement.

## *Immunophenotype*

The usual immunophenotype is similar to TAM. In the majority of cases, blasts show megakaryoblastic differentiation and are positive for CD117, CD13, CD33, CD7, CD4 dim, CD42, CD36, CD61, CD71, TPO-R, and IL-3R. Myeloperoxidase is negative. HLA-DR expression is variable. In contrast to TAM, CD34 is negative in 50% of cases, and CD56 and CD41 are negative in 30% [5].

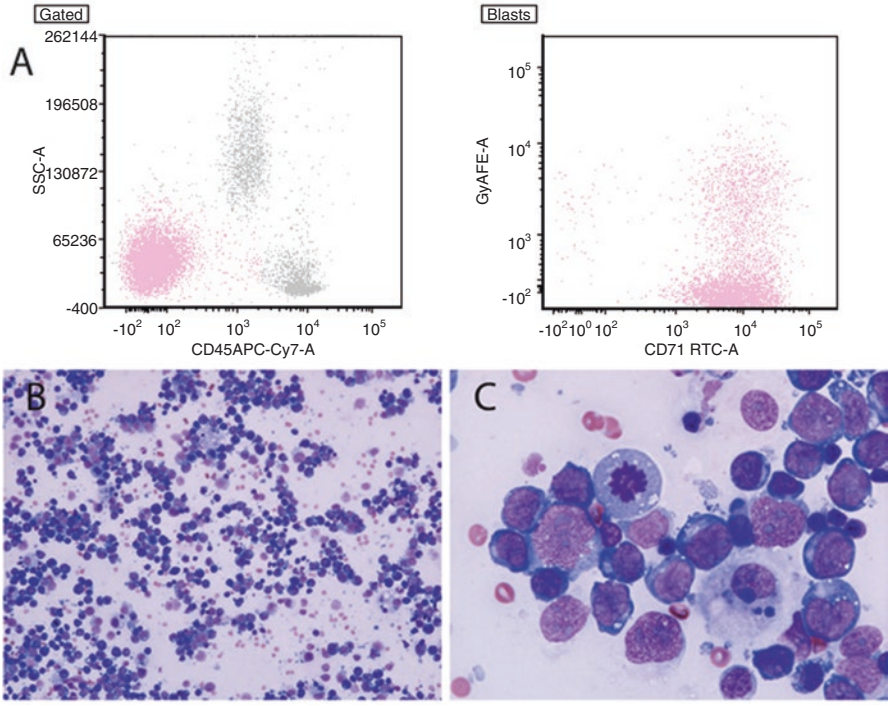


**Fig. 14.5** Pure erythroid leukemia arising in a 2-year-old boy with Down syndrome. (**a**) Flow cytometry dot plots showing 62% blasts which are CD45 negative with co-expression of CD71. Glycophorin A is positive in a small subset. The blasts also express CD34 (subset), CD117, CD7 (partial), CD99, and CD33 (variable). (**b**, **c**) Aspirate smears show numerous erythroblasts with deeply basophilic cytoplasm and round nuclei with fine chromatin and one or more nucleoli. Cytoplasmic vacuolization is present (**b** Wright-Giemsa 200×, **c** Wright-Giemsa 1000×)

## *Cytogenetics and Molecular Findings*

Karyotypic abnormalities may indicate clonal evolution of TAM to DS-ML [11]. Cytogenetic changes in DS-ML appear to be distinct from those observed in other subtypes of AML. Trisomy 8 commonly occurs in 13–44% of cases [28, 29]. Other less common abnormalities include dup(1q), del(6q), del(7p), dup(7q), del(16q), der(3q), +8, +11, +21, and −7 [21, 27, 30]. Interestingly, an additional nonconstitutional chromosome 21 is more frequent in DS-ML than in conventional AML. The recurrent cytogenetic abnormalities in conventional AML such as t(8;21), 11q23 translocations, and inv(16) are significantly less common in DS-ML. The most common ploidy levels are low hyperdiploidy and pseudodiploidy [30].

As previously elucidated in the previous section, patients with Down syndrome have trisomy 21 (first step) as a pre-existing event that disturbs normal hematopoiesis. *GATA1* mutations (second step), as a required pre-leukemic event, are found in all TAM. Additional molecular events (third step), however, are almost certainly required for progression from TAM to AML, the details of which have not been fully elucidated (Fig. 14.3).

A Down Syndrome Critical Region (DSCR) on chromosome 21 has been identified, which is a 4.3–5.4 Mb region located at 21q22 that contains several candidate genes involved in leukemogenesis including *ERG*, *ETS2*, *RUNX1*, *GABPA*, *BACH1,* and *DYRK1A* [7, 12]. Among these, *ERG* and *RUNX1* have received the most attention.

*ERG* expression is a poor prognostic indicator in adult T-ALL and cytogenetically normal AML, but its role in childhood leukemia is uncertain [31]. Overexpression has been demonstrated in non-DS acute megakaryoblastic leukemia, but it is not overexpressed in induced pleuripotent stem cell (iPS)-derived Down syndrome progenitors, an in vitro model of trisomy 21 [13, 32]. *ERG* causes immortalization of hematopoietic progenitor cells in *GATA1* mutant mice through JAK/STAT pathway activation, and a TMD-like effect has been demonstrated in mice models (Ts65Dn) [33, 34]. Furthermore, Ts65Dn mice that are converted from trisomy *ERG* to disomy show complete reversal of the myeloproliferative phenotype [35]. While its direct role in leukemogenesis is unclear, *ERG* and *DYRK1A* are important mediators of abnormal megakaryopoiesis [2].

*RUNX1* plays an etiologic role in a subset of AML, but there is conflicting evidence for its role in DS-ML, especially since *RUNX1* trisomy does not appear to be required for the development of DS-ML [36]. *RUNX1* has a joint role with *GATA1* in megakaryopoiesis [37]. Increased expression has been demonstrated in acute megakaryoblastic leukemia, but it is not overexpressed in iPS-derived Down syndrome progenitors [13, 38].

Several other candidate genes have been proposed for which excellent reviews are available elsewhere [7]. MicroRNAs including miR-125b-2 on chromosome 21 and miR-486-5p on chromosome 8 have been implicated. Epigenetic targets on chromosome 21 including *BRWD1*, *HLCS*, and *HMGN1* may play a role [39, 40]. Candidate genes not located on chromosome 21 include *JAK3*, *MYCN*, *MYC*, *PRAME, CDKN2C,* and *EZH2*. *JAK3* mutations have been detected in a small subset of DS-ML [20]. *CDKN2C* and *PRAME* transcripts were shown to be increased in DS-ML, while *MYCN* transcripts are increased in TAM [23]. This latter observation seems to have the most potential thus far for distinguishing cases of DS-ML from TAM, although current diagnostic algorithms do not incorporate molecular testing.

The first comprehensive molecular landscape of DS-ML was achieved by Yoshida et al. whereby the authors demonstrated clonal evolution of TAM to DS-ML through acquisition of additional mutations. The major evolutional targets identified in this

study were as follows: cohesin components genes (i.e., *RAD21*, *STAG2*, *SMC3,* and *SMC1A*) (53%), *CTCF* (20%); *EZH2*, *KANSL1* and other epigenetic regulators (45%); and common signaling pathways, such as the *JAK* family kinases, *MPL*, *SH2B3* (*LNK*), and *RAS* pathway genes (47%) [41].

The molecular pathogenesis involved in DS-ML has yet to be worked out, but current knowledge supports *GATA1* mutation as an essential pre-leukemic event in the development of DS-ML. Additional transforming events, possibly involving genes in the DSCR region, lead to progression to acute leukemia [2, 7].

## *Prognosis and Therapy*

Prognosis is overall good, and response to therapy is superior in DS-ML in comparison to non-DS AML, with 88% versus 42% 4-year disease-free survival [27]. Age greater than 4 years is associated with worse outcomes and is thought to represent a different cohort of patients [42]. In contrast to conventional AML, white blood cell count at diagnosis is not predictive of outcome [27].

Cytarabine and anthracyclines are key therapeutic agents in treatment protocols for DS-ML. This is based on the apparent increased sensitivity to DNA-damaging chemotherapeutic agents in this population [8, 43]. The increased sensitivity profile has been attributed to oxidative stress and altered folate metabolism in DS-ML, yielding deficient DNA repair mechanisms, a notion supported by the observed overexpression of genes involved in oxidative/folate metabolism including cystathionine beta synthase (*CBS*) and zinc-copper superoxide dismutase (*SOD1*) [43]. It has been proposed that this environment of oxidative stress and disrupted folate metabolism may in fact contribute to the development of *GATA1* mutations in the DS population [43]. Other proposed mechanisms contributing to the increased sensitivity include altered drug metabolism [7]. The observed sensitivity profile to these agents has permitted dose-reduction strategies in the DS-ML population that achieve the same cure rates with less treatment-related toxicity [43]. Targeted therapies have not yet been investigated in this population.

## *Conclusion*

DS-ML may occur in individuals with Down syndrome, and trisomy 21 serves as a pre-existing event (step 1). *GATA1* mutations serve as an additional required pre-leukemic event (step 2). Additional molecular alterations must occur for promotion of leukemogenesis, the details of which are not yet fully characterized (step 3). The proposed three-step model (Fig. 14.3) is the best model for leukemogenesis based on the most recent available studies.

## Down Syndrome-Associated Acute Lymphoblastic Leukemia

### *Introduction*

There is a 20-fold increased risk of developing acute lymphoblastic leukemia (ALL) in children with Down syndrome compared to the general population. In contrast to DS-ML, there is not sufficient molecular or clinical evidence to support Down syndrome-associated ALL (DS-ALL) as a distinct biologic entity by the current WHO classification [5, 44]. However, recent advances including the discovery of *CRLF2* translocations in B-ALL have shed light on the molecular pathogenesis in at least a large proportion of DS-ALL.

### *Epidemiology*

Children with Down syndrome have a 20-fold increased risk for ALL compared to children without Down syndrome [1]. In contrast to the general population where the ALL:AML ratio is 4–6.5:1, from birth to 15 years of age, ALL occurs with only minimally higher frequency than acute myeloid leukemia (ALL:AML ratio of 1.2–1.7:1) in children with Down syndrome [7].

### *Morphology and Immunophenotyping*

Morphologic findings are identical to non-DS-ALL. Typical lymphoblasts have scant agranular cytoplasm, coarse-to-fine chromatin, and often indistinct nucleoli, but variations in morphology may be seen.

### *Immunophenotype*

More than 90% of DS-ALL exhibit a precursor B phenotype. Precursor B cell neoplasms express CD45 (dim) and B-lineage markers CD19, CD22, CD20 (often weak or absent), CD24, CD79a, and PAX-5. TdT is expressed in the majority of cases. Expression of immunoglobulin is variable but often absent. CD10, CD133, CD34, HLA-DR, and CD99 may be seen. Myeloid antigens such as CD11b, CD13, CD15, and CD33 may be seen in DS-B-ALL. A minority of DS-ALL are of precursor T cell origin with expression profiles identical to non-DS T-ALL [5, 45].

## *Cytogenetics and Molecular Findings*

A normal karyotype (aside from constitutional trisomy 21) is observed in 40% DS-ALL, which is a much greater proportion compared to non-DS pediatric ALL (7%). Rearrangements of *CRLF2* are reported in a large proportion of DS-ALL (discussed below). Favorable cytogenetic profiles including t(12;21)(p13;q22);*ETV6-RUNX1*, double trisomy of 4 and 8, and high hyperdiploidy are significantly less common in DS-ALL; t(12;21)(p13;q22) and hyperdiploidy were found in 10% of cases in the largest series [30]. Unfavorable cytogenetic findings, including *BCR-ABL1* fusion and *MLL* rearrangements, are also less common. The largest series to date reported three abnormalities +X, t(8;14)(q11;q32), and del(9p) as unique changes seen in DS-ALL although these findings have not been definitively corroborated as recurrent abnormalities [30]. Acquired +21 is fairly frequent in DS-ALL, although less so than non-DS-ALL.

Three predominant molecular aberrations have been described in DS-ALL: *CRLF2* overexpression, *JAK2* alterations, and *RAS* mutations. Constitutional trisomy 21 likely plays a role, a theory corroborated by data from non-DS ALL with intrachromosomal amplification of chromosome 21 (iAMP21).

### CRLF2 Overexpression

Dysregulation of the cytokine receptor-like factor 2 (*CRLF2*) is found in 60% of DS-ALL compared to 10% of non-DS pediatric ALL [46–49]. *CRLF2* is located on the X and Y chromosomes, at Xp22.3 and Yp11.3. The protein product of *CRLF2* forms a heterodimer receptor for thymic stromal lymphopoietin (TSLP) in combination with interleukin-7 receptor subunit alpha (IL-7Rα) [1]. Receptor binding of TSLP results in activation of the STAT3, STAT5, and JAK2 pathways. Dysregulation of this pathway may result from translocations, deletions, or point mutations of the *CRLF2* gene. Translocations resulting in *CRLF2* rearrangement with *IGH* on chromosome 14 result in *CRLF2* gene overexpression [46, 48]. By an alternate mechanism, deletions in the pseudoautosomal region 1 (PAR1) of Xp22.3/Yp11.3 result in a *P2RY8-CRLF2* fusion, leading to *CRLF2* overexpression [47]. The latter mechanism is more frequent. Lastly, point mutations at codon 232 (F232C) result in *CRLF2* overexpression and have been documented in 9% of DS-ALL patients and 21% of adult B-ALL patients [1]. Of note, interleukin-7 receptor (IL-7R) gene mutations have been reported in B- and T-ALL in association with aberrant *CRLF2* overexpression, although such mutations have not specifically been described in DS-ALL [50]. A subset of DS-B-ALL patients who carry *CRLF2* gene translocation may be classified as "*BCR-ABL1*-like ALL," which is a provisional entity as proposed by the 2016 updated WHO classification schema [26].

### JAK2 Mutations

*JAK2* mutations may occur in combination with *CRLF2* fusions, conferring a likely cooperative effect [1]. JAK2 is a cytoplasmic protein-tyrosine kinase that catalyzes the transfer of the gamma-phosphate group of adenosine triphosphate to the hydroxyl groups of specific tyrosine residues in signal transduction molecules [51, 52]. The downstream effectors of JAK2 are a family of transcription factors called signal transducers and activators of transcription (STAT) proteins. JAK-STAT signaling plays an important role in B lymphopoiesis. Deletion of five amino acid residues from positions 682 to 686 within the JH2 pseudokinase domain on exon 14 of *JAK2* (JAK2ΔIREED) or other mutations in the R683 residue on exon 16 lead to activated JAK2 signaling in some B-ALLs [53, 54]. The majority of R683 mutations reported lead to replacement of arginine, a basic residue, with a neutral amino acid, which leads to altered interactions at the C-terminal kinase domain and results in constitutive JAK2 activation [54]. *JAK2* R683 abnormalities have been reported in 18–20% in DS-ALL [54–56]. Mutations in the *JAK2* kinase domain, as well as the *JAK1* pseudokinase domain, have also been reported with much less frequency [47]. Interestingly, *JAK2* V617F, mutations occurring in the pseudokinase domain at a different site compared to *JAK2* R683, are common mutations in myeloproliferative neoplasms but are not seen in DS-ALL [1].

### Ras Mutations

Mutations in *NRAS* and *KRAS* have been reported to occur with a frequency similar to *JAK2* mutations and *P2RY8-CRLF2* fusions (15 of 42 cases in one study), and often occur as later events [57]. *RAS* and *JAK2* mutations are mutually exclusive events. Current data support the idea that *CRLF2* fusions serve as the initiating event in leukemogenesis, while *JAK2* or *RAS* mutations may arise later in the disease as subclones [57].

### Contribution of Constitutional Trisomy 21

The contribution of constitutional trisomy 21 to the development of DS-ALL is less clear but it almost certainly plays a role. Interestingly, there is an overall reduction in committed B-lineage progenitors in the trisomy 21 mouse model (Ts1Rhr) with a relative increase in less mature forms. These trisomic pro-B-cells display the capability for increased self-renewal [58]. Two candidate genes located on chromosome 21, *HMGN1* and *DYRK1A*, have received the most attention. The protein product of *HMGN1* is a nucleosome-binding protein implicated in histone H3 modifications and it has been shown to alter B-cell development in Ts1Rhr mice [reviewed in 1]. Epigenetic changes related to *HMGN1* expression may lead to B cell proliferation

and leukemogenesis. *DYRK1A*, a member of the CMGC superfamily of protein kinases and located in the Down syndrome critical region (DSCR) of chromosome 21, is also one of the candidate genes implicated in DS-ML. The protein product of *DYRK1A* is essential for lymphoid, but not myeloid, development where it is involved in cell cycle regulation and appears to be important for shifting lymphocytes from a proliferative to a quiescent state [59].

### iAMP21

Intrachromosomal amplification of chromosome 21 (iAMP21) occurs in 2% of non-DS pediatric ALL and has similarities to DS-ALL, further supporting the contribution of constitutional trisomy 21 to the pathogenesis of DS-ALL [reviewed in 1]. Many of the genes amplified in iAMP21 are the same genes implicated in constitutional trisomy 21, namely, *RUNX1* and miR-802, and possibly *DYRK1A* and *ETS2*. Other molecular abnormalities observed in the iAMP21 group include *IKZF* deletions (16%), *PAX5* deletions (8%), *CDKN2A* deletions (13%), *ETV6* deletions (15%), gain of X-chromosome (20%), and *P2RY8-CRLF2* fusions (17%). Among these, gain of X-chromosome and *P2RY8-CRLF2* fusions have been reported in DS-ALL in 24% and 22% of cases, respectively. A notable difference between the iAMP21 group and DS-ALL is the lack of *JAK2* mutations in the former.

## *Prognosis and Therapy*

Higher relapse rates and overall inferior outcomes are observed in DS-ALL compared to the general population. While different biologic, cytogenetic, and molecular profiles may certainly contribute to this trend, individuals with Down syndrome appear to have a higher incidence of methotrexate toxicity, which has led to dose-adjusted protocols in this population [1, 60]. It has been suggested that the higher relapse rates are at least partially attributable to the difference in treatment protocols, which appear necessary due to the excessive morbidity and mortality observed with conventional protocols designed for the general pediatric population [1]. *JAK2* mutations are associated with high-risk features and relapsed disease in non-DS-ALL but apparently not in DS-ALL, while *KRAS* mutations were associated with poor outcome for DS-ALL in one study [57].

Hematopoietic stem cell transplantation is an option for high-risk or relapsed ALL in the general population, but data for DS-ALL is limited [1]. While targeted therapy does not yet play a major role, several new agents have been proposed as potential therapies in the DS and non-DS ALL populations, especially in the relapsed/refractory disease setting. Such agents include chimeric antigen receptor T cells, the CD19/CD3 bispecific antibody blinatumomab that redirects cytotoxic T cells to CD19-expressing leukemic cells, and agents targeting JAK2 (i.e., ruxolitinib, momelotinib) or mTOR (i.e., temsirolimus, everolimus) pathways [1].

## Conclusions

There is an increased risk of developing ALL in individuals with Down syndrome. The molecular pathogenesis of DS-ALL involves three predominant molecular aberrations – *CRLF2* overexpression, *JAK2* alterations, and *RAS* mutations, with likely contributions from constitutional trisomy 21. Inferior outcomes are seen in DS-ALL when compared to the general population. While the WHO 2016 does not recognize DS-ALL as a distinct entity, the different clinical, cytogenetic, and molecular characteristics underscore the complexity and differences among DS-ALL and ALL in general pediatric population.

## References

1. Lee P, Bhansali R, Izraeli S, Hijiya N, Crispino JD. The biology, pathogenesis and clinical aspects of acute lymphoblastic leukemia in children with Down syndrome. Leukemia. 2016;30(9):1816–23.
2. Bhatnagar N, Nizery L, Tunstall O, Vyas P, Roberts I. Transient Abnormal Myelopoiesis and AML in Down Syndrome: an Update. Curr Hematol Malig Rep. 2016;11:333–41.
3. Roberts I, Alford K, Hall G, et al. GATA1-mutant clones are frequent and often unsuspected in babies with Down syndrome: identification of a population at risk of leukemia. Blood. 2013;122:3908–17.
4. Roy A, Roberts I, Norton A, Vyas P. Acute megakaryoblastic leukaemia (AMKL) and transient myeloproliferative disorder (TMD) in Down syndrome: a multi-step model of myeloid leukaemogenesis. Br J Haematol. 2009;147:3–12.
5. Swerdlow S, Campo E, Harris NL, et al., editors. WHO classification of haematopoietic and lymphoid tissue (IARC WHO classification of tumours). 4th ed. Geneva: World Health Organization; 2008.
6. Chen CP, Lin SP, Tsai FJ, et al. Transient abnormal myelopoiesis of mosaic trisomy 21 presenting fetoplacental cytogenetic discrepancy, hepatosplenomegaly, oligohydramnios and abnormal hematological findings. Genet Couns. 2008;19:255–8.
7. Mateos MK, Barbaric D, Byatt SA, Sutton R, Marshall GM. Down syndrome and leukemia: insights into leukemogenesis and translational targets. Transl Pediatr. 2015;4:76–92.
8. Gamis AS, Alonzo TA, Gerbing RB, et al. Natural history of transient myeloproliferative disorder clinically diagnosed in Down syndrome neonates: a report from the Children's Oncology Group Study A2971. Blood. 2011;118:6752–9; quiz 6996.
9. Gamis AS, Smith FO. Transient myeloproliferative disorder in children with Down syndrome: clarity to this enigmatic disorder. Br J Haematol. 2012;159:277–87.
10. Kitoh T, Taki T, Hayashi Y, Nakamura K, Irino T, Osaka M. Transient abnormal myelopoiesis in a Down syndrome newborn followed by acute myeloid leukemia: identification of the same chromosomal abnormality in both stages. Cancer Genet Cytogenet. 2009;188:99–102.
11. Bombery M, Vergilio JA. Transient abnormal myelopoiesis in neonates: GATA get the diagnosis. Arch Pathol Lab Med. 2014;138:1302–6.
12. Roy A, Roberts I, Vyas P. Biology and management of transient abnormal myelopoiesis (TAM) in children with Down syndrome. Semin Fetal Neonatal Med. 2012;17:196–201.
13. Maclean GA, Menne TF, Guo G, et al. Altered hematopoiesis in trisomy 21 as revealed through in vitro differentiation of isogenic human pluripotent cells. Proc Natl Acad Sci U S A. 2012;109:17567–72.

14. Tunstall-Pedoe O, Roy A, Karadimitris A, et al. Abnormalities in the myeloid progenitor compartment in Down syndrome fetal liver precede acquisition of GATA1 mutations. Blood. 2008;112:4507–11.
15. Liu B, Filippi S, Roy A, Roberts I. Stem and progenitor cell dysfunction in human trisomies. EMBO Rep. 2015;16:44–62.
16. Pine SR, Guo Q, Yin C, Jayabose S, Druschel CM, Sandoval C. Incidence and clinical implications of GATA1 mutations in newborns with Down syndrome. Blood. 2007;110:2128–31.
17. Zipursky A. Transient leukaemia–a benign form of leukaemia in newborn infants with trisomy 21. Br J Haematol. 2003;120:930–8.
18. Ahmed M, Sternberg A, Hall G, et al. Natural history of GATA1 mutations in Down syndrome. Blood. 2004;103:2480–9.
19. Hollanda LM, Lima CS, Cunha AF, et al. An inherited mutation leading to production of only the short isoform of GATA-1 is associated with impaired erythropoiesis. Nat Genet. 2006;38:807–12.
20. Norton A, Fisher C, Liu H, et al. Analysis of JAK3, JAK2, and C-MPL mutations in transient myeloproliferative disorder and myeloid leukemia of Down syndrome blasts in children with Down syndrome. Blood. 2007;110:1077–9.
21. Klusmann JH, Creutzig U, Zimmermann M, et al. Treatment and prognostic impact of transient leukemia in neonates with Down syndrome. Blood. 2008;111:2991–8.
22. Massey GV, Zipursky A, Chang MN, et al. A prospective study of the natural history of transient leukemia (TL) in neonates with Down syndrome (DS): Children's Oncology Group (COG) study POG-9481. Blood. 2006;107:4606–13.
23. McElwaine S, Mulligan C, Groet J, et al. Microarray transcript profiling distinguishes the transient from the acute type of megakaryoblastic leukaemia (M7) in Down's syndrome, revealing PRAME as a specific discriminating marker. Br J Haematol. 2004;125:729–42.
24. Chisholm KM, Rivetta CV, Heerema-McKenney A. PRAME immunohistochemical staining in transient abnormal myelopoiesis and myeloid leukemia associated with Down syndrome. Ann Clin Lab Sci. 2015;45:121–7.
25. Hasle H, Clemmensen IH, Mikkelsen M. Risks of leukaemia and solid tumours in individuals with Down's syndrome. Lancet. 2000;355:165–9.
26. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127:2391–405.
27. Lange BJ, Kobrinsky N, Barnard DR, et al. Distinctive demography, biology, and outcome of acute myeloid leukemia and myelodysplastic syndrome in children with Down syndrome: Children's Cancer Group Studies 2861 and 2891. Blood. 1998;91:608–15.
28. Hasle H, Niemeyer CM, Chessells JM, et al. A pediatric approach to the WHO classification of myelodysplastic and myeloproliferative diseases. Leukemia. 2003;17:277–82.
29. Henderson R, Spence L. Down syndrome with myelodysplasia of megakaryoblastic lineage. Clin Lab Sci. 2006;19:161–4.
30. Forestier E, Izraeli S, Beverloo B, et al. Cytogenetic features of acute lymphoblastic and myeloid leukemias in pediatric patients with Down syndrome: an iBFM-SG study. Blood. 2008;111:1575–83.
31. Tsuzuki S, Taguchi O, Seto M. Promotion and maintenance of leukemia by ERG. Blood. 2011;117:3858–68.
32. Malinge S, Bliss-Moreau M, Kirsammer G, et al. Increased dosage of the chromosome 21 ortholog Dyrk1a promotes megakaryoblastic leukemia in a murine model of Down syndrome. J Clin Invest. 2012;122:948–62.
33. Birger Y, Goldberg L, Chlon TM, et al. Perturbation of fetal hematopoiesis in a mouse model of Down syndrome's transient myeloproliferative disorder. Blood. 2013;122:988–98.
34. Stankiewicz MJ, Crispino JD. ETS2 and ERG promote megakaryopoiesis and synergize with alterations in GATA-1 to immortalize hematopoietic progenitor cells. Blood. 2009;113:3337–47.
35. Ng AP, Hyland CD, Metcalf D, et al. Trisomy of Erg is required for myeloproliferation in a mouse model of Down syndrome. Blood. 2010;115:3966–9.

36. Kirsammer G, Jilani S, Liu H, et al. Highly penetrant myeloproliferative disease in the Ts65Dn mouse model of Down syndrome. Blood. 2008;111:767–75.
37. Elagib KE, Goldfarb AN. Regulation of RUNX1 transcriptional function by GATA-1. Crit Rev Eukaryot Gene Expr. 2007;17:271–80.
38. Rainis L, Toki T, Pimanda JE, et al. The proto-oncogene ERG in megakaryoblastic leukemias. Cancer Res. 2005;65:7596–602.
39. Chou ST, Byrska-Bishop M, Tober JM, et al. Trisomy 21-associated defects in human primitive hematopoiesis revealed through induced pluripotent stem cells. Proc Natl Acad Sci U S A. 2012;109:17573–8.
40. Letourneau A, Santoni FA, Bonilla X, et al. Domains of genome-wide gene expression dysregulation in Down's syndrome. Nature. 2014;508:345–50.
41. Yoshida K, Toki T, Okuno Y, et al. The landscape of somatic mutations in Down syndrome-related myeloid disorders. Nat Genet. 2013;45:1293–9.
42. Sorrell AD, Alonzo TA, Hilden JM, et al. Favorable survival maintained in children who have myeloid leukemia associated with Down syndrome using reduced-dose chemotherapy on Children's Oncology Group trial A2971: a report from the Children's Oncology Group. Cancer. 2012;118:4806–14.
43. Kolb EA, Meshinchi S. Acute myeloid leukemia in children and adolescents: identification of new molecular targets brings promise of new therapies. Hematology Am Soc Hematol Educ Program. 2015;2015:507–13.
44. Swerdlow SH, Campo E, Pileri SA, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood. 2016;127:2375–90.
45. Proytcheva MA, editor. Diagnostic pediatric hematopathology. Cambridge: Cambridge University Press; 2011.
46. Hertzberg L, Vendramini E, Ganmore I, et al. Down syndrome acute lymphoblastic leukemia, a highly heterogeneous disease in which aberrant expression of CRLF2 is associated with mutated JAK2: a report from the International BFM Study Group. Blood. 2010;115:1006–17.
47. Mullighan CG, Collins-Underwood JR, Phillips LA, et al. Rearrangement of CRLF2 in B-progenitor- and Down syndrome-associated acute lymphoblastic leukemia. Nat Genet. 2009;41:1243–6.
48. Russell LJ, Capasso M, Vater I, et al. Deregulated expression of cytokine receptor gene, CRLF2, is involved in lymphoid transformation in B-cell precursor acute lymphoblastic leukemia. Blood. 2009;114:2688–98.
49. Yoda A, Yoda Y, Chiaretti S, et al. Functional screening identifies CRLF2 in precursor B-cell acute lymphoblastic leukemia. Proc Natl Acad Sci U S A. 2010;107:252–7.
50. Shochat C, Tal N, Bandapalli OR, et al. Gain-of-function mutations in interleukin-7 receptor-alpha (IL7R) in childhood acute lymphoblastic leukemias. J Exp Med. 2011;208:901–8.
51. James C, Ugo V, Le Couedic JP, et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. Nature. 2005;434:1144–8.
52. Vainchenker W, Constantinescu SN. A unique activating mutation in JAK2 (V617F) is at the origin of polycythemia vera and allows a new classification of myeloproliferative diseases. Hematology Am Soc Hematol Educ Program. 2005;2005:195–200.
53. Malinge S, Ben-Abdelali R, Settegrana C, et al. Novel activating JAK2 mutation in a patient with Down syndrome and B-cell precursor acute lymphoblastic leukemia. Blood. 2007;109:2202–4.
54. Bercovich D, Ganmore I, Scott LM, et al. Mutations of JAK2 in acute lymphoblastic leukaemias associated with Down's syndrome. Lancet. 2008;372:1484–92.
55. Gaikwad A, Rye CL, Devidas M, et al. Prevalence and clinical correlates of JAK2 mutations in Down syndrome acute lymphoblastic leukaemia. Br J Haematol. 2009;144:930–2.
56. Kearney L, Gonzalez De Castro D, Yeung J, et al. Specific JAK2 mutation (JAK2R683) and multiple gene deletions in Down syndrome acute lymphoblastic leukemia. Blood. 2009;113:646–8.
57. Nikolaev SI, Garieri M, Santoni F, et al. Frequent cases of RAS-mutated Down syndrome acute lymphoblastic leukaemia lack JAK2 mutations. Nat Commun. 2014;5:4654.

58. Lane AA, Chapuy B, Lin CY, et al. Triplication of a 21q22 region contributes to B cell transformation through HMGN1 overexpression and loss of histone H3 Lys27 trimethylation. Nat Genet. 2014;46:618–23.
59. Thompson BJ, Bhansali R, Diebold L, et al. DYRK1A controls the transition from proliferation to quiescence during lymphoid development by destabilizing Cyclin D3. J Exp Med. 2015;212:953–70.
60. Buitenkamp TD, Mathot RA, de Haas V, Pieters R, Zwaan CM. Methotrexate-induced side effects are not due to differences in pharmacokinetics in children with Down syndrome and acute lymphoblastic leukemia. Haematologica. 2010;95:1106–13.

# Chapter 15
# Inherited and Acquired Myeloid Neoplasms of Childhood

**Kevin E. Fisher and M. Monica Gramatges**

## Introduction

Myeloid neoplasms occurring in childhood encompass a broad spectrum of diseases that include the myeloproliferative neoplasms (MPN), myelodysplastic syndromes (MDS), myelodysplastic/myeloproliferative neoplasms (MDS/MPN), and acute myeloid leukemia (AML). MPNs result from an underlying mutation or gene fusion that is associated with constitutive activation of a signaling pathway, leading to enhanced cell survival or excessive proliferation of relatively differentiated hematopoietic cells. MDS, in contrast, is a clonal disorder affecting progenitor cells of myeloid, erythroid, or megakaryocytic lineage, and in children is typically associated with significant cytopenias and a rapidly progressive disease course. MDS/MPNs are unique in sharing some morphologic and molecular features of both MDS and MPNs. Lastly, AML is an aggressive cancer restricted to the abnormal proliferation and differentiation of myeloid precursors.

In this chapter, we will discuss the epidemiology, clinical features, and associated pathology for myeloid neoplasms observed in childhood, including recent advances in our understanding of genetic contributors to disease risk and pathophysiology. Given the intimate association of childhood MPN/MDS/AML and inherited susceptibility, mutations associated with genetic predisposition will be discussed, as well as diagnostic implications for potentially at-risk family members. The focus of this chapter will be on both inherited and acquired pediatric MDS, but for MDS/MPN, MPN, and AML will be restricted to inherited risk, as many of these

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disease entities are discussed in greater detail elsewhere. Notably, according to the 2016 revision of the World Health Organization (WHO) classification of myeloid neoplasms, current recommendations are that underlying germline mutations, if present, be included in the diagnostic classification [1].

## Inherited Myeloproliferative Neoplasms and Myelodysplastic/ Myeloproliferative Neoplasms of Childhood

MPNs are a heterogeneous group of clonal hematopoietic stem cell disorders characterized by proliferation in one or more of the myeloid lineages [2]. They include disorders discussed in more detail in other chapters, such as chronic myeloid leukemia (CML), chronic neutrophilic leukemia, chronic eosinophilic leukemia, not otherwise specified, MPN unclassifiable, mastocytosis, polycythemia vera (PV), primary myelofibrosis (PMF), and essential thrombocythemia (ET). After mastocytosis, which has a strong childhood predilection, CML is the most common MPN encountered in pediatric patients, comprising 2–5% of pediatric leukemias [3]. Despite their rarity in children, there are several well-recognized genetic changes associated with hereditary MPNs that merit discussion here.

The morphologic features of childhood MPNs are virtually indistinguishable from their adult counterparts and they share a similar clinical presentation with a few notable exceptions. Though not the focus of this section, there are notable dissimilarities in the spectrum of acquired mutations observed in children diagnosed with MPN compared with adults. For example, acquired Janus Kinase 2 (*JAK2*) p.V617F or *JAK2* exon 12 mutations are detected in essentially all adult cases of PV [4], but occur in only 30% of pediatric PV cases [5]. Also, a large subset of adult onset ET and PMF cases can be characterized by somatic calreticulin (*CALR*) exon 9 frameshift mutations leading to aberrant translation of the CALR protein C-terminus in adult populations [6, 7]. However, *CALR* exon 9 mutations are rarely detected in pediatric MPN cases [8–10]. In contrast, acquired mutations in the thrombopoietin receptor gene *MPL* are detected in approximately 5% of adult ET, and are equally rare in pediatric ET [11]. However, inherited *MPL* mutations are associated with congenital amegakaryocytic thrombocytopenia (discussed below), and dominant-negative *MPL* mutations can cause a rare form of familial ET [12].

Next-generation sequencing (NGS) discovery efforts have provided some insight into the somatic mutation spectrum of pediatric MPNs. Investigators recently identified novel and recurrent mutations in the *IRF8* gene, a gene that encodes a transcription factor in the interferon regulatory factor family, in addition to mutations in *JAK2* and *CALR*, and *MPL*, in a population of children diagnosed with MPN. Fewer than expected mutations in genes involved in epigenetic regulation were also observed in comparison with adult MPN populations. Moreover, in this study, no mutations were detected in one third of pediatric MPN, compared with 8% of adult MPN [13]. Thus, despite efforts, the somatic alterations that drive MPNs of childhood remain largely uncharacterized.

Although a vast majority of MPNs are thought to arise de novo, there is growing evidence that a significant proportion are associated with inherited predisposition. A large epidemiological study from Sweden demonstrated a five- to seven-fold elevated risk of MPNs among first-degree relatives of MPN patients [14]. The *JAK2* 46/1 "GGCC" haplotype, a region of *JAK2* spanning introns 10–15 characterized by four single nucleotide polymorphisms (SNPs) in complete linkage disequilibrium, is associated with a predisposition to *JAK2*- and *MPL*-positive MPNs [15, 16]. Two SNPs in *JAK2* and *MECOM* (MDS1 and EVI1 complex locus), respectively, are associated with an increased risk of *JAK2*-negative MPNs [17]. Activating *JAK2* mutations are key initiating molecular events in the pathogenesis of PV, although additional mutations are required to cause disease [18]. The rarity of childhood PV and acquired *JAK2* mutations, as well as the discovery of germline MPN susceptibility variants, is consistent with this observation. Additional germline mutations associated with increased risk of MPN include an intronic SNP in the telomerase reverse transcriptase (*TERT*) gene associated with a twofold risk of developing MPN [19, 20], and mutations in retinoblastoma binding protein 6 (*RBBP6*), believed to contribute to approximately 5% of familial MPN [21].

Therapeutic interventions for pediatric MPNs are generally less aggressive, likely due to the limited knowledge of disease natural history in these rare conditions, as well as limited evidence for use of investigational agents in a pediatric rare disease population. For example, in pediatric ET, asymptomatic children are generally observed without intervention, and in PV, phlebotomy is typically recommended as an initial intervention. However, when diagnosing and treating MPN in pediatric patients, consideration should be given to genetic syndromes predisposing to specific MPN subtypes, in particular Noonan syndrome, neurofibromatosis type 1, and Down syndrome (each discussed in more detail below).

## *Juvenile Myelomonocytic Leukemia in Association with Noonan Syndrome and Neurofibromatosis Type 1*

Children with both Noonan syndrome and neurofibromatosis type 1 (NF1) are at increased risk of developing an MPN that is similar to JMML. Noonan syndrome is a genetic disorder characterized by heart defects, short stature, learning problems, and specific facial features. Children with Noonan syndrome are at an increased risk of developing a JMML-like disorder (~10%) [22]. JMML in the setting of Noonan syndrome presents far earlier compared with sporadic JMML (<2 months vs. 1.8 years) and is characterized by a polyclonal myelopoiesis stemming from germline mutations in *PTPN11* [23]. *PTPN11* encodes a protein tyrosine phosphatase (protein tyrosine phosphatase, non-receptor type 11) that regulates mitogenic activation, metabolic control, transcription regulation, and cell migration. Differentiating sporadic JMML from JMML in association with Noonan syndrome is critical, as sporadic JMML is universally fatal in the absence of stem cell transplantation (SCT), whereas JMML in association with Noonan syndrome typically undergoes

spontaneous regression. Thus, in the setting of Noonan syndrome, chemotherapy and/or SCT are typically not recommended.

NF1 (von Recklinghausen disease) is an inherited autosomal dominant neurocutaneous genetic disorder characterized by café-au-lait macules, neurofibromas, skinfold freckling, iris hamartomas (Lisch nodules), optic gliomas, and skeletal deformities [24]. Neurofibromatosis occurs as a result of inactivating germline mutations in the neurofibromin 1 gene (*NF1*), leading to hyperactive RAS signaling and downstream ERK activation. Children with this diagnosis have a 200- to 500-fold increased risk of developing a myeloid malignancy [25], and comprise 10–15% of children diagnosed with JMML [26].

Neurofibromatosis, Noonan syndrome, and JMML are all considered "RASopathies" due to their shared inheritance of RAS pathway gene mutations and propensity for malignancy. The other RASopathies such as Noonan-like CBL syndrome, Costello syndrome, cardiofaciocutaneous (CFC) syndrome, and Legius syndrome similarly predispose to a range of malignancies and abnormal myelopoiesis in infancy [27]. Another example is the association between germline mutations in *CBL*, a gene that encodes an E3 ubiquitin ligase and acts as a key negative regulator of the RAS signaling pathway, and increased risk of development of JMML [28, 29].

Importantly, however, activating mutations in RAS pathway genes are also detected in nonmalignant conditions. For example, identical *KRAS* and *NRAS* mutations are observed in both JMML and in Ras-associated autoimmune leukoproliferative disorder (RALD). Although RALD shares clinical and morphologic features with JMML and CMML, this disease follows an invariably indolent course. The acquisition of additional cooperating somatic mutations in JMML is hypothesized to contribute to the disparate clinical manifestations of these entities [30].

## *Transient Abnormal Myelopoiesis in Association with Down Syndrome*

Children with Down syndrome due to trisomy 21 are at increased risk of developing transient abnormal myelopoiesis (TAM), which is characterized by a clonal proliferation of myeloid blasts and is associated with acquired mutations in *GATA1* (the gene that encodes GATA Binding Protein 1) [31, 32]. Although patients may present with hepatomegaly, splenomegaly, jaundice, or effusions, infants are asymptomatic in approximately 10–25% of cases. Of note, TAM may be the first clinical manifestation of Down syndrome.

Blasts in TAM typically exhibit characteristic features of megakaryoblastic differentiation including round to ovoid nuclei, dispersed chromatin, small nucleoli, and deeply basophilic cytoplasm with cytoplasmic blebbing. Blasts express stem cell, myeloid, nonlineage, and megakaryocytic/blastic markers immunophenotypically, although no blast percentage threshold has been established for the diagnosis of TAM [33]. If TAM is suspected, somatic mutations in exons 2 and 3 of *GATA1* support a clonal proliferation, and constitutional testing to exclude trisomy 21 mosaicism should also be considered.

The majority of Down syndrome infants diagnosed with TAM experience spontaneous regression within 1–3 months and do not require chemotherapeutic intervention [34]. However, 20–30% of Down syndrome infants diagnosed with TAM progress to develop acute megakaryoblastic leukemia by 5 years of age [35], a risk that is likely associated with additional acquired driver mutations [36]. Therefore, following a diagnosis of TAM, close clinical monitoring is warranted at least until the age of 5 years.

In conclusion, the absence of consistent genetic drivers, both germline and somatic, and differences in disease pathophysiology distinguish pediatric MPN and pediatric MDS/MPN from adult MPN and adult MDS/MPN. These observations underscore the necessity of cooperative patient registries for these rare diseases, and require additional investigation to elucidate the molecular pathogenesis and clinical outcomes of childhood MPNs and MDS/MPNs. Until that time, the accurate diagnosis and clinical management of these myeloid neoplasms in children requires comprehensive clinical and laboratory data, a differential diagnosis that includes underlying constitutional syndromes (e.g., Noonan syndrome), and the meticulous exclusion of reactive secondary disorders [37].

## *Acquired Childhood Myelodysplastic Syndrome*

The overall incidence of myelodysplastic syndrome (MDS) of childhood is quite rare, with only 1–2 cases per million. The disease has an equal male/female distribution and a median age at presentation of 6.8 years [38, 39]. Pediatric MDS often presents with clinical symptoms associated with cytopenias such as fatigue, infection, and bleeding. However, unlike adults who commonly present with isolated anemia [40], children with MDS more commonly present with two or more cytopenias [41]. Moreover, in contrast to the relatively indolent disease course observed in adult MDS, pediatric MDS is often progressive, transforming to AML in 10–20% of cases [42].

Marked bone marrow hypocellularity is a hallmark of MDS in childhood. Nearly half of the cases are classified as refractory cytopenia of childhood (RCC), an MDS subtype characterized by dysplastic changes in 2 hematologic lineages, or >10% dysplasia in one lineage, with marked decreases in megakaryocytes [2]. The diagnostic criteria of RCC, a provisional entity in the 2016 WHO revision, are discussed in detail elsewhere. Other non-neoplastic disorders are often morphologically indistinguishable from RCC, such as viral infections, vitamin B12/folate deficiencies, metabolic disorders, and inherited bone marrow failure disorders [40]. Thus, initial evaluation should always exclude possible secondary causes. Careful attention should also be paid to both physical exam features and family history, as childhood MDS may present with congenital anomalies or bone marrow failure suggestive of an inherited bone marrow failure syndrome (IBMFS), and should trigger a genetic evaluation for such syndromes (e.g., Fanconi anemia, dyskeratosis congenita, and Shwachmann-Diamond syndrome, discussed in more detail below).

Monosomy 7 is the most common acquired cytogenetic abnormality in childhood MDS, although RCC typically exhibits a normal karyotype. Unlike adult MDS, mutations in genes encoding RNA spliceosome machinery are rare events in pediatric MDS [43]. However, mutations in genes involved in kinase signaling and transcriptional regulators such as *RAS* and *GATA2*, respectively, are often detected [44]. Of note, germline *GATA2* mutations have been detected in ~10% of a pediatric MDS cohort lacking features of an underlying disorder [45], suggesting a need for germline genetic testing when rendering a diagnosis of de novo MDS in a pediatric patient (discussed in more detail below) [46].

Prognosis of pediatric MDS is generally poor, with the only curative therapy being SCT. Steadily worsening cytopenias, transfusion dependence, multilineage dysplasia, increasing blast count, or detection of a clonal cytogenetic abnormality suggests heightened risk of progression to AML, such that proceeding to SCT is recommended as soon as possible [47]. This is because SCT outcomes are best when performed early, without pre-SCT chemotherapy, and prior to disease progression [48, 49]. Meanwhile, standard supportive care should be offered with transfusions and antimicrobial prophylaxis, as well as a high index of suspicion and rapid empiric therapy for infections related to neutropenia.

## *Acute Myeloid Leukemia and Myelodysplastic Syndrome with Germline Predisposition*

Less than 5% of all cases of AML or MDS may be attributed to genetic predisposition conferred either by an underlying diagnosis of an inherited bone marrow failure syndrome (IBMFS) or by a single gene defect that has been associated with significantly increased risk of hematologic malignancy. A significant update to the 2016 revision of the WHO classification now includes myeloid neoplasms with germline predisposition, such as MDS, MDS/MPN, and acute leukemias that occur in the setting of a predisposing germline mutation. It is recommended that the specific underlying genetic defect or predisposition syndrome be noted as part of the pathologic diagnosis [1]. This section will discuss the IBMFS and disorders of DNA repair that are associated with an increased risk of MDS/AML among other cancers, as well as genetic alterations predisposing to familial risk of MDS/AML. A summary of each of these disorders is provided in Table 15.1.

### Inherited Bone Marrow Failure Syndromes

The IBMFS are a heterogeneous group of rare disorders characterized by ineffective hematopoiesis and cancer predisposition. The etiology of these disorders ranges from telomere dysfunction (dyskeratosis congenita) to defects in DNA repair (Fanconi anemia) or ribosome assembly/function (Diamond-Blackfan anemia and

**Table 15.1** Conditions with hereditary predisposition toward acute myeloid leukemia and myelodysplastic syndrome

| | Mode of inheritance | Gene | Percentage of affected individuals who develop AML/MDS | Associated hematologic findings |
|---|---|---|---|---|
| Inherited bone marrow failure syndromes | | | | |
| Diamond-Blackfan anemia | AD | *RPS7*<br>*RPS17*<br>*RPS19*<br>*RPS24*<br>*RPL5*<br>*RPL11*<br>*RPL35A* | 2–5% | Anemia and reticulocytopenia, macrocytosis, mild neutropenia, elevated HbF, elevated erythrocyte adenosine deaminase activity |
| Shwachman-Diamond syndrome | AR | *SBDS* | 10–30% | Intermittent neutropenia, anemia, elevated HbF, thrombocytopenia |
| Severe congenital neutropenia | AD | *ELANE* | 20–40% | Neutropenia |
| | AR | *CSF3R*[a]<br>*HAX1*<br>*GF11*<br>*G6PC3*<br>*STK4* | | |
| | XLR | *TAZ*<br>*WAS* | | |

(continued)

**Table 15.1** (continued)

| | Mode of inheritance | Gene | Percentage of affected individuals who develop AML/MDS | Associated hematologic findings |
|---|---|---|---|---|
| Congenital amegakaryocytic thrombocytopenia | AR | *MPL* | Unknown | Thrombocytopenia with normal size platelets |
| Dyskeratosis congenita | XL | *DKC1* | 15–30% (90% lifetime prevalence of bone marrow failure) | Cytopenias |
| | AD | *TERC*<br>*TINF2* | | |
| | AR | *NOP10*<br>*CTC1*<br>*PARN*<br>*WRAP53*<br>*NHP2* | | |
| | AR or AD | *ACD*<br>*TERT*<br>*TREL1*<br>*ACD*<br>*RTEL1*<br>*TERT* | | |

| | | | | |
|---|---|---|---|---|
| DNA repair deficiency syndromes | | | | |
| Fanconi anemia | AR | *FANCA*<br>*FANCC*<br>*BRCA2 (FANCD1)*<br>*FANCD2*<br>*FANCE*<br>*FANCF*<br>*FANCG*<br>*FANCI*<br>*FANCJ*<br>*FANCL*<br>*FANCM*<br>*PALB2 (FANCN)*<br>*RAD51C (FANCO)*<br>*SLX4* | 40–50% | Cytopenias, elevated HbF, macrocytosis, or none prior to hematologic malignancy |
| | XLR | *FANCB* | | |
| Bloom syndrome | AR | *BLM* | 25% | None prior to hematologic malignancy |
| Li-Fraumeni syndrome | AD | *TP53* | 5–10% | None prior to hematologic malignancy |
| Familial AML/MDS syndromes | | | | |
| Familial MDS and AML with mutated *GATA2* | AD | *GATA2* | 70% | Mild cytopenias including chronic neutropenia.<br>Commonly B-/NK-cell lymphopenia, and monocytopenia |
| Familial monosomy 7 | AD | Multiple | Unknown | Cytopenias |
| Familial platelet disorder with propensity to myeloid malignancy (FDP/AML) | AD | *RUNX1* | 20–60% | Mild to moderate thrombocytopenia<br>Normal platelet size ± platelet function abnormalities |

(continued)

**Table 15.1** (continued)

| | Mode of inheritance | Gene | Percentage of affected individuals who develop AML/MDS | Associated hematologic findings |
|---|---|---|---|---|
| Thrombocytopenia 2 | AD | *ANKRD26* | Unknown | Chronic thrombocytopenia Normal platelet size with platelet dysfunction increased plasma TPO |
| Thrombocytopenia 5 | AD | *ETV6* | Unknown | Chronic thrombocytopenia with normal platelet size, macrocytosis |
| Familial aplastic anemia/ MDS with *SRP72* mutation | AD | *SRP72* | Unknown | None prior to hematologic malignancy |
| Familial AML with *CEPBA* mutation | AD | *CEBPA* | 90%, AML only | None prior to hematologic malignancy |
| Familial AML with mutated *DDX41* | AD | *DDX41* | Unknown | None prior to hematologic malignancy |

*Abbreviations*: *AML* acute myeloid leukemia, *MDS* myelodysplastic syndrome, *AD* autosomal dominant, *AR* autosomal recessive, *XLR* X-linked recessive, *BMF* bone marrow failure, *AA* aplastic anemia, *TPO* thrombopoietin, *HbF* hemoglobin F

[a]Inherited *CSF3R* mutations are rare, acquired mutations are much more frequent [50, 51]

Shwachman-Diamond syndrome). Other disorders falling into this category, albeit with less definitive risk of MDS/AML, include severe congenital neutropenia and congenital amegakaryocytic thrombocytopenia.

Clinical presentation is typically with one or more cytopenias, which may or may not be associated with clinical features classic of the specific disorder. In all cases, the treatment of choice is SCT as early as possible from a matched related donor who is not a carrier of the disease. Therefore, it is imperative that all potential familial donors be screened for the genetic defect identified in the index case. Disease-specific recommendations for SCT should also be considered [52]. In the absence of an appropriate matched related or unrelated donor, supportive care may be offered in the form of transfusion support and antimicrobial prophylaxis, as appropriate.

### Diamond-Blackfan Anemia

Diamond-Blackfan anemia (DBA) is a rare congenital disorder characterized by a pure red cell aplasia, first noted by Hugh Josephs in 1936, and later defined as congenital hypoplastic anemia by Drs. Diamond and Blackfan in 1938 [53]. In 1999, a variety of mutations associated with this disease were identified in a cohort of unrelated DBA patients, all occurring in a single gene encoding ribosomal protein S19 (*RPS19*) [54]. Mutations in *RPS19* are now recognized as comprising 25% of the genetic etiology in DBA, although haploinsufficiency in at least 10 different ribosomal proteins, including both the small, 40S subunit and the large, 60S subunit are described [55]. Of note, 40% of DBA cases do not harbor mutations in these genes, suggesting that additional undescribed mutations may contribute to the pathophysiology of this disorder.

The diagnostic criteria for DBA include anemia presenting at less than 1 year of age with near normal, but variable, neutrophil and/or platelet counts, reticulocytopenia, macrocytosis, and normal marrow cellularity with a paucity of red cell precursors [56]. The mean age of DBA presentation is early, between 2 and 3 months of age, with 50% demonstrating associated physical abnormalities that are predominantly craniofacial, including flat nasal bridge, hypertelorism, and high arched palate [57]. Anomalies of the thumb may also be observed. Acquired transient erythroblastopenia of childhood (TEC) can mimic DBA, but TEC is generally observed in older patients (greater than 1 year of age) and lacks associated congenital anomalies. In addition, in TEC the mean corpuscular volume is typically normal. In making a diagnosis of DBA, the presence of other inherited syndromes or immune-mediated diseases, infectious agents, and drugs and toxins should also be excluded [58].

The estimated incidence of malignancy in DBA is approximately 3–5%, with a median age of first cancer diagnosis at 15 years [range 1–43 years]. Approximately half of all described malignancies are AML or MDS. Individuals with DBA who do not receive a SCT are also at an increased risk of solid tumors [59, 58]. In approximately 75% of DBA patients, the anemia responds, at least initially, to steroid therapy, reducing the need for transfusions [60]. Furthermore, spontaneous remission

or improvement to a mild macrocytic anemia may occur even late in the disease course [61]. SCT may be limited to steroid refractory cases, though in cases of children with a matched related donor lacking the disease, upfront early SCT should be considered [52].

### Dyskeratosis Congenita

Dyskeratosis congenita (DC) is a disorder of telomere maintenance characterized by exceedingly short telomeres and predisposition to bone marrow failure and cancer. Telomeres are the DNA-protein repeats forming protective caps on chromosome ends, serving to protect against DNA degradation, fusions, or recombination. Mutations in telomerase reverse transcriptase (*TERT*), the reverse transcriptase responsible for counteracting the telomere loss that occurs with DNA replication, were first described in association with DC in 1999 [62]. However, numerous related proteins are required for telomerase assembly, trafficking, and recruitment to telomeres. Therefore, although 50% of the underlying mutations associated with this disease are in *TERT*, *TERC*, *DKC1*, and *TINF2*, causative mutations have thus far been identified in 11 genes involved in telomere maintenance and extension (Table 15.1) [63].

Like the other IBMFS, DC is a rare disease described in approximately 400 families worldwide. The classic clinical triad consists of mucocutaneous abnormalities including nail defects, a lacy reticular rash of the neck and upper chest, and oral leukoplakia. Patterns of inheritance for DC are varied, and specific to the causative gene (Table 15.1). Interestingly in the case of specific genes, there is evidence for disease anticipation with shorter telomeres and increasingly severe symptoms as well as earlier disease presentation observed in successive generations [64].

Individuals with DC have a near 50% cumulative lifetime risk of malignancy that includes solid tumors, particularly oropharyngeal cancers, and hematologic malignancies. Notably, DC patients are at 200-fold risk of developing AML and at 1500-fold risk of developing MDS compared with the general population [65]. Bone marrow failure is highly prevalent, with an over 90% lifetime risk. Bone marrow failure, or even AML, may be the presenting feature with or without the associated classic clinical features.

Clinical presentation with cytopenias should prompt bone marrow evaluation, as well as a thorough physical exam that includes the nails, skin, and oropharynx. The bone marrow findings in DC range from normal to variable stages of aplasia depending on the stage of the disease and may be indistinguishable from aplastic anemia [66]. Obtaining a family history for malignancy and related telomere biology disorders, including pulmonary fibrosis and hepatic cirrhosis, is also recommended. If a high index of suspicion for DC exists, blood should be sent for leukocyte telomere length testing using multicolor flow fluorescence in situ hybridization (telomere flow FISH). This test is highly suggestive of DC, as telomere length less than the first percentile for age in lymphocytes is 97% sensitive and 91% specific for this diagnosis [67]. Follow-up testing should then include molecular

testing for a causative mutation, either by serial single-gene testing or by use of a multi-gene panel. However, even in patients meeting clinical criteria, an underlying genetic mutation is only discovered in approximately 60% of cases.

If a diagnosis of DC is made and an unaffected matched related donor is available, SCT with reduced conditioning is recommended as initial therapy, due to the highly prevalent feature of bone marrow failure and significantly increased cancer risk [52, 68]. If no donor is available, supportive care should be offered in addition to cancer surveillance and consideration of androgens to mitigate transfusion requirements [69].

### Fanconi Anemia

Fanconi anemia (FA) was first described in 1927 in three brothers with fatal refractory anemia [70]. The disease is now known to arise from mutations in genes involved in double-stranded DNA repair and homologous recombination, and over 15 genes are now associated with the FA phenotype (see Table 15.1). Mutations in the Fanconi Anemia Complementation Group A (*FANCA*) gene account for almost two-thirds of cases, *FANCC* and *FANCG* for 25%, and *FANCE* and *FANCF* an additional 8% [71]. The molecular hallmarks of FA include chromosomal cytogenetic instability, increased chromosomal breakage, and defective DNA repair. FA cells demonstrate hypersensitivity to DNA cross-linking agents, a feature that can be leveraged to assess for FA by culturing peripheral blood T lymphocytes or fibroblasts in the presence of cross-linking agents mitomycin C or diepoxybutane, and quantifying the number of chromosomal aberrations observed in metaphase spreads [72].

FA is inherited in an autosomal recessive pattern, with the sole exception being X-linked recessive inheritance of *FANCB* mutations. The estimated incidence is 1 in 360,000 in the general population and 1 in 30,000 in the Ashkenazi Jewish population. The ethnic background of the proband has implications for constitutional genetic testing. For example, homozygous splice site mutations of *FANCC* c.711+4A>T are detected in 80% of FA patients of Ashkenazi Jewish descent, so testing strategies often prioritize mutation testing based on positive predictive value [73]. However, de novo missense, nonsense, and frameshift mutations are described in many genes, so comprehensive assessment typically involves a combination of sequential targeted and broad sequencing strategies [74]. Clinical findings can be discordant among siblings, so all family members should be genetically tested for FA mutations to identify at-risk individuals [75].

FA patients are phenotypically characterized by short stature, cytopenias, skeletal (radial) anomalies, and a high risk of BMF and myeloid malignancies. FA patients have a 600-fold increase in risk of AML and more than a 5000-fold risk increase for MDS compared to the general population [76]. Leukemia often presents in childhood and adolescence (median 11–14 years), and almost all cases arise before the age of 25 years. As has been shown in registry data, those cases lacking congenital anomalies are at highest risk of developing both AML and solid tumors,

and are therefore more likely to experience cancer as their initial event [77]. A diagnosis of FA may also be suspected following diagnosis of leukemia with associated complex cancer cytogenetics or excessive therapy-related toxicities [78].

Bone marrow failure is a prominent hematologic manifestation of FA that usually presents within the first two decades of life. A detailed initial cytogenetic bone marrow analysis is critical, and should specifically include FISH for gains of chromosome 3q [71]; gains of 3q are directly associated with progression from MDS to AML [79]. Other worrisome chromosomal aberrations include 1q gains, 7q losses, and cryptic *RUNX1* translocations, deletions, or mutations [80]. Yearly bone marrow morphology and cytogenetics monitoring is appropriate for FA patients without cytopenias, dysplasia, or karyotypic abnormalities [81]. Early SCT is generally recommended, though tissue damage should be limited through the use of reduced conditioning in order to reduce the risk of additional malignancies [52].

### Shwachman-Diamond Syndrome

Shwachman-Diamond syndrome (SDS) is an autosomal recessive disease caused by mutations in the Shwachman-Bodian-Diamond syndrome (*SBDS*) gene [82]. The estimated prevalence worldwide is 1:350,000, with a distinct female/male predilection of 1.7:1. The *SBDS* gene is involved in ribosome biogenesis, and mutations cause defective ribosome assembly, maturation, and function [83]. Deleterious *SBDS* mutations may manifest as bone marrow failure, exocrine pancreatic insufficiency, and skeleton abnormalities; the syndrome was first described in association with pancreatic insufficiency in 1964 [84]. Mutations can be missense, nonsense, or frameshift, and sequencing of exon 2 detects at least one mutation in up to 90% of SDS patients [85].

SDS patients have a 10–30% lifetime risk of developing AML/MDS, often presenting in childhood and adolescence (median age of 14 years, range 1.5–43 years). Although AML/MDS can be of any subtype, acute erythroid leukemia is particularly common, occurring in about 30% of cases with a classifiable leukemia. Interestingly, there does not appear to be an increased risk of developing solid tumors or other malignancies. Almost all SDS patients (90–100%) present with either a chronic or intermittent neutropenia initially. A normochromic-normocytic or macrocytic anemia with reticulocytopenia and/or thrombocytopenia are also common presenting symptoms. Other associated clinical features include immunologic, hepatic, and cardiac disorders [86–88].

Bone marrow findings are variable and may reveal a hypocellular, normocellular, or hypercellular marrow [86]. Cytogenetic abnormalities of chromosome 7 and deletions of 20q are common, seen in approximately 60% and 15% of SDS patients, respectively. Importantly, isochromosome 7q [i(7q)] is an extremely uncommon cytogenetic abnormality in non-SDS MDS, AML, or ALL patients, suggesting that it is a fairly specific marker for this syndrome. However, i(7q) appears to be an indolent chromosome 7 abnormality with a limited risk of progression to AML, whereas other chromosome 7 abnormalities are associated with progression to AML [89, 90]. Thus, i(7q) may not represent a true clonal aberration, but may rep-

resent breakage of a fragile chromosomal site secondary to mutated SBDS and/or its pseudogene SBDSP [87].

As with the other IBMFS, close monitoring for cytopenias is recommended, with bone marrow biopsy as indicated. In the setting of marrow failure, SCT prior to the development of AML/MDS has been associated with better outcomes [91]. Supportive care strategies for managing neutropenia, including use of granulocyte colony-stimulating factor, may be considered. Given the associated clinical manifestations of SDS, patients should also be followed by a gastroenterologist for management of exocrine pancreatic insufficiency, as well as an endocrinologist to address any underlying endocrine problems [86].

### Severe Congenital Neutropenia

Severe congenital neutropenia (SCN) is a rare disease with an estimated incidence of 1:200,000, which was originally described as infantile agranulocytosis by Rolf Kostmann in 1956 [92]. SCN is most frequently associated with autosomal dominant, heterozygous mutations in the neutrophil elastase gene (*ELANE*) [93]. However, similarly to other IBMFS, mutations in numerous other genes related to myeloid differentiation are associated with the disease, and the inheritance pattern can be autosomal dominant, autosomal recessive, or X-linked recessive, depending on the affected gene (Table 15.1) [94]. In approximately one-third of cases, the genetic cause is unknown, and sporadic cases may also occur. Mutations in *ELANE* often result in a fully translated polypeptide; therefore, rather than haploinsufficiency, alternative hypotheses for disease pathophysiology have been proposed, including protein mislocalization or misfolding [94]. Although both cyclic neutropenia and SCN predominantly result from heterozygous mutations in *ELANE*, only SCN is associated with profound neutropenia and clinical features such as aphthous stomatitis and bacterial infections, though clinical severity is variable.

The risk of MDS/AML in SCN approaches 40% at 10 years, with an increased risk observed in those receiving chronic granulocyte colony-stimulating factor therapy [95]. Acquired *CSF3R* (colony-stimulating factor 3 receptor) mutations are detected in approximately 80% of SCN patients who develop MDS/AML, and together with *RAS* mutations, are implicated in leukemogenesis [96–98]. Germline *ELANE* p.C151Y and p.G214R mutations confer the highest risk of evolution to AML [99]. Thus, early SCT should be considered, especially if these specific inherited mutations are present and there is an unaffected related donor available.

### Congenital Amegakaryocytic Thrombocytopenia

Congenital amegakaryocytic thrombocytopenia (CAMT) is a rare autosomal recessive cause of inherited bone marrow failure secondary to germline mutations in *MPL*, the gene that encodes the thrombopoietin (TPO) receptor [100–102]. There have been approximately 100 cases reported in the literature since the first description of “congenital essential thrombocytopenia” by Greenwald and Sherman

in 1929 [103]. A founder c.79+2T>A splice site mutation in the Ashkenazi Jewish population confers a predicted incidence of CAMT in this ethnic group of approximately 1 in 22,500 pregnancies [104]. There is some evidence that CAMT predisposes to AML/MDS, but given the rarity of this condition, the association is not as definitive as with other bone marrow failure syndromes [105].

The *MPL* mutation spectrum correlates with distinct clinical phenotypes. Type I CAMT is more severe, with constantly low platelet counts and early onset of pancytopenia. Conversely, type II CAMT is characterized by a transient increase in platelet counts during the first year of life and or little or no development of pancytopenia [102]. Type I CAMT occurs as a result of deletions, splice site, frameshift, and nonsense mutations in *MPL* that lead to complete loss of function of the TPO receptor, whereas missense mutations that retain some degree of TPO receptor function are associated with type II [102].

Bone marrow aspirates from infants with CAMT demonstrate reduced or absent megakaryocytes in an otherwise normocellular marrow, with the remaining megakaryocytes appearing small or immature [102]. Later in the course of the disease, patients develop pan hypocellular marrow with decreased progenitors in all lineages so that it is difficult to distinguish CAMT from other forms of aplastic anemia. Plasma TPO levels in children with CAMT are typically very high, often tenfold or more above controls. Clinical management focuses on primarily supportive management of thrombocytopenia and SCT for definitive treatment [106].

### DNA Repair Disorders

An increased risk of MDS/AML in the absence of other hematologic features is associated with certain disorders of DNA repair, including Bloom syndrome and Li-Fraumeni syndrome.

### Bloom Syndrome

Bloom syndrome is a rare autosomal recessive genetic disorder typically seen in the Ashkenazi Jewish population, with approximately 300 known cases. David Bloom first described the condition in 1954 in 3 children with short stature, skin rash, and photosensitive bullous lip lesions [107]. Homozygous mutations in the *BLM* gene encoding the DNA repair enzyme Bloom Syndrome RecQ-like helicase lead to impaired DNA replication and repair. These defects predispose patients to AML, lymphoma, gastrointestinal and genitourinary malignancies, as well as cutaneous tumors. Characteristic narrow facial features, elongated limbs, and several dermatologic complications including photosensitivity, poikiloderma, and telangiectatic erythema are also hallmarks of this disease. Notably, acute leukemia is the most common malignancy that presents before the age of 20 [108]. Both AML and MDS can arise in Bloom syndrome, and loss or partial loss of chromosome 7 is a common cytogenetic finding [109].

### Li-Fraumeni Syndrome

Li-Fraumeni syndrome (LFS) is a hereditary cancer predisposition syndrome originally described in a cohort of 24 families by Frederick Li, Joseph Fraumeni, and colleagues in 1988 [110]. The overall incidence is unknown, but approximately 400 families are described in the literature to date. LFS is caused by autosomal dominant germline mutations in the Tumor Protein p53 (*TP53*) gene [111]. *TP53* is a tumor suppressor involved in cell cycle arrest, apoptosis, senescence, and DNA repair. Over 250 familial missense, nonsense, and frameshift mutations have been described throughout the *TP53* gene, though the majority occur within exons 5–8, leading to a functional impairment of the p53 DNA-binding domain [112]. The lifetime risk of malignancy is 90% for women and 70% for men. Soft tissue and bone sarcomas, breast, and central nervous system tumors are the 3 most common malignancies observed [113]. Leukemia, primarily of lymphoid origin, occurs rarely in LFS patients, with an approximate incidence of 5% [110, 114]. However, both AML and therapy-related MDS arising have been reported in LFS pedigrees [115].

### Familial MDS/AML with a Single Gene Conferring Inherited Risk

Lastly, in the past few decades, research and clinical application of next-generation sequencing strategies to familial AML/MDS pedigrees has uncovered causative mutations in genes previously unrecognized as contributing to disease pathogenesis. Though inherited AML/MDS remains relatively rare, these discoveries of single gene disorders provide an opportunity to better understand key pathways underlying disease susceptibility and risk of progression. These disorders are discussed in greater detail below.

### Familial Myelodysplastic Syndrome and Acute Leukemia with Mutated *GATA2*

Heterozygous germline mutations in *GATA2*, which encodes GATA Binding Protein 2, a zinc finger transcription factor that regulates hematopoiesis and lymphatic development, contribute to a significant subset of familial MDS/AML and pediatric MDS [116–119]. Germline GATA2 haploinsufficiency is also implicated in monocytopenia and mycobacterial infections; dendritic cell, monocyte, B, and natural killer lymphoid deficiency; and Emberger syndrome (primary lymphedema with MDS) and their accompanying infectious, pulmonary, dermatologic, neoplastic, and vascular/lymphatic manifestations [120]. Familial AML/MDS secondary to *GATA2* germline mutations will be discussed here; inclusion of other manifestations of GATA2 deficiency is beyond the scope of this chapter.

The high association of germline *GATA2* mutations in pediatric MDS (*GATA2*-MDS) was recently described following sequencing of 500 children and adolescents diagnosed with primary or secondary MDS. Germline *GATA2* mutations were found in 7% of primary MDS cases, now considered the most common germline defect predisposing to pediatric MDS [45]. Moreover, *GATA2* mutations were more common in cases of advanced disease, comprising 15% of this MDS subset, and were highly prevalent in cases with concomitant monosomy 7. Mutations in *GATA2* were rarely observed in MDS secondary to therapy or with acquired aplastic anemia. Lastly, *GATA2* mutations were detected in individuals lacking features of GATA2 haploinsufficiency, highlighting the importance of screening for deleterious germline variants when rendering the diagnosis of pediatric AML/MDS [45, 121, 122].

Individuals with *GATA2* germline mutations have a significantly increased lifetime risk of developing MDS/AML; approximately 70% of affected individuals will develop MDS/AML by age 29 years (median, range 0.4–78 years) [123]. Regular peripheral blood testing and a bone marrow biopsy with cytogenetic analysis are recommended at baseline, and should be repeated if there are any changes in the CBC worrisome for MDS/AML. Most cases of *GATA2*-MDS are accompanied by acquired monosomy 7, and recent evidence suggests that cooperating acquired oncogenic mutations in AML driver genes like *ASXL1*, *NRAS*, *RUNX1, SETPB1,* and *TP53* may arise in the background of *GATA2*-MDS and facilitate transformation to AML [44, 124]. The natural course of *GATA2*-MDS is relatively rapid transformation to AML, thus the prognosis is poor with the best outcomes observed for those able to undergo allogeneic SCT [125].

### Familial Monosomy 7

Familial monosomy 7 is a rare disease, with only 14 kindreds reported [126]. This diagnosis should be suspected in individuals presenting with BMF, MDS, or AML characterized by this cytogenetic feature, and with a family history of a relative with hematologic malignancy and monosomy 7. The mode of inheritance is not known at this time, and it is also unclear whether a single gene or multiple genes on chromosome 7 contribute to the pathogenesis of this disorder.

Disease presentation is often with cytopenias in early childhood, with rapid progression to AML/MDS. Children in familial monosomy 7 kindreds may initially demonstrate a normal karyotype in cells of the hematopoietic compartment, later developing mosaicism for monosomy 7. Thus, such children should be routinely screened for development of cytopenias, even if monosomy 7 has not yet been detected. Therapeutic recommendations include SCT prior to the emergence of a leukemic clone [127]. Given that the pathogenic variant is not known, and the disease has been observed to emerge in relatives previously found to have normal karyotype, in these cases, an unrelated donor choice for SCT may be preferred [127].

### Hereditary *RUNX1* Mutations: Familial Platelet Disorder with Propensity to Myeloid Malignancy

Familial platelet disorder with propensity to myeloid malignancy (FPD-AML) is an autosomal dominant familial MDS/AML syndrome caused by mutations in Runt Related Transcription Factor 1 (*RUNX1*). The clinical entity was first described by Dowton and colleagues in 1985 in a kindred of 22 affected individuals with platelet dysfunction, 6 of whom developed hematologic malignancies [128]. Song and colleagues identified the causative gene in 1999 [129]. Phenotypic manifestations include mild to moderate thrombocytopenia, bleeding time prolongation, and abnormal platelet aggregation. Platelet survival time is typically normal.

Nonsense, missense, or frameshift mutations, or insertions, deletions, or translocations can all lead to loss of function or dominant-negative impairment of the wild-type RUNX1 protein [125]. The most notable phenotypic manifestations of this syndrome are quantitative and qualitative platelet defects, and an approximately 40% increased lifetime risk of developing AML/MDS. However, several patients with normal platelet counts and platelet function are described [130], so the diagnosis of FPD/AML does not require a platelet disorder but rather demonstration of a germline *RUNX1* mutation. *RUNX1* mutations are acquired events in approximately 30% of AML/MDS, so their detection alone is not sufficient evidence for a familial syndrome. Allele fractions consistent with heterozygosity and/or two individual *RUNX1* mutations suggestive of an acquired and germline mutation may prompt a hereditary inquiry in cases with absent clinical manifestations of FPD/AML.

The average age of AML/MDS onset in affected individuals is 33 years (range 6–76 years), and an association with risk for T-cell acute lymphoblastic leukemia (T-ALL) is also described [129, 130]. Progression to MDS/AML requires acquisition of additional driver mutations, often including additional somatic *RUNX1* mutations, and may explain the varying phenotypes and penetrance seen in this condition. Affected individuals typically do not require treatment for incidental thrombocytopenia but may require monitoring during surgical or obstetrical procedures. No clinical or laboratory markers are currently available to predict the risk of progression to AML/MDS, so the current recommendations include a baseline bone marrow biopsy with cytogenetic analysis, complete blood count (CBC), and clinical exams at regular intervals. Any significant changes in the CBC warrant repeat bone marrow evaluation [123].

### Hereditary *ANKRD26* Mutations: Thrombocytopenia 2

Autosomal dominant germline mutations in the 5′ untranslated region (UTR) of the Ankyrin Repeat Domain 26 (*ANKRD26*) gene cause Thrombocytopenia 2, an inherited disorder characterized by moderate thrombocytopenia, elevated TPO levels, platelet aggregations defects, and dysmegakaryopoiesis with micromegakaryocytes and hypolobated nuclei [131, 132]. Isolated cases reports of this disorder first surfaced in the literature in the mid-1960s, and the first presumed case series was

reported by Bithell and colleagues in 1965 [133]. Almost all described mutations are point mutations in the *ANKRD26* 5′ UTR promoter that disrupt assembly of the co-repressors RUNX1 and FLI1, causing increased gene transcription and enhanced signaling through the TPO receptor pathway [134]. The prevalence of Thrombocytopenia 2 is unknown, but in one study of families with an inherited thrombocytopenia of unknown origin, *ANKRD26* mutations were detected in 23 of 215 individuals (11%) [135].

Affected individuals have a 30-fold increased risk of developing AML/MDS [136], but it is diagnostically challenging to distinguish individuals with germline *ANKRD26* mutations from the dysmegakaryopoiesis related to development of de novo MDS. Thus, AML/MDS in the clinical context of a patient with long-standing thrombocytopenia, particularly a patient with idiopathic thrombocytopenic purpura, should prompt investigation for *ANKRD26* germline mutations [131, 136]. If confirmed, genetic counseling, appropriate screening, and management options should be extended to the patient and at-risk family members. The surveillance recommendations are similar to patients with FPD/AML with germline *RUNX1* mutations.

### Hereditary *ETV6* Mutations: Thrombocytopenia 5

Another inherited autosomal dominant MDS/AML predisposition syndrome is attributable to mutations in the ETS Variant 6 (*ETV6*) gene. Both the clinical characteristics and causative mutations were described by two independent groups in 2015 [137, 138]. The causative missense germline mutations underlying Thrombocytopenia 5 encode aberrant dominant-negative ETV6 proteins that disrupt transcription of platelet-associated genes. ETV6 is an ETS-family transcription factor essential to hematopoiesis and implicated in the pathogenesis of multiple malignancies.

Affected individuals present with variable degrees of moderate thrombocytopenia and mild-to-moderate bleeding tendencies, and in addition to AML/MDS, are at risk of other hematologic malignancies such as chronic myelomonocytic leukemia, B-ALL, plasma cell myeloma, and early onset colorectal cancer [123]. The actual incidence of AML/MDS is unknown, but the relative frequency of *ETV6*-related thrombocytopenia is approximately 5% in families with known forms of inherited thrombocytopenia. There are no definitive clinical or laboratory features specific to this disease, but it should be noted that platelets are typically not enlarged [139]. Surveillance recommendations are similar to patients with *RUNX1* and *ANKRD26* germline mutations.

### Familial Aplastic Anemia/Myelodysplastic Syndrome with *SRP72* Mutations

One of the rarest causes of familial aplastic anemia and MDS is attributable to autosomal dominant mutations in the Signal Recognition Particle 72kDa (*SRP72*) gene. SRP72 is a component of a ribonucleoprotein complex that mediates the targeting

of secretory proteins to the endoplasmic reticulum. To date, only two familial pedigrees have been described, harboring unique missense and frameshift mutations [140]. Given its rarity, there is very little known regarding diagnosis, incidence, prognosis, or treatment. However, additional data are likely forthcoming now that *SRP72* mutations – and possibly other members of its protein complex – are implicated in familial MDS.

### Familial Acute Myeloid Leukemia with *CEBPA* Mutations

Autosomal dominant germline mutations in the CCAAT/enhancer binding protein alpha gene (*CEBPA*) contribute to a distinct form of familial AML. Smith and colleagues originally described the condition in 2004 [141], and subsequent familial pedigrees have been reported [142–145]. AML appears to be the only hematologic malignancy associated with this disorder, and the median age of presentation is approximately 24.5 years (range 1.75–46 years) [146].

Affected individuals often inherit frameshift mutations in the 5′ region of *CEBPA* that encode a truncated CEBPA protein deficient in target gene promoter and protein interactions [147]. Individuals invariably acquire a "second-hit" in the remaining wild-type allele, typically 3′ to the germline mutation, disrupting all CEBPA protein function and leading to overt AML [146]. Leukemic blasts are characterized by aberrant CD7 expression, a normal karyotype, and frequent Auer rods, similar to sporadic AML with biallelic *CEPBA* mutations [125, 148]. Thus, a family history of AML and/or development of AML with biallelic *CEBPA* mutations are key findings supporting the possible diagnosis; no additional preceding blood count abnormalities or physical features are known [125]. Interestingly, routine sequencing for somatic *CEPBA* mutations reveals previously unknown germline mutations in ~1% of patients, suggesting that the incidence may be higher than is reported [149].

Patients typically respond to standard induction chemotherapy and have a favorable prognosis with durable responses to secondary therapy, but there is a high cumulative incidence of relapse [146]. Contrary to the management of sporadic AML with biallelic *CEBPA* mutations, familial syndrome patients are also at risk of the development of additional leukemias, so consideration of allogeneic stem cell transplantation for definitive cure is necessary [125]. It is also critical to diagnose familial AML with mutated *CEBPA* in order to identify potentially afflicted biologic family members, particularly given the autosomal dominant pattern of inheritance.

### Familial Acute Myeloid Leukemia with Mutated *DDX41*

Polprasert and colleagues recently described a familial MDS/AML syndrome characterized by autosomal dominant germline mutations in the DEAD-box helicase 41 (*DDX41*) gene [150]. Approximately 75% of patients with *DDX41* germline mutations harbor somatic point mutations or deletion of the other *DDX41* locus. It is

hypothesized that loss of DDX41 function causes altered pre-mRNA splicing and RNA processing, although the precise mechanisms by which *DDX41* mutations exert their pro-leukemogenic defects are unclear [150]. Most mutations are frameshift mutations, but familial pedigrees with inherited point mutations and splice site mutations are described [151, 152]. The true prevalence is unknown, but in 289 cases of suspected familial inherited hematologic malignancies, 3% ($n = 9$) were found to harbor germline mutations in *DDX41* [152].

In this disorder, most unaffected carriers have a normal CBC, bone marrow morphology and cellularity, and karyotype well into adulthood. Carriers who develop MDS or AML most often present with leukopenia. The most notable morphologic feature at diagnosis is a hypocellular bone marrow with prominent erythroid dysplasia and/or erythroleukemia and a normal karyotype. A small proportion of carriers may present with granulomatous and immune disorders prior to malignancy [152].

AML with *DDX41* germline mutation is of particular importance because unlike other AML predisposition syndromes, it is characterized by long latency (median age of diagnosis is approximately 62 years), advanced disease (high-risk MDS/AML), and poor prognosis [123, 150, 152]. Also, germline *DDX41* mutations seem to predispose to somatic mutations in *DDX41*, so the detection of a *DDX41* mutation in a de novo AML sample should prompt evaluation to exclude a germline origin [150]. Lastly, germline *DDX41* mutations may predispose to other hematologic malignancies such as Hodgkin and non-Hodgkin lymphoma, CML, and multiple myeloma [152]. Bone marrow biopsies, cytogenetic analysis, and CBCs are recommended at regular intervals for known carriers [47].

## Recognition and Clinical Diagnosis of Inherited Myeloid Disorders

The phenotypic abnormalities associated with myeloid malignancy predisposition syndromes are well characterized, though broad in scope and variably penetrant. Suggestive features are often recognized during thorough physical examination or assessment of family history. Although tumor NGS testing for leukemia samples is intended to detect somatic alterations, sequencing peripheral blood and bone marrow samples will also readily detect inherited mutations. An individual with a presumed somatic mutation in any of the familial predisposition genes may be offered germline testing on matched buccal swabs or cultured fibroblasts, a step that is especially important for patients with biallelic *CEBPA* mutations, *GATA2* mutations, or *RUNX1* mutations, for the reasons outlined above. A familiarity with the causative genes predisposing to myeloid malignancies will facilitate accurate diagnosis and may impact diagnostic work-up, clinical management, and genetic counseling of potentially at-risk family members [153].

## Conclusion

It is clear that although the inherited and acquired myeloid neoplasms of childhood are rare, there are key clinical and molecular/genetic components to each diagnosis. Practitioners who diagnose and/or treat childhood MPN, MDS, or AML should be aware of the close association of these disorders with inherited conditions, and perform a thorough work-up to ensure detection of any underlying predisposition [153]. As our knowledge evolves, it is likely that additional genes predisposing to these conditions will be uncovered, as well as molecular markers assisting in both diagnosis and prognostic determinations. In the interim, a working understanding of the biology, epidemiology, and natural history of these rare disorders serves as an indispensable companion to diagnostic practice.

## References

1. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–405.
2. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pilari SA, Stein H, et al. WHO classification of tumours of haematopoietic and lymphoid tissues. Lyon: International Agency for Research on Cancer; 2008.
3. Belgaumi AF, Al-Shehri A. Clinical characteristics and treatment outcome of pediatric patients with chronic myeloid leukemia. Haematologica. 2010;95(9):e4.
4. James C, Ugo V, Le Couedic JP, Staerk J, Delhommeau F, Lacout C, et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. Nature. 2005;434(7037):1144–8.
5. Giona F, Teofili L, Moleti ML, Martini M, Palumbo G, Amendola A, et al. Thrombocythemia and polycythemia in patients younger than 20 years at diagnosis: clinical and biologic features, treatment, and long-term outcome. Blood. 2012;119(10):2219–27.
6. Klampfl T, Gisslinger H, Harutyunyan AS, Nivarthi H, Rumi E, Milosevic JD, et al. Somatic mutations of calreticulin in myeloproliferative neoplasms. N Engl J Med. 2013;369(25):2379–90.
7. Nangalia J, Massie CE, Baxter EJ, Nice FL, Gundem G, Wedge DC, et al. Somatic CALR mutations in myeloproliferative neoplasms with nonmutated JAK2. N Engl J Med. 2013;369(25):2391–405.
8. An W, Wan Y, Guo Y, Chen X, Ren Y, Zhang J, et al. CALR mutation screening in pediatric primary myelofibrosis. Pediatr Blood Cancer. 2014;61(12):2256–62.
9. Giona F, Teofili L, Capodimonti S, Laurino M, Martini M, Marzella D, et al. CALR mutations in patients with essential thrombocythemia diagnosed in childhood and adolescence. Blood. 2014;123(23):3677–9.
10. Langabeer SE, Haslam K, McMahon C. CALR mutations are rare in childhood essential thrombocythemia. Pediatr Blood Cancer. 2014;61(8):1523.
11. Sekiya Y, Okuno Y, Muramatsu H, Ismael O, Kawashima N, Narita A, et al. JAK2, MPL, and CALR mutations in children with essential thrombocythemia. Int J Hematol. 2016;104(2):266–7.
12. Ding J, Komatsu H, Wakita A, Kato-Uranishi M, Ito M, Satoh A, et al. Familial essential thrombocythemia associated with a dominant-positive activating mutation of the c-MPL gene, which encodes for the receptor for thrombopoietin. Blood. 2004;103(11):4198–200.

13. Karow A, Nienhold R, Lundberg P, Peroni E, Putti MC, Randi ML, et al. Mutational profile of childhood myeloproliferative neoplasms. Leukemia. 2015;29(12):2407–9.
14. Landgren O, Goldin LR, Kristinsson SY, Helgadottir EA, Samuelsson J, Bjorkholm M. Increased risks of polycythemia vera, essential thrombocythemia, and myelofibrosis among 24,577 first-degree relatives of 11,039 patients with myeloproliferative neoplasms in Sweden. Blood. 2008;112(6):2199–204.
15. Hermouet S, Vilaine M. The JAK2 46/1 haplotype: a marker of inappropriate myelomonocytic response to cytokine stimulation, leading to increased risk of inflammation, myeloid neoplasm, and impaired defense against infection? Haematologica. 2011;96(11):1575–9.
16. Olcaydu D, Harutyunyan A, Jager R, Berg T, Gisslinger B, Pabinger I, et al. A common JAK2 haplotype confers susceptibility to myeloproliferative neoplasms. Nat Genet. 2009;41(4):450–4.
17. Tapper W, Jones AV, Kralovics R, Harutyunyan AS, Zoi K, Leung W, et al. Genetic variation at MECOM, TERT, JAK2 and HBS1L-MYB predisposes to myeloproliferative neoplasms. Nat Commun. 2015;6:6691.
18. Langabeer SE, Smith OP, McMahon C. The JAK2 V617F mutation in pediatric myeloproliferative neoplasms: how and when? Pediatr Hematol Oncol. 2014;31(2):138–9.
19. Jager R, Harutyunyan AS, Rumi E, Pietra D, Berg T, Olcaydu D, et al. Common germline variation at the TERT locus contributes to familial clustering of myeloproliferative neoplasms. Am J Hematol. 2014;89(12):1107–10.
20. Oddsson A, Kristinsson SY, Helgason H, Gudbjartsson DF, Masson G, Sigurdsson A, et al. The germline sequence variant rs2736100_C in TERT associates with myeloproliferative neoplasms. Leukemia. 2014;28(6):1371–4.
21. Harutyunyan AS, Giambruno R, Krendl C, Stukalov A, Klampfl T, Berg T, et al. Germline RBBP6 mutations in familial myeloproliferative neoplasms. Blood. 2016;127(3):362–5.
22. Choong K, Freedman MH, Chitayat D, Kelly EN, Taylor G, Zipursky A. Juvenile myelomonocytic leukemia and Noonan syndrome. J Pediatr Hematol Oncol. 1999;21(6):523–7.
23. Tartaglia M, Mehler EL, Goldberg R, Zampino G, Brunner HG, Kremer H, et al. Mutations in PTPN11, encoding the protein tyrosine phosphatase SHP-2, cause Noonan syndrome. Nat Genet. 2001;29(4):465–8.
24. Shofty B, Constantini S, Ben-Shachar S. Advances in molecular diagnosis of neurofibromatosis type 1. Semin Pediatr Neurol. 2015;22(4):234–9.
25. Niemeyer CM, Arico M, Basso G, et al. Chronic myelomonocytic leukemia in childhood: a retrospective analysis of 110 cases. European Working Group on Myelodysplastic Syndromes in Childhood (EWOG-MDS). Blood. 1997;89(10):3534–43.
26. Side LE, Emanuel PD, Taylor B, et al. Mutations of the NF1 gene in children with juvenile myelomonocytic leukemia without clinical evidence of neurofibromatosis, type 1. Blood. 1998;92(1):267–72.
27. Niemeyer CM. RAS diseases in children. Haematologica. 2014;99(11):1653–62.
28. Niemeyer CM, Kang MW, Shin DH, et al. Germline CBL mutations cause developmental abnormalities and predispose to juvenile myelomonocytic leukemia. Nat Genet. 2010;42(9):794–800.
29. Perez B, Mechinaud F, Galambrun C, et al. Germline mutations of the CBL gene define a new genetic syndrome with predisposition to juvenile myelomonocytic leukaemia. J Med Genet. 2010;47(10):686–91.
30. Calvo KR, Price S, Braylan RC, et al. JMML and RALD (Ras-associated autoimmune leukoproliferative disorder): common genetic etiology yet clinically distinct entities. Blood. 2015;125(18):2753–8.
31. Hitzler JK, Cheung J, Li Y, Scherer SW, Zipursky A. GATA1 mutations in transient leukemia and acute megakaryoblastic leukemia of Down syndrome. Blood. 2003;101(11):4301–4.
32. Xu G, Nagano M, Kanezaki R, Toki T, Hayashi Y, Taketani T, et al. Frequent mutations in the GATA-1 gene in the transient myeloproliferative disorder of Down syndrome. Blood. 2003;102(8):2960–8.

33. Bombery M, Vergilio JA. Transient abnormal myelopoiesis in neonates: GATA get the diagnosis. Arch Pathol Lab Med. 2014;138(10):1302–6.
34. Massey GV, Zipursky A, Chang MN, Doyle JJ, Nasim S, Taub JW, et al. A prospective study of the natural history of transient leukemia (TL) in neonates with Down syndrome (DS): Children's Oncology Group (COG) study POG-9481. Blood. 2006;107(12):4606–13.
35. Malinge S, Izraeli S, Crispino JD. Insights into the manifestations, outcomes, and mechanisms of leukemogenesis in Down syndrome. Blood. 2009;113(12):2619–28.
36. Nikolaev SI, Santoni F, Vannier A, Falconnet E, Giarin E, Basso G, et al. Exome sequencing identifies putative drivers of progression of transient myeloproliferative disorder to AMKL in infants with Down syndrome. Blood. 2013;122(4):554–61.
37. Hofmann I. Myeloproliferative neoplasms in children. J Hematop. 2015;8(3):143–57.
38. Passmore SJ, Chessells JM, Kempski H, Hann IM, Brownbill PA, Stiller CA. Paediatric myelodysplastic syndromes and juvenile myelomonocytic leukaemia in the UK: a population-based study of incidence and survival. Br J Haematol. 2003;121(5):758–67.
39. Luna-Fineman S, Shannon KM, Atwater SK, Davis J, Masterson M, Ortega J, et al. Myelodysplastic and myeloproliferative disorders of childhood: a study of 167 patients. Blood. 1999;93(2):459–66.
40. Niemeyer CM, Baumann I. Classification of childhood aplastic anemia and myelodysplastic syndrome. Hematol Am Soc Hematol Educ Program. 2011;2011:84–9.
41. Hasle H, Niemeyer CM, Chessells JM, Baumann I, Bennett JM, Kerndrup G, et al. A pediatric approach to the WHO classification of myelodysplastic and myeloproliferative diseases. Leukemia. 2003;17(2):277–82.
42. Woods WG, Kobrinsky N, Buckley J, Neudorf S, Sanders J, Miller L, et al. Intensively timed induction therapy followed by autologous or allogeneic bone marrow transplantation for children with acute myeloid leukemia or myelodysplastic syndrome: a Childrens Cancer Group pilot study. J Clin Oncol Off J Am Soc Clin Oncol. 1993;11(8):1448–57.
43. Hirabayashi S, Flotho C, Moetter J, Heuser M, Hasle H, Gruhn B, et al. Spliceosomal gene aberrations are rare, coexist with oncogenic mutations, and are unlikely to exert a driver effect in childhood MDS and JMML. Blood. 2012;119(11):e96–9.
44. Wang X, Muramatsu H, Okuno Y, Sakaguchi H, Yoshida K, Kawashima N, et al. GATA2 and secondary mutations in familial myelodysplastic syndromes and pediatric myeloid malignancies. Haematologica. 2015;100(10):e398–401.
45. Wlodarski MW, Hirabayashi S, Pastor V, Stary J, Hasle H, Masetti R, et al. Prevalence, clinical characteristics, and prognosis of GATA2-related myelodysplastic syndromes in children and adolescents. Blood. 2016;127(11):1387–97.
46. Collin M, Dickinson R, Bigley V. Haematopoietic and immune defects associated with GATA2 mutation. Br J Haematol. 2015;169(2):173–87.
47. Churpek JE, Lorenz R, Nedumgottil S, Onel K, Olopade OI, Sorrell A, et al. Proposal for the clinical detection and management of patients and their family members with familial myelodysplastic syndrome/acute leukemia predisposition syndromes. Leuk Lymphoma. 2013;54(1):28–35.
48. Smith AR, Christiansen EC, Wagner JE, Cao Q, MacMillan ML, Stefanski HE, et al. Early hematopoietic stem cell transplant is associated with favorable outcomes in children with MDS. Pediatr Blood Cancer. 2013;60(4):705–10.
49. Stary J, Locatelli F, Niemeyer CM, European Working Group on Myelodysplastic S, Pediatric Diseases Working Party of the E. Stem cell transplantation for aplastic anemia and myelodysplastic syndrome. Bone Marrow Transplant. 2005;35(Suppl 1):S13–6.
50. Triot A, Jarvinen PM, Arostegui JI, Murugan D, Kohistani N, Dapena Diaz JL, et al. Inherited biallelic CSF3R mutations in severe congenital neutropenia. Blood. 2014;123(24):3811–7.
51. Klimiankou M, Klimenkova O, Uenalan M, Zeidler A, Mellor-Heineke S, Kandabarau S, et al. GM-CSF stimulates granulopoiesis in a congenital neutropenia patient with loss-of-function biallelic heterozygous CSF3R mutations. Blood. 2015;126(15):1865–7.
52. Peffault de Latour R, Peters C, Gibson B, Strahm B, Lankester A, de Heredia CD, et al. Recommendations on hematopoietic stem cell transplantation for inherited bone marrow failure syndromes. Bone Marrow Transplant. 2015;50(9):1168–72.

53. Diamond LK, Blackfan KD. Hypoplastic anemia. Am J Dis Child. 1938;56:464.
54. Draptchinskaia N, Gustavsson P, Andersson B, Pettersson M, Willig TN, Dianzani I, et al. The gene encoding ribosomal protein S19 is mutated in Diamond-Blackfan anaemia. Nat Genet. 1999;21(2):169–75.
55. Horos R, von Lindern M. Molecular mechanisms of pathology and treatment in Diamond Blackfan Anaemia. Br J Haematol. 2012;159(5):514–27.
56. Diamond LK, Wang WC, Alter BP. Congenital hypoplastic anemia. Adv Pediatr. 1976;22:349–78.
57. Ball S. Diamond Blackfan anemia. Hematol Am Soc Hematol Edu Program. 2011;2011: 487–91.
58. Vlachos A, Ball S, Dahl N, Alter BP, Sheth S, Ramenghi U, et al. Diagnosing and treating Diamond Blackfan anaemia: results of an international clinical consensus conference. Br J Haematol. 2008;142(6):859–76.
59. Vlachos A, Rosenberg PS, Atsidaftos E, Alter BP, Lipton JM. Incidence of neoplasia in Diamond Blackfan anemia: a report from the Diamond Blackfan anemia registry. Blood. 2012;119(16):3815–9.
60. Gasser C. Aplastic anemia (chronic erythroblastophthisis) and cortisone. Schweiz Med Wochenschr. 1951;81(50):1241–2.
61. Allen DM, Diamond LK. Congenital (erythroid) hypoplastic anemia: cortisone treated. Am J Dis Child. 1961;102:416–23.
62. Mitchell JR, Wood E, Collins K. A telomerase component is defective in the human disease dyskeratosis congenita. Nature. 1999;402(6761):551–5.
63. Savage SA. Dyskeratosis Congenita. In: Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, LJH B, et al., editors. GeneReviews®. Seattle: University of Washington; 1993.
64. Savage SA, Bertuch AA. The genetics and clinical manifestations of telomere biology disorders. Genet Med. 2010;12(12):753–64.
65. Alter BP, Giri N, Savage SA, Rosenberg PS. Cancer in dyskeratosis congenita. Blood. 2009;113(26):6549–57.
66. Leguit RJ, van den Tweel JG. The pathology of bone marrow failure. Histopathology. 2010;57(5):655–70.
67. Alter BP, Rosenberg PS, Giri N, Baerlocher GM, Lansdorp PM, Savage SA. Telomere length is associated with disease severity and declines with age in dyskeratosis congenita. Haematologica. 2012;97(3):353–9.
68. Dietz AC, Orchard PJ, Baker KS, Giller RH, Savage SA, Alter BP, et al. Disease-specific hematopoietic cell transplantation: nonmyeloablative conditioning regimen for dyskeratosis congenita. Bone Marrow Transplant. 2011;46(1):98–104.
69. Savage SA, Dokal I, Armanios M, Aubert G, Cowen EW, Domingo DL, et al. Dyskeratosis congenita: the first NIH clinical research workshop. Pediatr Blood Cancer. 2009;53(3):520–3.
70. Fanconi G. Familial infantile pernicious-like anemia. Jarbuch fur Kinderheilkunde. 1927;117:257–80.
71. Schneider M, Chandler K, Tischkowitz M, Meyer S. Fanconi anaemia: genetics, molecular biology, and cancer – implications for clinical management in children and adults. Clin Genet. 2015;88(1):13–24.
72. Oostra AB, Nieuwint AW, Joenje H, de Winter JP. Diagnosis of fanconi anemia: chromosomal breakage analysis. Anemia. 2012;2012:238731.
73. Gille JJ, Floor K, Kerkhoven L, Ameziane N, Joenje H, de Winter JP. Diagnosis of Fanconi anemia: mutation analysis by multiplex ligation-dependent probe amplification and PCR-based sanger sequencing. Anemia. 2012;2012:603253.
74. Ameziane N, Errami A, Leveille F, Fontaine C, de Vries Y, van Spaendonk RM, et al. Genetic subtyping of Fanconi anemia by comprehensive mutation screening. Hum Mutat. 2008;29(1):159–66.
75. Koc A, Pronk JC, Alikasifoglu M, Joenje H, Altay C. Variable pathogenicity of exon 43del (FAA) in four Fanconi anaemia patients within a consanguineous family. Br J Haematol. 1999;104(1):127–30.

76. Shimamura A, Alter BP. Pathophysiology and management of inherited bone marrow failure syndromes. Blood Rev. 2010;24(3):101–22.
77. Rosenberg PS, Alter BP, Ebell W. Cancer risks in Fanconi anemia: findings from the German Fanconi Anemia Registry. Haematologica. 2008;93(4):511–7.
78. Seif AE. Pediatric leukemia predisposition syndromes: clues to understanding leukemogenesis. Cancer Genet. 2011;204(5):227–44.
79. Tonnies H, Huber S, Kuhl JS, Gerlach A, Ebell W, Neitzel H. Clonal chromosomal aberrations in bone marrow cells of Fanconi anemia patients: gains of the chromosomal segment 3q26q29 as an adverse risk factor. Blood. 2003;101(10):3872–4.
80. Quentin S, Cuccuini W, Ceccaldi R, Nibourel O, Pondarre C, Pages MP, et al. Myelodysplasia and leukemia of Fanconi anemia are associated with a specific pattern of genomic abnormalities that includes cryptic RUNX1/AML1 lesions. Blood. 2011;117(15):e161–70.
81. Peffault de Latour R, Soulier J. How I treat MDS and AML in Fanconi anemia. Blood. 2016;127(24):2971–9.
82. Boocock GR, Morrison JA, Popovic M, Richards N, Ellis L, Durie PR, et al. Mutations in SBDS are associated with Shwachman-Diamond syndrome. Nat Genet. 2003;33(1):97–101.
83. Burwick N, Coats SA, Nakamura T, Shimamura A. Impaired ribosomal subunit association in Shwachman-Diamond syndrome. Blood. 2012;120(26):5143–52.
84. Shwachman H, Diamond LK, Oski FA, Khaw KT. The syndrome of pancreatic insufficiency and bone marrow dysfunction. J Pediatr. 1964;65:645–63.
85. Costa E, Santos R. Hematologically important mutations: Shwachman-Diamond syndrome. Blood Cells Mol Dis. 2008;40(2):183–4.
86. Burroughs L, Woolfrey A, Shimamura A. Shwachman-Diamond syndrome: a review of the clinical presentation, molecular pathogenesis, diagnosis, and treatment. Hematol Oncol Clin North Am. 2009;23(2):233–48.
87. Dror Y. Shwachman-Diamond syndrome. Pediatr Blood Cancer. 2005;45(7):892–901.
88. Shimamura A. Shwachman-Diamond syndrome. Semin Hematol. 2006;43(3):178–88.
89. Cunningham J, Sales M, Pearce A, Howard J, Stallings R, Telford N, et al. Does isochromosome 7q mandate bone marrow transplant in children with Shwachman-Diamond syndrome? Br J Haematol. 2002;119(4):1062–9.
90. Maserati E, Pressato B, Valli R, Minelli A, Sainati L, Patitucci F, et al. The route to development of myelodysplastic syndrome/acute myeloid leukaemia in Shwachman-Diamond syndrome: the role of ageing, karyotype instability, and acquired chromosome anomalies. Br J Haematol. 2009;145(2):190–7.
91. Yusuf U, Frangoul HA, Gooley TA, Woolfrey AE, Carpenter PA, Andrews RG, et al. Allogeneic bone marrow transplantation in children with myelodysplastic syndrome or juvenile myelomonocytic leukemia: the Seattle experience. Bone Marrow Transplant. 2004;33(8):805–14.
92. Kostmann R. Infantile genetic agranulocytosis; agranulocytosis infantilis hereditaria. Acta Paediatr Suppl. 1956;45(Suppl 105):1–78.
93. Horwitz M, Benson KF, Person RE, Aprikyan AG, Dale DC. Mutations in ELA2, encoding neutrophil elastase, define a 21-day biological clock in cyclic haematopoiesis. Nat Genet. 1999;23(4):433–6.
94. Horwitz MS, Corey SJ, Grimes HL, Tidwell T. ELANE mutations in cyclic and severe congenital neutropenia: genetics and pathophysiology. Hematol Oncol Clin North Am. 2013;27(1):19–41, vii.
95. Rosenberg PS, Zeidler C, Bolyard AA, Alter BP, Bonilla MA, Boxer LA, et al. Stable long-term risk of leukaemia in patients with severe congenital neutropenia maintained on G-CSF therapy. Br J Haematol. 2010;150(2):196–9.
96. Germeshausen M, Ballmaier M, Welte K. Incidence of CSF3R mutations in severe congenital neutropenia and relevance for leukemogenesis: results of a long-term survey. Blood. 2007;109(1):93–9.
97. Germeshausen M, Kratz CP, Ballmaier M, Welte K. RAS and CSF3R mutations in severe congenital neutropenia. Blood. 2009;114(16):3504–5.

98. Klimiankou M, Mellor-Heineke S, Zeidler C, Welte K, Skokowa J. Role of CSF3R mutations in the pathomechanism of congenital neutropenia and secondary acute myeloid leukemia. Ann N Y Acad Sci. 2016;1370(1):119–25.
99. Makaryan V, Zeidler C, Bolyard AA, Skokowa J, Rodger E, Kelley ML, et al. The diversity of mutations and clinical outcomes for ELANE-associated neutropenia. Curr Opin Hematol. 2015;22(1):3–11.
100. Ballmaier M, Germeshausen M, Schulze H, Cherkaoui K, Lang S, Gaudig A, et al. c-mpl mutations are the cause of congenital amegakaryocytic thrombocytopenia. Blood. 2001;97(1):139–46.
101. van den Oudenrijn S, Bruin M, Folman CC, Peters M, Faulkner LB, de Haas M, et al. Mutations in the thrombopoietin receptor, Mpl, in children with congenital amegakaryocytic thrombocytopenia. Br J Haematol. 2000;110(2):441–8.
102. King S, Germeshausen M, Strauss G, Welte K, Ballmaier M. Congenital amegakaryocytic thrombocytopenia: a retrospective clinical analysis of 20 patients. Br J Haematol. 2005;131(5):636–44.
103. Greenwald HM, Sherman I. Congenital essential thrombocytopenia. Am J Dis Child. 1929;38(6):1245–51.
104. Jalas C, Anderson SL, Laufer T, Martimucci K, Bulanov A, Xie X, et al. A founder mutation in the MPL gene causes congenital amegakaryocytic thrombocytopenia (CAMT) in the Ashkenazi Jewish population. Blood Cells Mol Dis. 2011;47(1):79–83.
105. Alter BP. Bone marrow failure syndromes in children. Pediatr Clin N Am. 2002;49(5):973–88.
106. Geddis AE. Congenital amegakaryocytic thrombocytopenia. Pediatr Blood Cancer. 2011;57(2):199–203.
107. Bloom D. Congenital telangiectatic erythema resembling lupus erythematosus in dwarfs; probably a syndrome entity. AMA. Am J Dis Child. 1954;88(6):754–8.
108. Arora H, Chacon AH, Choudhary S, McLeod MP, Meshkov L, Nouri K, et al. Bloom syndrome. Int J Dermatol. 2014;53(7):798–802.
109. Poppe B, Van Limbergen H, Van Roy N, Vandecruys E, De Paepe A, Benoit Y, et al. Chromosomal aberrations in Bloom syndrome patients with myeloid malignancies. Cancer Genet Cytogenet. 2001;128(1):39–42.
110. Li FP, Fraumeni JF Jr, Mulvihill JJ, Blattner WA, Dreyfus MG, Tucker MA, et al. A cancer family syndrome in twenty-four kindreds. Cancer Res. 1988;48(18):5358–62.
111. Malkin D, Li FP, Strong LC, Fraumeni JF Jr, Nelson CE, Kim DH, et al. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science (New York, NY). 1990;250(4985):1233–8.
112. Kamihara J, Rana HQ, Garber JE. Germline TP53 mutations and the changing landscape of Li-Fraumeni syndrome. Hum Mutat. 2014;35(6):654–62.
113. Gonzalez KD, Noltner KA, Buzin CH, Gu D, Wen-Fong CY, Nguyen VQ, et al. Beyond Li Fraumeni Syndrome: clinical characteristics of families with p53 germline mutations. J Clin Oncol. 2009;27(8):1250–6.
114. Olivier M, Goldgar DE, Sodha N, Ohgaki H, Kleihues P, Hainaut P, et al. Li-Fraumeni and related syndromes: correlation between tumor type, family structure, and TP53 genotype. Cancer Res. 2003;63(20):6643–50.
115. Talwalkar SS, Yin CC, Naeem RC, Hicks MJ, Strong LC, Abruzzo LV. Myelodysplastic syndromes arising in patients with germline TP53 mutation and Li-Fraumeni syndrome. Arch Pathol Lab Med. 2010;134(7):1010–5.
116. Hsu AP, Johnson KD, Falcone EL, Sanalkumar R, Sanchez L, Hickstein DD, et al. GATA2 haploinsufficiency caused by mutations in a conserved intronic element leads to MonoMAC syndrome. Blood. 2013;121(19):3830–7, S1–7.
117. Kazenwadel J, Secker GA, Liu YJ, Rosenfeld JA, Wildin RS, Cuellar-Rodriguez J, et al. Loss-of-function germline GATA2 mutations in patients with MDS/AML or MonoMAC syndrome and primary lymphedema reveal a key role for GATA2 in the lymphatic vasculature. Blood. 2012;119(5):1283–91.

118. Ostergaard P, Simpson MA, Connell FC, Steward CG, Brice G, Woollard WJ, et al. Mutations in GATA2 cause primary lymphedema associated with a predisposition to acute myeloid leukemia (Emberger syndrome). Nat Genet. 2011;43(10):929–31.
119. Hahn CN, Chong CE, Carmichael CL, Wilkins EJ, Brautigan PJ, Li XC, et al. Heritable GATA2 mutations associated with familial myelodysplastic syndrome and acute myeloid leukemia. Nat Genet. 2011;43(10):1012–7.
120. Spinner MA, Sanchez LA, Hsu AP, Shaw PA, Zerbe CS, Calvo KR, et al. GATA2 deficiency: a protean disorder of hematopoiesis, lymphatics, and immunity. Blood. 2014;123(6):809–21.
121. Zhang MY, Keel SB, Walsh T, Lee MK, Gulsuner S, Watts AC, et al. Genomic analysis of bone marrow failure and myelodysplastic syndromes reveals phenotypic and diagnostic complexity. Haematologica. 2015;100(1):42–8.
122. Keel SB, Scott A, Sanchez-Bonilla M, Ho PA, Gulsuner S, Pritchard CC, et al. Genetic features of myelodysplastic syndrome and aplastic anemia in pediatric and young adult patients. Haematologica. 2016;101(11):1343–50.
123. Bannon SA, DiNardo CD. Hereditary Predispositions to Myelodysplastic Syndrome. Int J Mol Sci. 2016;17(6). pii: E838.
124. Bodor C, Renneville A, Smith M, Charazac A, Iqbal S, Etancelin P, et al. Germ-line GATA2 p.THR354MET mutation in familial myelodysplastic syndrome with acquired monosomy 7 and ASXL1 mutation demonstrating rapid onset and poor survival. Haematologica. 2012;97(6):890–4.
125. West AH, Godley LA, Churpek JE. Familial myelodysplastic syndrome/acute leukemia syndromes: a review and utility for translational investigations. Ann N Y Acad Sci. 2014;1310:111–8.
126. Gaitonde S, Boumendjel R, Angeles R, Rondelli D. Familial childhood monosomy 7 and associated myelodysplasia. J Pediatr Hematol Oncol. 2010;32(6):e236–7.
127. Morrissette JJD, Wertheim G, Olson T. Familial Monosomy 7 Syndrome. In: Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, LJH B, et al., editors. GeneReviews ®. Seattle: University of Washington; 1993.
128. Dowton SB, Beardsley D, Jamison D, Blattner S, Li FP. Studies of a familial platelet disorder. Blood. 1985;65(3):557–63.
129. Song WJ, Sullivan MG, Legare RD, Hutchings S, Tan X, Kufrin D, et al. Haploinsufficiency of CBFA2 causes familial thrombocytopenia with propensity to develop acute myelogenous leukaemia. Nat Genet. 1999;23(2):166–75.
130. Owen CJ, Toze CL, Koochin A, Forrest DL, Smith CA, Stevens JM, et al. Five new pedigrees with inherited RUNX1 mutations causing familial platelet disorder with propensity to myeloid malignancy. Blood. 2008;112(12):4639–45.
131. Noris P, Perrotta S, Seri M, Pecci A, Gnan C, Loffredo G, et al. Mutations in ANKRD26 are responsible for a frequent form of inherited thrombocytopenia: analysis of 78 patients from 21 families. Blood. 2011;117(24):6673–80.
132. Pippucci T, Savoia A, Perrotta S, Pujol-Moix N, Noris P, Castegnaro G, et al. Mutations in the 5′ UTR of ANKRD26, the ankirin repeat domain 26 gene, cause an autosomal-dominant form of inherited thrombocytopenia, THC2. Am J Hum Genet. 2011;88(1):115–20.
133. Bithell TC, Didisheim P, Cartwright GE, Wintrobe MM. Thrombocytopenia inherited as an autosomal dominant trait. Blood. 1965;25:231–40.
134. Bluteau D, Balduini A, Balayn N, Currao M, Nurden P, Deswarte C, et al. Thrombocytopenia-associated mutations in the ANKRD26 regulatory region induce MAPK hyperactivation. J Clin Invest. 2014;124(2):580–91.
135. Noris P, Favier R, Alessi MC, Geddis AE, Kunishima S, Heller PG, et al. ANKRD26-related thrombocytopenia and myeloid malignancies. Blood. 2013;122(11):1987–9.
136. Godley LA. Inherited predisposition to acute myeloid leukemia. Semin Hematol. 2014;51(4):306–21.
137. Zhang MY, Churpek JE, Keel SB, Walsh T, Lee MK, Loeb KR, et al. Germline ETV6 mutations in familial thrombocytopenia and hematologic malignancy. Nat Genet. 2015;47(2):180–5.

138. Noetzli L, Lo RW, Lee-Sherick AB, Callaghan M, Noris P, Savoia A, et al. Germline mutations in ETV6 are associated with thrombocytopenia, red cell macrocytosis and predisposition to lymphoblastic leukemia. Nat Genet. 2015;47(5):535–8.
139. Melazzini F, Palombo F, Balduini A, De Rocco D, Marconi C, Noris P, et al. Clinical and pathogenetic features of ETV6 related thrombocytopenia with predisposition to acute lymphoblastic leukemia. Haematologica. 2016;101(11):1333–42.
140. Kirwan M, Walne AJ, Plagnol V, Velangi M, Ho A, Hossain U, et al. Exome sequencing identifies autosomal-dominant SRP72 mutations associated with familial aplasia and myelodysplasia. Am J Hum Genet. 2012;90(5):888–92.
141. Smith ML, Cavenagh JD, Lister TA, Fitzgibbon J. Mutation of CEBPA in familial acute myeloid leukemia. N Engl J Med. 2004;351(23):2403–7.
142. Pathak A, Seipel K, Pemov A, Dewan R, Brown C, Ravichandran S, et al. Whole exome sequencing reveals a C-terminal germline variant in CEBPA-associated acute myeloid leukemia: 45-year follow up of a large family. Haematologica. 2016;101(7):846–52.
143. Nanri T, Uike N, Kawakita T, Iwanaga E, Mitsuya H, Asou N. A family harboring a germline N-terminal C/EBPalpha mutation and development of acute myeloid leukemia with an additional somatic C-terminal C/EBPalpha mutation. Genes Chromosomes Cancer. 2010;49(3):237–41.
144. Renneville A, Mialou V, Philippe N, Kagialis-Girard S, Biggio V, Zabot MT, et al. Another pedigree with familial acute myeloid leukemia and germline CEBPA mutation. Leukemia. 2009;23(4):804–6.
145. Carmichael CL, Wilkins EJ, Bengtsson H, Horwitz MS, Speed TP, Vincent PC, et al. Poor prognosis in familial acute myeloid leukaemia with combined biallelic CEBPA mutations and downstream events affecting the ATM, FLT3 and CDX2 genes. Br J Haematol. 2010;150(3):382–5.
146. Tawana K, Wang J, Renneville A, Bodor C, Hills R, Loveday C, et al. Disease evolution and outcomes in familial AML with germline CEBPA mutations. Blood. 2015;126(10):1214–23.
147. Pabst T, Mueller BU. Complexity of CEBPA dysregulation in human acute myeloid leukemia. Clin Cancer Res. 2009;15(17):5303–7.
148. Owen C, Barnett M, Fitzgibbon J. Familial myelodysplasia and acute myeloid leukaemia–a review. Br J Haematol. 2008;140(2):123–32.
149. Pabst T, Eyholzer M, Haefliger S, Schardt J, Mueller BU. Somatic CEBPA mutations are a frequent second event in families with germline CEBPA mutations and familial acute myeloid leukemia. J Clin Oncol. 2008;26(31):5088–93.
150. Polprasert C, Schulze I, Sekeres MA, Makishima H, Przychodzen B, Hosono N, et al. Inherited and somatic defects in DDX41 in myeloid neoplasms. Cancer Cell. 2015;27(5):658–70.
151. Li R, Sobreira N, Witmer PD, Pratz KW, Braunstein EM. Two novel germline DDX41 mutations in a family with inherited myelodysplasia/acute myeloid leukemia. Haematologica. 2016;101(6):e228–31.
152. Lewinsohn M, Brown AL, Weinel LM, Phung C, Rafidi G, Lee MK, et al. Novel germ line DDX41 mutations define families with a lower age of MDS/AML onset and lymphoid malignancies. Blood. 2016;127(8):1017–23.
153. Churpek JE, Godley LA. How I diagnose and manage individuals at risk for inherited myeloid malignancies. Blood. 2016;pii:blood-2016-05-670240. [Epub ahead of print]. doi:10.1182/blood-2016-05-670240.

# Chapter 16
# Myeloid and Lymphoid Neoplasms with Eosinophilia and Abnormalities of *PDGFRA*, *PDGFRB*, *FGFR1,* or t(8;9)(p22;p24.1);*PCM1-JAK2*

**Joanna M. Chaffin and Natasha M. Savage**

## Hematopoietic Neoplasms Associated with Eosinophilia and Abnormalities of *PDGFRA*

### *Introduction*

For years, the majority of patients with eosinophilia and abnormalities of *PDGFRA* fell into the nebulous category of hypereosinophilic syndrome (HES) [1]. With no abnormalities by karyotype and no other infectious or allergic causes clinically found to explain the persistent eosinophilia, patients were left with little explanation and a diagnosis of exclusion [2]. If clonality could be proven or if increased myeloblasts were present in peripheral blood or bone marrow, then a diagnosis of chronic eosinophilic leukemia (CEL) could be given, though little was available in the way of effective treatment [2]. This changed in 2001, when a subset of patients with HES was found to show responsiveness to tyrosine kinase inhibitors (TKIs), specifically imatinib [1, 3]. Molecular investigation of the tyrosine kinases within these patients revealed a cryptic deletion resulting in fusion of *PDGFRA* to *FIP1L1* [1]. As more fusion partners were identified for *PDGFRA* as well as other tyrosine kinases, *PDGFRB* and *FGFR1*, a new category was created for hematopoietic neoplasms with eosinophilia with these related molecular findings and recognized in the fourth edition of the World Health Organization's (WHO) Classification of Tumors of Hematopoietic and Lymphoid Tissues [4].

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Among patients with "HES," between 10% and 25% will have abnormalities of *PDGFRA* [3]. The overwhelming majority of these patients are male, with a 17:1 male to female ratio [4]. The median age of onset is 40, with a range of 7–77 years reported.

Patients with hematopoietic neoplasms involving *PDGFRA* often present with fatigue or pruritic rash [1, 4]. Tissue infiltration by eosinophils (and to some extent mast cells) leads to multi-organ tissue damage, including respiratory, gastrointestinal, or cardiac sequelae [4]. Cardiomyopathy secondary to endomyocardial fibrosis, in particular, is associated with a poor outcome. Physical examination reveals splenomegaly in the majority of patients and hepatomegaly within a minority. Elevated serum tryptase is also often present [5].

## *Morphology and Immunophenotyping*

Peripheral blood and marrow samples are most often consistent with CEL [4]. Eosinophilia is the most predominant feature on peripheral blood. However, eosinophilia may be mild or even absent in patients with certain rare variants [6]. Eosinophils are largely mature, with only rare eosinophilic myelocytes [4]. Atypia may include sparse granulation, cytoplasmic vacuolation, small granules, or abnormal segmentation or may be completely absent (Fig. 16.1). Anemia and thrombocytopenia are common, while monocytosis and basophilia are infrequent [7]. Bone marrow trephine biopsies are hypercellular with increased eosinophils and precursors (Fig. 16.2) [4]. These may show evidence of activation by immunohistochemistry, with CD23, CD25, or CD69 positivity. Reticulin is also increased (Fig. 16.3).

Mast cells are often increased as well, in a loose fashion or in cohesive clusters [4]. These mast cells may have atypical features such as spindled morphology and may be confused with systemic mastocytosis (SM). The immunophenotype is variable, with mast cells most often being CD2-negative CD25-positive, but occasionally

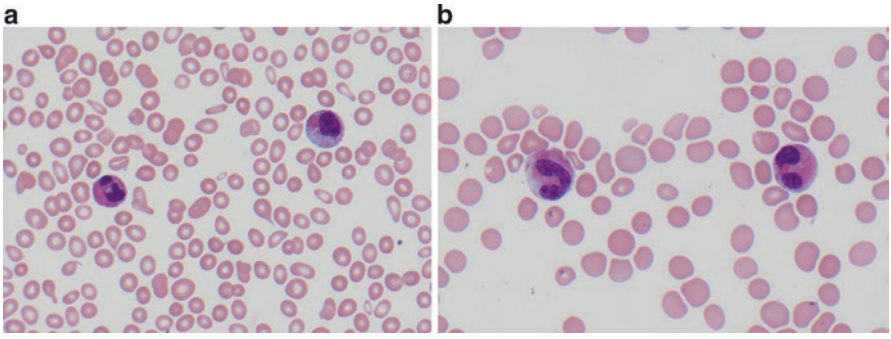


**Fig. 16.1** (**a**, **b**) Wright stained peripheral blood smear, 500× and 1000×, respectively. Peripheral eosinophils with atypia, including abnormal segmentation (a) and sparse, pooling granules (**a**, **b**)

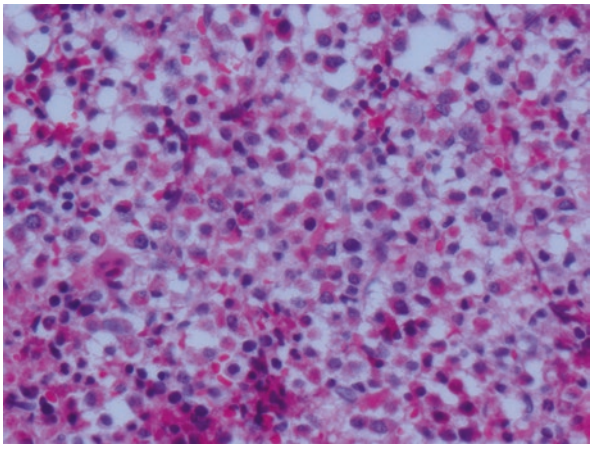

**Fig. 16.2** Hematoxylin and eosin stained biopsy, 500×. Hypercellular marrow with increased eosinophilic precursors

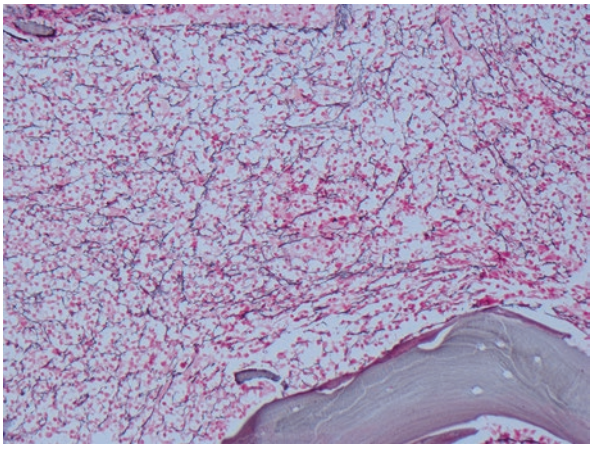

**Fig. 16.3** Reticulin stain, 200×. Increased reticulin deposition in bone marrow trephine biopsy

CD2-negative CD25-negative or CD2-positive CD25-positive as well. Although aberrant mast cell immunophenotypes and morphology may be noted, *KIT* D816V mutation is not present and serum tryptase levels are typically less than 20 ng/mL, and therefore diagnostic criteria for SM is not met.

While most patients have a CEL phenotype, the associated hematopoietic neoplasm can be quite varied [4]. Occasional patients present with acute myeloid leukemia (AML) or T-cell lymphoblastic leukemia/lymphoma (T-ALL) [4, 8]. Rarely, myeloid sarcoma has been reported [9]. Even when patients present with a phenotype other than CEL, peripheral eosinophilia usually remains a consistent feature [8, 9].

Since the majority of patients resemble HES, diagnostic workup is first directed at excluding other causes of eosinophilia, such as parasitic infection, hypersensitivity, drug reactions, and non-myeloid malignancies producing cytokines promoting eosinophilic differentiation [3]. Once secondary eosinophilia is excluded, bone

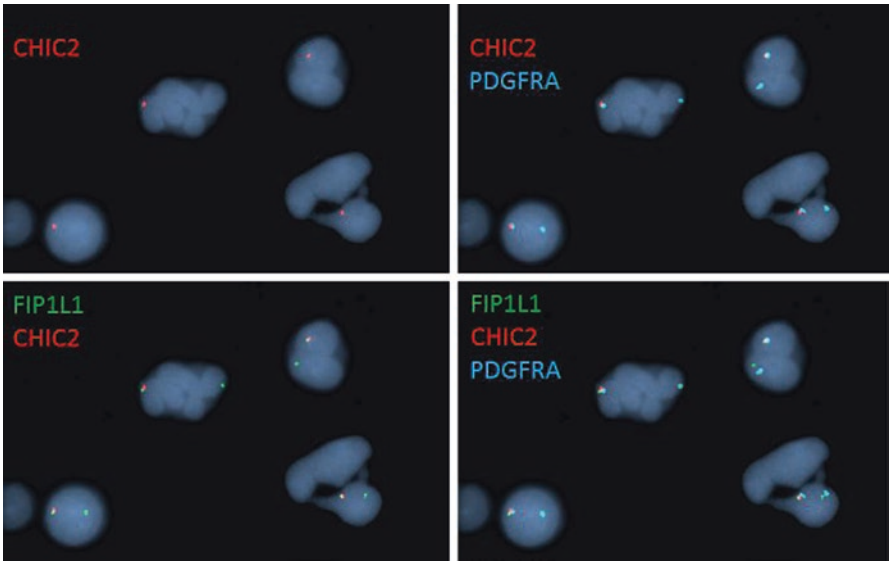


**Fig. 16.4** Images of FISH revealing *FIP1L1-PDGFRA* fusion gene due to deletion of *CHIC2* gene. This patient had a normal karyotype (figure courtesy of Dr. Robert Jenkins of the Mayo Clinic)

marrow aspirate and biopsy with cytogenetic analysis should be performed along with screening for *PDGFRA* abnormality by fluorescence in situ hybridization (FISH) or reverse transcription polymerase chain reaction (RT-PCR) (Fig. 16.4).

## *Cytogenetics and Molecular Findings*

*PDGFRA* encodes one of two mammalian receptor tyrosine kinases that interact with the family of platelet-derived growth factors (PDGFs) [10]. Platelet-derived growth factor receptor α (PDGFRα) comprises five extracellular immunoglobulin loops and a split intracellular tyrosine kinase domain (Fig. 16.5). Ligand binding leads to PDGFRα dimerization and initiates signaling. PDGFs and their receptors are crucial during development, particularly organogenesis, but have limited known functions within the adult. In addition to hematopoietic neoplasms, alterations of *PDGFRA* have been implicated in a host of solid tumors, including gastrointestinal stromal tumor (GIST) [11], glioblastoma [12, 13], inflammatory fibroid polyp [14], and malignant peripheral nerve sheath tumor [15].

Although a number a fusion partners for PDGFRA have been identified (Table 16.1), the most common is *FIP1L1* by far [4]. *FIP1L1* codes for the protein pre-mRNA 3′-end-processing factor FIP1, which interacts with poly(A) polymerase and other factors to bring about cleavage and poly(A) addition [16]. The *FIP1L1-PDGFRA*

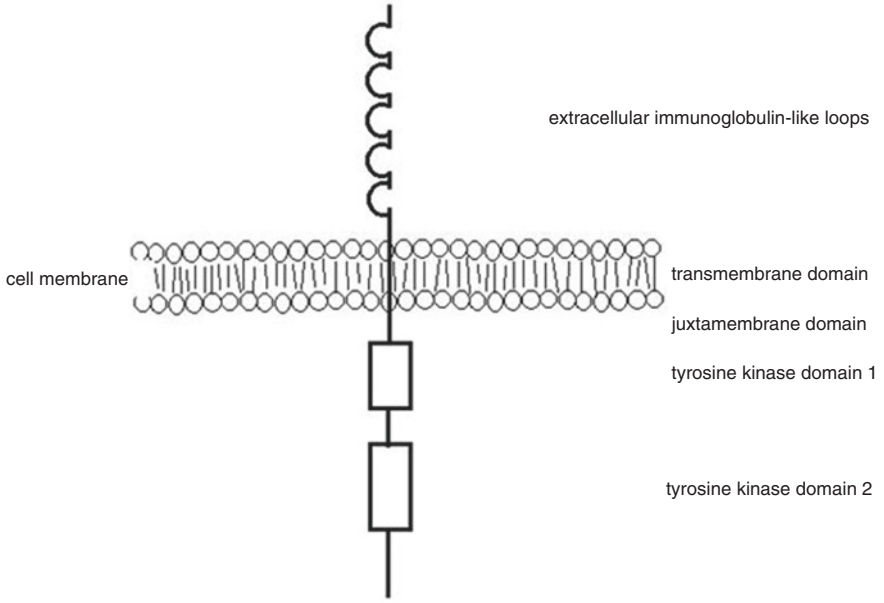


**Fig. 16.5** Schematic representation of PDGFRα protein

**Table 16.1** Myeloid and lymphoid neoplasms with eosinophilia and abnormalities of *PDGFRA*: fusion partners

| Fusion partner gene name | Fusion partner gene location |
|---|---|
| *FIP1L1* | 4q12 |
| *BCR* | 22q11 |
| *ETV6* | 12p13 |
| *STRN* | 2p22 |
| *CDK5RAP2* | 9q33 |
| *KIF5B* | 10p11 |
| *FOXP1* | 3p13 |

fusion gene was first described by Cools et al. in 2003 [1, 7]. It is created by an interstitial deletion on chromosome 4q12 [1]. This deletion includes cysteine-rich hydrophobic domain 2 (*CHIC2*) (Fig. 16.6) [17]. Although the deletion is most often cryptic [4], it may be caused by chromosomal rearrangement. t(1;4)(q44;q12) and t(4;10)(q12;p11) have been reported in such cases [18, 19].

The breakpoint of *FIP1L1* is variable, involving mostly introns 7 through 13 [20]. However, the *PDGFRA* breakpoint is much more highly conserved [20]. In all cases of *FIP1L1-PDGFRA* thus far reported, the *PDGFRA* breakpoint has been consistently located within exon 12, which contains the auto-inhibitory juxtamembrane (JM) domain. Two tryptophan (W) residues within the JM domain have consistently been truncated within *FIP1L1-PDGFRA*. Disruption of the WW residues leads to constituent kinase activation that is independent of dimerization. This is in contrast to other chimeric tyrosine kinases which require self-association domains

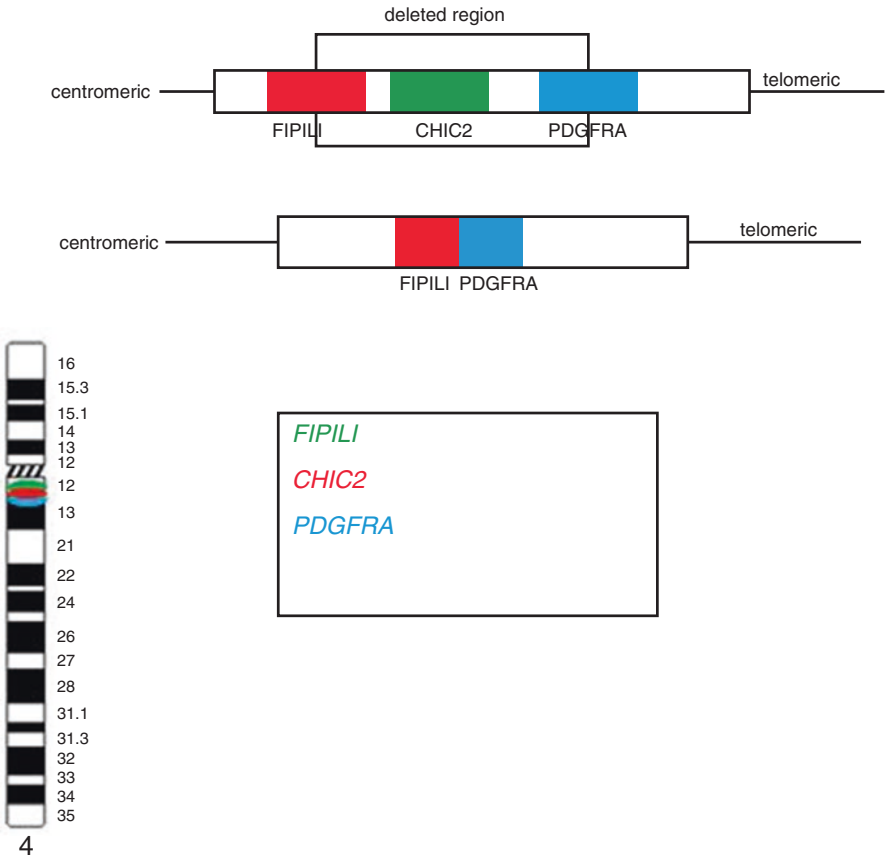


**Fig. 16.6** Schematic representations of *FIP1L1-PDGFRA* fusion gene due to deletion of *CHIC2* gene (second representation courtesy of Dr. Robert Jenkins of the Mayo Clinic)

on their fusion partners to enforce homodimerization (Fig. 16.7). PDGFRα is capable of dimerization-independent activation solely by loss of the auto-inhibitor JM domain. Although commonly present, *FIP1L1* is not required for this activation. This exon 12 breakpoint is conserved for patients with CEL, AML, or T-ALL phenotypes [8, 21].

*BCR-PDGFRA* is the second most commonly reported fusion protein associated with *PDGFRA*, following *FIP1L1* [4, 6, 18, 22]. Located on 22q11, the *BCR* gene encodes for the breakpoint cluster region protein [23]. The protein has serine/threonine kinase activity and is a GTPase-activating protein for p21rac [23]. *BCR* is most famous for its involvement as the partner of *ABL* in t(9;22), the defining translocation of chronic myelogenous leukemia (CML) [24]. Although extensively studied, the function of *BCR's* gene product is not clear.

*BCR-PDGFRA* typically resembles atypical chronic myelogenous leukemia (aCML) morphologically [18, 22]. Those treated with imatinib have achieved complete

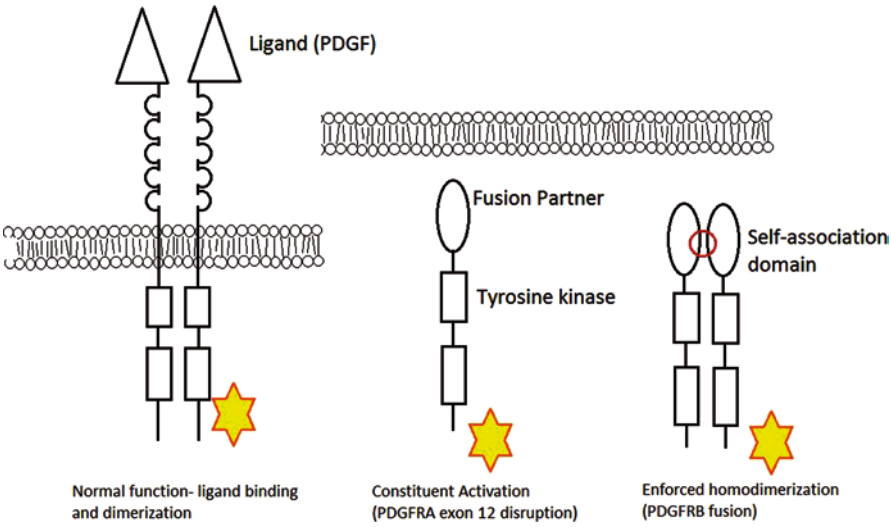


**Fig. 16.7** Schematic contrasting abnormalities of PDGFRA and PDGFRB

hematologic remission. Although the first reported cases of *BCR-PDGFRA* involved exon 12 of *PDGFRA*, interruption of exon 13 has also been reported [6]. The patient involved in this case demonstrated no eosinophilia [6], in contrast to other *BCR-PDGFRA* presentations [18, 22]. A T-ALL phenotype without eosinophilia has also been reported; the exon involved in the *PDGFRA* breakpoint was not specified in this case [25].

The *ETV6* gene is located at 12p13, and is a member of the ETS family of transcription factors [26]. *ETV6* is frequently involved in myeloid and lymphoid malignancies. *ETV6* is also a fusion partner with *PDGFRB* in other hematopoietic neoplasms associated with eosinophilia [4]. *ETV6-PDGFRA* has been described by Curtis et al. in a 51-year-old male with asthma, diabetes, and marked eosinophilia [26]. Bone marrow was suggestive of a myeloproliferative neoplasm (MPN). Conventional cytogenetics revealed a t(4;12)(q2?3;p1?2) in all metaphase cells. Sequencing of the fusion gene by PCR revealed breakpoints of intron 6 for *ETV6* and intron 11 for *PDGFRA*. Despite the variant breakpoint for *PDGFRA*, the patient still achieved complete cytogenetic remission following imatinib therapy.

Localized to the short-arm of chromosome 2 [26], *STRN* encodes for the protein striatin [27]. Striatin possesses four protein-protein interaction domains including a caveolin-binding domain, a coiled-coil domain, a Ca2 + −calmodulin-binding domain, and a tryptophan-aspartate-repeat domain. It is found most abundantly in the central and peripheral nervous system, as well as the lung, liver, kidney, and other tissues. Striatin is broadly implicated in neuronal function and may play a role in dendritic growth and remodeling. Musto et al. first described a 64-year-old male who presented with pruritus and a 2-year history of eosinophilia [28]. Bone marrow was consistent with an MPN [26]. A t(2;4)(p24;q12) was identified in 60% of meta-

phases by conventional cytogenetics. *PDGFRA* exon 12 was once again implicated in the breakpoint of the fusion gene, along with *STRN* intron 6. Although the patient initially achieved molecular remission on imatinib, eosinophilia recurred 14 months following treatment discontinuation.

*CKD5RAP2* encodes a protein comprising mostly coiled-coil domains [29]. The CKD5RAP2 protein stably associates with the centrosome and is present in all stages of the cell cycle. Mutations in *CKD5RAP2* have been associated with congenital microcephaly and intellectual disability [30]. Walz et al. first described the *CDK5RAP2-PDGFRA* fusion gene in a 79-year-old female [31]. She presented with splenomegaly, constitutional symptoms, spontaneous hematomas, and marked eosinophilia. Bone marrow was consistent with accelerated phase of CEL. Conventional cytogenetics demonstrated an ins(9;4)(q33;q12q25) in 5 of 21 cells. This insertion involved *CDK5RAP2* exon 13, an inverted portion of *PDGFRA* intron 9, as well as a truncated *PDGFRA* exon 12. The patient initially responded to imatinib, but subsequently relapsed with imatinib-resistant AML; no *CDK5RAP2-PDGFRA* was detectable at relapse.

*KIF5B* encodes the protein kinesin family member 5b [32]. This microtubule-based motor protein is involved in transportation of organelles. The fusion gene *KIF5B-PDGFRA* was identified by Score et al. in a 54-year-old male presenting with B-symptoms, headaches, and marked eosinophilia. Bone marrow revealed increased myeloid cells and eosinophilia. Conventional cytogenetics revealed a complex karyotype in eight out of 16 metaphases, 46XY, del(3)(p21), add(4)(q12), −10,13q?, +der(?)(? → cen →?::4q12 → 4q28.3::10q11.2 → 10qter). Sequencing of the fusion gene revealed involvement of *KIF5B* exon 23 and *PDGFRA* exon 12. Imatinib therapy resulted in complete cytogenetic and molecular response.

*FOXP1* encodes the forkhead box protein P1 which is an essential factor in B-cell development, regulating transition from resting follicular cells to activated germinal cells [33]. *FOXP1* fusion genes have also been implicated in B-cell lymphoblastic lymphoma (B-ALL), and single nucleotide deletions have been found in MPN. Sugimoto et al. described the *FOXP1-PDGFRA* fusion in a patient presenting with a wet cough. Peripheral blood demonstrated marked eosinophilia, neutrophilia, and erythrocytosis. Bone marrow biopsy was consistent with an MPN. An abnormal 46, XY, t(3;4)(p13;q12) was identified by conventional cytogenetics, with breakpoints in exon 23a of *FOXP1* and exon 12 of *PDGFRA*. The patient achieved sustained molecular remission for 3 years following imatinib treatment.

Although myeloid neoplasms with eosinophilia and abnormalities of *PDGFRA* comprise rearrangements with resultant fusion proteins, some point mutations have been identified within *PDGFRA* in patients with HES [34]. In one study of 87 hypereosinophilic patients with no evidence of *PDGFRA* rearrangement, 8% demonstrated point mutations within *PDGFRA*. Further investigation is needed to determine the response of such cases to imatinib.

Exon 12 disruption is a recurring theme for most *PDGFRA*-associated fusion genes. The only exception seems to be instances in which *PDGFRA* is paired with a gene already associated with hematopoietic neoplasms, such as *BCR* and *ETV6* [4, 26]. Regardless of partner gene, most *PDGFRA*-associated hematopoietic

neoplasms respond well to imatinib therapy. Thus it may be inferred that the exact fusion is of little clinical significance so long as *PDGFRA* exon 12 disruption is confirmed.

Since the majority of *PDGFRA*-related hematopoietic neoplasms are the result of a cryptic 4q12 deletion, karyotyping is inadequate for detection [4]. FISH, on the other hand, has proven useful for detecting *PDGFRA* rearrangements [35]. An early strategy used a single probe directed at the *CHIC2* locus [17]. Newer methods use multiple probes directed at various loci, including *FIP1L1*, *PDGFRA*, and the intervening regions [36].

RT-PCR also has utility in diagnosis of *PDGFRA*-rearranged hematopoietic neoplasms [36]. RT-PCR is almost equivalent to FISH in diagnosis of *PDGFRA* rearrangement, largely due to the highly conserved nature of exon 12 involvement, although, theoretically, a fusion variant involving a different *PDGFRA* locus might be missed. For monitoring purposes, however, RT-PCR may be more suitable, since autofluorescence in interphase FISH can make it difficult to determine the exact percentage of *FIP1L1-PDGFRA* positive cells. Furthermore, RT-PCR would be able to detect lower disease burden.

## *Therapy, Prognosis, and Monitoring*

Since the discovery of hematopoietic neoplasms with abnormalities of *PDGFRA*, imatinib has been the therapy of choice for this entity [1]. An initial dose of 100 mg per day leads to complete hematologic response in the majority of patients [37]. Occasionally, imatinib is discontinued secondary to adverse reactions or rarely due to resistance, but it can be replaced by second or third generation TKIs. While on maintenance therapy, >90% of patients will achieve complete molecular response [37, 38].

Although the majority of eosinophilic *PDGFRA*-related neoplasms demonstrate a rapid and durable response to imatinib therapy [39], some initially responding patients may develop resistance. This is most often due to the secondary mutation, T674I *FIP1L1-PDGFRA* [40]. This missense mutation located in ATP binding region of the kinase domain also leads to resistance to second generation TKIs, nilotinib and sorafenib [40, 41]. Rebastanib and ponatinib, both third generation TKIs originally designed to treat secondarily resistant CML, have shown promise in treatment of secondary resistance in mice models, but still require safety or efficacy trials [40].

Another missense mutation associated with secondary imatinib resistance is D842V *PDGFRA* [42, 43]. This substitution within the kinase activation loop favors the active conformation of PDGFRα, while imatinib is only able to bind to the inactive conformation [11]. While this mutation has been known to confer primary resistance to imatinib in some GISTs [11], it has only been reported following TKI therapy in *PDGFRA* myeloid neoplasms [42, 43].

Primary resistance to imatinib has only been reported in one case [44]. This was reported in a 59-year-old man diagnosed with idiopathic HES 15 years prior. RT-PCR and sequencing revealed two missense mutations within the kinase domain,

S601P and L629P. Subsequent investigation suggested that S601P was likely the mutation responsible for imatinib resistance and that the L629P was incidental [45].

The natural history of *PDGFRA*-associated hematopoietic neoplasms in the absence of treatment is difficult to elucidate due to the simultaneous characterization of the disease with discovery of its primary treatment. However, those maintained on continuous imatinib therapy have largely done well thus far, maintaining complete molecular response [37, 38]. Some have even attained complete molecular response after discontinuation of imatinib. Rarely, patients relapse while on therapy or may transform to a more aggressive neoplasm [38]. Transformation to B-ALL has been reported following TKI therapy [46]. However, despite these rare occurrences, the majority of patients have a good prognosis if maintained on therapy, with most morbidity and mortality being attributable to end-organ damage prior to treatment [37, 38].

### *Conclusion*

The elucidation of the *FIP1L1-PDGFRA* fusion gene and related *PDGFRA* rearrangements dramatically highlights how advances in our understanding of the molecular causes of disease can help direct patient therapy. Regardless of the fusion gene involved, imatinib remains an effective therapy for the majority of patients. For those rare patients with resistance, new TKIs are continuing development. Other options for treatment beyond TKIs may also be available in the future. For instance, the involvement of nuclear factor –κB in eosinophilic proliferation and differentiation has provided a possible target for new treatments [47].

## Myeloid and Lymphoid Neoplasms with Eosinophilia and Abnormalities of *PDGFRB*

### *Introduction*

Gene fusions with *PDGFRB* were first described by Golub et al. in 1994 in a patient with features consistent with chronic myelomonocytic leukemia (CMML) [48]. Since that time, over 20 fusion partners have been described [1, 49]. Myeloid and lymphoid neoplasms with eosinophilia and abnormalities of *PDGFRB* are rare, accounting for less than 2% of all myelodysplastic/myeloproliferative neoplasms (MDS/MPN) [49]. Adult males are most commonly affected with a median age of onset in the late forties; however, children have also been rarely affected.

Patients typically present with splenomegaly; hepatomegaly is less frequent. Lymphadenopathy may also be seen. Skin and cardiac infiltration may be present at diagnosis with resulting cardiac damage. Serum tryptase levels may be predictively elevated.

## *Morphology and Immunophenotyping*

In patients with abnormalities of *PDGFRB,* peripheral blood and bone marrow is almost always involved. Leukocytosis is typical with monocytosis and eosinophilia. Rarely, basophilia is also prominent. Anemia and thrombocytopenia may also be present. Overall, the features are typically suggestive of CMML with eosinophilia; however, some patients present with features more in keeping with aCML or CEL. Rarely, they present with features of ALL, AML, and juvenile myelomonocytic leukemia (JMML).

The bone marrow is typically hypercellular with accompanying fibrosis. As in cases with *PDGFRA* abnormalities, mast cell aggregates (not meeting criteria for SM) can be seen [1].

## *Cytogenetics and Molecular Findings*

The structure of *PDGFRB* is very similar to *PDGFRA*, sharing 30% amino acid similarity [50]. It too encodes a plasma membrane-spanning receptor with five extracellular immunoglobulin-like loops for ligand binding and a split intracellular tyrosine kinase domain. Signal transduction is very similar, with ligand binding inducing dimerization and autophosphorylation of the tyrosine kinase. In addition to its role in embryonic development, *PDGFRB* mediates chemotactic responses of monocytes, macrophages, and platelets to inflammatory processes. Overexpression has been implicated in solid tumors, such as medulloblastoma and chordoma.

Like *PDGFRA*, *PDGFRB* has a frequently observed common breakpoint, albeit not as highly conserved as *PDGFRA* exon 12. Unlike *PDGFRA*, however, this breakpoint is located in intron 10; therefore, most fusion proteins begin the *PDGFRB* portion with an intact exon 11 [51]. Rarely, the *PDGFRB* breakpoint is found elsewhere, most commonly within exon 12 [52–54]. As in *PDGFRA*, this exon contains the juxtamembrane domain and a WW-like domain with inhibitory function. While thus far, this breakpoint has not been proven to be sufficient for inducing transformation to malignancy, it may still have an important regulatory role in kinase activation [55]. Since the auto-inhibitory juxtamembrane domain is unaffected in the majority of fusions, oligomerization is still required for activation of the tyrosine kinase. This oligomerization is facilitated by the structure of the various partner genes. The prototypic fusion partner, *ETV6*, possesses a helix-loop-helix domain (also called the pointed domain), which functions as the primary site of oligomerization [56]. Moreover, the grand majority of the less common fusion partners possess at least one coiled-coil domain instead, which likely serves the same purpose. Occasionally, an additional motif aiding in dimerization may be found in the form of a leucine zipper [57–59] or other oligomerization domain [60].

Of the tyrosine kinase receptors discussed in this chapter, *PDGFRB* is the most promiscuous regarding fusions producing hematologic malignancy with eosinophilia,

**Table 16.2** Myeloid and lymphoid neoplasms with eosinophilia and abnormalities of *PDGFRB*: fusion partners and various associations

| Fusion partner gene name | Fusion partner gene location | Association |
|---|---|---|
| *ETV6* | 12p12 | CMML-E, CEL |
| *CCDC88C* | 14q32 | CMML-E |
| *CCDC6* | 10q21 | aCML-E, MPN-E |
| *TRIP11* | 14q32 | Only reported in patients of Asian ancestry |
| *TPM3* | 1q21 | CEL |
| *CAPRIN1* | 1p11 | CEL |
| *GIT2* | 12q24 | CEL |
| *RABEP1* | 17p13 | CMML, T-ALL |
| *CEP85L* | 6q22 | MPN-E, T-ALL |
| *PRKG2* | 4q21 | Chronic basophilic leukemia |
| *COL1A1* | 17q21 | |
| *NDE1* | 16p13 | CMML |
| *SPTBN1* | 2p21 | |
| *PDE4DIP* | 1q21 | MDS/MPN-E |
| *TP53BP1* | 15q15-q21 | aCML-E |
| *SPECC1* | 17p11 | JMML |
| *GOLGA4* | 3p22 | aCML-E, MPN-E |
| *HIP1* | 7q11 | CMML-E |
| *BIN2* | 12q13 | |
| *MYO18A* | 17q11 | |
| *NIN* | 14q22 | aCML-E |
| *SART3* | 12q23 | |
| *ERC1* | 12p13 | |
| *WDR48* | 3p21 | CEL |
| *DTD1* | 20p11 | |
| *KANK1* | 9p24 | ET |

with at least 26 fusion partners currently described (Table 16.2). As previously alluded to, by far the most common is *ETV6-PDGFRB*. Fusion results in the joining of the N-terminal domain of *ETV6* to the tyrosine kinase-containing C-terminal of *PDGFRB* (Fig. 16.8) [55]. This fusion leads to oligomerization at the pointed domain, constituently active phosphorylation, and activation of STAT proteins. This fusion was first described in 1987 by Keene et al. as a case series of four patients, two with MPN, one with ALL, one with CEL, and all with significant eosinophilia [61]. The t(5;12)(9q33;p12) usually results in RT-PCR detectable fusion of *ETV6* exon 4 and *PDGFRB* exon 11 [51]. As previously stated, *ETV6* is often involved in myeloid and lymphoid malignancies. In addition to infrequent fusion with *PDGFRA*, *ETV6* rearrangements are also involved in B-ALL and undifferentiated AML [26].

The second most commonly reported fusion is *CCDC88C-PDGFRB*. This fusion gene is typically the result of t(5;14)(q33;q32), however, a t(5;17;14)(q33;q11;q32)

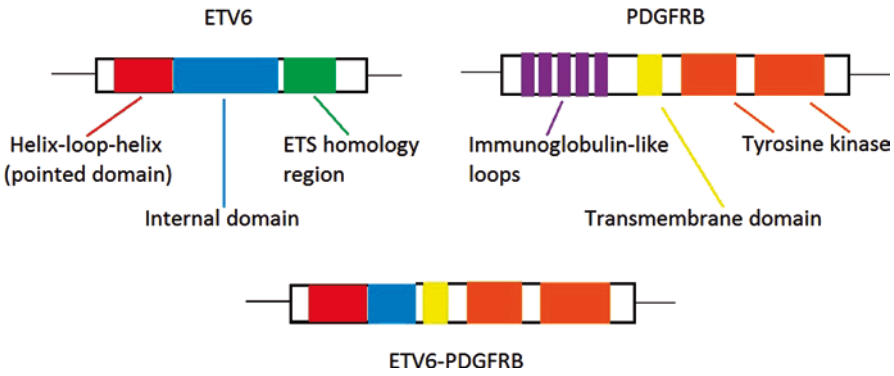


**Fig. 16.8** Schematic representation of *ETV6-PDGFRB* fusion gene

has also been reported [52]. Although the breakpoint for *CCDC88C* is variable, located anywhere between introns 9 and introns 26, the *PDGFRB* breakpoint is more highly conserved—only reported to deviate from the typical intron 10 once [52, 62, 63]. *CCDC88C* encodes a protein widely expressed, with highest levels found in bone marrow, spleen, and thymus [62]. Its N-terminal includes a highly conserved coiled-coil domain. Interestingly enough, two of the five reported cases have included women presenting with a CEL phenotype, remarkable for differing from both the usually predominant gender and the typical CMML phenotype [63, 64]. *CCDC88C-PDGFRB* is one of two fusions that have rarely shown resistance to imatinib, although this was overcome following the addition of prednisolone to the treatment regimen [64].

Four patients have been identified bearing the *CCDC6-PDGFRB* fusion gene. All patients have been male, of a CMML or aCML phenotype, demonstrated a t(5;10)(q33;q21), and shown breakpoints of *CCDC6* intron 7 and *PDGFRB* intron 10 [57, 65–67]. Although ubiquitously expressed, the protein product of *CCDC6* is of unknown function [57]. The gene has been implicated in papillary thyroid carcinoma, specifically when an inv.(10)(q22q21) leads to *CCDC6-RET* fusion in 25% of patients [57, 68]. The N-terminal possesses coiled-coil domains and a leucine zipper, both of which have been proven necessary for IL3-independent growth [57, 69]. Resistance to imatinib has also been rarely reported in *CCDC6-PDGFRB* [68].

*TRIP11-PDGFRB* fusion has, thus far, only been reported in patients of Asian ancestry [58, 70–72]. The first reported case was of a 21-year-old Japanese woman with a history of relapsed AML associated with new onset eosinophilia and t(5;14)(q33;q32) [58, 72]. Since then, it has been identified in a Korean and a Chinese patient as well [70, 71]. RT-PCR demonstrated fusion of *TRIP11* exon 16 to *PDGFRB* exon 11 [70]. Curiously, in two of the three cases, the fusion only arose as a therapy related myeloid neoplasm following remission of AML (acute promyelocytic leukemia in the case of the Korean patient) [58, 70]. *TRIP11*'s product, golgi-microtubule-associated protein of 210 kDa, also known as thyroid hormone receptor interactor 11, is a ubiquitous member of the golgin family that possesses a long

coiled-coil domain [58, 73]. The protein also possesses a leucine zipper structure, a motif important in dimerization and DNA binding [58].

While most *PDGRFB*-rearranged hematopoietic neoplasms have a CMML phenotype, *TPM3-PDGFRB* has thus far only been reported in patients with CEL [74, 75]. One case involved a 21-year-old male, and the other involved an 8-year-old male. Two other fusion genes have presented with a CEL phenotype, *GIT2* and *CAPRIN1*. The patient with fusion of *CAPRIN1* to *PDGFRB* had a complex karyotype with der(1)t(1;5)(p34;q33), der(5)t(1;5)(p34;q15), der(11) ins(11;5)(p12;q15q33) [54]. Caprin-1 is a cytoplasmic protein implicated in cellular activation and proliferation which is highly expressed in hematopoietic cells. The *GIT2-PDGFRB* fusion had a t(5;12)(q31–33;q24) by karyotype. *GIT2*'s product is ubiquitously expressed and involved in reorganization of the cytoskeleton for cell motility. Although its protein does not contain any coiled-coil domains, it does possess an ankyrin domain at the N-terminus, which may be involved in protein-protein interactions.

Two fusion genes have been reported associated with a T-ALL phenotype, in addition to *ETV6-PDGFRB* [76]. The *RABEP1-PDGFRB* fusion gene is the product of t(5;17)(q33;p13) [77, 78]. *RABEP1* encodes rabaptin-5, a cytosolic protein-regulating endocytosis with a coiled-coil structure [78]. Although it has been associated with a CMML phenotype, *RABEP1-PDGFRB* has also been seen in a 64-year-old man presenting with T-ALL [77]. *CEP85L-PDGFRB* fusion with t(5;6)(q33–34;q22–23) has also infrequently been reported, with both MPN and T-ALL with peripheral eosinophilia as initial presentation [53, 77, 79]. Exceptionally, rapid amplification of cDNA ends (RACE) and single step RT-PCR identified in frame fusion of *CEP85L* exon 11 and *PDGFRB* exon 12, rather than the typical exon 11 [53, 79]. The product of *CEP85L*, centrosomal protein 85 kDa-like, as yet has no clearly identified function, but has been linked to the QT interval and was initially reported as a breast cancer antigen [79].

*PRKG2-PDGFRB* has been found as the result of a variety of fusions, including t(4;5)(q21;q33), t(4;5)(q21.1;q31.3), and t(4;5;5)(q23;q31;q33) [54, 80, 81]. The exon 12 breakpoint disrupted the WW-like auto-inhibitory domain of *PDGFRB*, similar to the highly conserved breakpoint in exon 12 of *PDGFRA* [54], and could be postulated to be capable of constituent activation in the absence of dimerization. *PKRG2* encodes a cGMP-dependent protein kinase involved in multiple functions including neuronal adaption, bone growth, and renin secretion, to name a few.

Although clinical information is limited, *COL1A1-PDGFRB* has been reported in a 44- year-old male and an 86-year-old female with t(5;17;22)(q32;q21;q21) and complex +X, +1, +7, +9, +10, +11, −-13, +14, +15, del (17)(p12){7}/der(17)t(5;17)(q33;q21), +18, +19, +20, +21, +22, +3mar {8}, respectively [82]. Defects of collagen type I alpha 1, the primary subunit of type 1 collagen, have been implicated in osteogenesis imperfecta, Ehlers-Danlos syndrome, and osteoporotic conditions [83]. Although not possessing a coiled-coil domain, collagen type I alpha 1 does have a central triple-helix domain, which one might speculate to serve as a site for dimerization.

A t(5;12)(q33;p13) has led to *NDE1-PDFGRB* fusion in a 35-year-old woman with Noonan syndrome presenting with CMML [84]. *NDE1* encodes for a centro-

somal protein-regulating movement of kinetochore proteins to spindle poles, and is almost always disrupted in *CBFB/MYH11* positive AML [84]. The underlying cause of her Noonan syndrome was a germline missense *PTPN11* mutation, whose product regulates intracellular signaling of a number of growth factors, including *PDGFRB*. It is unclear what role this germline mutation may have played in her disease presentation.

*SPTBN1-PDGFRB* fusion has been identified in a 73-year-old woman with multiple endocrine neoplasia (MEN) type 1 [81]. She presented with an MPN phenotype, and cytogenetics demonstrated t(2;5)(p21;q33). *SPTBN1* encodes a nonerythroid spectrin participating in cell-cell and cell-matrix adhesion.

Numerous other fusion partners are described for *PDGFRB*, usually limited to single case reports. In those cases in which imatinib therapy was initiated, the patients entered hematologic or molecular remission. The following cases demonstrated either a *PDGFRB* intron 10 breakpoint or fusion of *PDGFRB* exon 11 to the corresponding gene transcript by RT-PCR or RACE. *PDE4DIP-PDGFRB* has been identified in a 11-month-old female with t(1;5)(q23;q33) [85]. *PDE4DIP* encodes the protein myomegalin, and in this case, the fusion protein involved the isoform which predominantly consists of coiled-coil structures. *TP53BP1-PDGFRB*, another infrequently reported fusion, was first described in a 79-year-old male presenting with t(5;15)(q33;q22) [86]. *TP53BP1* encodes the tumor protein P53 binding protein 1, which binds to wild-type p53 and contributes to the cellular response to DNA damage. *SPECC1-PDGFRB* was identified in an 18-month-old male with t(5;17)(q33;p11.2) who presented with JMML [87]. *SPECC1*, previously called *HCMOGT-1*, encodes for a sperm antigen and is not typically implicated in malignancy. *GOLGA4-PDGFRB* fusion has been reported twice, once in a 67- year-old male and again in a 13-month-old male presenting with aCML and MPN, respectively [88]. Cytogenetics revealed t(3;5)(p21–25;q31–35) [88]. *GOLGA4*, another member of the golgin family, encodes a golgi-localized protein with extensive coiled-coil regions. *HIP1-PDGFRB* fusions have infrequently been reported and was first described in a 54-year-old male with t(5;7)(q33;q11.2) presenting with CMML [89]. *HIP1* encodes a Huntingtin interacting protein, possesses three areas mediating protein-protein interaction, including a leucine zipper, an opened coiled-coil, and a domain with homology to talin, a cytoskeletal protein implicated in cell-cell and cell-matrix interactions [59, 90].

Many other cases have rarely been described with breakpoints other than *PDGFRB* intron 10. *PDGFRB* fusion to *BIN2*, which is expressed predominantly in hematopoietic cells during granulocytic differentiation, has been reported once [88]. Cytogenetic analysis showed a t(5;12)(q33;q13), and RACE revealed fusion of *BIN2* exon 9 to a truncated *PDFGRB* exon 12. *MYO18A-PDGFRB* has been reported in a 51-year-old male with t(5;17)(q33–34;q11.2) and features resembling CMML; *PDGFRB* breakpoint was at intron 9 [91]. *MYO18A,* also a reported fusion partner for *FGFR1,* is a member of the myosin superfamily originally identified in hematopoietic stromal lines and whose function is not well understood. A *NIN-PDGFRB* fusion with t(5;14)(q33;q24) was reported in a 35-year-old male presenting with aCML with fusion of NIN exon 28 to *PDGFRB* exon 12 [92]. Ninein, the product

of the *NIN* gene, is a centrosomal protein with numerous coiled-coils. The protein's structure closely resembles centrosomal protein 110 kDa (CEP110), which is the gene product of *CNTRL*, a reported fusion partner of *FGFR1*. Fusion between *PDGFRB* exon 12 and *SART3* exon 15 has also been identified in a patient with MPN with peripheral eosinophilia [93]. Karyotyping was uninformative in this case due to severe myelofibrosis and limited metaphase cells present. *SART3* encodes an RNA binding nuclear protein initially identified in a myeloid cell line and highly expressed in cancerous cell. *PDGFRB* fusion to *ERC1*, a gene just telomeric to *ETV6*, was found in a 36-year-old man with relapsed AML when karyotype revealed t(5;12)(q33;p13), but FISH failed to show *ETV6* rearrangement; *ERC1* exon 15 was fused to *PDGFRB* exon 10 [94]. A three-way t(1;3;5)(p36;p21;q33) has resulted in *WDR48-PDGFRB* fusion in a 65-year-old female with long-standing chronic MPN [88]. Breakpoints included *PDGFRB* intron 11 and *WDR48* intron 10. *WDR48* encodes an endosomal protein including a carboxyl coiled-coil region and an amino terminal WD repeat region. The *DTD1-PDGFRB* gene has been reported in a 43-year-old male presenting with a CEL phenotype [52]. Cytogenetics identified t(5;20)(q33;p12), and RACE demonstrated cDNA fusion of *DTD1* exon 4 and a truncated *PDGFRB* exon 12. The product of *DTD1* has a similar sequence to histidyl-tRNA synthetase and is speculated to play a role in defense against harmful effects of D-tyrosine. *KANK1-PDGFRB* has been reported in a 67-year-old man presenting with thrombocytosis, but without prominent eosinophilia [95]. Karyotype showed t(5;9)(q31–33;p22-?24.3), and RACE revealed fusion of *KANK1* exon 2 to *PDGFRB* exon 9. *KANK1*, a potential tumor suppressor gene, has been implicated in various malignancies, including ALL.

Unlike identifying the *FIP1L1-PDGFRA* gene, conventional cytogenetics readily identifies 5q33 rearrangement and is useful at the time of initial presentation. Multicolor FISH has been useful in recognizing *PDGFRB* rearrangement or confirming suspected fusion on karyotype, using probes that closely flank both ends of the gene [96]. Other probes can be added once a suspected fusion partner is identified. The heterogeneity of fusion partners makes it difficult to specific RT-PCR assays [93]. However, generic quantitative PCR can successfully screen for *PDGFRB* rearrangement by either comparing the relative expression between *PDGFRB* and *ABL* [93], or by comparing expression of *PDGFRB's* tyrosine kinase domain to the region containing all described breakpoints [75]. Once overexpression of *PDGFRB* is identified, the partner gene can be identified by sequencing of amplified cDNA scripts [93]. Actual identification of the partner gene, however, may be largely of academic interest, due to the almost universal response to imatinib.

## *Therapy, Prognosis, and Monitoring*

Prior to therapy with imatinib, this neoplasm was regarded as an aggressive disease with a median survival of less than 2 years. However, most patients are now known to have excellent morphologic and molecular response to imatinib with a recent

study citing a 10-year overall survival of 90% [97]. Furthermore, earlier diagnosis due to recognition of this entity will result in earlier initiation of appropriate therapy, preventing cardiac damage and blast phase. Primary and secondary resistance is uncommon; however, initial response typically occurs within 2 months, and if not seen by 3 months, consideration of another therapy is suggested. Currently, it is not known if therapy can be stopped in patients with long term molecular remission; however, a recent article cited one patient in remission 4 years after therapy cessation [98].

### *Conclusion*

Similar to myeloid and lymphoid neoplasms with eosinophilia and abnormalities of *PDGFRA*, this entity highlights the great strides that have been made in regard to subclassification of hematopoietic neoplasms allowing for more efficacious therapy. Rearrangements of *PDGFRB* are most common in adult males who present with features of CMML and eosinophilia, although other presentations are described. Over 20 fusion partners have been identified, and most rearrangements will be detected by conventional karyotyping, but FISH and PCR methodologies exist. Treatment with imatinib is the current mainstay of therapy with an excellent prognosis.

## Myeloid and Lymphoid Neoplasms with Eosinophilia and Abnormalities of *FGFR1*

### *Introduction*

Myeloid and lymphoid neoplasms with eosinophilia and abnormalities of *FGFR1* (*fibroblast growth factor receptor-1*) were initially described by Macdonald and colleagues in 1995 [99]. In this review, they identified 13 case reports in the literature. These patients presented with an MPN with eosinophilia and lymphadenopathy with a high incidence of T-cell non-Hodgkin lymphoma (NHL) and progression to AML. They identified 3 unique translocations (t(8;13)(p11–12;q11–12), t(8;9)(p11;q32–34) and t(6;8)(q27;p12)) and coined the term "8p11 myeloproliferative syndrome" (EMS). Other synonyms have included "8p11 stem cell leukemia/lymphoma" and "8p11 stem cell syndrome" [100]. However, in the 2008 World Health Organization of Tumors of the Hematopoietic and Lymphoid Tissues, the designation of "myeloid and lymphoid neoplasm with FGFR1 abnormalities" was recognized, which is under the umbrella diagnosis of myeloid and lymphoid neoplasms with eosinophilia and abnormalities of *PDGFRA*, *PDGFRB*, or *FGFR1* [4]. Although first officially described in 1995, a review of older literature reveals several case reports that likely describe EMS, but in which cytogenetics/molecular evaluation was not performed. The first such case report was likely by Manthorpe et al. in 1977,

**Table 16.3** Myeloid and lymphoid neoplasms with eosinophilia and abnormalities of *FGFR1*: more common fusion partners and various associations

| Fusion partner gene name | Fusion partner gene location | Association |
|---|---|---|
| *ZMYM2 (ZNF198)* | 13q12 | A subset with lymphadenopathy and "bilineal lymphoma" |
| *CNTRL* | 9q33–34 | Monocytosis, tonsillar involvement |
| *FGFR1OP* | 6q27 | Older age, more prominent eosinophilia, erythrocytosis |
| *BCR* | 8q11 | Older age, leukocytosis with neutrophilia and basophilia |
| Not stated | 1q25 | Peripheral monocytosis and myeloproliferative neoplasm-like findings in bone marrow |

wherein they describe a patient with T-ALL, granulocytic hyperplasia in bone marrow, and increased abnormal eosinophils [101].

This neoplasm can occur across a wide age distribution with one case report describing a 3-year-old child with EMS and another describing an 84-year-old patient with EMS [102, 103]. The median age of onset, however, is 44 years of age [100]. There is only a slight male predominance.

Given the heterogeneous nature of this neoplasm, clinical presentation is variable. Many patients present with systemic symptoms including fatigue, fever, night sweats, and/or weight loss. Nonetheless, many patients, reportedly approximately 20%, are asymptomatic at time of diagnosis when complete blood counts, obtained at routine clinic visits, etc., reveal abnormal results. At time of diagnosis, lymphadenopathy and/or organomegaly (hepatomegaly, splenomegaly, hepatosplenomegaly) is a common finding. However, mediastinal lymphadenopathy, typical of T-ALL among other lymphomas, is not frequently seen. Other clinical findings such as tonsillar involvement, etc., vary depending on exact fusion partner (to be discussed in greater detail below- Table 16.3) [1].

## *Morphology and Immunophenotyping*

In the peripheral blood, leukocytosis is typically with neutrophilia and a left shift [100]. Eosinophilia (>1.5 × $10^9$/L) is common (85% of cases), but not as common as that described in myeloid and lymphoid neoplasms with *PDGFRA* rearrangements [104]. Monocytosis is also a common feature, particularly in patients with t(8;9)(p11;q33–34) [105]. In these patients, tonsillar involvement is also typical. Basophilia, typical of *BCR-ABL1* positive CML, is not frequent but may be seen in patients with t(8;22)(p11;q11) resulting in a diagnostic challenge [106]. Erythrocytosis may also be present, mimicking polycythemia vera (PV), with cases harboring t(6;8)(q27;p11–12) frequently showing increased hemoglobin levels [107, 108]. These patients also tend to be older at time of diagnosis. Platelet count is quite variable [100].

The bone marrow is usually hypercellular due to granulocyte hyperplasia, and morphologic findings may raise the differential of CML, aCML (*BCR-ABL1* negative), and/or CMML. Blast counts are variable, but a subset of cases will present in frank acute leukemia with AML, mixed phenotype acute leukemia, and ALL described. Although not as common as cases with *PDGFRA/B* rearrangements, rarely atypical mast cell aggregates with aberrant immunophenotype have been identified [105].

As previously mentioned, lymphadenopathy is a common feature in patients with EMS. In patients who undergo lymph node biopsy, T-ALL and myeloid sarcoma have been frequently reported. The involvement may be subtle with only a portion of the lymph node replaced. In these cases, mature eosinophils are often intermingled with the lymphoblasts. A case series by Vega et al. describes a small series of EMS cases with t(8;13)(p11;q12), in which lymph nodes were replaced by T-ALL as well as atypical myeloid proliferations with abundant eosinophilic cytoplasm surrounding blood vessels or follicles [109]. These cells expressed variable myeloid markers, but also expressed CD3 and CD43; the term "bilineal lymphoma" was suggested. Given the heterogeneity of this neoplasm, the immunophenotypic features are in turn heterogeneous. However, cases presenting with T-ALL typically express pan T-cell markers, TdT, and possibly CD1a.

## *Cytogenetics and Molecular Findings*

The *fibroblast growth factor receptor 1 gene* is located on chromosome 8p11–12 and has 19 exons allowing for variant splicing and several isoforms. The longest protein product encodes for a receptor with 2 or 3 immunoglobulin-like extracellular domains, a single transmembrane domain, and an intracellular tyrosine kinase domain. In the presence of appropriate ligands, FGFR1 undergoes conformational change, induces dimerization, and then autophosphorylation of intracellular tyrosine residues. These function as specific docking sites for target proteins involved in downstream pathways.

*FGFR1* is a member of a family of 4 high-affinity, highly conserved receptor tyrosine kinases (fibroblast growth factor receptor family) that function in embryonic development, by controlling growth, differentiation, and migration of diverse cell types. They differ from one another in regard to their ligand affinities and tissue distribution.

Mutations in this gene have been associated with a host of constitutional disorders including Pfeiffer syndrome, a disorder resulting in premature fusion of skull bones. Furthermore, abnormalities in this family of genes have been identified in numerous hematopoietic and non-hematopoietic neoplasms.

In EMS, few partner genes have been described to date. Thus far, all translocations involving *FGFR1* have a similar structure with a 5′ gene partner translocating to the 3′ *FGFR1* at exon 9. The fusions encode large proteins containing the N-terminus of the translocation partner with a dimerization domain and the tyrosine kinase domain of *FGFR1* in the C-terminus. The partners all serve to promote

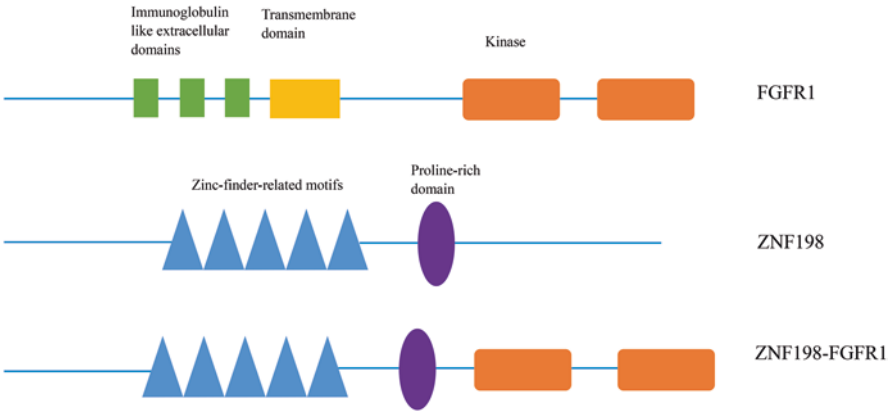


**Fig. 16.9** Schematic representation of ZMYM2-FGFR1 fusion product

dimerization. Abnormal activation of FGFR1 results in malignant potential and leukemogenesis. As previously mentioned, the fusion partner influences clinical and morphologic findings.

The first translocation identified in EMS, and still the most common, is the t(8;13)(p11;q12), where *FGFR1* fuses with *ZMYM2* (previously *ZNF198*) at 13q12, resulting in a ZMYM2-FGFR1 fusion protein and constitutive activation of the FGFR1 tyrosine kinase (Fig. 16.9). In cases with t(8;9)(p11;q33), the second most common cytogenetic finding, the partner gene is centrosomal protein 110 kDa, centriolin (*CNTRL*) and monocytosis and tonsillar involvement is well described [110]. FGFR1 oncogenic partner 1 (*FGFR1OP*) is the fusion partner in cases with t(6;8)(q27;p11–12), which is the 3rd most common cytogenetic finding. Leucine-rich domains of FGFR1OP lead to dimerization of the fusion protein FGFR1OP-FGFR1, resulting in constitutive activation of FGFR1 tyrosine kinases and downstream signaling pathways, which include BCL2 overexpression [111]. In these patients, eosinophilia may be more prominent and patients may present at older ages with features of PV [107, 108].

t(8;22) (p11;q11) includes the *BCR* fusion gene. Interestingly, these patients often present at an older age with leukocytosis, neutrophilia, and basophilia, thereby mimicking CML. In addition, a few patients have presented with B-ALL [106, 112].

Three cases of EMS with t(1;8)(q25;p11.2) have been described. All three presented with peripheral monocytosis and bone marrow features consistent with an MPN [113]. However, despite abnormal karyotype, *FGFR1* rearrangement could not be confirmed in 2 of these 3 cases due to negative FISH and/or RT-PCR. The remaining fusions are less frequent than the aforementioned and often include only a single reported case (Table 16.4).

To date, t(8;19)(p12;q13.3) has been identified once in the literature, in which the patient presented with AML with features suggestive of a background MPN including

**Table 16.4** Myeloid and lymphoid neoplasms with eosinophilia and abnormalities of *FGFR1*: rarer fusion partners

| Fusion partner gene name | Fusion partner gene location | Association |
|---|---|---|
| *CUX1* | 7q22 | T-ALL |
| *NUP98* | 11p15 | AML |
| *HERVK* | 19q13.3 | AML secondary to MDS/MPN |
| *FGFR1OP2* | 12p11 | T-LBL with eosinophilia |
| *TIF1* | 7q34 | |
| *LRRFIP1* | 2q37 | MDS, RAEB |
| *MYO18A* | 17q23 | MDS/MPN with eosinophilia and basophilia |

polycythemia and atypical megakaryocytes [114]. Subsequent evaluation identified the fusion gene to be human endogenous retrovirus gene (*HERVK*) [115]. ins(12;8)(p11;p11p22) has also been described once in the literature in a patient presenting with T-ALL [116]. In this patient, fibroblast growth factor receptor oncogene partner 2 (*FGFR1OP2*) was identified as the fusion gene. t(7;8)(q34;p11), also described once in the literature, results in a fusion gene including *TIF1* on 7q34 [117]. This patient presented with monocytosis, and bone marrow revealed a myelomonocytic blast proliferation as well as a B lymphoblast proliferation. Peripheral eosinophilia was absent, but marrow eosinophilia was present.

In a patient with t(2;8)(q37;p11), *LRRFIP1* (leucine-rich repeat flightless-interacting protein 1) gene was the identified fusion gene, and the patient presented with findings consistent with refractory anemia with excess blasts; this progressed to AML after 6 years of supportive care [118]. Monocytosis, eosinophilia, and basophilia were reported in a patient with t(8;17)(p11;q23) where *MYO18A* was the associated fusion gene [119]. EMS with t(8;12) appears to have been reported 3 times in the literature but with different breakpoints, including t(8;12)(p11;q15), dic(8;12)(p11.2;p11.2), and t(8;12)(q11;p12) [120–122]. All cases appeared to have eosinophilia and T-ALL at initial diagnosis.

The literature cites one case of EMS with t(8;11)(p11;p15) in which the patient presented with AML, and the fusion gene partner was identified as *NUP98* [120]. A 29-year-old woman presented with T-ALL without eosinophilia; t(7;8)(q22;p11) resulting in a *CUX1-FGFR1* fusion gene was identified [123]. Lastly, a literature review identifies one patient with t(3;8;9)(p25;p21;q34) [124]. This patient presented with eosinophilia and T-ALL and later developed B-ALL.

Conventional karyotyping has identified all cases of EMS reported in the literature to date except one case [125]. Therefore, karyotyping remains the mainstay of identification as it is relatively sensitive and capable of identifying all fusion partners. In addition to karyotyping, reference laboratories offer FISH analysis to detect translocations of *FGFR1* (dual-color break-apart probe set). Due to the rarity of EMS, RT-PCR is not routinely used outside of research settings.

### *Therapy, Prognosis, and Monitoring*

EMS is an aggressive disease; most patients either present with acute leukemia or subsequently progress. Cytogenetic evolution has been described in patients with EMS, and acquisition of 21 is a frequent occurrence [100]. Overall survival is poor at approximately 15 months, and long term survival has only been documented in those receiving allogeneic transplantation. Current therapy includes typical AML or ALL induction regimens, as appropriate, followed by transplantation. Unlike patients with abnormalities of *PDGFRA* and *PDGFRB*, no well-established targeted therapy is currently available. However, a few documented responses with targeted therapies have been identified in the literature [123, 126–128]. A murine model was recently developed, which provides an opportunity to develop therapeutic strategies [129].

### *Conclusion*

EMS is a rare disorder with variable clinical presentation, which is a result of constitutive activation of FGFR1. Various fusion partner genes have been identified and may influence the clinical presentation in this heterogeneous disorder. Many patients present with features of MPN with eosinophilia. Presentation with or development of acute leukemia (AML, ALL, or mixed phenotype) is typical. Identification of the abnormal fusion product gene is usually via conventional karyotyping, but FISH analysis is available at reference laboratories. To date, no targeted therapy is available, but research is on-going.

## Myeloid and Lymphoid Neoplasms with Eosinophilia and T(8;9)(P22;P24.1); *PCM1-JAK2*

### *Introduction*

In the 2016 revision of the WHO classification of tumors of hematopoietic and lymphoid tissues, myeloid and lymphoid neoplasms with eosinophilia and t(8;9)(p22;p24.1);*PCM1-JAK2* will be recognized as a new provisional entity [130]. This continues to exemplify the theme of subclassification based on cytogenetic and molecular findings to allow for better characterization of disease, better therapy, and eventually prolonged survival. This rare entity was first described in 1990 by Steward et al. [131] with the *PCM1-JAK2* fusion gene being identified in 2005 [132]. Currently over 30 cases have been described with a marked male predominance noted [133]. Affected individuals have ranged from 12 years to 75 years of age, with a median age of 47. The patients often present with hepatosplenomegaly.

## *Morphology and Immunophenotyping*

Initially, most cases were felt to represent an MPN or an MDS/MPN, with CEL, aCML, and primary myelofibrosis (PMF) being the most frequent subclassifications. The peripheral blood often displays eosinophilia, but monocytosis is uncommon. The bone marrow is typically hypercellular, often with accompanying fibrosis. A subset of cases presented in blast phase, either AML, T-ALL, or B-ALL. Furthermore, subsequent progression to blast phase is common.

## *Cytogenetics and Molecular Findings*

As previously mentioned, t(8;9)(p22;p24.1) results in fusion of *PCM1 to JAK2* [134]. JAK2 is a member of the Janus family of tyrosine kinases, which are non-receptor tyrosine kinases that play a significant role in signal transduction pathways involved in regulation of cell survival, proliferation, differentiation, and apoptosis. The protein has seven domains including the JH1 domain located at the carboxyl terminus, with features of a typical tyrosine kinase domain and the JH2 domain located in exon 14, with a negative autoregulatory function. JAK2 activation results in neoplastic transformation and abnormal cell proliferation in various malignancies, with the prototype being PV due to V617F point mutation.

*PCM1* encodes a large protein containing several potential coiled-coil domains in its aminoterminal part. It plays a role in the assembly of centrosomal proteins, microtubule organization, and progression of cell cycle. Due to the *PCM1-JAK2* gene fusion, the coiled-coil domains of PCM1 mediate an oligomerization that brings together the linked JAK2 domains resulting in a constitutively activated tyrosine kinase domain of JAK2.

The t(8;9)(p22;p24.1) is identified via conventional karyotyping; however, FISH analysis as well as PCR have also been utilized to detect this aberration. Finally, other *JAK2*-rearranged neoplasms are less common and are not currently included in the 2016 WHO as distinct entities [130].

## *Therapy, Prognosis, and Monitoring*

Currently, therapy for this newly recognized entity is not standardized. Prognosis was previously poor with many patients requiring stem cell transplantation [133]. However, a recent article describes long term cytogenetic and molecular remission in two patients with janus kinase inhibitor, ruxolitinib [135]. One patient died 36 months after therapy with ruxolitinib for unrelated issues and was last noted to be in molecular remission prior to death. Another patient is still in molecular remission 46 months after initiation of therapy. These cases suggest a significant role for JAK2 inhibitors in treatment; continued research is needed.

## *Conclusion*

Myeloid and lymphoid neoplasms with eosinophilia and t(8;9)(p22;p24.1);*PCM1-JAK2* is a rare newly recognized provisional entity occurring most frequently in males with a wide age distribution. Patients typically present with features of MPN or MDS/MPN with eosinophilia commonly noted. The translocation can be detected via routine karyotyping, but FISH analysis and PCR have proven to be successful as well. Therapy is not standardized, but JAK2 inhibitors will likely play a substantial role.

## References

1. Cools J, DeAngelo DJ, Gotlib J, Stover EH, Legare RD, Cortes J, Kutok J, Clark J, Galinsky I, et al. A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 .Genes as a therapeutic target of imatinib in idiopathic Hypereosinophilic syndrome. N Engl J Med. 2003;348(13):1201–14.
2. Bain BJ, Gilliland DG, Vardiman JW, Horny HP. Chronic eosinophilic leukemia, not otherwise specified. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al., editors. World Health Organization classification of Tumours. Pathology and Genetics or Tumours of Haematopoietic and lymphoid tissues. Lyon: IARC Press; 2008. p. 51–3.
3. Gotlib J. World Health Organization-defined eosinophilic disorders: 2015 update on diagnosis, risk stratification, and management. Am J Hematol. 2015;90(11):1078–89.
4. Bain BJ, Gilliland DG, Horny HP, Vardiman JW. Myeloid and lymphoid neoplasms with eosinophilia and abnormalities of PDGFRA, PDGFRB or FGFR1. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al., editors. World Health Organization classification of tumours. Pathology and genetics or tumours of haematopoietic and lymphoid tissues. Lyon: IARC Press; 2008. p. 68–73.
5. Klion AD, Noel P, Akin C, Law MA, Gilliland DG, Cools J, et al. Elevated serum tryptase levels identify a subset of patients with a myeloproliferative variant of idiopathic hypereosinophilic syndrome associated with tissue fibrosis, poor prognosis, and imatinib responsiveness. Blood. 2003;101(12):4660–6.
6. Trempat P, Villalva C, Laurent G, Armstrong F, Delsol G, Dastugue N, et al. Chronic myeloproliferative disorders with rearrangement of the platelet-derived growth factor α receptor: a new clinical target for STI571/Glivec. Oncogene. 2003;22(36):5702–6.
7. Savage NM, George TI, Gotlib J. Myeloid neoplasms associated with eosinophilia and rearrangement of PDGFRA, PDGFRB, and FGFR1: a review. Int Jnl Lab Hem. 2013;35:491–500.
8. Metzgeroth G, Walz C, Score J, Siebert R, Schnittger S, Haferlach C, et al. Recurrent finding of the FIP1L1-PDGFRA fusion gene in eosinophilia-associated acute myeloid leukemia and lymphoblastic T-cell lymphoma. Leukemia. 2007;21(6):1183–8.
9. Chen D, Bachanova V, Ketterling RP, Begna KH, Hanson CA, Viswanatha DS. A case of nonleukemia myeloid sarcoma with FIP1L1-PDGFRA rearrangement: an unusual presentation of a rare disease. Am J Surg Pathol. 2013;37(1):147–51.
10. Andrae J, Gallini R, Betsholtz C. Role of platelet-derived growth factors in physiology and medicine. Genes Dev. 2008;22(10):1276–312.
11. Yoo C, Ryu MH, Jo J, Park I, Ryoo BY, Kang YK. Efficacy of Imatinib in Patients with Platelet-Derived Growth Factor Receptor Alpha–Mutated Gastrointestinal Stromal Tumors. Cancer Res Treat. 2016;48(2):546–52.
12. Ozawa T, Brennan CW, Wang L, Squatrito M, Sasayama T, Nakada M, et al. PDGFRA gene rearrangements are frequent genetic events in PDGFRA-amplified glioblastomas. Genes Dev. 2010;24(19):2205–18.

13. Verhaak RG, Hoadley KA, Purdom E, Wang V, Qi Y, Wilkerson MD, et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer Cell. 2010;17(1):98–110.
14. Huss S, Wardelmann E, Goltz D, Binot E, Hartmann W, Merkelbach-Bruse S, et al. Activating PDGFRA mutations in inflammatory fibroid polyps occur in exons 12, 14, and 18 and are associated with tumor localization. Histopathology. 2012;61(1):59–68.
15. Holtkamp N, Okuducu AF, Mucha J, Afanasieva A, Hartmann C, Atallah I, et al. Mutation and expression of PDGFRA and KIT in malignant peripheral nerve sheath tumors, and its implications for imatinib sensitivity. Carcinogenesis. 2006;27(3):664–71.
16. Kaufmann I, Martin G, Friedlein A, Langen H, Keller W. Human Fip1 is a subunit of CPSF that binds to U-rich RNA elements and stimulates poly(A) polymerase. EMBO J. 2004;23(3):616–26.
17. Pardanani A, Ketterling RP, Brockman SR, Flynn HC, Paternoster SF, Shearer BM, et al. CHIC2 deletion, a surrogate for FIP1L1-PDGFRA fusion, occurs in systemic mastocytosis associated with eosinophilia and predicts response to imatinib mesylate therapy. Blood. 2003;102(9):3093–6.
18. Baxter EJ, Hochhaus A, Bolufer P, Reiter A, Fernandez JM, Senent L, et al. The t(4;22)(9q12;q11) in atypical chronic myeloid leukaemia fuses BCR to PDGFRA. Hum Mol Genet. 2002;11(12):1391–7.
19. Tashiro H, Shirasaki R, Noguchi M, Gotoh M, Kawasugi K, Shirafuji N. Molecular analysis of chronic eosinophilic leukemia with t(4;10) showing good response to imatinib mesylate. Int Jnl Hem. 2006;83(5):433–8.
20. Stover EH, Chen J, Folens C, Lee BH, Mentens N, Marynen P, et al. Activation of FIP1L1-PDGFRα requires disruption of the juxtamembrane domain of PDGFRα and is FIP1L1 independent. Proc Natl Acad Sci U S A. 2006;103(21):8078–83.
21. Capovilla M, Cayuela JM, Bilhou-Nabera C, Gardin C, Letestu R, Baran-Marzak F, et al. Synchronous FIP1L1-PDGFRA-positive chronic eosinophilic leukemia and T-cell lymphoblastic lymphoma: a bilineal clonal malignancy. Eur J Haematol. 2007;80(1):81–6.
22. Safley AM, Sebastian S, Collins TS, Tirado CA, Stenzel TT, Gong JZ, et al. Molecular and cytogenetic characterization of a novel translocation t(4;22) involving the breakpoint cluster region and platelet-derived growth factor receptor-alpha genes in a patient with a typical chronic myeloid leukemia. Genes Chromosom Cancer. 2004;40(1):44–50.
23. M K, Mahon GM, Cheng L, Whitehead IP. p38 MAPK-mediated activation of NF-kappaB by the RhoGEF domain of Bcr. Oncogene. 2002;21(30):4601–12.
24. Vardiman JW, Melo JV, Baccarani M, Thiele J. Chronic Myelogenous leukemia, BCR-ABL positive. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al., editors. World Health Organization classification of tumours. Pathology and genetics or tumours of haematopoietic and lymphoid tissues. Lyon: IARC Press; 2008. p. 32–7.
25. Yigit N, WW W, Subramaniyam S, Mathew S, Geyer JT. BCR-PDGFRA fusion in a T lymphoblastic leukemia/lymphoma. Cancer Gene Ther. 2015;208(7–8):404–7.
26. Curtis CE, Grand FH, Musto P, Clark A, Murphy J, Perla G, et al. Two novel imatinib-responsive PDGRFA fusion genes in chronic eosinophilic leukaemia. Br J Haematol. 2007;138(1):77–81.
27. Hwang J, Pallas DC. STRIPAK complexes: structure, biological function, and involvement in human disease. Int J Biochem Cell Biol. 2014;47:118–48.
28. Musto P, Falcone A, Sanpaolo G, Bodenizza C, Perla G, Minervini MM, et al. Heterogeneity of response to imatinib-mesylate (Glivec) in patients with hypereosinophilic syndrome: implications for dosing and pathogenesis. Leuk Lymphoma. 2004;45(6):1219–22.
29. Fong K, Choi Y, Rattner JB, Qi RZ. CDK5RAP2 is a Pericentriolar protein that functions in centrosomal attachment of the γ-tubulin ring complex. Mol Biol Cell. 2008;19(1):115–25.
30. Tan CA, Topper S, Ward Melver C, Stein J, Reeder A, Arndt K, et al. The first case of CDK5RAP2-related primary microcephaly in a non-consanguineous patient identified by next generation sequencing. Brain Dev. 2014;36(4):351–5.

31. Walz C, Curtis C, Schnittger S, Schultheis B, Metzgeroth G, Schoch C, et al. Transient response to imatinib in a chronic eosinophilic leukemia associated with ins(9;4)(q33;q12q25) and a CKD5RAP2-PDGFRA fusion Gene. Genes Chromosom Cancer. 2006;45(10):950–6.
32. Score J, Curtis C, Waghorn K, Stalder M, Jotterand M, Grand FH, et al. Identification of a novel imatinib responsive KIF5B-PDGFRA fusion gene following screening for PDGFRA overexpression in patients with hypereosinophilia. Leukemia. 2006;20(5):827–32.
33. Sugimoto Y, Sada A, Shimokariya Y, Monma F, Ohishi K, Masuya M, et al. A novel FOXP1-PDGFRA fusion gene in myeloproliferative neoplasm with eosinophilia. Cancer Gene Ther. 2015;208(10):508–12.
34. Elling C, Erben P, Walz C, Frickenhaus M, Schemionek M, Stehling M, et al. Novel imatinib sensitive PDGRFA-activating point mutations in hypereosinophilic syndrome induce growth factor independence and leukemia-like disease. Blood. 2011;117(10):2935–43.
35. La Starza R, Specchia G, Cuneo A, Beacci D, Nozzoli C, Luciano L, et al. The hypereosinophilic syndrome: fluorescence in situ hybridization detects the del(4)(q12)-FIP1L1/PDGFRA but not other genomic rearrangements of other tyrosine kinases. Haematologica. 2005;90(5):596–601.
36. Vandenberghe P, Wlodarska I, Michaux L, Zachée P, Boogaerts M, Vanstraelen D, et al. Clinical and molecular features of FIP1L1-PDGFRA (+) chronic eosinophilic leukemias. Leukemia. 2004;18(4):734–42.
37. Legrand F, Renneville A, Macintyre E, Mastrilli S, Ackermann F, Cayuela JM, et al. The spectrum of FIP1L1-PDGFRA-associated chronic eosinophilic leukemia: new insights based on a survey of 44 cases. Medicine (Baltimore). 2013;92(5):e1–9.
38. Pardanani A, D'Souza A, Knudson RA, Hanson CA, Ketterling RP, Tefferi A. Long-term follow-up of FIP1L1-PDGFRA-mutated patients with eosinophilia: survival and clinical outcome. Leukemia. 2012;26(11):2439–41.
39. von Bubnoff N, Gorantla SP, Engh RA, Oliveira TM, Thöne S, Aberg E, et al. The low frequency of clinical resistance to PDGFR inhibitors in myeloid neoplasms with abnormalities of PDGFRA might be related to the limited repertoire of possible PDGFRA kinase domain mutations in vitro. Oncogene. 2011;30(8):933–43.
40. Shen Y, Shi X, Pan J. The conformational control inhibitor of tyrosine kinases DCC-2036 is effective for imatinib-resistant cells expressing T674I FIP1L1-PDGFRα. PLoS One. 2013;8(8):e73059.
41. Metzgeroth G, Erben P, Martin H, Mousset S, Teichmann M, Walz C, et al. Limited clinical activity of nilotinib and sorafenib in FIP1L1-PDGFRA positive chronic eosinophilic leukemia with imatinib-resistant T674I mutation. Leukemia. 2012;26(1):162–4.
42. Lierman E, Michaux L, Beullens E, Pierre P, Marynen P, Cools J, et al. FIP1L1-PDGFRα D842V, a novel panresistant mutant, emerging after treatment of FIP1L1-PDGFRα T674I eosinophilic leukemia with single agent sorafenib. Leukemia. 2009;23(5):845–51.
43. Score J, Walz C, Jovanovic JV, Jones AV, Waghorn K, Hidalgo-Curtis C, et al. Detection and molecular monitoring of FIP1L1-PDGFRA-positive disease by analysis of patient-specific genomic DNA fusion junctions. Leukemia. 2009;23(2):332–9.
44. Somin D, Salemi S, Yousefi S, Simon HU. Primary resistance to imatinib in Fip-like 1-platelet-derived growth factor receptor α- positive eosinophilic leukemia. J Allergy Clin Immunol. 2008;121(4):1054–6.
45. Salemi S, Yousefi S, Simon D, Schmid I, Moretti L, Scapozza L, et al. A novel FIP1L1-PDGFRA mutant destabilizing the inactive conformation of the kinase domain in chronic eosinophilic leukemia/hypereosinophilic syndrome. Allergy. 2009;64(6):913–8.
46. Huang Q, Snyder DS, Chu P, Gaal KK, Chang KL, Weiss LM. PDGFRA rearrangement leading to hyper-eosinophilia, T-lymphoblastic lymphoma, myeloproliferative neoplasm and precursor B-cell acute lymphoblastic leukemia. Leukemia. 2011;25(2):371–5.
47. Montano-Almendras CP, Essaghir A, Schoemans H, Varis I, Noël LA, Velghe AI, et al. ETV6-PDGFRB and FIP1L1-PDGFRA stimulate human hematopoietic progenitor cell proliferation and differentiation into eosinophils: the role of nuclear factor-κB. Haematologica. 2012;97(7):1064–72.

48. Golub TR, Barker GF, Lovett M, Gilliland DG. Fusion of PDGF receptor-beta to a novel Ets-like gene, Tel, in chronic myelomonocytic leukemia with t(512) chromosomal translocation. Cell. 1994;77(2):307–16.
49. Vega F, Medeiros LJ, Bueso-Ramos CE, Arboleda P, Miranda RN. Hematolymphoid neoplasms associated with rearrangements of PDGFRA, PDGFRB, and FGFR1. Am J Clin Pathol. 2015;144(3):377–92.
50. Jones AV, Cross NC. Oncogenic derivatives of platelet-derived growth factor receptors. Cell Mol Life Sci. 2004;61(23):2912–23.
51. Curtis CE, Grand FH, Waghorn K, Sahoo TP, George J, Cross NC. A novel ETV6-PDGFRB fusion transcript missed by standard screening in a patient with an imatinib responsive chronic myeloproliferative disease. Leukemia. 2007;21(8):1839–41.
52. Gosenca D, Kellert B, Metzgeroth G, Haferlach C, Fabarius A, Schwaab J, et al. Identification and functional characterization of imatinib-sensitive DTD1-PDGFRB and CCDC88C-PDGFRB fusion genes in eosinophilia-associated myeloid/lymphoid neoplasms. Genes Chromosom Cancer. 2014;53(5):411–21.
53. Chmielecki J, Peifer M, Viale A, Hutchinson K, Giltnane J, Socci ND, et al. Systematic screen for tyrosine kinase rearrangements identifies a novel C6orf204-PDGFRB fusion in a patient with recurrent T-ALL and an associated myeloproliferative neoplasm. Genes Chromosom Cancer. 2012;51(1):54–65.
54. Walz C, Metzgeroth G, Haferlach C, Schmitt-Graeff A, Fabarius A, Hagen V, et al. Characterization of three new imatinib-responsive fusion genes in chronic myeloproliferative disorders generated by disruption of the platelet-derived growth factor receptor beta gene. Haematologica. 2007;92(2):163–9.
55. Chen J, Williams IR, Kutok JL, Duclos N, Anastasiadou E, Masters SC, et al. Positive and negative regulatory roles of the WW-like domain in TEL-PDGFbetaR transformation. Blood. 2004;104(2):535–42.
56. Carroll M, Tomasson MH, Barker GF, Golub TR, Gilliland DG. The TEL/platelet-derived growth factor beta receptor (PDGF beta R) fusion in chronic myelomonocytic leukemia is a transforming protein that self-associates and activates PDGF beta R kinase-dependent signaling pathways. Proc Natl Acad Sci U S A. 1996;93(25):14845–50.
57. J S, Anastasiadou E, Cain D, Kutok J, Wojiski S, Williams IR, et al. H4(D10S170), a gene frequently rearranged in papillary thyroid carcinoma, is fused to the platelet-derived growth factor receptor beta gene in atypical chronic myeloid leukemia with t(5;10)(q33;q22). Blood. 2001;97(12):3910–8.
58. Abe A, Emi N, Tanimoto M, Terasaki H, Marunouchi T, Saito H. Fusion of the platelet-derived growth factor receptor beta to a novel gene CEV14 in acute myelogenous leukemia after clonal evolution. Blood. 1997;90(11):4271–7.
59. Ross TS, Gilliland DG. Transforming properties of the Huntingtin interacting protein 1/platelet-derived growth factor beta receptor fusion protein. J Biol Chem. 1999;274(32):22328–36.
60. Cavazzini F, Bardi A, Ciccone M, Rigolin GM, Gorello P, La Starza R, et al. Trisomy 8 in PDGFRB-negative cells in a patient with imatinib-sensitive chronic myelomonocytic leukemia and t(5;16)(q33;p13), PDGFRB-NDE1 fusion. Cancer Genet Cytogenet. 2009;194(1):67–9.
61. Keene P, Mendelow B, Pinto MR, Bezwoda W, MacDougall L, Falkson G, et al. Abnormalities of chromosome 12p 13 and malignant proliferation of eosinophils: a nonrandom association. Br J Haematol. 1987;67(1):25–31.
62. Levine RL, Wadleigh M, Sternberg DW, Wlodarska I, Galinsky I, Stone RM, et al. KIAA1509 is a novel PDGFRB fusion partner in imatinib-responsive myeloproliferative disease associated with a t(5;14)(q33;q32). Leukemia. 2005;19(1):27–30.
63. Albano F, Anelli L, Zagaria A, Lonoce A, La Starza R, Liso V, et al. Extramedullary molecular evidence of the 5′KIAA1509/3′PDGFRB fusion gene in chronic eosinophilic leukemia. Leuk Res. 2008;32(2):347–51.
64. Wang JR, Yen CC, Gau JP, Hsiao LT, Liu CY, Pai JT, et al. A case of myeloid neoplasm associated with eosinophilia and KIAA1509-PDGFRβ responsive to combination treatment with imatinib mesylate and prednisolone. J Clin Pharm Ther. 2010;35(6):733–6.

65. Kulkarni S, Heath C, Parker S, Chase A, Iqbal S, Pocock CF, et al. Fusion of H4/D10S170 to the platelet-derived growth factor receptor β in BCR-ABL-negative myeloproliferative disorders with a t(5;10)(q33;q21). Cancer Res. 2000;60(13):3592–8.
66. Drechsler M, Hildebrandt B, Kündgen A, Germing U, Royer-Pokora B. Fusion of H4/D10S170 to PDGFRbeta in a patient with chronic myelomonocytic leukemia and long-term responsiveness to imatinib. Ann Hematol. 2007;86(5):353–4.
67. Garcia JL, Font de Mora J, Hernandez JM, Queizan JA, Gutierrez NC, Hernandez JM, et al. Imatinib mesylate elicits positive clinical response in atypical chronic myeloid leukemia involving the platelet-derived growth factor receptor beta. Blood. 2003;102(7):2699–700.
68. Bastie JN, Garcia I, Terré C, Cross NC, Mahon FX, Castaigne S. Lack of response to imatinib mesylate in a patient with accelerated phase myeloproliferative disorder with rearrangement of the platelet-derived growth factor receptor beta-gene. Acta Haematol. 2002;107(2):113–22.
69. Tong Q, Li Y, Smanik PA, Fithian LJ, Xing S, Mazzaferri EL, et al. Characterization of the promoter region and oligomerization domain of H4 (D10S170), a gene frequently rearranged with the ret proto-oncogene. Oncogene. 1995;10(9):1781–7.
70. Kim HG, Jang JH, Koh EH. TRIP11-PDGFRB fusion in a patient with a therapy-related myeloid neoplasm with t(5;14)(q33;q32) after treatment for acute promyelocytic leukemia. Mol Cytogenet. 2014;7(1):103.
71. Gong SL, Guo MQ, Tang GS, Zhang CL, Qiu HY, XX H, et al. Fusion of platelet-derived growth factor receptor β to CEV14 gene in chronic myelomonocytic leukemia: a case report and review of the literature. Oncol Lett. 2016;11(1):770–4.
72. Abe A, Tanimoto M, Towatari M, Matsuoka A, Kitaori K, Kato H, et al. Acute myeloblastic leukemia (M2) with translocation (7;11) followed by marked eosinophilia and additional abnormalities of chromosome 5. Cancer Genet Cytogenet. 1995;83(1):37–41.
73. Ríos RM, Sanchís A, Tassin AM, Fedriani C, Bornens M. GMAP-210 recruits gamma-tubulin complexes to cis-Golgi membranes and is required for Golgi ribbon formation. Cell. 2004;118(3):323–35.
74. Rosati R, La Starza R, Luciano L, Gorello P, Matteucci C, Pierini V, et al. TPM3/PDGFRB fusion transcript and its reciprocal in chronic eosinophilic leukemia. Leukemia. 2006;20(9):1623–4.
75. Li Z, Yang R, Zhao J, Yuan R, Lu Q, Li Q, et al. Molecular diagnosis and targeted therapy of a pediatric chronic eosinophilic leukemia patient carrying TPM3-PDGFRB fusion. Pediatr Blood Cancer. 2011;56(3):463–6.
76. Chang H, Chuang WY, Sun CF, Barnard MR. Concurrent acute myeloid leukemia and T lymphoblastic lymphoma in a patient with rearranged PDGFRB genes. Diagn Pathol. 2012;7:19.
77. Ondrejka SL, Jegalian AG, Kim AS, Chabot-Richards DS, Giltnane J, Czuchlewski DR, et al. PDGFRB-rearranged T-lymphoblastic leukemia/lymphoma occurring with myeloid neoplasms: the missing link supporting a stem cell origin. Haematologica. 2014;99(9):e148–51.
78. Magnusson MK, Meade KE, Brown KE, Arthur DC, Krueger LA, et al. Rabaptin-5 is a novel fusion partner to platelet-derived growth factor beta receptor in chronic myelomonocytic leukemia. Blood. 2001;98(8):2518–25.
79. Winkelmann N, Hidalgo-Curtis C, Waghorn K, Score J, Dickinson H, Jack A, et al. Recurrent CEP85L-PDGFRB fusion in patient with t(5;6) and imatinib-responsive myeloproliferative neoplasm with eosinophilia. Leuk Lymphoma. 2013;54(7):1527–31.
80. Lahortiga I, Akin C, Cools J, Wilson TM, Mentens N, Arthur DC, et al. Activity of imatinib in systemic mastocytosis with chronic basophilic leukemia and a PRKG2-PDGFRB fusion. Haematologica. 2008;93(1):49–56.
81. Gallagher G, Horsman DE, Tsang P, Forrest DL. Fusion of PRKG2 and SPTBN1 to the platelet-derived growth factor receptor beta gene (PDGFRB) in imatinib-responsive atypical myeloproliferative disorders. Cancer Genet Cytogenet. 2008;181(1):46–51.
82. Arefi M, García JL, Peñarrubia MJ, Queizán JA, Hermosín L, López-Corral L, et al. Incidence and clinical characteristics of myeloproliferative neoplasms displaying a PDGFRB rearrangement. Eur J Haematol. 2012;89(1):37–41.

83. Stover DA, Verrelli BC. Comparative vertebrate evolutionary analyses of type I collagen: potential of COL1a1 gene structure and intron variation for common bone-related diseases. Mol Biol Evol. 2011;28(1):533–42.
84. Folens C, Cools J, Marynen P, Martelli MF, Mecucci C, Cuneo A. A new NDE1/PDGFRB fusion transcript underlying chronic myelomonocytic leukaemia in Noonan Syndrome. Leukemia. 2007;21(4):830–3.
85. Wilkinson K, Velloso ER, Lopes LF, Lee C, Aster JC, Shipp MA, et al. Cloning of the t(1;5)(q23;q33) in a myeloproliferative disorder associated with eosinophilia: involvement of PDGFRB and response to imatinib. Blood. 2003;102(12):4187–90.
86. Grand FH, Burgstaller S, Kühr T, Baxter EJ, Webersinke G, Thaler J, et al. p53-Binding protein 1 is fused to the platelet-derived growth factor receptor beta in a patient with a t(5;15)(q33;q22) and an imatinib-responsive eosinophilic myeloproliferative disorder. Cancer Res. 2004;64(20):7216–9.
87. Morerio C, Acquila M, Rosanda C, Rapella A, Dufour C, Locatelli F, et al. HCMOGT-1 is a novel fusion partner to PDGFRB in juvenile myelomonocytic leukemia with t(5;17)(q33;p11.2). Cancer Res. 2004;64(8):2649–51.
88. Hidalgo-Curtis C, Apperley JF, Stark A, Jeng M, Gotlib J, Chase A, et al. Fusion of PDGFRB to two distinct loci at 3p21 and a third at 12q13 in imatinib-responsive myeloproliferative neoplasms. Br J Haematol. 2010;148(2):268–73.
89. Ross TS, Bernard OA, Berger R, Gilliland DG. Fusion of Huntingtin interacting protein 1 to platelet-derived growth factor beta receptor (PDGFbetaR) in chronic myelomonocytic leukemia with t(5;7)(q33;q11.2). Blood. 1998;91(12):4419–26.
90. Niu Q, Ybe JA. Crystal structure at 2.8 A of Huntingtin-interacting protein 1 (HIP1) coiled-coil domain reveals a charged surface suitable for HIP-protein interactor (HIPPI). J Mol Biol. 2008;375(5):1197–205.
91. Walz C, Haferlach C, Hänel A, Metzgeroth G, Erben P, Gosenca D, et al. Identification of a MYO18A-PDGFRB fusion gene in an eosinophilia-associated atypical myeloproliferative neoplasm with a t(5;17)(q33-34;q11.2). Genes Chromosom Cancer. 2009 Feb;48(2):179–83.
92. Vizmanos JL, Novo FJ, Román JP, Baxter EJ, Lahortiga I, Larráyoz MJ, et al. NIN, a gene encoding a CEP110-like centrosomal protein, is fused to PDGFRB in a patient with a t(5;14)(q33;q24) and an imatinib-responsive myeloproliferative disorder. Cancer Res. 2004;64(8):2673–6.
93. Erben P, Gosenca D, Müller MC, Reinhard J, Score J, Del Valle F, et al. Screening for diverse PDGFRA or PDGFRB fusion genes is facilitated by generic quantitative reverse transcriptase polymerase chain reaction analysis. Haematologica. 2010;95(5):738–44.
94. Gorello P, La Starza R, Brandimarte L, Trisolini SM, Pierini V, Crescenzi B, et al. A PDGFRB-positive acute myeloid malignancy with a new t(5;12)(q33;p13.3) involving the ERC1 gene. Leukemia. 2008;22(1):216–8.
95. Medves S, Duhoux FP, Ferrant A, Toffalini F, Ameye G, Libouton JM, et al. KANK1, a candidate tumor suppressor gene, is fused to PDGFRB in an imatinib-responsive myeloid neoplasm with severe thrombocythemia. Leukemia. 2010;24(5):1052–5.
96. Baxter EJ, Kulkarni S, Vizmanos JL, Jaju R, Martinelli G, Testoni N, et al. Novel translocations that disrupt the platelet-derived growth factor receptor beta (PDGFRB) gene in BCR-ABL-negative chronic myeloproliferative disorders. Br J Haematol. 2003;120(2):251–6.
97. Cheah CY, Burbury K, Apperley JF, Huguet F, Pitini V, Gardembas M, et al. Patients with myeloid malignancies bearing PDGFRB fusion genes achieve durable long-term remissions with imatinib. Blood. 2014;123(23):3574–7.
98. Cerrano M, Crisà E, Gottardi E, Aguzzi C, Boccadoro M, Ferrero D. Long-term therapy-free remission in a patient with platelet-derived growth factor receptor beta (PDGFRB)-rearranged myeloproliferative neoplasm. Am J Hematol. 2016;91(9):E353. [Epub ahead of print]
99. Macdonald D, Aguiar RC, Mason PJ, Goldman JM, Cross NC. A new myeloproliferative disorder associated with chromosomal translocations involving 8p11: a review. Leukemia. 1995;9(10):1628–30.

100. Jackson CC, Medeiros LJ, Miranda RN. 8p11 myeloproliferative syndrome: a review. Hum Pathol. 2010;41(4):461–76.
101. Manthorpe R, Egeberg J, Hesselvik M, Videbaek A. Unique eosinophil granules in a case of T-cell lymphoma. Scand J Haematol. 1977;19(2):129–44.
102. Kuskonmaz B, Kafali C, Akcoren Z, Karabulut HG, Akalin I, Tuncer MA. The 8p11 myeloproliferative syndrome in a 3-year-old child. Leuk Res. 2008;32(1):198–9.
103. Friedhoff F, Rajendra B, Moody R, Alapatt T. Novel reciprocal translocation between chromosomes 8 and 9 found in a patient with myeloproliferative disorder. Cancer Genet Cytogenet. 1983;9(4):391–4.
104. Patnaik MM, Gangat N, Knudson RA, Keefe JG, Hanson CA, Pardanani A, et al. Chromosome 8p11.2 translocations: prevalence, FISH analysis for FGFR1 and MYST3, and clinicopathologic correlates in a consecutive cohort of 13 cases from a single institution. Am J Hematol. 2010;85(4):238–42.
105. Savage NM, Johnson RC, Gotlib J, George TI. Myeloid and lymphoid neoplasms with FGFR1 abnormalities: diagnostic and therapeutic challenges. Am J Hematol. 2013;88(5):427–30.
106. Demiroglu A, Steer EJ, Heath C, Taylor K, Bentley M, Allen SL, et al. The t(8;22) in chronic myeloid leukemia fuses BCR to FGFR1: transforming activity and specific inhibition of FGFR1 fusion proteins. Blood. 2001;98(13):3778–83.
107. Popovici C, Zhang B, Grégoire MJ, Jonveaux P, Lafage-Pochitaloff M, Birnbaum D, et al. The t(6;8)(q27;p11) translocation in a stem cell myeloproliferative disorder fuses a novel gene, FOP, to fibroblast growth factor receptor 1. Blood. 1999;93(4):1381–9.
108. Vizmanos JL, Hernández R, Vidal MJ, Larráyoz MJ, Odero MD, Marín J, et al. Clinical variability of patients with the t(6;8)(q27;p12) and FGFR1OP-FGFR1 fusion: two further cases. Hematol J. 2004;5(6):534–7.
109. Vega F, Medeiros LJ, Davuluri R, Cromwell CC, Alkan S, Abruzzo LV. t(8;13)-positive bilineal lymphomas: report of 6 cases. Am J Surg Pathol. 2008;32(1):14–20.
110. Hu S, He Y, Zhu X, Li J, He H. Myeloproliferative disorders with t(8;9)(p12;q33): a case report and review of the literature. Pediatr Hematol Oncol. 2011;28(2):140–6.
111. Guasch G, Ollendorff V, Borg JP, Birnbaum D, Pebusque MJ. 8p12 stem cell myeloproliferative disorder: the FOP-fibroblast growth factor receptor 1 fusion protein of the t(6;8) translocation induces cell survival mediated by mitogen-activated protein kinase and phosphatidylinositol 3-kinase/Akt/mTOR pathways. Mol Cell Biol. 2001;21(23):8129–42.
112. Matikas A, Tzannou I, Oikonomopoulou D, Bakiri M. A case of acute myelogenous leukaemia characterised by the BCR-FGFR1 translocation. BMJ Case Rep. 2013;pii:bcr2013008834
113. Kim WS, Park SG, Park G, Jang SJ, Moon DS, Kang SH. 8p11 myeloproliferative syndrome with t(1;8)(q25;p11.2): a case report and review of the literature. Acta Haematol. 2015;133(1):101–5.
114. Mugneret F, Chaffanet M, Maynadié M, Guasch G, Favre B, Casasnovas O, et al. The 8p12 myeloproliferative disorder. t(8;19)(p12;q13.3): a novel translocation involving the FGFR1 gene. Br J Haematol. 2000;111(2):647–9.
115. Guasch G, Popovici C, Mugneret F, Chaffanet M, Pontarotti P, Birnbaum D, et al. Endogenous retroviral sequence is fused to FGFR1 kinase in the 8p12 stem-cell myeloproliferative disorder with t(8;19)(p12;q13.3). Blood. 2003;101(1):286–8.
116. Grand EK, Grand FH, Chase AJ, Ross FM, Corcoran MM, Oscier DG, et al. Identification of a novel gene, FGFR1OP2, fused to FGFR1 in 8p11 myeloproliferative syndrome. Genes Chromosom Cancer. 2004;40(1):78–83.
117. Belloni E, Trubia M, Gasparini P, Micucci C, Tapinassi C, Confalonieri S, et al. 8p11 myeloproliferative syndrome with a novel t(7;8) translocation leading to fusion of the FGFR1 and TIF1 genes. Genes Chromosom Cancer. 2005;42(3):320–5.
118. Soler G, Nusbaum S, Varet B, Macintyre EA, Vekemans M, Romana SP, et al. LRRFIP1,a new FGFR1 partner gene associated with 8p11 myeloproliferative syndrome. Leukemia. 2009;23(7):1359–61.
119. Walz C, Chase A, Schoch C, Weisser A, Schlegel F, Hochhaus A, et al. The t(8;17)(p11;q23) in the 8p11 myeloproliferative syndrome fuses MYO18A to FGFR1. Leukemia. 2005;19(6):1005–9.

120. Shvidel L, Sigler E, Vorst E, Feldberg E, Voskoboinic N, Shtalrid M, et al. A novel cytogenetic aberration found in stem cell leukemia/lymphoma syndrome. Leukemia. 2008;22(3):644–6.
121. Sohal J, Chase A, Mould S, Corcoran M, Oscier D, Iqbal S, et al. Identification of four new translocations involving FGFR1 in myeloid disorders. Genes Chromosom Cancer. 2001;32(2):155–63.
122. Bae SY, Kim JS, Han EA, Lee HJ, Ryeu BJ, Lee KN, et al. Cytogenetic abnormality involving 8p11.2 in T-lymphoblastic lymphoma: report of a new case. Cancer Genet Cytogenet. 2009;191(1):57–8.
123. Wasag B, Lierman E, Meeus P, Cools J, Vandenberghe P. The kinase inhibitor TKI258 is active against the novel CUX1-FGFR1 fusion detected in a patient with T-lymphoblastic leukemia/lymphoma and t(7;8)(q22;p11). Haematologica. 2011;96(6):922–6.
124. Post GR, Holloman D, Christiansen L, Smith J, Stuart R, Lazarchick J. Translocation t(3;8;9)(p25;p21;q34) in a patient with features of 8p11 myeloproliferative syndrome: a unique case and review of the literature. Leuk Res. 2010;34(11):1543–4.
125. Abruzzo LV, Jaffe ES, Cotelingam JD, Whang-Peng J, Del Duca V, Medeiros LJ. T-cell lymphoblastic lymphoma with eosinophilia associated with subsequent myeloid malignancy. Am J Surg Pathol. 1992;16(3):236–45.
126. Chen J, Deangelo DJ, Kutok JL, Williams IR, Lee BH, Wadleigh M, et al. PKC412 inhibits the zinc finger 198-fibroblast growth factor receptor 1 fusion tyrosine kinase and is active in treatment of stem cell myeloproliferative disorder. Proc Natl Acad Sci U S A. 2004;101(40):14479–84.
127. Martinez-Climent JA, Vizcarra E, Benet I, Marugan I, Terol MJ, Solano C, et al. Cytogenetic response induced by interferon alpha in the myeloproliferative disorder with eosinophilia, T cell lymphoma and the chromosomal translocation t(8;13)(p11;q12). Leukemia. 1998;12(6):999–1000.
128. Zhou L, Fu W, Yuan Z, Hou J. Complete molecular remission after interferon alpha treatment in a case of 8p11 myeloproliferative syndrome. Leuk Res. 2010;34(11):306–7.
129. Ren M, Qin H, Wu Q, Savage NM, George TI, Cowell JK. Development of ZMYM2-FGFR1 driven AML in human CD34+ cells in immunocompromised mice. Int J Cancer. 2016;139(4):836–40.
130. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–405.
131. Stewart K, Carstairs KC, Dubé ID, Keating A. Neutrophilic myelofibrosis presenting as Philadelphia chromosome negative BCR non-rearranged chronic myeloid leukemia. Am J Hematol. 1990;34(1):59–63.
132. Reiter A, Walz C, Watmore A, Schoch C, Blau I, Schlegelberger B, et al. The t(8;9)(p22;p24) is a recurrent abnormality in chronic and acute leukemia that fuses PCM1 to JAK2. Cancer Res. 2005;65(7):2662–7.
133. Bain BJ, Ahmad S. Should myeloid and lymphoid neoplasms with PCM1-JAK2 and other rearrangements of JAK2 be recognized as specific entities? Br J Haematol. 2014;166(6):809–17.
134. Patterer V, Schnittger S, Kern W, Haferlach T, Haferlach C. Hematologic malignancies with PCM1-JAK2 gene fusion share characteristics with myeloid and lymphoid neoplasms with eosinophilia and abnormalities of PDGFRA, PDGFRB, and FGFR1. Ann Hematol. 2013;92(6):759–69.
135. Rumi E, Milosevic JD, Selleslag D, Casetti I, Lierman E, Pietra D, et al. Efficacy of ruxolitinib in myeloid neoplasms with PCM1-JAK2 fusion gene. Ann Hematol. 2015;94(11):1927–8.

# Chapter 17
# Mixed Phenotype Acute Leukemia

**Olga K. Weinberg**

## Introduction

Mixed phenotype acute leukemia is a rare disease and comprises 2–5% of all acute leukemias. These disorders have been historically labeled by a variety of names, such as mixed-lineage leukemia, bilineal leukemia, and biphenotypic leukemia [1]. Both the earlier 2008 and more recent 2016 World Health Organization (WHO) classifications have proposed a simpler diagnostic algorithm to define mixed phenotype acute leukemia (MPAL), which includes both biphenotypic and bilineal acute leukemias.

## Clinical Presentation

Presenting clinical symptoms in MPAL are similar to other acute leukemias and include fatigue, infections, and bleeding disorders [2]. Usually, the white blood cell count is high and most patients will have a high number of circulating blasts [2].

## Morphology and Immunophenotype

Morphologically, MPAL blasts appear most often as undifferentiated medium-sized blasts with fine chromatin and indistinct-to-prominent nucleoli; however, these blasts can show classical lymphoid features and appear smaller in size with variably condensed

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nuclear chromatin and very high nuclear-to-cytoplasmic ratios, or myeloid features with cytoplasmic granules, very fine nuclear chromatin, and large prominent nucleoli.

However, the diagnosis of MPAL rests on the immunophenotypic features of these blasts rather than morphology. Flow cytometry is the preferred method for recognizing MPAL. Even when there are not 2 distinctly separable populations, most cases of MPAL will show heterogeneity of expression of some antigens. For example, MPO expression will be expressed on the subset of blasts that show relatively brighter expression of myeloid markers and lower intensity of B-cell–associated markers. Figure 17.1 is an example of MPAL where the blasts are small with

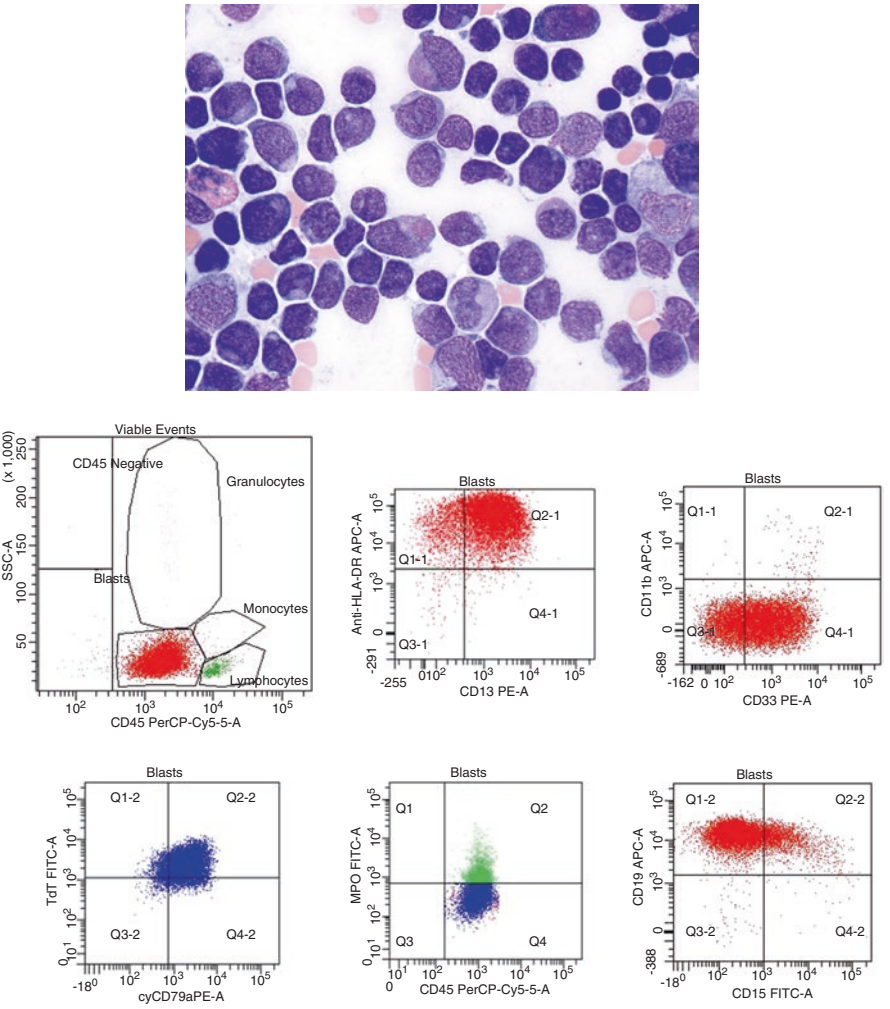


**Fig. 17.1** Case of B/myeloid mixed phenotype acute leukemia. Blasts are mostly small with moderate cytoplasm (part **a**) and expressed CD34, CD13, CD33, CD19, CD79a, TdT, and MPO (part **b**)

moderate cytoplasm and express myeloid markers (CD13, CD33, MPO) as well as strong CD19 and CD79a.

One of the first major attempts to define MPAL was the scoring criteria proposed by the European Group for the Immunological Characterization of Leukemias (EGIL) (Table 17.1) [3]. A numerical value, ranging from 0.5 to 2, was assigned for individual myeloid-associated or lymphoid-associated markers expressed by the blasts, and a biphenotypic acute leukemia was defined when a score over 2 points was achieved for each lineage [3]. In later years, the 2001 World Health Organization (WHO) classification of hematopoietic and lymphoid neoplasms incorporated the EGIL scoring system when defining acute leukemias of ambiguous lineage [4].

Then, in 2008, the WHO classification proposed a simpler diagnostic algorithm to define MPAL, which relies on fewer, more lineage-specific markers [5] (Table 17.2). Myeloid lineage requires the presence of myeloperoxidase as detected by flow cytometry, immunohistochemistry or cytochemistry, or evidence of monocytic differentiation (with at least 2 of the following markers being positive: non-specific esterase cytochemistry, CD11c, CD14, and CD64). T-lineage can be shown with cytoplasmic or surface CD3, at least as intense as background reactive T-cells, and multiple antigens are required for B-lineage including CD19, CD79a, CD22, and CD10. All possible combinations of MPAL can be observed including B/myeloid,

**Table 17.1** EGIL scoring system for biphenotypic acute leukemia

| Points | Myeloid lineage | B lineage | T lineage |
|---|---|---|---|
| 2 | MPO | CD79a | CD3 (cyt/m) |
| | lysozyme | Cyt IgM | anti-TCR α/β |
| | | Cyt CD22 | anti-TCR γ/δ |
| 1 | CD13 | CD19 | CD2 |
| | CD33 | CD10 | CD5 |
| | CD65 | CD20 | CD8 |
| | CD117 | | CD10 |
| 0.5 | CD14 | TdT | TdT |
| | CD15 | CD24 | CD7 |
| | CD64 | | CD1a |

**Table 17.2** 2008 WHO classification: acute leukemia of ambiguous lineage

| Lineage | Markers |
|---|---|
| Myeloid | Myeloperoxidase OR Monocytic differentiation (at least 2 of the following: NSE, CD11c, CD14, CD64, lysozyme |
| T lineage | Cytoplasmic CD3 OR Surface CD3 |
| B lineage | Strong CD19 *and* at least 1 of the following with strong expression: CD79a, cytoplasmic CD22 or CD10<br>OR<br>Weak CD *and* at least 2 of the following with strong expression: CD79a, cytoplasmic CD22 or CD10 |

T/myeloid, B/T, or even rarely B/T/Myeloid [5]. MPAL with t(9;22) and MLL rearrangement have been separated out as distinct subtypes. Acute leukemia of ambiguous lineage is reserved for cases of acute leukemia that show no clear evidence of differentiation along a single lineage.

In the 2016 revision to the WHO classification, no new entities were defined within this group of leukemias [6]. Although the list of lineage-specific markers is unchanged, it is now emphasized that in cases with 2 distinct blast populations, each population should meet criteria for B-lymphoblastic leukemia (B-ALL), T-ALL, or acute myeloid leukemia but it is not necessary that specific markers are present [6]. It is also now more specifically stated that cases of otherwise typical B-ALL with only low-level expression of MPO (without other evidence of myeloid differentiation) should not be classified as MPAL. Furthermore, a specific statement is now included that cases of otherwise typical ALL or AML do not need to meet the strict lineage defining criteria listed for MPAL.

## *MPAL with BCR-ABL Fusion Gene*

Two genetic lesions are frequent enough in MPAL to now be considered as separate entities. The first is MPAL with t(9;22)(q34;q11.2) or *BCR-ABL1* rearrangement. The t(9;22)(q34;q11.2) translocation results in a *BCR-ABL1* fusion gene located on the Philadelphia chromosome (Ph), causing a constitutively active BCR-ABL1 tyrosine kinase. Acute leukemia with t(9;22) and blast phase of chronic myeloid leukemia (CML) have very similar clinical presentations and morphologic features. The 2008 WHO classification suggests caution when making the diagnosis of MPAL with t(9;22) [4]. Splenomegaly, peripheral leukocytosis due to maturing myeloid precursors and mature neutrophils, absolute basophilia, and a clinical history of CML may support the diagnosis of blast phase of CML with MPAL phenotype [4]. De novo MPAL with *BCR-ABL* rearrangement generally occurs more frequently in older patients. Although most studies found the frequency of MPAL with t(9;22) to be 28–35%, pediatric studies report it to be much lower at 3% [7]. Many of these cases show a dimorphic population of blasts, with most showing B and myeloid lineage [7]. Some studies suggest that this subtype of MPAL has a worse outcome [8].

## *MPAL with MLL Rearrangement*

The second most frequent genetic lesion in MPAL is translocations involving MLL gene. *MLL* rearrangement juxtaposes the amino-terminus of the histone methyltransferase MLL to a variety of fusion partners, with the most common partner gene being AF4 on chromosome 4 band q21.35 in MPAL [9]. This tends to occur more commonly in children and is more frequent in infancy [9]. One study showed frequency of 10% in adults to 12–18% in pediatric MPAL [1]. These cases also tend to

present with a dimorphic blast population, one resembling lymphoblasts and the other resembling monoblasts. By flow cytometry, the lymphoblasts usually have a CD19-positive, CD10-negative, B-precursor immunophenotype and are frequently positive for CD15. Usually, the flow cytometry identifies a separate population of myeloid blasts with monocytic differentiation. The prognosis of MPAL patients with MLL rearrangement is also poor [10].

## Mixed Phenotype Acute Leukemia, Not Otherwise Specified

### *Cytogenetics and Molecular Findings*

In a recent study, Yan et al., found that of 92 MAPL patients assessed, 64% presented with cytogenetic abnormalities [11]. The most prevalent aberration was the complex karyotype found in 24% of patients, followed by the t(9;22) chromosome in 15% (all B-myeloid phenotype) and translocations involving MLL gene at 11q23 in 4.3% of patients [11]. A specific reference was made in the 2008 WHO classification to exclude cases that can be classified in another category, either by genetic or clinical features. For instance, AML with t(8;21), t(15;17), and inv. [11] can express lymphoid-associated markers but should be classified as AML with recurrent genetic abnormalities. Cases of chronic myelogenous leukemia (CML) in blast crisis, AML with myelodysplasia-related changes, and therapy-related AML should be classified as their respective entities even if they happen to have a mixed phenotype.

In a study of 61 MPAL patients, Weinberg et al., found that 23 of 61 patients were under 21 years of age (38%), most showed a B/myeloid phenotype (67%), and had normal cytogenetics (44% of patients with cytogenetic information) [12]. Seven patients (or 22%) had t(9;22) or MLL rearrangement. This is a similar distribution to what Matutes et al., found in their study [13]. However, both Matutes et al., and Yan et al., included MPAL patients with complex karyotype (~24–32% of all their patients) in their series [11, 13]. In the 2008 WHO classification, the presence of a complex karyotype would be considered as AML with myelodysplasia-related changes if defined by cytogenetics alone, and such cases were excluded from the study by Weinberg et al.

Rubnitz et al., analyzed gene expression patterns in 13 pediatric patients with MPAL (as defined by EGIL) and found that 8 patients displayed gene expression patterns that were different from AML and ALL [14]. In contrast, using microRNA profiling studies, de Leeuw et al., demonstrated that 16 cases had microRNA expression profiles that clustered with AML or ALL [15]. Heesch et al., noted a higher expression of *BAALC* and *ERG* in 26 cases of MPAL as compared with other cases of AML [16]. Array-based comparative genomic hybridization analysis in 12 patients with MPAL demonstrated that all patients had at least 1 abnormality, including deletions of *CDKN2A, IKZF1, MEF2C, BCOR, EBF1, KRAS, LEF1, MBNL1, PBX3,* and *RUNX1* [14].

Information regarding the mutational landscape of MPAL is based on small patient numbers. Yan et al., analyzed 31 patients with MPAL and reported that 12 patients

(39%) were found to harbor a known mutation [11]. These included *IKZF1* deletion in 4 patients (all B-myeloid phenotype with evidence of BCR-ABL1 fusion gene), *EZH2* in 3 (B- or T-myeloid; one case showing complex karyotype and another showing loss of chromosome 7), *ASXL1* in 2 (both B-myeloid), *TET2* in one (B-myeloid), and *ETV6* and *NOTCH1* in 1 patient each (both T-myeloid) [11]. A high rate of mostly biallelic mutations *DNMT3A* mutations were reported in 10 of 18 adults with T-myeloid MPAL [14]. No evidence of mutations in C*BL, DNMT3A, FBXW7, FLT3, IDH1, IDH2, KIT, NPM1, PHF6, RUNX1,* and *WT1* were found in Yan's study [11].

Whole-exome sequencing in 23 adult and pediatric patients with MPAL demonstrated that 35% patients had mutations in epigenetic regulatory genes ([17], Table 17.3). *DNMT3A* was the most common mutation (23%) followed by *IDH2 (9%), TET3 (4%), and EZH2 (9%).* All of the *DNMT3A* mutations involved the methyltransferase domain, three of which were missense mutations at Arg882, the hotspot common in AML. *DNMT3A* occurred in all immunophenotypic subtypes examined. Similar to reports in AML, MPAL patients with mutation in *DNMT3A* trended toward being older and having a normal cytogenetics [17]. Tumor

**Table 17.3** Summary of mutations from whole-exome sequencing of 23 MPAL samples

| | Gene | Frequency (%) |
|---|---|---|
| Epigenetic | *DNMT3A* | 6 (23%) |
| | *IDH2* | 2 (9%) |
| | *TET3* | 1 (4%) |
| | *EZH2* | 2 (9%) |
| Activated signaling | *NRAS* | 4 (17%) |
| | *KRAS* | 3 (13%) |
| | *NF1* | 2 (8%) |
| | *FLT3* | 3 (13%) |
| | *JAK2* | 1 (4%) |
| | *JAK3* | 1 (4%) |
| Tumor suppressor | *TP53* | 5 (22%) |
| | *WT1* | 3 (13%) |
| | *PHF6* | 2 (8%) |
| | *PTCH11* | 2 (8%) |
| | *CDKN2A* | 1 (4%) |
| Transcription factors | *NOTCH1* | 5 (22%) |
| | *RUNX1* | 4 (17%) |
| | *GATA2* | 1 (4%) |
| | *IKZF1* | 1 (4%) |
| Splicing | *SF3A1* | 1 (4%) |
| Cohesin | *RAD21* | 1 (4%) |
| | *SMC1A* | 1 (4%) |
| Others | *CDKN2B* | 1 (4%) |
| | *LEF1* | 1 (4%) |

Data from Eckstein et al. [17]

suppressors were also frequently mutated and 5 patients (22%) had *TP53* mutations ([17], Table 17.3). Mutations of DNMT3A and tumor suppressors showed high variant allele frequency (VAF), suggesting that these mutations arise early in the disease. Sixty-one percent of the patients also had mutually exclusive mutations of activating signaling genes including *NRAS, KRAS, and NF1* [18]. *NOTCH1* mutations were present in 5 of 16 (32%) with T-myeloid and B/T leukemia. Three samples (13%) also had *WT1* mutations. In another series, clustering of *FLT3 ITD* and *TKD* mutations was reported in patients with T-myeloid MPAL. Seven of 15 patients (47%) were positive for FLT3 mutations (mostly ITD), all of which were CD117+ [19].

### *Prognosis and Therapy*

There is no set therapy for MPAL patients, which is a result of the absence of prospective trials. In the few larger retrospective series of MPAL, the median overall survival is reported to range from 14.8 to 18 months and the rate of achieving long-term survival in patients with adult MPAL is poor (<20%) [17, 19, 20]. Most of the retrospective case series suggest that the complete remission rates are higher with ALL therapy or an ALL/AML combined regimen than with AML-type therapy [21, 22]. Children with MPAL are suggested to do better, although they do have inferior outcome compared with those diagnostic with typical ALL [22]. A few studies compared outcome of MPAL patients with that of matched control ALL or AML groups and most found that MPAL patients did worse than AML or ALL [1]. In a study of 61 patients, Weinberg et al., found that when compared with 177 patients with acute myeloid leukemia (AML), MPAL patients had better overall survival ($P$ = .0003) and progression-free survival ($P$ = .0001). However, no difference in overall survival between MPAL and 387 patients with acute lymphoblastic leukemia was present ($P$ = .599) [12]. For patients with t(9;22)-positive MPAL, a tyrosine kinase inhibitor (TKI) is usually added to treatment [23]. In his review, Wolach et al. suggested that the best approach for the non-t(9;22) MPAL patient is to treat with an ALL regimen and consolidate with an allogeneic stem cell transplant if a donor is available [23]. Shimizu H et al., have suggested that allogeneic hematopoietic stem cell transplantation may be an effective treatment for MPAL patients, especially early in the disease course [24].

## Conclusion

Overall, acute leukemias with mixed phenotypes are uncommon and comprise 2–5% of all acute leukemias. Molecular studies showed frequent mutations in epigenetic regulatory genes and tumor suppressors in MPAL patients. The outcome of MPAL patients remains poor and mutations have been identified in this disease that are

potentially targetable by agents that are currently available or are being tested in clinical trials, including epigenetically targeted agents, tyrosine kinase pathway inhibitors, and NOTCH1 inhibitors. Studies suggest that the best treatment of non-t(9;22) MPAL patient is to treat with an ALL regimen and consolidate with an allogeneic stem cell transplant if a donor is available. More studies are needed to address the biology and treatment of MPAL patients.

## References

1. Weinberg OK, Arber DA. Mixed-phenotype acute leukemia: historical overview and a new definition. Leukemia. 2010;24(11):1844–51.
2. Weir EG, Borowitz MJ. Acute leukemias of ambiguous lineage. In: Jaffe ES, Harris NL, Vardiman JW, Campo E, Arber DA, editors. Hamtopathology. Philadelphia: Elsevier; 2010.
3. Bene MC, Castoldi G, Knapp W, et al. Proposals for the immunological classification of acute leukemias. European Group for the Immunological Characterization of Leukemias (EGIL). Leukemia. 1995;9:1783–6.
4. Jaffe E, Harris N, Stein HVJ, et al. World Health Organization classification of tumours. Pathology and genetics of tumors of hematopoietic and lymphoid tissues 2nd printing. Lyon: IARC Press; 2001.
5. Borowitz MJ, Bene MC, Harris NL, et al. Acute leukemias of ambiguous lineage. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW, editors. WHO classification of tumours of haematopoietic and lymphoid tissues. Lyon: IARC Press; 2008. p. 150–5.
6. Arber DA, Orazi A, Hasserjian R, Thiele T, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–405.
7. Al-Seraihy AS, Owaidah TM, Ayas M, El-Solh H, Al-Mahr M, Al-Ahmari A, et al. Clinical characteristics and outcome of children with biphenotypic acute leukemia. Haematologica. 2009;94:1682–90.
8. Killick S, Matutes E, Powles RL, Hamblin M, Swansbury J, Treleaven JG, et al. Outcome of biphenotypic acute leukemia. Haematologica. 1999;84:699–706.
9. XQ X, Wang JM, Lü SQ, Chen L, Yang JM, Zhang WP, et al. Clinical and biological characteristics of adult biphenotypic acute leukemia in comparison with that of acute myeloid leukemia and acute lymphoblastic leukemia: a case series of a Chinese population. Haematologica. 2009;94:919–27.
10. Owaidah TM, Al Beihany A, Iqbal MA, Elkum N, Roberts GT. Cytogenetics, molecular and ultrastructural characteristics of biphenotypic acute leukemia identified by the EGIL scoring system. Leukemia. 2006;20:620–6.
11. Yan L, Ping N, Zhu M, et al. Clinical, immunophenotypic, cytogenetic, and molecular genetic features in 117 adult patients with mixed-phenotype acute leukemia defined by WHO-2008 classification. Haematologica. 2012;97(11):1708–12.
12. Weinberg OK, Seetharam M, Ren L, Alizadeh A, Arber DA. Mixed phenotype acute leukemia: a study of 61 cases using World Health Organization and European Group for the Immunological Classification of Leukaemias criteria. Am J Clin Pathol. 2014;142(6):803–8.
13. Matutes E, Pickl WF, Van't Veer M, et al. Mixed-phenotype acute leukemia: clinical and laboratory features and outcome in 100 patients defined according to the WHO 2008 classification. Blood. 2011;117(11):3163–71.
14. Rubnitz JE, Onciu M, Pounds S, et al. Acute mixed lineage leukemia in children: the experience of St Jude Children's Research Hospital. Blood. 2009;113(21):5083–9.

15. de Leeuw DC, van den Ancker W, Denkers F, et al. MicroRNA profiling can classify acute leukemias of ambiguous lineage as either acute myeloid leukemia or acute lymphoid leukemia. Clin Cancer Res. 2013;19(8):2187–96.
16. Heesch S, Neumann M, Schwartz S, et al. Acute leukemias of ambiguous lineage in adults: molecular and clinical characterization. Ann Hematol. 2013;92(6):747–58.
17. Eckstein OS, Wang L, Punia JN, Kornblau SM, Andreeff M, Wheeler DA, Goodell MA, Rau RE, et al. Mixed-phenotype acute leukemia (MPAL) exhibits frequent mutations in DNMT3A and activated signaling genes. Exp Hematol. 2016;44(8):740–4.
18. Kern W, Grossmann V, Roller A, et al. Mixed Phenotype Acute Leukemia, T/Myeloid, NOS (MPAL-TM) has a high DNMT3A mutation frequency and carries further genetic features of both AML and T-ALL: results of a comprehensive next-generation sequencing study analyzing 32 genes. Blood. 2012;120:403.
19. Hoehn D, Medeiros LJ, Chen SS, et al. CD117 expression is a sensitive but nonspecific predictor of FLT3 mutation in T acute lymphoblastic leukemia and T/myeloid acute leukemia. Am J Clin Pathol. 2012;137(2):213–9.
20. Deffis-Court M, Alvarado-Ibarra M, Ruiz-Argüelles GJ, et al. Diagnosing and treating mixed phenotype acute leukemia: a multicenter 10-year experience in México. Ann Hematol. 2014;93(4):595–601.
21. Liu QF, Fan ZP, MQ W, et al. Allo-HSCT for acute leukemia of ambiguous lineage in adults: the comparison between standard conditioning and intensified conditioning regimens. Ann Hematol. 2013;92(5):679–87.
22. Gerr H, Zimmermann M, Schrappe M, et al. Acute leukaemias of ambiguous lineage in children: characterization, prognosis and therapy recommendations. Br J Haematol. 2010;149(1):84–92.
23. Wolach O, Stone RM. How I treat mixed-phenotype acute leukemia. Blood. 2015;125(16):2477–85.
24. Shimizu H, Saitoh T, Machida S, Kako S, Doki N, Mori T, Sakura T, Kanda Y, Kanamori H, Miyawaki S, Okamoto S, Kanto Study Group for Cell Therapy (KSGCT). Allogeneic hematopoietic stem cell transplantation for adult patients with mixed phenotype acute leukemia: results of a matched-pair analysis. Eur J Haematol. 2015;95(5):455–60.

# Chapter 18
# Blastic Plasmacytoid Dendritic Cell Neoplasm

**Michael J. Cascio and Robert S. Ohgami**

## Introduction

First described in 1994 [1], blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare, aggressive hematopoietic neoplasm. It has been variably known as blastic NK-cell lymphoma, agranular CD4+ natural killer cell leukemia, and agranular CD4+/CD56+ hematodermic neoplasm.

The normal cellular counterpart to BPDCN is the precursor plasmacytoid dendritic cell (pDCs). These cells play central roles in infectious and inflammatory conditions, primarily through secretion of type I interferons which stimulate T-cells and B-cells, resulting in effective augmentation of anti-viral immune responses, or in the case of autoimmune conditions, generation of abnormally autoreactive T-cells and B-cells, via overstimulated antigen presenting cells [2]. Reactive pDCs are increased in the lymph nodes of patients with inflammatory disorders such as Kikuchi-Fujimoto lymphadenitis and hyaline vascular Castleman disease [3, 4].

Given its relationship to normal plasmacytoid dendritic cells, BPDCN is classified as a precursor neoplasm related to acute myeloid leukemia (AML) in the World Health Organization Classification of Tumours of Haematopoietic and Lymphoid Tissues [5].

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## Epidemiology

In large retrospective reviews, BPDCN accounts for less than 1% of all acute leukemias and lymphomas, although, until recently, markers that help to distinguish BPDCN from AML were not widely available. There is a slight male predominance (3:1), with no ethnic or racial predisposition [6]. Mean age at presentation is 60–70 years, but a large age distribution is observed, including rare congenital cases. A pre-existing myeloid neoplasm, such as myelodysplastic syndrome or chronic myelomonocytic leukemia, has been noted in 5–10% of patients [6].

## Etiology

The pathogenesis of BPDCN is poorly understood, and as such, no known environmental exposures or genetic predispositions have been described.

## Clinical Features

The majority of patients have cutaneous involvement at the time of diagnosis, characterized by nodules or purpuric plaque-like skin lesions (Fig. 18.1). BPDCN may also present as a leukemic infiltrate in the blood or marrow, show involvement of the lymph nodes or spleen, or demonstrate simultaneous tissue and blood/marrow involvement. In cases where the neoplasm appears confined to the skin, dissemination to peripheral blood and bone marrow occurs shortly thereafter. Thrombocytopenia, anemia, and absolute neutropenia are commonly found on peripheral blood evaluation. Clinical features are summarized in Table 18.1.

## Morphology and Immunophenotyping

### *Morphologic Features*

The morphologic features of neoplastic cells of BPDCN can be quite variable, but in the most classic cases, cells are medium-sized with rounded to slightly irregular nuclei, fine chromatin, absent or inconspicuous small nucleoli, and scant to moderate amounts of cytoplasm; small cytoplasmic vacuoles may be present (Figs. 18.2 and 18.3). In cutaneous tissues, BPDCN may show perivascular, periadnexal, or sheet-like pattern of growth in the dermis and subcutis, with sparing of the overlying epidermis (Fig. 18.2). Bone marrow evaluation may reveal patchy involvement or complete effacement.

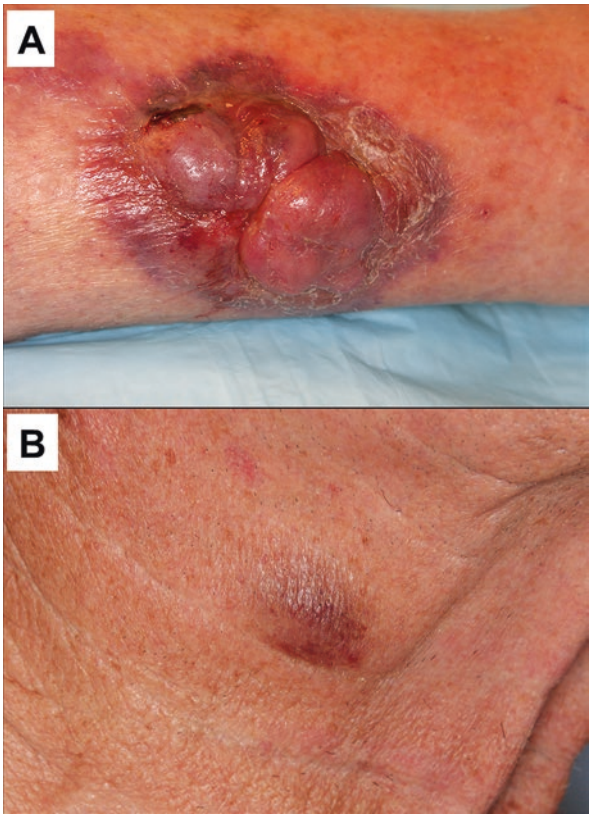


**Fig. 18.1** Cutaneous manifestations of blastic plasmacytoid dendritic cell neoplasm (BPDCN). (**a**) Violaceous nodules can be seen with skin infiltration by BPDCN. (**b**) Plaques are also commonly seen (Images courtesy of Dr. Youn Kim, Stanford University)

**Table 18.1** Clinical features of BPDCN

| |
|---|
| *Age* |
| Median 65 year (range, 0–95) |
| *Gender* |
| M:F 3:1 |
| *Sites involved at diagnosis* |
| Skin 70–90% |
| Peripheral blood 50–70% |
| Bone marrow 60–90% |
| Lymph node 50–70% |
| Spleen 40–60% |
| *Other clinical findings* |
| Cytopenias 70–90% |

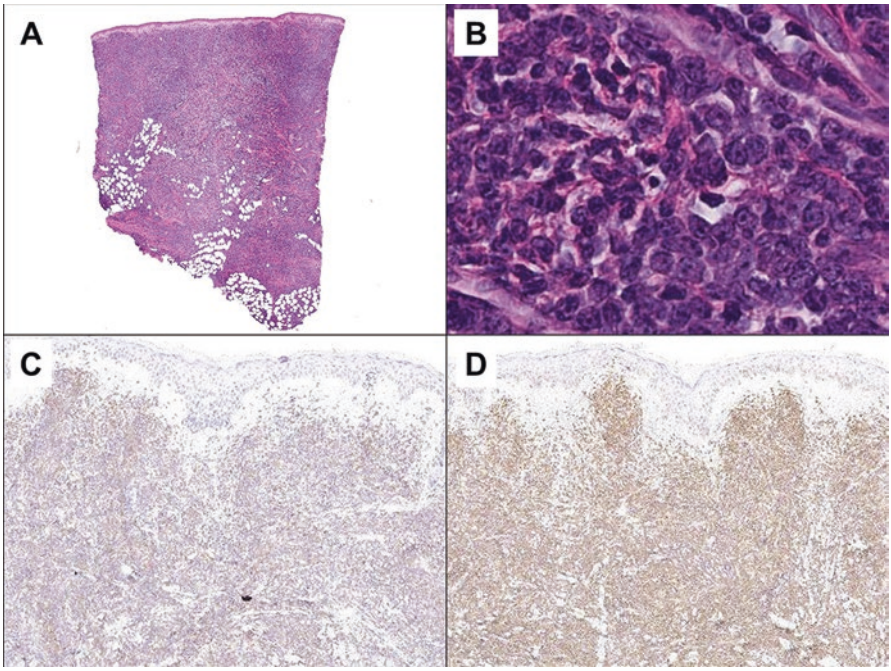


**Fig. 18.2** Morphologic and immunohistochemical features of skin infiltration by blastic plasmacytoid dendritic cell neoplasm (BPDCN). (**a**) Sheet-like infiltration of mononuclear cells with sparing of the epidermis (H&E, 1×) (**b**) With higher power magnification showing neoplastic cells to have blastic chromatin with small to indistinct nucleoli (H&E, 400×). Positive immunohistochemical stains for (**c**) CD123 (40×), and (**d**) TCL1 (40×) are shown

## *Immunophenotype*

Immunophenotypic analysis is critical in order to make the diagnosis of BPDCN and distinguish it from other entities. These proliferations consistently show expression of CD4 and CD56; however, this combination is nonspecific, as it can also be seen in the setting of NK/T-cell malignancies and AML, particularly those with monocytic differentiation. CD45 intensity can be dim or moderate by flow cytometry, also raising the possibility of AML or lymphoblastic leukemia/lymphoma. Bright CD123 expression is a hallmark of BPDCN and a useful feature in distinguishing it from AML. While expression of the myeloid-associated antigens CD13 and CD33 can be present, other markers of the myelomonocytic lineage (CD14, CD163, myeloperoxidase, lysozyme) are uniformly absent. Expression of CD303/BDCA-2, CLA/CD162, TCL1, and TdT is variable, which some investigators speculate may reflect the stage of maturation of the pDC that gives rise to the neoplastic clone [7]. T-cell-associated antigens CD2 and CD7 were found to be expressed in 37% and 11% of cases studied in the largest series [8]. There are isolated reports of

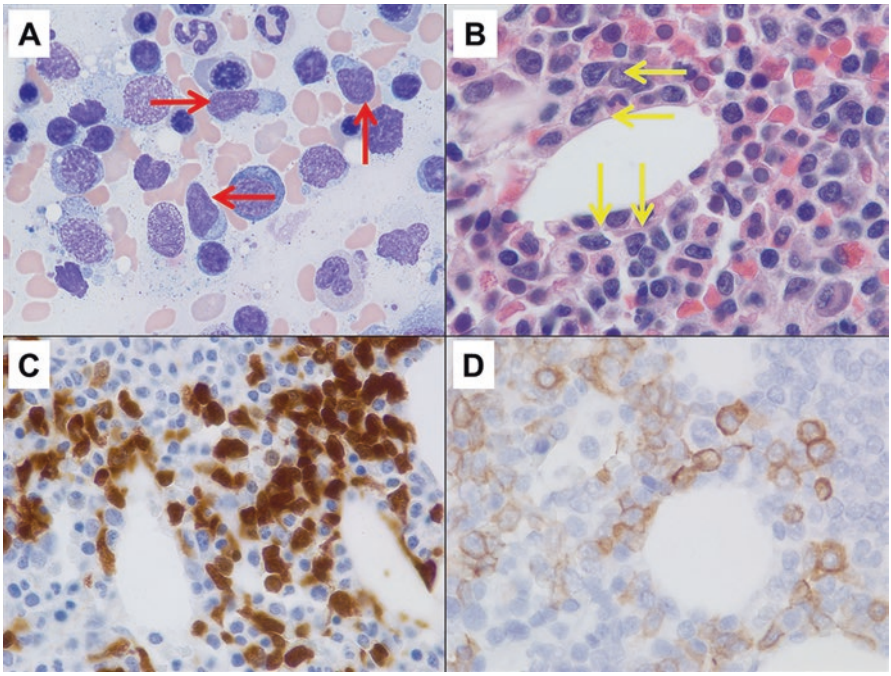


**Fig. 18.3** Features of bone marrow infiltration by blastic plasmacytoid dendritic cell neoplasm (BPDCN). (**a**) Morphologic evaluation of a bone marrow aspirate shows scattered mononuclear cells with fine/blastic chromatin and small to indistinct nucleoli, and eccentric nuclei (*red arrows*; Wright-Giemsa, 1000×). (**b**) An H&E stained bone marrow core biopsy shows BPDCN cells (*yellow arrows*; H&E, 400×). Positive immunohistochemical stains for (**c**) TCL1 (400×) and (**d**) CD123 are shown (400×)

cytoplasmic CD3 expression in BPDCN; however, it has been attributed to the use of a polyclonal antibody and consensus guidelines suggest that the presence of CD3 should exclude a diagnosis of BPDCN [9]. Expression of B-lineage-associated antigens (CD19, CD20, PAX5) has not been described. CD22, a B-lineage marker, has been documented in a few cases when using the s-HCL-1 antibody clone; however, evaluation of 5 additional anti-CD22 clones showed no significant staining, indicating a clone-specific phenomenon [10].

Because of the clinical, morphologic, and immunophenotypic overlap with AML, a panel of antibodies is commonly performed to arrive at the correct diagnosis. Several groups have attempted to define the optimal antibody panel for distinguishing AML from BPDCN. Sangle and coworkers determined that a panel comprising CD4, CD56, CD123, lysozyme, myeloperoxidase, TCL1, and MxA proved useful in discriminating AML from BPDCN [11]. BPDCN was strongly associated with positive staining for CD4, CD56, CD123, TCL1, and MxA expression. Further, the expression of MPO and lysozyme confirmed the diagnosis of AML. CLA/CD162 and CD303 expression showed no significant association

with the reference diagnosis, although only 7 of 17 cases of BPDCN were studied with these two markers. Julia et al. studied the immunoprofile of 91 BPDCN cases and propose that a diagnosis of BPDCN can be established when at least 4 of 5 markers (CD4, CD56, CD123, CD303, and TCL1) are expressed, in agreement with a prior study by Cronin and colleagues [8, 12]. None of the cases showed simultaneous absence of CD4 and CD56. CD303, a specific marker of plasmacytoid dendritic cells, was found to be expressed in 63% of the 85 cases tested. Recently, myeloid cell nuclear differentiation antigen (MNDA) expression was found to be expressed in the majority of AML and uniformly negative BPDCN, providing another marker to help sort out the differential diagnosis. [13]. Immunohistochemical markers useful in differentiating BPDCN from mimics are summarized in Table 18.2.

## Cytogenetics and Molecular Features

Karyotypic abnormalities are a common finding, with up to 80% of BPDCN showing complex (≥3) clonal abnormalities [14]. A few recurring chromosomal loci are deleted in BPDCN, namely 5q, 12p, 13q, 6q, 15q, and 9 (Table 18.3) [14–16]. In a handful of cases, a t(6;8)(p21;q24) translocation involving *MYC* has been identified [14, 17–19].

Sapienza and investigators subjected 27 BPDCN samples from untreated patients to gene expression profiling (GEP), which revealed that BPDCN appears significantly more related to normal myeloid precursors than lymphoid precursors, and closely resembles resting pDCs [20]. Further analysis showed that BPDCN tended to look more similar to AML on the molecular level, but shared patterns of gene deregulation that overlapped with both AML and ALL. GEP studies have demonstrated altered expression of tumor suppressors (*RB1, LATS2, CDC14B, DBC1, SYK, KPNA3*) and oncogenes (*HES6, RUNX2, FLT3*) [21].

Recently, whole-exome sequencing of BPDCN by Menezes identified 38 genes of interest [22]. Interrogation of 28 cases yielded mutations in a number of genes with known pathogenic effects in myeloid malignancies, including genes involved in DNA methylation (*TET2, DNMT3A, IDH1, IDH2)*, chromatin remodeling (*ASXL1*), cell proliferation (*NRAS, KRAS*), transcription factors (*ETV6, IKZF1/2/3*, *RUNX1*), splicing machinery (*SF3B1, SRSF2, U2AF1, ZRSR2),* protein kinases (*FLT3, JAK2, KIT)*, tumor suppressors (*TP53*), and ubiquitination (*CBLB, CBLC, UBE2G2).* Subsequently, 33 additional cases of BPDCN were subjected to massively paralleled sequencing, identifying many of the same molecular aberrations [23]. However, none of these mutations is specific for BPDCN and the prognostic significance of these mutations remains to be determined. A summary of pathogenic mutations detected by sequencing methods is presented in Table 18.4. Below we review in detail some of the more commonly described abnormalities and possible pathogenetic mechanisms.

**Table 18.2** Immunohistochemical profile of BPDCN

| Marker | CD2 | CD4 | CD7 | CD13 | CD33 | CD34 | CD43 | CD56 | CD68 | CD117 | CD123 | CD163 | CD303 | Lysozyme | MPO | TCL1 | TdT | MX-1 | S100 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| # of cases tested | 92 | 193 | 96 | 12 | 30 | 15 | 38 | 193 | 102 | 28 | 185 | 9 | 113 | 14 | 40 | 172 | 161 | 85 | 82 |
| % Positive | 38 | 99 | 21 | 0 | 73 | 0 | 97 | 94 | 79 | 11 | 97 | 0 | 50 | 0 | 0 | 95 | 29 | 67 | 32 |

Composite from Julia [8], Marafioti [54], Sangle [11], Johnson [13], Alayed [29], Herling [53], and Boiocchi [55]

**Table 18.3** Chromosomal regions frequently deleted in BPDCN

| Chromosome locus involved | Frequency (%) |
|---|---|
| 5q | 70 |
| 12p | 60 |
| 13q | 60 |
| 6q | 50 |
| 15q | 40 |
| 9 | 30 |

Adapted from Leroux et al. [14]

**Table 18.4** Summary of somatic point mutations and insertion/deletion mutations identified by sequencing

| Gene | Frequency of mutation (%) |
|---|---|
| DNA methylation | |
| *TET2* | 30–50 |
| *IDH1/2* | 10 |
| *DNMT3A* | 10 |
| Chromatin remodeling | |
| *ASXL1* | 30 |
| RAS family | |
| *NRAS* | 10–30 |
| *KRAS* | 10 |
| Transcription factors | |
| *ETV6* | 10 |
| *RUNX1* | Rare (<5) |
| *IKZF1/2/3* | 20 |
| Splicing machinery | |
| *SF3B1* | 10 |
| *SRSF2* | 10 |
| *U2AF1* | 0–10 |
| *ZRSR2* | 10 |
| Protein kinases | |
| *FLT3* | 0–10 |
| *ATM* | 20 |
| *KIT* | 0–10 |
| Tumor suppressors | |
| *TP53* | 5–10 |
| *RB1* | 5–10 |

Adapted from Stenzinger et al. [23] and Menezes et al. [22]

## *Cell Cycle Genes (RB1, CDK Inhibitors, IKZF1, TP53)*

Loss of gene loci important in the normal function of the cell cycle is common in BPDCN. *RB1* is a cell cycle gene located at 13q13.1-q14.3 and deletion or down-regulation of *RB1* has been identified in approximately half of cases studied (13/26, 50%) [14, 21, 24]. Normally, Rb1 prevents cells from transitioning from the gap 1 (G1) phase (G1) of the cell cycle into the synthesis (S) phase. Thus, in the case of

BPDCN, with loss of Rb1, the deletion or downregulation of its activities is believed to alleviate the block from G1 to S phases.

CDK inhibitors are also frequently (23/30, 77%) disrupted in BPDCN, resulting in unimpeded entry into the cell cycle [15, 24]. *CDKN1B* ($p27^{Kip1}$), *CDKN2A* ($p16^{INK4A}$), and *CDKN2B* ($p15^{INK4B}$) are CDK inhibitors that each play a role in controlling the G1/S-phase transition in the cell cycle. Deletion of the 9p21.3 locus (including *CDKN2A/CDKN2B*) and 12p13.2-p13.1 locus (including *CDKN1B*) was discovered in 67% and 57% of BPDCN studied by array-based comparative genomic hybridization (aCGH) [24]. Although this was a small series of nonuniformly treated patients, multivariate analysis suggested that the presence of homozygous 9p21.3 deletion was an independent prognostic factor. Jardin et al. identified loss of *CDKN2A/CDKN2B* and *CDKN1B* loci in a similar proportion of cases, suggesting that these alterations are important in the pathogenesis of BPDCN.

In about 20% of BPDCN cases, a locus on 7p12.2 that contains *IKZF1* is deleted [24]. Furthermore, Menezes and coworkers found frame shift and missense mutations in the *IKZF* gene family in an additional 20% of cases [22]. *IKZF1* encodes Ikaros, a DNA- and protein-binding transcription factor with zinc finger binding motifs. Ikaros plays a crucial role in the cell cycle regulation and cell differentiation, including an important role in lymphocyte development [25]. The significance of *IKZF1* mutations and deletions in BPDCN is still unknown.

Mutations of *TP53,* or loss of the chromosome region, 17p13, encompassing *TP53* are seen in many cases of BPDCN [15, 26]. The protein product of *TP53*, p53, is a tumor suppressor, and is activated during cellular stress and exerts antiproliferative effects at the G1/S and G2/M checkpoints in the cell cycle primarily through activating the CDK inhibitor p21. P53 also functions as a pro-apoptotic protein by activating *BAX*. Germline *TP53* mutations are seen in Li-Fraumeni syndrome (LFS). In LFS, patients have a 25-fold increased risk of developing cancer by age 50. Breast and adrenal carcinomas, gliomas, sarcoma, and leukemia are the most common neoplasms encountered in this setting.

## *Genes Involved in Hematopoiesis (ETV6, TET2, FLT3, ASXL1)*

In slightly more than half of the BPDCN cases studied, Leroux et al. found deletions in the locus surrounding *ETV6*, suggesting that loss of transcriptional repression by *ETV6* may play an important role in BPDCN pathogenesis [14]. In addition, a rare case of BPDCN harboring an *ETV6* rearrangement with an unknown partner gene has also been reported [27].

*ETV6* encodes a protein that mediates cell proliferation and differentiation, and is required for establishing embryonic hematopoiesis in the bone marrow [28]. How deletions and mutations affect the pathogenesis of BPDCN is still unknown, though the *ETV6* locus is frequently translocated in cases of AML and B-lymphoblastic leukemia (B-LBL). *ETV6* functional effects in translocations are dependent on its partner and ultimately result in oncogenesis.

*TET2* (Ten-Eleven Translocation-2) is part of a family of dioxygenases that promote DNA demethylation and mutations have been found in approximately 50% of the BPDCN cases interrogated [22, 26, 29]. These mutations are heterozygous, with most occurring in exons 3 and 11 as frame shift or nonsense mutations. Changes in *TET2* expression and function can lead to alterations in posttranscriptional modification of histones and ultimately changes in gene expression.

Yet another gene mutated in BPDCN, *FLT3*, encodes the protein FMS-like tyrosine kinase 3, which is a receptor tyrosine kinase that transmits signals important for cellular proliferation and survival. *FLT3* is critical for normal development of the hematopoietic systems, including pDCs [30–33]. A significant proportion of cases of AML harbor *FLT3* mutations, either in the form of internal tandem duplications (ITD) involving the juxtamembrane domain or tyrosine kinase domain (TKD) mutations and affect prognosis [34]. While BPDCN shows some morphologic, immunophenotypic, and genetic overlap with AML, only rare published cases have demonstrated *FLT3* mutations, including both ITD and TKD mutations [22, 26]. *FLT3* inhibitors are being evaluated in the treatment of AML and may be of utility in *FLT3*-mutated BPDCN [35].

*ASXL1* is mutated in roughly one-third of the BPDCN cases interrogated [22]. *ASXL1* mutations are not unique to BPDCN, as they are found in a number of myeloid malignancies, including chronic myelomonocytic leukemia, myelodysplastic syndrome, and AML. *ASXL1* can act independently and in concert with BRCA1-associated protein (BAP1) to promote deubiquitination of histone proteins, some of which are involved in cell proliferation. Although this pathway is thought to be important for regulating myelopoiesis, specific mechanisms of tumorigenesis in the setting of *ASXL1* mutations are largely unknown [36–40].

## *DNA Repair Genes (HINT1, EWSR1, NPM1)*

Studies of the 5q commonly deleted region in BPDCN have yielded a few known cancer-related genes, including *HINT1* [41]. *HINT1* (histidine triad nucleotide-binding protein 1) encodes a purine phosphoramidase that inhibits transcriptional activity of activation protein-1 (AP-1), α-catenin, MITF, and USF2 (upstream transcription factor 2, c-fos interacting). *HINT1* deficiency impairs acetylation of the ATM protein, which inhibits DNA repair mechanisms [42]; its functional role in BPDCN is unknown.

*EWSR1* is a member of the *TET2* family of genes; it binds DNA/RNA and has a general role in gene expression and cell signaling. Additionally, *EWSR1* plays a role in controlling DNA-damage-induced alternative splicing of some oncogenic proteins, such as *BRCA1* [43]. *EWSR1* gene fusions are common in sarcomas, including the Ewing sarcoma family of tumors. A single case of BPDCN has shown *EWSR1* rearrangement with an unidentified translocation partner, suggesting that this locus may play a role in the pathogenesis of some cases of BPDCN [44].

*NPM1* encodes the protein nucleophosmin, which mediates a number of different cellular processes, including DNA repair, regulation of the *TP53* tumor suppressor pathway, and cell cycle events. *NPM1* mutations have been identified in 20% of

the cases of BPDCN analyzed, and include frame shift, nonsense, and missense mutations [22]. *NPM1* is familiar in the context of AML, where it is mutated in approximately 50% of cases, and can affect prognosis [45]; however, the role of *NPM1* mutations in BPDCN is unclear.

## Therapy and Prognosis

As a rare entity, a uniform approach to therapy for BPDCN has not been devised. Non-Hodgkin lymphoma (hyper-CVAD, CHOP, or CHOP-like), AML, and ALL-type regimens have been employed [46–49]. While the majority of patients are able to achieve complete remission (CR), nearly all relapse, regardless of therapy, with a median overall survival (OS) of approximately 12 months [5].

Pagano et al. retrospectively identified 43 patients with BPDCN [50]. Of those treated with induction therapy, 60% were treated with an AML regimen and 35% were treated with an ALL regimen. CR was obtained in 17 patients, and though ALL-directed therapies showed significantly better initial remission rates than those that received AML-directed treatment ($p = 0.02$), patients treated with ALL therapy were more likely to experience eventual relapse. Hypomethylating agents (e.g., 5-azacitidine), which are commonly used in the treatment of myeloid malignancies, have been used in the treatment of a few BPDCN patients; however, despite good initial responses, the patients showed dismal outcomes [51]. Hyper-CVAD has also been shown to have some efficacy with median OS of 18 months and median CR of 21 months in at least one study [49].

The role of allogeneic and autologous stem cell transplant is still not well understood but in some instances, prolonged survival can be seen in patients [49, 50]. In the study by Pagano et al., the median OS of 6 allogeneic hematopoietic stem cell transplant recipients was 23 months, compared to 7 months in the 35 patients who did not undergo transplant. In a separate study by Pemmaraju et al., allogeneic and autologous stem cell transplant (SCT) patients had an overall similar median OS (18 months), compared to non-SCT patients treated with hyper-CVAD, CHOP, and other therapies (23 months).

Given the expression of CD123 and CD56 in BPDCN, clinical trials with anti-CD123 therapy (SL-401) and anti-CD56 (lorvotuzamab) are underway. In a prospective study of SL-401 therapy, Frankel et al. found that 7/9 patients had objective response, with 3 patients alive and in remission (3, 7, and 20 months follow-up) [52]. Clinical trials using lorvotuzamab for BPDCN are ongoing [49].

## Conclusion

BPDCN is a rare, aggressive malignancy derived from immature plasmacytoid dendritic cells that shows a characteristic CD4+/CD56+/CD123+ immunophenotype. Even with aggressive therapy, including stem cell transplant, the prognosis is

dismal. Although there is significant morphologic, immunophenotypic, mutational overlap with AML, recent gene expression profiling studies have shown distinct differences between these two entities, possibly providing new avenues for developing targeted therapies. While there are notable differences, the spectrum of gene alterations discovered by sequencing methods suggests at least a partial overlap with AML and implies that therapeutic strategies targeting aberrant methylation, chromatin remodeling, and splicing machinery in AML should also be investigated in BPDCN. Early results with anti-CD123 therapy have shown promising results; however, additional investigation will be required to determine whether this provides improved recurrence-free or overall survival. Increased awareness of this entity and further investigation of pathogenic mechanisms using modern techniques will serve to better define and devise optimal treatment strategies for BPDCN.

## References

1. Adachi M, Maeda K, Takekawa M, Hinoda Y, Imai K, Sugiyama S, et al. High expression of CD56 (N-CAM) in a patient with cutaneous CD4-positive lymphoma. Am J Hematol. 1994;47(4):278–82.
2. Banchereau J, Pascual V. Type I interferon in systemic lupus erythematosus and other autoimmune diseases. Immunity. 2006;25(3):383–92.
3. Pilichowska ME, Pinkus JL, Pinkus GS. Histiocytic necrotizing lymphadenitis (Kikuchi-Fujimoto disease): lesional cells exhibit an immature dendritic cell phenotype. Am J Clin Pathol. 2009;131(2):174–82.
4. Rollins-Raval MA, Marafioti T, Swerdlow SH, Roth CG. The number and growth pattern of plasmacytoid dendritic cells vary in different types of reactive lymph nodes: an immunohistochemical study. Hum Pathol. 2013;44(6):1003–10.
5. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW. WHO classification of tumours of haematopoietic and lymphoid tissues. 4th ed. Lyon, France: IARC Press; 2008.
6. Facchetti F, Cigognetti M, Fisogni S, Rossi G, Lonardi S, Vermi W. Neoplasms derived from plasmacytoid dendritic cells. Mod Pathol. 2016;29(2):98–111.
7. Martín-Martín LLA, Vidriales B, Caballero MD, Rodrigues AS, Ferreira SI, Lima M, Almeida S, Valverde B, Martínez P, Ferrer A, Candeias J, Ruíz-Cabello F, Buadesa JM, Sempere A, Villamor N, Orfao A, Almeida J. Classification and clinical behavior of blastic plasmacytoid dendritic cell neoplasms according to their maturation-associated immunophenotypic profile. Oncotarget. 2015;6(22):19204–16.
8. Julia F, Dalle S, Duru G, Balme B, Vergier B, Ortonne N, et al. Blastic Plasmacytoid dendritic cell neoplasms: Clinico-immunohistochemical correlations in a series of 91 patients. Am J Surg Pathol. 2014;38(5):673–80.
9. Herling M, Jones D. CD4+/CD56+ hematodermic tumor: the features of an evolving entity and its relationship to dendritic cells. Am J Clin Pathol. 2007;127(5):687–700.
10. Reineks EZ, Osei ES, Rosenberg A, Auletta J, Meyerson HJ. CD22 expression on blastic plasmacytoid dendritic cell neoplasms and reactivity of anti-CD22 antibodies to peripheral blood dendritic cells. Cytometry B Clin Cytom. 2009;76((4):237–48.
11. Sangle NA, Schmidt RL, Patel JL, Jeffrey Medeiros L, Agarwal AM, Perkins SL, et al. Optimized immunohistochemical panel to differentiate myeloid sarcoma from blastic plasmacytoid dendritic cell neoplasm. Mod Pathol (An Official Journal of the United States and Canadian Academy of Pathology, Inc). 2014;27(8):1137–43.

12. Cronin DM, George TI, Reichard KK, Sundram UN. Immunophenotypic analysis of myeloperoxidase-negative leukemia cutis and blastic plasmacytoid dendritic cell neoplasm. Am J Clin Pathol. 2012;137(3):367–76.
13. Johnson RCKJ, Natkunam Y, Sundram U, Freud AG, Gammon B, Cascio MJ. Myeloid cell nuclear differentiation antigen (MNDA) expression distinguishes Extramedullary presentations of myeloid leukemia from Blastic Plasmacytoid dendritic cell neoplasm. Am J Surg Pathol. 2016;40(4):502–9.
14. Leroux DMF, Callanan M, Radford-Weiss I, Dastugue N, Feuillard J, Le Mée F, Plessis G, Talmant P, Gachard N, Uettwiller F, Pages MP, Mozziconacci MJ, Eclache V, Sibille C, Avet-Loiseau H, Lafage-Pochitaloff M. CD4(+), CD56(+) DC2 acute leukemia is characterized by recurrent clonal chromosomal changes affecting 6 major targets: a study of 21 cases by the Groupe Français de Cytogénétique Hématologique. Blood. 2002;99(11):4154–9.
15. Jardin F, Callanan M, Penther D, Ruminy P, Troussard X, Kerckaert JP, et al. Recurrent genomic aberrations combined with deletions of various tumour suppressor genes may deregulate the G1/S transition in CD4+CD56+ haematodermic neoplasms and contribute to the aggressiveness of the disease. Leukemia. 2009;23(4):698–707.
16. Wiesner T, Obenauf AC, Cota C, Fried I, Speicher MR, Cerroni L. Alterations of the cell-cycle inhibitors p27(KIP1) and p16(INK4a) are frequent in blastic plasmacytoid dendritic cell neoplasms. J Invest Dermatol. 2010;130(4):1152–7.
17. Nakamura Y, Kayano H, Kakegawa E, Miyazaki H, Nagai T, Uchida Y, et al. Identification of SUPT3H as a novel 8q24/MYC partner in blastic plasmacytoid dendritic cell neoplasm with t(6;8)(p21;q24) translocation. Blood cancer journal. 2015;5:e301.
18. Takiuchi YMH, Aoki K, Kato A, Ono Y, Nagano S, Arima H, Inoue D, Mori M, Tabata S, Yanagita S, Matsushita A, Nishio M, Imai Y, Imai Y, Ito K, Fujita H, Kadowaki N, Ishikawa T, Takahashi T. Leukemic manifestation of blastic plasmacytoid dendritic cell neoplasm lacking skin lesion : a borderline case between acute monocytic leukemia. J Clin Exp Hematop. 2012;52(2):107–11.
19. Momoi ATK, Kawai K, Tsuchiyama J, Suzuki N, Yano T, Uesugi Y, Takahashi M, Aizawa Y. Cutaneous lymphoblastic lymphoma of putative plasmacytoid dendritic cell-precursor origin: two cases. Leuk Res. 2002;26(7):693–8.
20. Sapienza MR, Fuligni F, Agostinelli C, Tripodo C, Righi S, Laginestra MA, et al. Molecular profiling of blastic plasmacytoid dendritic cell neoplasm reveals a unique pattern and suggests selective sensitivity to NF-kB pathway inhibition. Leukemia. 2014;28(8):1606–16.
21. Dijkman R, van Doorn R, Szuhai K, Willemze R, Vermeer MH, Tensen CP. Gene-expression profiling and array-based CGH classify CD4+CD56+ hematodermic neoplasm and cutaneous myelomonocytic leukemia as distinct disease entities. Blood. 2007;109(4):1720–7.
22. Menezes J, Acquadro F, Wiseman M, Gomez-Lopez G, Salgado RN, Talavera-Casanas JG, et al. Exome sequencing reveals novel and recurrent mutations with clinical impact in blastic plasmacytoid dendritic cell neoplasm. Leukemia. 2014;28(4):823–9.
23. Stenzinger AEV, Pfarr N, Andrulis M, Jöhrens K, Klauschen F, Siebolts U, Wolf T, Koch PS, Schulz M, Hartschuh W, Goerdt S, Lennerz JK, Wickenhauser C, Klapper W, Anagnostopoulos I, Weichert W. Targeted ultra-deep sequencing reveals recurrent and mutually exclusive mutations of cancer genes in blastic plasmacytoid dendritic cell neoplasm. Oncotarget. 2014;5(15):6404–13.
24. Lucioni M, Novara F, Fiandrino G, Riboni R, Fanoni D, Arra M, et al. Twenty-one cases of blastic plasmacytoid dendritic cell neoplasm: focus on biallelic locus 9p21.3 deletion. Blood. 2011;118(17):4591–4.
25. Georgopoulos K, Winandy S, Avitahl N. The role of the Ikaros gene in lymphocyte development and homeostasis. Annu Rev Immunol. 1997;15:155–76.
26. Jardin FRP, Parmentier F, Troussard X, Vaida I, Stamatoullas A, Leprêtre S, Penther D, Duval AB, Picquenot JM, Courville P, Capiod JC, Tilly H, Bastard C, Marolleau JP. TET2 and TP53 mutations are frequently observed in blastic plasmacytoid dendritic cell neoplasm. Br J Haematol. 2011;153(3):413–6.

27. Gao NA, Wang XX, Sun JR, WZ Y, Guo NJ. Blastic plasmacytoid dendritic cell neoplasm with leukemic manifestation and ETV6 gene rearrangement: a case report. Exp Ther Med. 2015;9(4):1109–12.
28. Wang LCSW, Fujiwara Y, Davidson L, Visvader J, Kuo F, Alt FW, Gilliland DG, Golub TR, Orkin SH. The TEL/ETV6 gene is required specifically for hematopoiesis in the bone marrow. Genes Dev. 1998;12(15):2392–402.
29. Alayed K, Patel KP, Konoplev S, Singh RR, Routbort MJ, Reddy N, et al. TET2 mutations, myelodysplastic features, and a distinct immunoprofile characterize blastic plasmacytoid dendritic cell neoplasm in the bone marrow. Am J Hematol. 2013;88(12):1055–61.
30. Watowich SS, Liu YJ. Mechanisms regulating dendritic cell specification and development. Immunol Rev. 2010;238(1):76–92.
31. Lyman SD, Jacobsen SE. C-kit ligand and Flt3 ligand: stem/progenitor cell factors with overlapping yet distinct activities. Blood. 1998;91(4):1101–34.
32. Adolfsson J, Borge OJ, Bryder D, Theilgaard-Mönch K, Astrand-Grundström I, Sitnicka E, et al. Upregulation of Flt3 expression within the bone marrow Lin(−)Sca1(+)c-kit(+) stem cell compartment is accompanied by loss of self-renewal capacity. Immunity. 2001;15(4):659–69.
33. Maraskovsky E, Daro E, Roux E, Teepe M, Maliszewski CR, Hoek J, et al. Vivo generation of human dendritic cell subsets by Flt3 ligand. Blood. 2000;96(3):878–84.
34. Berman E, Maloy M, Devlin S, Jhanwar S, Papadopoulos E, Jakubowski A. Stem cell transplantation in adults with acute myelogenous leukemia, normal cytogenetics, and the FLT3-ITD mutation. Leuk Res. 2016;40:33–7.
35. Wander S, Levis M, Fathi A. The evolving role of FLT3 inhibitors in acute myeloid leukemia: quizartinib and beyond. Ther Adv Hematol. 2014;5(3):65–77.
36. Abdel-Wahab O, Dey A. The ASXL-BAP1 axis: new factors in myelopoiesis, cancer and epigenetics. Leukemia. 2013;27(1):10–5.
37. Daou S, Hammond-Martel I, Mashtalir N, Barbour H, Gagnon J, Iannantuono NV, et al. The BAP1/ASXL2 histone H2A Deubiquitinase complex regulates cell proliferation and is disrupted in cancer. J Biol Chem. 2015;290(48):28643–63.
38. LaFave LM, Béguelin W, Koche R, Teater M, Spitzer B, Chramiec A, et al. Loss of BAP1 function leads to EZH2-dependent transformation. Nat Med. 2015;21(11):1344–9.
39. Micol JB, Abdel-Wahab O. The role of additional sex combs-like proteins in cancer. Cold Spring Harb Perspect Med. 2016;6:a026526.
40. Sahtoe DD, van Dijk WJ, Ekkebus R, Ovaa H, Sixma TK. BAP1/ASXL1 recruitment and activation for H2A deubiquitination. Nat Commun. 2016;7:10292.
41. Fu Y, Fesler M, Mahmud G, Bernreuter K, Jia D, Batanian JR. Narrowing down the common deleted region of 5q to 6.0 Mb in blastic plasmacytoid dendritic cell neoplasms. Cancer Genet. 2013;206(7–8):293–8.
42. Li H, Balajee AS, Su T, Cen B, Hei TK, Weinstein IB. The HINT1 tumor suppressor regulates both gamma-H2AX and ATM in response to DNA damage. J Cell Biol. 2008;183(2):253–65.
43. Paronetto MP, Miñana B, Valcárcel J. The Ewing sarcoma protein regulates DNA damage-induced alternative splicing. Mol Cell. 2011;43(3):353–68.
44. Cao QLF, Niu G, Xue L, Han A. Blastic plasmacytoid dendritic cell neoplasm with EWSR1 gene rearrangement. J Clin Pathol. 2014;67(1):90–2.
45. Oelschlaegel U, Mohr B, Schaich M, Schakel U, Kroschinsky F, Illmer T, et al. HLA-DRneg patients without acute promyelocytic leukemia show distinct immunophenotypic, genetic, molecular, and cytomorphologic characteristics compared to acute promyelocytic leukemia. Cytometry B Clin Cytom. 2009;76((5):321–7.
46. Feuillard J, Jacob MC, Valensi F, Maynadie M, Gressin R, Chaperot L, et al. Clinical and biologic features of CD4(+)CD56(+) malignancies. Blood. 2002;99(5):1556–63.
47. Gruson B, Vaida I, Merlusca L, Charbonnier A, Parcelier A, Damaj G, et al. L-asparaginase with methotrexate and dexamethasone is an effective treatment combination in blastic plasmacytoid dendritic cell neoplasm. Br J Haematol. 2013;163(4):543–5.
48. Gilis L, Lebras L, Bouafia-Sauvy F, Espinouse D, Felman P, Berger F, et al. Sequential combination of high dose methotrexate and L-asparaginase followed by allogeneic transplant: a first-line strategy for CD4+/CD56+ hematodermic neoplasm. Leuk Lymphoma. 2012;53(8):1633–7.

49. Pemmaraju N, Kantarjian HM, Cortes JE, Duvic M, Khoury JD, Patel K, Daver N, O'Brien S, Pierce S, Garcia-Manero G, Jabbour E, Jain N, Faderl S, Thomas D, Frankel AE, Qazilbash MH, Konopleva M. Blastic Plasmacytoid dendritic cell neoplasm (BPDCN): a large single-center experience: analysis of clinical and molecular characteristics and patient outcomes. Blood. 2015;126(23):3746.
50. Pagano L, Valentini CG, Pulsoni A, Fisogni S, Carluccio P, Mannelli F, et al. Blastic plasmacytoid dendritic cell neoplasm with leukemic presentation: an Italian multicenter study. Haematologica. 2013;98(2):239–46.
51. Laribi K, Denizon N, Ghnaya H, Atlassi M, Besancon A, Pineau-Vincent F, et al. Blastic plasmacytoid dendritic cell neoplasm: the first report of two cases treated by 5-azacytidine. Eur J Haematol. 2014;93(1):81–5.
52. Frankel AE, Woo JH, Ahn C, Pemmaraju N, Medeiros BC, Carraway HE, et al. Activity of SL-401, a targeted therapy directed to interleukin-3 receptor, in blastic plasmacytoid dendritic cell neoplasm patients. Blood. 2014;124(3):385–92.
53. Herling M, Teitell MA, Shen RR, Medeiros LJ, Jones D. TCL1 expression in plasmacytoid dendritic cells (DC2s) and the related CD4+ CD56+ blastic tumors of skin. Blood. 2003;101(12):5007–9.
54. Marafioti TPJ, Ballabio E, Reichard KK, Tedoldi S, Hollowood K, Dictor M, Hansmann ML, Pileri SA, Dyer MJ, Sozzani S, Dikic I, Shaw AS, Petrella T, Stein H, Isaacson PG, Facchetti F, Mason DY. Novel markers of normal and neoplastic human plasmacytoid dendritic cells. Blood. 2008;111(7):3778–92.
55. Boiocchi L, Lonardi S, Vermi W, Fisogni S, Facchetti F. BDCA-2 (CD303): a highly specific marker for normal and neoplastic plasmacytoid dendritic cells. Blood. 2013;122(2):296–7.

# Chapter 19
# Existing and Emerging Molecular Technologies in Myeloid Neoplasms

**Eric Q. Konnick and David Wu**

## The Era of Genomic Medicine

Genomic medicine has had a long and rich history. In the 1970s, the discovery of restriction endonucleases first allowed scientists to cleave DNA in a reproducible manner, allowing for the probing of specific alterations of DNA sequence at these sites [1]. As additional restriction endonucleases were discovered, simultaneous interrogation of multiple nucleotides at specific genomic positions soon became possible leading to key technological advances such as DNA cloning, DNA sequencing, and in situ hybridization.

The discovery of recurrent translocations in many neoplasms offered the opportunity for identification of these genetic structural rearrangements by molecular methods. Advances in cytogenetic staining made karyotype the initial test of choice for structural genomic alterations, but that method was highly specialized, laborious, and required viable neoplastic cells. Subsequent development of molecular cloning allowed for the introduction of in situ hybridization methods, first with radioactive elements [2] and later with fluorescence labels [3], which then allowed for rapid and specific identification of recurrent chromosomal alterations. Around this time, techniques to determine the sequence of DNA bases were developed by Frederick Sanger and colleagues using radiolabeled nucleotides [4–6]. Although the first use of these techniques was similarly laborious and time consuming, these approaches were critical in establishing foundational knowledge about the sequence and structure of genes. Other advances followed in rapid succession. Polymerase chain reaction (PCR) was invented in the mid-1980s by Kary B. Mullis [7] and was so revolutionary that clinical applications of this technology were adopted almost immediately thereafter [8]. The subsequent discovery and

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application of thermostable enzymes in PCR were further transformational [9, 10] and allowed for novel automation solutions that propelled the field forward, as the combination of these different techniques was powerful. For example, PCR allowed for the rapid amplification of specific nucleotide sequences and when combined with restriction enzyme technology offered a quick and simple way to identify point mutations in neoplastic tissue through the characterization of restriction fragment length polymorphisms (RFLP) [11]. During the 1980s and 1990s, our understanding of the role of genes in disease transformed with improvements in PCR technology, Sanger sequencing methods, and enhanced computing power. During this period, numerous genes were identified and associated with specific neoplastic conditions [12, 13]. These technological advancements led to rapid implementation of PCR-based assays in clinical laboratories for diagnosing diseases with well-known genetic predisposition [14] and infectious diseases associated with cancers [15, 16], and for characterizing alterations of specific neoplasms [17, 18]. PCR-based assays were also described as a possible method of residual disease detection when the genetic abnormalities were characteristic for a given disease [19]. By the end of the twentieth century, a massive expansion of robotic technology and DNA synthesis techniques allowed for the development of DNA microarrays in which the genome could be probed using hundreds of thousands of probes to identify copy number changes, single nucleotide polymorphisms, and quantification of RNA transcripts. At the same time, advancements in fluidics, digital imaging, and computational power allowed for the subsequent development of methods that allowed for genome-wide sequencing of millions of short sequences in a massively parallel manner.

These scientific and technologic advancements in laboratory techniques allowed physicians and scientists to apply insights from molecular biology toward a detailed understanding of hematological malignances due to the relative ease of obtaining viable neoplastic cells and working with such cells under a variety of conditions. As such, the application of the state-of-the-art techniques allowed for hematological disease classification to always reflect the best clinical, scientific and analytical data available. Thus, whereas the French–American–British (FAB) Cooperative Group proposed a system for classifying acute myeloid leukemia (AML) in 1976 using morphologic and cytochemical criteria to characterize the presumed stage of differentiation of myeloid neoplasms, as scientific insight and laboratory techniques improved, disease classification was improved when it was recognized that specific genetic lesions were associated with some entities. Additional knowledge obtained using advanced techniques revealed the importance of specific genetic alterations in not only diagnosis but also prognosis and prediction of therapeutic efficacy. With new data informing the field, the World Health Organization (WHO) developed a new hematologic disease classification approach that included genetic, immunophenotypic, biologic, and clinical features along with morphologic features. This comprehensive approach defined specific entities with diagnostic, prognostic, or therapeutic significance. Since the original WHO classification in 2001 [20], there have been two major revisions due to an evolving understanding of hematologic disease, with the first occurring in 2008 [21], and more recently in 2016 [22].

In this chapter, we attempt to describe some of the key techniques and laboratory approaches that were important in the development of our modern classification of myeloid neoplasia. As we move forward, new laboratory techniques are likely to further refine our understanding of the pathogenesis of neoplastic myeloid diseases, impact our approach for disease classification of myeloid neoplasia, and hopefully offer insight into new treatment opportunities for patients.

## Current Laboratory Methods in Common Use in Evaluation of Myeloid Neoplasia

### *Karyotype*

Nobel Prize winning work in the early twentieth century demonstrated that the exchange of genes was related to the exchange of chromosomal material [23, 24]. This work formed the basis for additional studies and experimentation that eventually led to the formalization of cytogenetics and karyotype analysis as the first, "whole-genome" diagnostic tool several decades later. Since that time, cytogenetic techniques and our understanding of its importance in neoplasia have improved, and currently, karyotype analysis is a standard part of the clinical evaluation of many myeloid neoplasms. Although high-resolution, 2000-band karyotypes are available that can identify abnormalities at 1–2 megabases (Mb) resolution, current clinical practice in many institutions is limited to the routine use of a 300-to-500-band karyotype, which is capable of resolution of 7–10 Mb alterations [25]. This resolution nevertheless allows for discernment of large structural changes to be readily identified. Many myeloid neoplasms demonstrate recurrent cytogenetic abnormalities, but a significant proportion, such as acute myeloid leukemia (AML), do not have significant cytogenetic abnormalities [26]. Although a subset of myeloid neoplasms do not have detectable aberrations by karyotype, this method nevertheless provides valuable diagnostic and prognostic information and is commonly employed clinically.

Karyotyping consists of several steps including the growth of cells in culture, arrest of cells in metaphase, treatment of cells with a hypotonic solution, fixation of cells, dropping the cells across a glass slide to disperse the fixed cells, staining of the genetic material, imaging of the stained materials, assembling of a karyogram, and interpretation. Multiple different properties of chromosomes can be described using current karyotyping techniques, including alterations in the absolute number, size, centromeric position, structure, and banding patterns of chromosomes (Fig. 19.1). From these characteristics, cytogeneticists can assess for aneuploidy, structural changes, and presence of unknown genetic material in satellite chromosomes. Abnormal karyotypes in the setting of myeloid neoplasms have been extensively described. As the technique became commonly used in the clinical evaluation of myeloid neoplasms, key observations were made that certain changes were recurrent

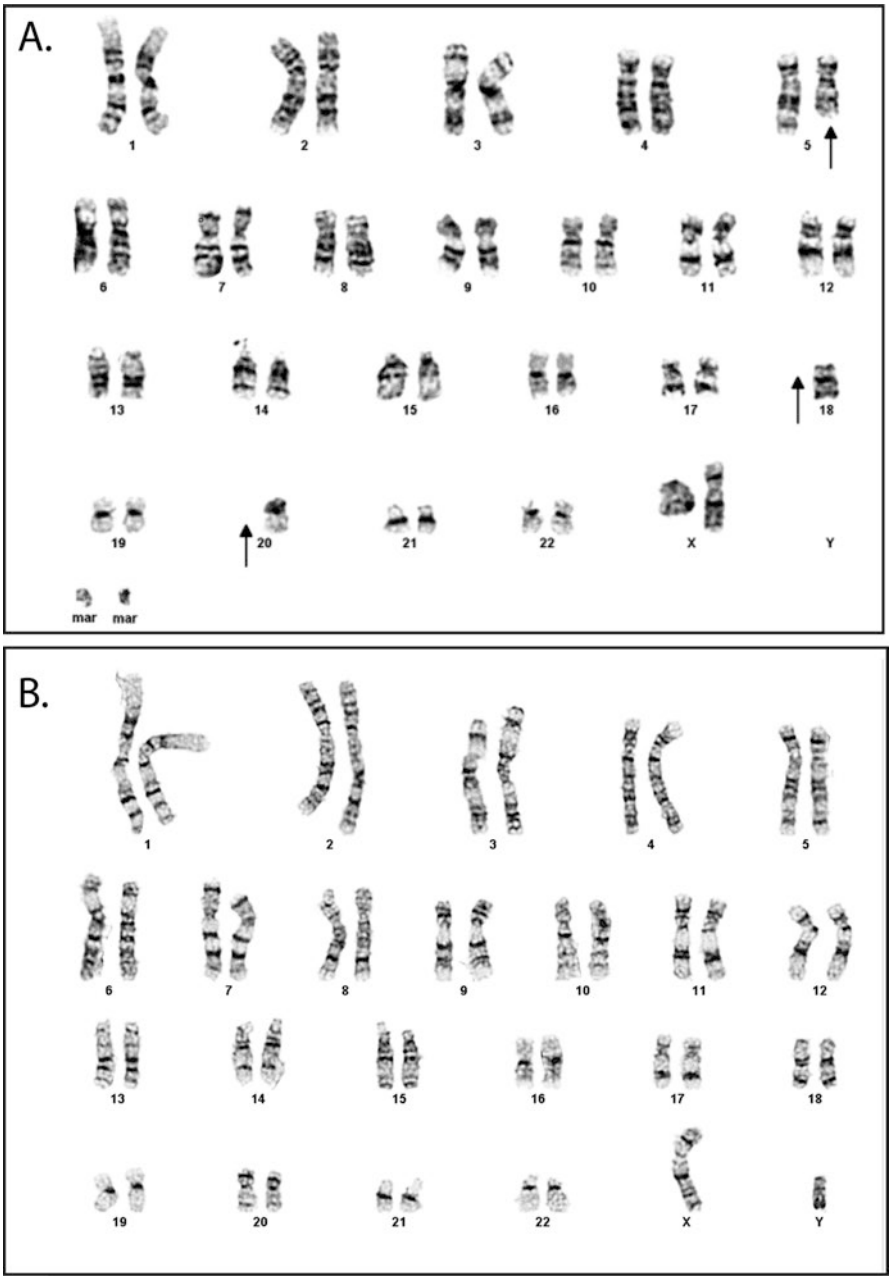


**Fig. 19.1** Examples of karyograms representing karyotypes at 300–500 band resolution (resolution of 7–10 mB). (**a**) Abnormal female karyogram from a bone marrow aspirate of a patient with myelodysplastic syndrome. The complex karyotype demonstrates multiple abnormalities (*arrows*), including loss of 5q, monosomy 18, and monosomy 20 in addition to two marker chromosomes of undetermined origin (mar). (**b**) Normal karyogram from a peripheral blood sample from a healthy male

and seemed to be associated with specific neoplastic features [27–29]. Additionally, it became apparent that if serial monitoring were performed clinically in patients during disease progression, there may be concurrent evolution of the karyotype as well [30]. Advantages of karyotypes include the ability to discern whole-genome duplication events, identification of large-scale chromosomal abnormalities, and assessment of changes in the karyotype over time. Currently, the processes for karyotyping have become well established and are generally available in commercial reference laboratories and in most academic medical centers. The karyotype relies on living cells to culture, and yield of viable cells may be limited by chemotherapy exposure or sampling. The presence of subtle genetic rearrangements may not be readily apparent on common preparations, and the sensitivity of the method to subclonal populations is poor. Important genetic changes such as substitution mutations or loss of heterozygosity cannot be resolved using karyotype analysis. Although karyotype has been described as a method for monitoring minimal residual disease, more sensitive methods are generally preferable, when possible [31]. Frequent abnormalities in karyotype are noted in several types of myeloid neoplasia [20, 21], and as such, karyotypic aberrations will likely continue to be considered in description and classification of myeloid neoplasms for many years to come.

## *Fluorescent In Situ Hybridization*

The cloning and restriction enzyme techniques that were developed in the 1970s allowed for a new set of tools that enabled detailed identification of structural and copy number changes in patient material. The first methods describing florescence in situ hybridization (FISH) were published in the early 1980s [32] and rapidly developed into a robust technique with numerous permutations. The technique is conceptually simple, consisting of hybridizing fluorescently labeled oligonucleotide probes complimentary to a sequence of interest to target-specific loci over the course of hours, washing away unbound probes, imaging the slide, and characterizing the binding pattern apparent in an appropriate number of cells. FISH can be used to enumerate specific types of genetic changes and rearrangements that occur in the setting of neoplasia. Assessment of copy gains or losses of whole chromosomes can be inferred using probes targeting the centromere of the chromosome of interest, while using a centromere probe combined with a probe for a gene of interest will allow the operator to determine if a specific gene is gained or lost or if such an event is due to a chromosome-level event. One of the most powerful permutations has been the assessment of chromosomal translocations or other structural rearrangements that are recurrent in neoplasia. Some of the commonly used methods employ fusion probes or break-apart probes. Fusion probes utilize two differently fluorescently-labeled probes that target genes involved in a translocation or other structural rearrangement. When the probes are spatially separated, the probes individually fluoresce at different wavelengths allowing the operator to discern separate signals for each probe. However, when the probes are in close proximity due

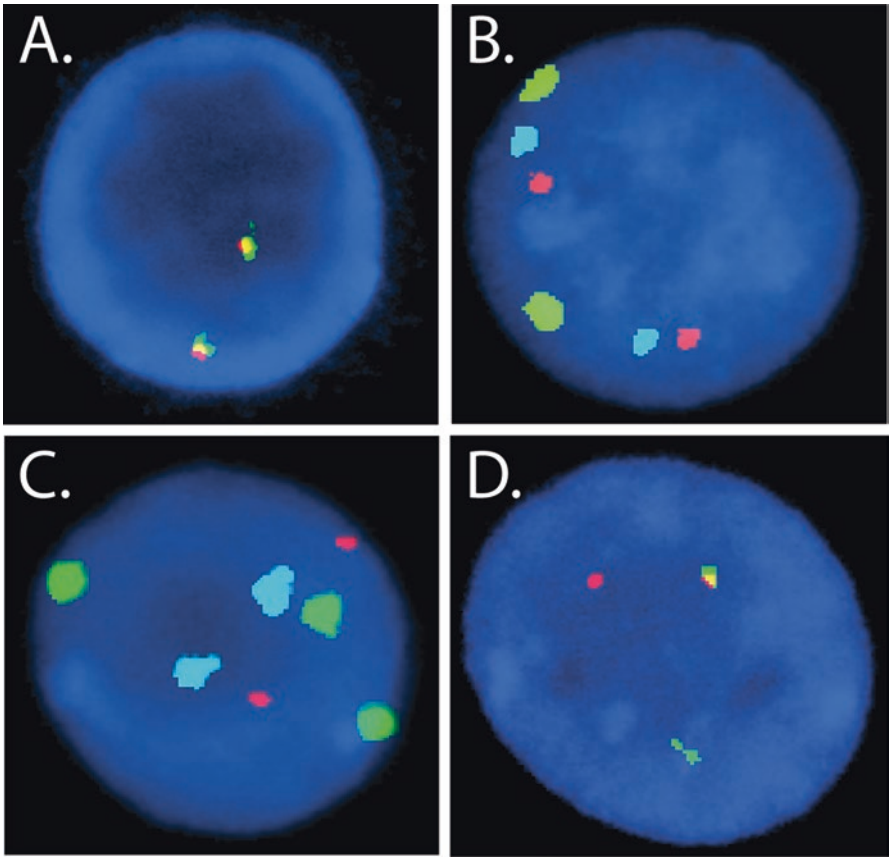


**Fig. 19.2** Examples of FISH studies from clinical samples demonstrating different types of FISH design. (**a**) Break-apart probe assay with intact probes. (**b**) No abnormalities – FISH assay using three probes: chromosome 12 centromere (*green* – two signals), 13p (*aqua* – two signals), 13q (*orange* – two signals). (**c**) Trisomy 12 – FISH assay using three probes: chromosome 12 centromere (*green* – three signals), 13p (*aqua* – two signals), 13q (*orange* – two signals). (**d**) Break-apart probe with single *red* and *green* signals representing the separated break-apart probes and a single *yellow* signal representing the intact locus (Images courtesy of Debra Dehoogh-Grigsby, University of Washington Cytogenetics Laboratory)

to a translocation event in which two different genomic loci are fused together, the signals can no longer be separately distinguished and the operator only can identify a composite signal of a color that is distinct from the individual fluorophores. In contrast, break-apart probes use a similar concept but consist of two probes that are on either side of a common translocation breakpoint of a single gene that is involved in the setting of structural rearrangements. In this design, when a given gene is intact, only a single, composite signal per chromosome is observed. When a gene is rearranged across a breakpoint that is between the two probes, the signals separate and either two separate-color signals or a single-color signal will be observed per disrupted chromosome (Fig. 19.2).

Fusion and break-apart probe designs have an inherent specificity built into their design, in which it is unlikely that a false-positive result will occur. When testing clinical formalin-fixed paraffin-embedded (FFPE) samples, it is possible that a subset of the evaluated nuclei will have been cut in such a way that the genomic material being probed will be present on separate planes and, as such, there will generally be a background level of artifactual "abnormal" signals that needs to be considered when the assay is evaluated. Fusion probes are a good design to use when rearrangements are observed between the same two partners in the setting of interest [33], but if a rearrangement occurs between one of the genes and an unknown partner, there is a possibility of a false negative result. One advantage of the break-apart FISH design is that it is agnostic to the partner of the interrogated gene, which is beneficial in diseases in which there are multiple possible rearrangement partners or when the main concern is identification of the presence of gene disruption and not the specific translocation partners. The disadvantage of such a design is that the knowledge of the second gene involved in the rearrangement is not known.

In general, FISH has several advantages, thereby making it useful for assessing large-scale genomic events in neoplasia. The detected signals can be very specific, the testing can be relatively inexpensive, rapid, and the assay can be quickly done on fresh, cultured, or FFPE samples. FISH studies on FFPE samples can often be accomplished in a much more rapid fashion than alternative techniques such as reverse-transcription PCR (RT-PCR) or next-generation sequencing (NGS). A key shortcoming of FISH, however, is that only a few targets can be evaluated per test, requiring selection of the appropriate probes before the test is performed. While such selection is often acceptable, rare cases where incorrect testing is performed can result in false-negative results that have important clinical implications. In cases where multiple targets need to be assessed for diagnostic, predictive, or prognostic uses, panels of simultaneous assays are often employed, and with each additional assay performed, the cost of testing increases linearly as does the labor of evaluating hundreds of cells per study for interpreting the results. In the setting of rearrangements, there are examples of cryptic rearrangements that may not be readily detected by standard FISH methods [34]. Finally, FISH can only identify large-scale genomic changes such as copy number alterations and structural rearrangements, and the technique will not detect small-scale mutations such as single-nucleotide variants and small insertions and deletions (indels). However, with proper understanding of the advantages and limitations of FISH and specific permutations, this technique is a rapid, sensitive, specific, and valuable method for assessing important and recurrent genomic events in the setting of neoplasia, particularly in the clinical laboratory.

## *Sanger Sequencing*

The initial description of sequencing by Frederick Sanger and colleagues used the concept of chain terminating nucleotides labeled with radioactive tracer molecules [4–6]. Separate reactions were used in which each reaction incorporated only a

single terminator nucleotide with a larger proportion of unlabeled nucleotides. After the sequencing reactions were completed, the four reactions were separated in separate lanes on an electrophoretic gel and visualized by autoradiography. As labeled terminator nucleotides were incorporated into a growing DNA strand, the strand would not be able to extend further and the base at that position could be inferred based on the position on the gel. This technology allowed the determination of each subsequent base in a sequence by incorporating nucleotides, specifically dideoxynucleotides that prevented further elongation of the DNA molecule and visualizing on an autoradiographic polyacrylamide gel after size separation.

With the introduction of PCR, Sanger sequencing became technically easier, but it was the introduction of fluorescently labeled nucleotide terminators and automation that greatly expanded the use of this technology, particularly in the clinical environment (Fig. 19.3). By utilizing separate fluorophores for each of the terminator nucleotides, a single reaction for the forward and reverse PCR product decreased the number of reactions necessary to obtain sequence data. Automated instruments,

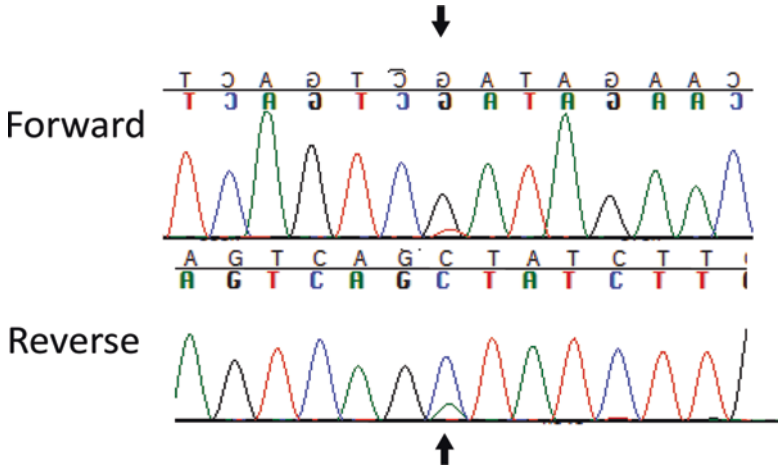


**Fig. 19.3** Screen capture of Sanger sequencing trace utilizing fluorescently labeled dideoxynucleotide terminators. In this method, a single reaction tube is required for the forward and reverse sequencing reaction for each sequenced region. Reactions include dideoxynucleotide terminators, each base labeled with fluorophores that emit at different wavelengths. After the sequencing reaction, the sequencing products are denatured and loaded onto an automated instrument which separates the DNA fragments based on size through the application of an electric field applied to a polyacrylamide gel or a sequencing capillary. Smaller-size DNA molecules move more quickly through the matrix and larger products more slowly. A laser or other excitation source and fluorescent detector are positioned at a fixed point along the electrophoresis apparatus. As DNA fragments pass by the detector window, the laser excites the florescent dye, which then returns to its resting state and emits a photon at a longer wavelength. This emission is captured through the detection apparatus and software determines the incorporated bases. Sequence data are represented by an electropherogram, which shows the fluorescence peaks of the labeled dideoxynucleotide terminators. Determination of the base pair composition can be accomplished with software algorithms, which allows for increased throughput. In this example, a G > T nucleotide substitution is identified (*arrows*) in approximately 15–20% of sequencing reads at the indicated position

initially as polyacrylamide slab-gel machines, and later capillary sequencers, allowed for the automation of electrophoresis and data acquisition [35, 36]. Developments during this time resulted in the ability to obtain DNA sequences of 500–1000 base pairs in a rapid and automated fashion.

With the rapid technological developments in the practice of Sanger sequencing, the technology had matured to the point that large-scale sequencing could be considered, and from 1990 to 2003 the Human Genome Project was undertaken to sequence the majority of several reference genomes obtained from volunteers [37]. The demands of this project resulted in numerous innovations in robotics, automation, and informatics that were rapidly adopted beyond the initial research initiative [38, 39]. In the late 1990s, the government-run effort was challenged by a private consortium using a novel approach of so-called, shotgun sequencing, which sheared DNA into random fragments and then used techniques to attach primers and sequence the intervening DNA [40, 41]. This technique relied heavily on the ability of computers to reassemble the DNA sequence by identifying regions that overlapped with one another. This situation led to a competition between the groups, which evolved into a collaborative effort resulting in the release of the first draft of the human genome in 2001 [42, 43].

Sanger sequencing is widely distributed and many laboratories are capable of generating high-quality sequence for research or clinical use with a high likelihood of success. This technique has some limitations, primarily that the technique can generally detect only minor sequence populations that are greater than 20% of the mixture, although recent developments suggest that more sensitive approaches may be available. While the output of these techniques has improved dramatically over the decades, the technology has limited throughput, often requiring dozens of reactions to fully analyze the exons of a single gene. This limitation makes large-scale analysis of genes difficult. Finally, while the informatics tools have improved dramatically, the commonly used methods still require a large amount of manual effort to review sequence data.

## *Polymerase Chain Reaction*

Polymerase chain reaction (PCR) was invented by Kary Mullis in the mid-1980s (Fig. 19.4) [7] and clinical uses of this powerful technique were implemented almost immediately [8]. PCR is accomplished through the combination of extracted sample DNA with oligonucleotide primers flanking the sequence of interest, deoxynucleotide triphosphates (dNTPs), a polymerase enzyme, buffers to allow enzyme function, and cations required for polymerase function. The reaction mixture is heated to denature the double-stranded DNA (denaturation) and then cooled to a temperature that allows binding of the primer oligonucleotides (primers) to the template strands. Because primers are much shorter than the template DNA, reaction kinetics favor the binding of these primers to the template molecules. In addition, primers are added in molar excess, which further favors the binding of primers to the

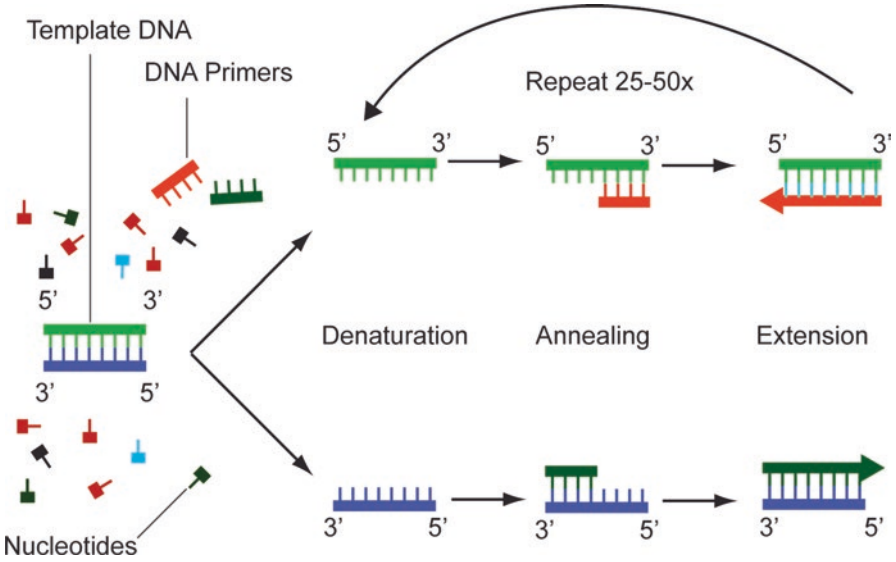


**Fig. 19.4** Schematic showing the key steps of the polymerase chain reaction. Template DNA is incubated with synthetic oligonucleotide primers specific for the sequence of interest, with dNTPs, and a thermostable *Thermus aquaticus (taq)* polymerase. Template DNA is denatured using high temperature and the reaction mixture is cooled to allow annealing of oligonucleotide primers. Primers are extended by *taq* polymerase. The process is repeated, with the amount of template DNA effectively doubling with each reaction cycle

template DNA molecules. A key to proper PCR assay design is the incorporation of one primer on each strand of the target sequence. After annealing, the reaction temperature is raised to the optimum temperature for the polymerase enzyme, which then incorporates deoxynucleotide triphosphates (dNTPs) into a complimentary DNA molecule. The process is then repeated for multiple cycles, with a theoretical doubling of PCR product with each cycle. As the cycles progress, DNA molecules created in the PCR process (amplicons) become the predominant species, with the size of the amplicon being determined by the positioning of PCR primers. The cycling process is repeated 25–50 times, creating billions of copies of the target sequence, which can be detected or further analyzed by additional techniques.

Initial implementations utilized polymerase enzymes that were not heat stable, requiring addition of fresh polymerase enzyme after each denaturation step, requiring large amounts of enzyme and constant attention by operators. However, the introduction of thermostable polymerases [9, 10] allowed for automation and rapid dissemination of the technique. PCR allowed for the rapid amplification of specific nucleotide sequences and, when combined with restriction enzyme technology (RFLP), offered a relatively quick and simple way to identify point mutations in neoplastic tissue [11]. During the 1980s and 1990s, PCR-based techniques had broad applications in the setting of myeloid neoplasia testing and they were widely used. Numerous adaptations of the core PCR method have been made to facilitate

increased flexibility, automation, and specificity. Some examples of these methods include the use of fluorescently labeled, sequence-specific probes that allow for identification of specific mutations or fluorescently labeled primers that allow for accurate sizing of PCR products that are amenable to analysis on capillary sequencers [44]. A key advantage of PCR-based techniques is the exquisite sensitivity of the method, which enables detection of rare transcripts with excellent specificity in the appropriate context, making such methods excellent approaches for minimal residual disease monitoring [45]. A weakness of PCR, however, is that it is best suited to situations in which targets have a limited spectrum of possible mutation sequences and in which primer-binding sequences are likely to be constant across all targets, neither of which is guaranteed in the setting of neoplasia.

### *Reverse-Transcription Polymerase Chain Reaction*

Reverse-transcription PCR methods have been developed to interrogate RNA molecules, first by using a reverse transcriptase enzyme to convert RNA to DNA and then by using conventional PCR to amplify the target of interest. This general approach has been extensively adapted, similar to PCR, to allow for numerous variations and automation to improve accuracy and throughput. Common uses for these techniques include fluorescently labeled, sequence-specific probes that allow for monitoring and quantitation of RT-PCR products that allow for rapid reporting of results [46] and interrogation of fusion transcripts to aid in diagnosis or monitoring of minimal residual disease [47–51]. Indeed, RT-PCR serves as important clinical tool for the evaluation of many chromosomal aberrations in routine clinical practice.

### *Microarray Testing*

At the turn of the century, there was an explosion of technical innovation that allowed for extensive probing of the genome at a much higher resolution than was previously available using karyotype or FISH. The concept of a DNA microarray originated as part of dot–blot methods in which one or more nucleic acid probes, specific for known nucleotide sequences, were adhered to membranes and sample DNA was allowed to hybridize against these probes. If there was a sequence complementarity of the sample to the target, the sample nucleic acid would remain bound to the probe and thus the membrane [52]. Using a variety of detection methods, including first radioactive and subsequently nonradioactive methods, the sequence of interest in a given sample could be inferred. The development of large-scale methods for cloning and oligonucleotide syntheses, combined with advances in robotics, allowed for a technological shift from membranes dotted with probe sequences to increasingly dense arrays of sequence-specific oligonucleotides

arrayed on solid substrates [25, 53, 54]. Current iterations of this technology allow for hybridization of DNA, RNA, or more complicated substrates, such as protein–nucleic acid complexes, to the dense arrays that can then generate signals, indicating if a given probe region has increased or decreased target binding compared to a reference sample. Data acquisition and translation are accomplished through the use of automated imaging and computational analysis.

Initial iterations of microarray technologies used differentially labeled DNA from the test sample and a well-characterized reference sample to compare the differences in signal between the two samples that were interpreted as the relative copy number of a specific genomic region on the test sample. After labeling and normalization, the products are hybridized to a solid substrate, allowed to equilibrate, and then residual unbound material is removed prior to imaging. Imaging allows the assessment of the relative signals of the test sample and the control sample, and in the situation in which equal amounts of DNA are present from each sample, the signal is interpreted as the two samples having equivalent genomic material. By contrast, when there is either a copy gain or loss of genetic material in the sample relative to the reference, the ratio between signals is greater than or lower than 1, respectively (Fig. 19.5a). With refinement of the technology, arrays were developed with a higher density of probes, allowing assessment of increasing numbers genomic loci, such that comparative samples were no longer necessary. Further, with knowledge of the human genome provided by the Human Genome Project, increased probe density allowed microarray platforms to identify copy-number variation with greater resolution, enabling the routine identification of genomic sites of microdeletion and gains.

Current copy-number arrays (CNAs) are capable of detecting gains and losses as small as 1 kilobase and as large as up to megabases in size and were critical for identifying normal copy number alterations that occur in the genome [55]. Recent advances have allowed the inclusion of probes capable of resolving single nucleotide variants at specified positions, which allows the assessment of single-nucleotide

**Fig. 19.5** (continued) amplification. (*2*) The prepared sample is hybridized onto a microarray "chip," allowing the sample DNA to bind specific spots with a prelabeled and known DNA sequence. (*3*) A higher magnification view of the individual spots on the microarray chip, when DNA is bound it results in a signal of varying intensity depending on the amount of bound DNA. (*4*) A simplified diagram of how the specific probes may be arranged on a given microarray chip. In this schematic, the CNA probes are placed in the lower three rows, while the SNP probes are placed in the other rows. The corresponding signals are seen with differential amounts of DNA binding. (**b**) Example of data from a high-resolution genomic microarray assay with both CNA and SNP probes. This view allows for visualization of the entire genome from chromosome 1 on the left to the gender chromosomes on the far right. There are two rows of data, the top row being the data generated from the spots with copy number probes, and the lower row generated from data with the SNP probes. (**c**) Example of virtual karyotype generated from high-resolution genomic microarray assay with both CNA and SNP probes. In this example, copy gains are highlighted in blue and shown to the right of the respective chromosome (1q), copy losses are shown in red and shown to the left of the respective chromosome (17p, 18q), and regions of copy neutral loss of heterozygosity (cnLOH) are highlighted in orange on top of the chromosome (4q)

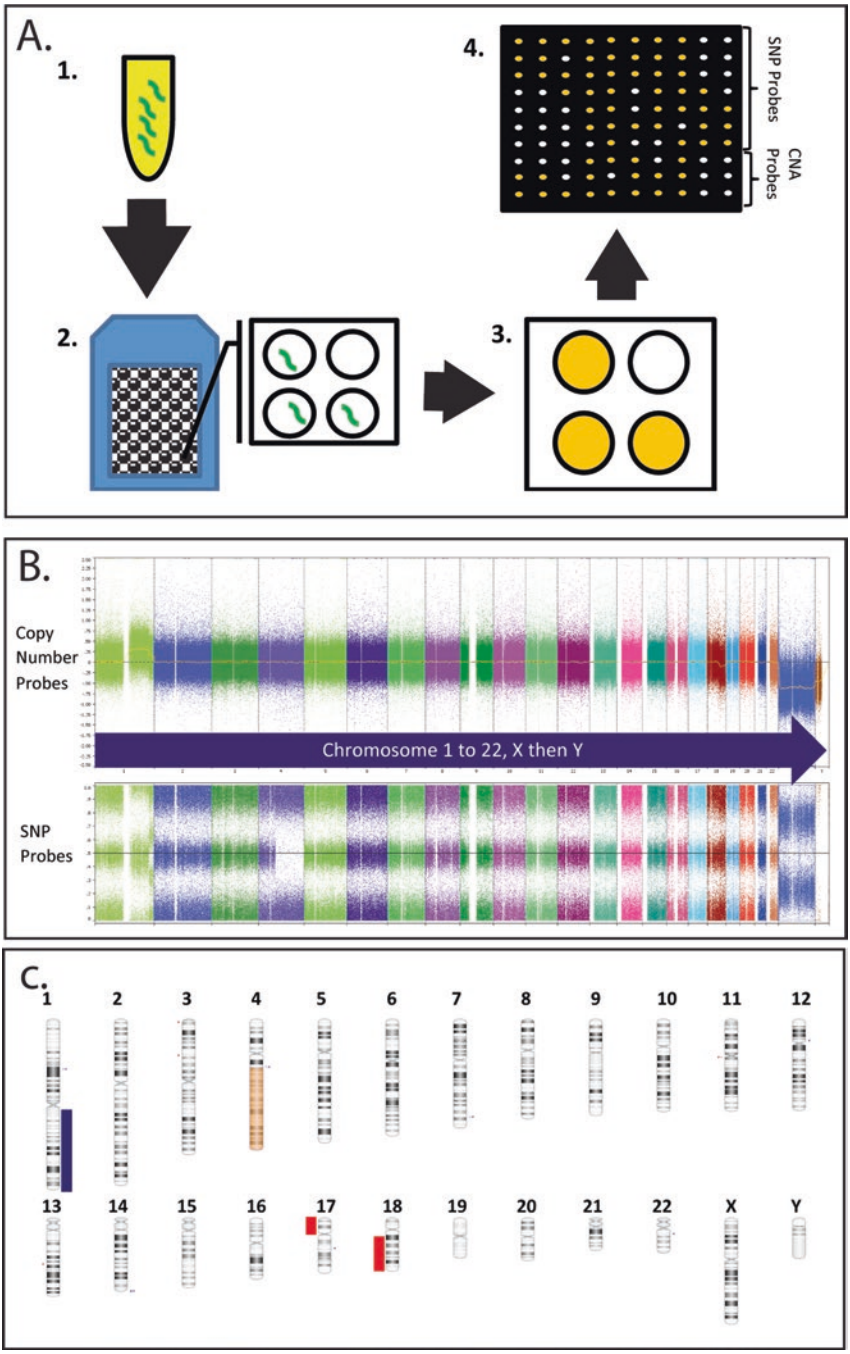


**Fig. 19.5** (**a**) A simplified schematic of newer generation high-resolution DNA microarray chips, many of which feature copy number alterations (CNA) and single nucleotide polymorphism (SNP) probes. (*1*) Sample DNA is processed through a number of preparative steps usually including

polymorphisms (SNPs) in samples. Such SNP arrays are useful in determining if copy neutral loss of heterozygosity (cnLOH) is present in samples, which is previously not detectable by other cytogenetic techniques. Copy-number and SNP arrays can be combined in a single platform to allow for assessment of copy number variants (CNVs) and LOH (Fig. 19.5b). Microarrays combining CNA and SNP probes are, therefore, able to provide high-resolution "virtual karyotype" with added cnLOH data that enable surveillance of the entire human genome for chromosomal aberrations (Fig. 19.5c). Various techniques and technologies have been applied to microarrays to allow the platform to interrogate different starting materials, such as DNA, messenger RNA (mRNA) expression, microRNA, protein–DNA complexes, and epigenetic modifications. DNA microarrays are highly customizable and custom arrays can be developed easily, allowing for customized, targeted platforms for research or clinical uses.

Microarrays are powerful tools that can be employed in the assessment of chromosomal losses and gains in the setting of myeloid neoplasia. The technique is capable of defining the chromosomal positions of gains and losses much more precisely than karyotype or FISH. Such information can leverage information on gene position to identify specific genes that are gained or lost in a given sample. SNP arrays are able to identify cnLOH in samples that appear karyotypically normal [56, 57], suggesting genes and genomic regions that may be important to disease pathogenesis. Automated instrumentation and software tools allow users to process the incredible amounts of data that can be generated by a microarray, but often the knowledge of specific regions that appear to have CNVs or LOH remains to be characterized. The technique is also not able to identify structural rearrangements that can commonly be identified using karyotype or FISH techniques, although some modifications may allow detection of balanced translocations [58]. The platform is sensitive to the proportion of sampled cells that possess the abnormal genomic complement, typically exceeding 20% of the sampled cells, limiting the use of the technique in evaluating subclonal populations or assessment of minimal-residual disease (MRD). While the technology is capable of identifying SNPs, it is not feasible to resolve all possible specific single-nucleotide mutations possible in a neoplastic genome. DNA microarrays, as currently available, complement other genomic techniques and offer a more granular interrogation of the genome than karyotype or FISH studies.

## *Massively Parallel Sequencing*

At the turn of the century, the first descriptions of massively parallel sequencing were published [59, 60]. Although these were different in approach and chemistry, their commonality was the ability to sequence millions of short DNA sequence reads, in parallel, without requiring a priori knowledge of the sequence. This technology has since been termed, massively parallel or "next-generation sequencing"

(NGS). These techniques rely on fragmentation of the DNA to be sequenced and ligation of common DNA sequences, which then allows amplification and enrichment of the sequences after a single DNA molecule isolation step, which are analogous to techniques used in shotgun sequencing approaches. Simultaneous sequencing of millions of small fragments of DNA is then accomplished in a parallel fashion, generating billions of bases of sequence data. The massive amount of short-read data require advanced computational approaches to assemble the data and align the sequences against the reference human genome, which was one product of the Human Genome Project. From the initial descriptions of the techniques in a research setting, the technology has evolved and become commercialized by multiple entities. NGS techniques have also proliferated, with numerous adaptations of the methods to allow for diverse applications, and with the maturation of the systems, there has been a rapid adoption of the techniques in research and clinical laboratories. As costs continue to decrease and analysis tools become more sophisticated, these techniques continue to find new uses and increasingly compete to displace some established techniques in clinical and research applications.

Of the published methods that have been employed in both research and clinical laboratories, there are several that have been widely used that warrant description. These approaches include sequencing by synthesis, pyrosequencing, sequencing by ligation, and semiconductor sequencing [61, 62]. Improvements in these techniques continue to occur and most certainly, new technologies will augment these approaches, such as demonstrated by recent advancements of third-generation technologies utilizing nanopore technologies. Each platform has strengths and limitations that must be considered. A brief description of these approaches is included below. However, this represents only a survey of the technologies and should not be considered a comprehensive review (Table 19.1).

In general, techniques that are widely used have the key common steps: isolation of genetic material, fragmentation of the genetic material into specific size ranges, ligation of sequence adapter molecules with known sequence (“barcodes”), enrichment for sequences of interest, massively parallel sequencing, bioinformatics pipeline data analysis, variant calling, and variant annotation. The use of bar-coded sequences, simply strings of nucleotides, allows for multiple samples to be sequenced together, with individual sequences attributed to specific samples through assessment of the barcode sequences through a demultiplex algorithm.

An innovation that allowed the use of NGS technologies was the implementation of bioinformatics pipelines that made the analysis of billions of bases of sequencing data a manageable task. These data analyses pipelines generally consist of multiple, separate, computer programs, which are linked together using additional programs such that sequence data can flow from one program to another or be analyzed for different features by different programs, either in a serial or parallel manner. After demultiplexing to separate and assign sequencing reads to respective samples through the use of sample-specific barcodes, individual sequences can be aligned against a reference genome that allows for the identification of variants

**Table 19.1.** Comparison of key features of commonly encountered sequencing platforms

| Method | Manufacturer | Read length (base pairs) | Accuracy, % | Time per run | Cost per 1 million bases (US$, approx.) | Advantages | Disadvantages |
|---|---|---|---|---|---|---|---|
| Sequencing by synthesis | Illumina/Solexa | 100–300 | 98 | Varies (1–10 d) | $0.07 | Potential for high sequence yield, depending upon sequencer model and desired application. Multiple scales of sequencing available. | Expensive equipment. Short read lengths. |
| Ion semiconductor | Ion Torrent | ≈400 | 98 | 2 h | $1 | Less expensive equipment. Fast. | Homopolymer errors. |
| Chain termination (Sanger) | Thermo Fisher (ABI) | Up to 1000 | 99.9 | 20 min −3 h | $2400 | Long individual reads. Useful for many applications. | High cost, low throughput. Low sensitivity to minor variant populations. |
| Nanopore | Oxford Nanopore | 10,000+ | 92–95 | Minutes to hours | Unknown | Compact instrumentation, long read lengths. | Lower accuracy, unknown cost, limited distribution. Frequent changes in hardware and chemistry. Requires high-quality DNA. |
| Single-molecule real-time sequencing | Pacific Biosciences | 10,000–15,000+ | 85 | 30 min – 4 h | $0.13–$0.60 | Longest read length. Fast. | Low accuracy. Moderate throughput. Expensive equipment. Requires high-quality DNA. |
| Pyrosequencing | 454/Roche | 700 | 99.9 | 24 h | $10 | Long read size. Fast. | Homopolymer errors, cost. Discontinued. |
| Sequencing by ligation | SOLiD | 50 + 35 or 50 + 50 | 99.9 | 1–2 week | $0.13 | Accuracy, low cost per base. | Short read assembly, difficulties with palindromes, slow. |

using bioinformatics programs referred to as variant callers (Fig. 19.6). In addition to programs that can identify single nucleotide changes from the reference sequence, additional computational approaches have been developed to detect insertion/deletions (indels), copy number variants (CNVs), and other structural alterations such as translocations [63]. Comparison studies have demonstrated that CNVs identified using some NGS assays show equivalency with results obtained using karyotype [64] or microarray [65], suggesting the possibility that NGS may be able to generate results similar to these well-established platforms.

Research groups and software developers are constantly producing new software packages and modifying existing programs to allow for improved performance and addition of new algorithms when possible. The cost of computational software, hardware, and the necessary technical expertise to implement such data analysis approaches is a critical consideration when developing and supporting NGS-based assays that should be considered from the outset [66]. While the hardware and software infrastructure required to support bioinformatics pipeline data analyses is a significant expense, the costs associated with maintaining the raw data acquired from NGS assays can quickly eclipse the initial sequencing expense, as the raw data can exceed hundreds of gigabytes per batch. Thus, it is not only a challenge to deal with the initial bolus of data but special consideration must be made for long-term storage of data because if such data are to be maintained over a long period of time as the resources for maintenance may exceed the initial cost of generating the data [67]. Researchers and clinical laboratories must therefore thoroughly evaluate and optimize their data analysis and storage methods. As this technology transitions from research laboratories to clinical laboratories, the pathology and laboratory medicine professionals who will most likely be responsible for clinical implementation and interpretation of such methods will require new skills and training programs in order to provide safe and effective tests for patient care [68, 69]. Although new training programs will enhance the abilities of new professionals, the wide range of experience in professionals who have previously completed training will likely benefit from decision support systems that can aid in the selection, interpretation, and reporting of genomic tests, as well as treatment decisions [70–72].

### Commonly Used Platforms for Next-Generation Sequencing

Multiple platforms have been developed to take advantage of the concept of massively parallel sequencing. Although multiple different approaches have been commercialized, the field has consolidated into a few dominant platforms. New sequencing technologies are always in development that promise to offer advantages over current technology, but are still in their infancy in comparison. In this next section, the main sequencing platforms used in current clinical testing and research are described, including several platforms that were previously widely employed and may be encountered in the literature.

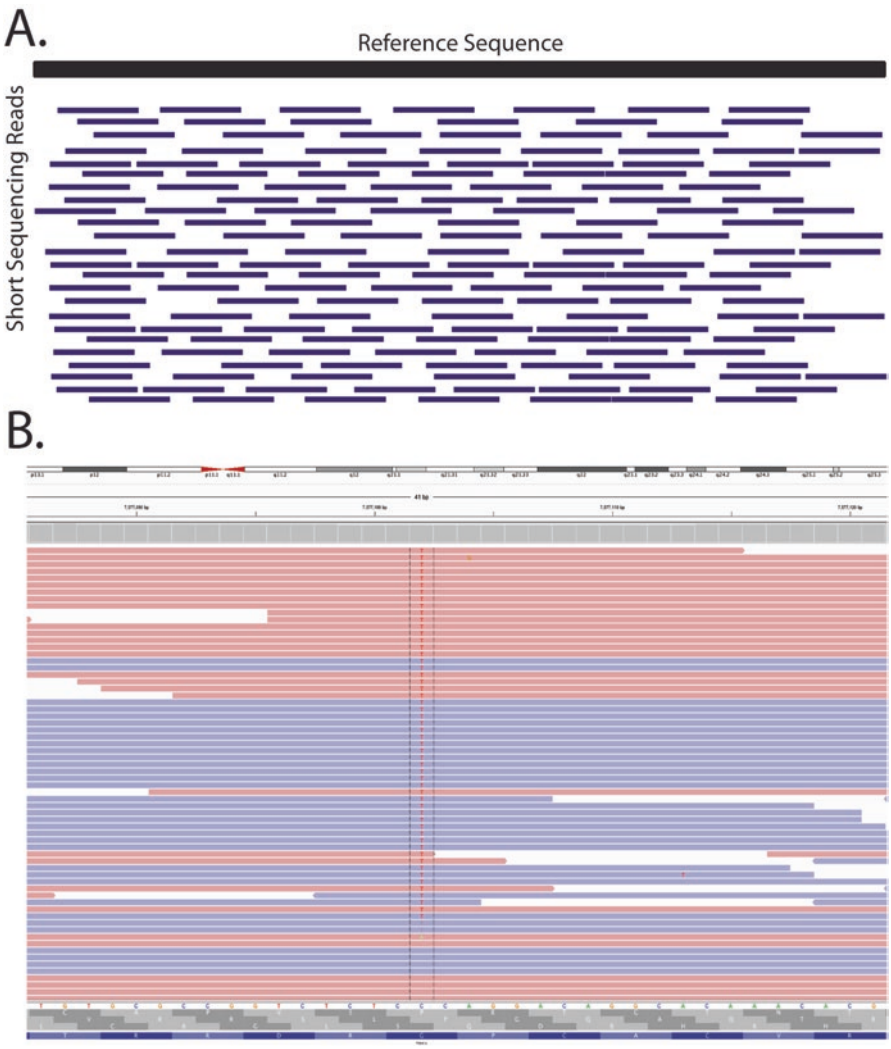


**Fig. 19.6** (**a**) Schematic showing the alignment of numerous short DNA sequencing reads to a template. Note the overlap in sequence from multiple short-read length fragments with often unique start and stop sites. When numerous fragments overlap at a given position, low-frequency variants may be identified in a small subset of the reads. Conversely, when there is low coverage of a given region of DNA, rare variants may not be detected because they represent the minor population of the sample. (**b**) Screen capture of clinical next generation sequence data as displayed using the Integrated Genomics Viewer (Broad Institute, Cambridge, MA, USA). The highlighted base is a variant that is identified in the *TP53* gene (p.G279E, NM_000546.5:c.836G > A)

### Sequencing by Synthesis (Solexa/Illumina)

The sequencing-by-synthesis (SBS) method, pioneered by Solexa, is a hybrid method combining fluorescent dideoxy Sanger sequencing and solid surface sequencing by synthesis [73, 74]. The use of fluorescently labeled, reversible dideoxy terminators [75] allows for the sequence-data acquisition to be decoupled from the sequencing chemical reaction. In this method, denatured DNA fragments with ligated adapters are flowed across a glass substrate ("flow cell") with oligonucleotides complimentary to the adapter molecules added to the DNA of interest during the library preparation stage. If the concentration of the sequencing library is correct, individual DNA molecules become spatially distributed on the flow cell, which will allow accurate interrogation at the sequencing stage. The attached sequences are then amplified in place using PCR such that products of the reaction are also attached to the solid substrate via the capture oligonucleotides, thus forming cluster of identical DNA fragments generated from the same initial library DNA fragment. Postamplification, fluorescently labeled, reversible terminators are added in bulk to the flow cell, a single base is incorporated into each bound molecule, and the residual reagent is washed away. The fluorescently tagged bound DNA molecules are excited via a laser, and digital imaging is used to scan the flow cell and identify incorporated bases in each DNA cluster based on fluorescent signal. After imaging, the reversible terminator is then cleaved from the captured DNA molecules, allowing for the incorporation of another fluorescently labeled nucleotide on the next cycle. Depending on the instrument, reagents, and DNA quality, the cycle can be repeated up to 150–300 times and then the unique indices are sequenced to establish the identity of each imaged cluster. After removing the sequencing products from the first round, a second round of amplification allows the sequencing of the captured molecule from the reverse direction and the confirmation of the identity of the cluster by sequencing a second index. A critical innovation of this technology was the decoupling of the enzymatic sequencing synthesis reaction from the acquisition of base identity, which allows for huge arrays of DNA library molecules to be sequenced simultaneously. This method has been shown to be susceptible to decreased read depth with increasing GC content [76–78] and may be susceptible to bias introduced via the multiple PCR amplifications utilized at different steps in the method [79]. Comparisons of available technologies have suggested that the SBS method has the highest sequencing throughput per batch coupled to the lowest error rates [80]. The need for specialized hardware and optics to identify the sequencing products, stability of the reagents, and the complicated fluidics has been cited as potentially limiting the read length and accuracy of the method [81]. Improved engineering, hardware upgrades, and innovation of software are likely to continue to improve the performance of this approach in the future. Currently, this technology dominates many of the research and clinical methods that have been used to evaluate the spectrum of variation that occurs in the neoplastic disease.

### Semiconductor-Based (Ion Torrent)

Semiconductor-based sequencing also relies on the combination of a solid substrate combined with numerous individual reactions targeting individual DNA molecules. In this method, microscopic reaction wells have been created with a semiconductor at the base of the reaction chamber. After DNA library preparation, the reaction device is flooded with droplets containing single strands of DNA [82]. Subsequently, the reaction device is sequentially treated with a single nucleotide, and if a given nucleotide is incorporated into a nascent strand of DNA, a hydrogen ion is liberated as part of the reaction. The released hydrogen molecule is then detected using a sensitive ion sensor located in the individual reaction well. A unique aspect of this design is that base incorporation is not limited to a single nucleotide, such that in the case of a homopolymer repeat region, multiple nucleotides will be incorporated in a single cycle. Such incorporation of multiple nucleotides results in a corresponding increase in the number of released hydrogen atoms, which then result in a proportionally greater electronic signal detected by the sensor. This aspect of the method can cause difficulty in assessing the true number of nucleotides within a homopolymer region and can result in a false-positive determination of insertion–deletion (indel) events. Nevertheless, numerous approaches have been developed to decrease the incidence of false-positive indel calls in commonly used bioinformatics pipelines, which may be useful in clinical laboratory settings [83–85]. A potential advantage that is often cited is the possibility of semiconductor sequencing to decrease in cost and improve in performance because the core technology is able to leverage the scale and infrastructure of the semiconductor industry [86]. Comparisons of semiconductor NGS methods to alternative technologies have demonstrated higher throughput than other methods [85].

## *"Third-Generation Sequencing" Sequencing Methods*

The majority of currently available NGS approaches that are in wide research and clinical use require one or more amplification steps where the nucleic acid sequences of interest are enriched before the actual sequencing reaction. This reliance on amplification prior to sequencing can have consequences such as bias and sequencing artifacts, which may limit the applicability in some situations. Additionally, the current generation of technologies in common use relies on interrogation of relatively short DNA sequences, which can hinder the evaluation of classes of mutation that are important in the setting of neoplasia, such as structural rearrangements or assignment of variants to a pseudogene. These factors have led to the development of computational methods to aid in the detection of such alterations [87–89]. Currently, several methods are available that sequence individual nucleic acids using approaches that allow contiguous sequence read lengths of several thousand kilobases or more. Although these methods have lower throughput compared to the commonly used platforms, it appears that fewer long-read sequences may result in

improved assemblies compared to short-read sequencing at higher depths [90, 91]. Additionally, these so-called third-generation sequencing technologies have been suggested to potentially further decrease sequencing costs [92], reduce bias in sequenced regions due to elimination of amplification steps, and improve sequencing of high GC-content DNA sequences [93]. In order to maximize the length of individual DNA molecules derived from a sample, high-quality samples and high-quality DNA extraction techniques must be employed in order to maximize the strengths of long-read nanopore sequencing approaches.

A major drawback of common NGS methods is the necessity for amplification of the source DNA prior to DNA sequencing. This approach can lead to amplification artifacts and biased coverage of the genome related to the G–C content or local structural properties of specific DNA sequences. Current NGS technologies produce relatively short reads, with median sequence lengths ranging from 100 to 300 base pairs (bp) for some methods (such as those by the manufacturer, Illumina) and up to 700 bp for the approach championed by the technology by 454. Short sequence reads are in general more difficult to align to a reference genome, especially in regions of high homology or in the context of pseudogenes [94, 95]. While short-length DNA sequencing may work approximately equally well with fresh or formalin-fixed, paraffin-embedded (FFPE) tissues, long-read sequencing of thousands of bases is unlikely to be successfully accomplished using the fragmented and lower-quality DNA found in clinical tissues due to formalin fixation. Use of abundant, high-quality DNA as is commonly found in hematological samples may make this sequencing approach an attractive research and diagnostic sequencing method in the near future.

### Single-Molecule, Real-Time Sequencing

Pacific Biosciences (PacBio) has developed a single-molecule sequencing method that utilizes a sequencing-by-synthesis approach combined with a zero-mode waveguide that allows for real-time sequencing of individual DNA molecules, which has been termed “single molecule, real time (SMRT)” sequencing [96]. The method uses small wells that have a diameter less than the wavelength of light chosen for interrogation (zero-mode waveguide) [97], where a DNA polymerase is bound to the bottom of each well. Nucleotides, each labeled with a different fluorophore, are added to the wells, and individual nucleotides are incorporated into a complementary DNA strand. As the fluorophore is released with each nucleotide addition, the zero-mode waveguide is used to detect the fluorophore released from the incorporated base, and thus the sequence within each waveguide is measured in real time.

SMRT sequencing does not require amplification prior to sequencing and has been reported to reduce compositional bias compared to other sequencing technologies [98], with median DNA sequence length reported in thousands of nucleotides, with substantial fractions of the DNA sequences greater than 10,000 base pairs [99]. Additionally, the lack of a DNA amplification step offers the potential for a shorter time between DNA extraction and sequence generation [100]. Such long-read

sequencing technology offers the potential of resolving pseudogenized genetic sequences [101], resolution of variant phasing, haplotype resolution, structural rearrangement determination, and indel identification [102, 103], all of which are problematic using short-read techniques.

The major drawback cited when describing SMRT sequencing is the relatively low accuracy of approximately 85% [99, 100], with indel errors predominating. Methods combining data from multiple sequencing modalities have been demonstrated to improve accuracy, albeit with increased costs and algorithm complexity [96, 104]. SMRT sequencing accuracy has also been demonstrated to improve when the same DNA molecule is sequenced multiple times within the same SMRT cell using a technique called "circular consensus sequencing" (CCS) [105, 106]. The principle of CCS is that by decreasing the length of the sequencing insert, the same DNA molecule will be sequenced multiple times, improving the overall accuracy of the consensus sequence because the sequencing errors occur randomly. Platform improvement and error-reduction optimization are ongoing.

### Nanopore Technologies

Nanopore sequencing of DNA uses techniques in which DNA molecules are passed through artificial nanoscale pore composed of organic or nonorganic molecules, and the DNA sequence order is determined based on electrical or other signals that are generated during passage of the DNA molecule through the pore [107–110]. In most nanopore methods, DNA passing through the nanopore results in changes in ion current, with multiple parameters impacting the measured current, with each nucleotide impacting the ion flow in such a way that the sequence can be gleaned from the measurements [111]. Similar to SMRT technology, these techniques offer the possibility of rapid progression from sample preparation to sequence generation, long sequencing reads, low cost and high speed with compact instrumentation [112].

Nanopore sequencing is based on the concept that single stranded nucleic acid (DNA or RNA) molecules can be forced through a biological pore by electrophoresis or other mechanisms in a linear fashion, with the determination of individual base composition at a specific position detected by a change in ionic current while the molecule is moving through the pore. Pores are created so that a detection mechanism is able recognize signals generated by different bases and a unique signal is generated corresponding to the sequence. Multiple different materials and designs for the nanopore have been developed, with differences in construction of the pore influencing the speed and accuracy of the sequencing process. Currently, the two classes of pore materials are solid state and protein based, each with different strengths and weaknesses. Protein nanopores are created from membrane protein complexes composed of alpha-hemolysin or *Mycobacterium smegmatis* Porin A (MspA) [107, 108, 111], while solid-state nanopores are created using synthetic materials. One theoretical advantage of synthetic materials is the possibility of customizing pore configurations specific to given applications, allowing for potentially

more stable chemical, mechanical, and thermal properties. Nanopore technologies are currently undergoing extensive ongoing development and testing and offer different performance characteristics that are still undergoing evaluation. In addition to the important nature of the pore materials and construction, precise control of the transport of the single-stranded nucleic acid through the pore is critical for accurate sequence determination, and multiple enzymes such as polymerases and exonucleases have been evaluated for this use [108].

Read lengths for some applications have been shown to be ~10 kb [113], but error rates for the technology have been shown to range between 5% and 8% for insertions, deletions, and substitutions for some methods [114]. Some evaluations of early instruments and chemistries have indicated that only a fraction of the sequencing reads are able to be mapped to the reference sequence of a known sequencing target [115]. These factors limit the current standalone, direct utility of the technologies in the diagnostic setting, but can be used as a method to generate a scaffold sequence that can be combined with other sequencing methods. Some analyses have suggested that accuracy can be improved through increasing the read coverage of individual sequenced molecules [116]. The methods may also require bioinformatics tools specific to nanopore sequencing [85].

## Summary

Although SMRT and nanopore sequencing technology have been available in select laboratories for several years, there is limited data on the use of such techniques in the setting of hematological malignancies and particularly in the clinical laboratory [117]. The currently described technologies capable of sequencing single-molecule nucleic acids with long-read techniques offer attractive features that would be useful in both research and diagnostic applications in myeloid neoplasia. Contiguous sequencing reads capable of routinely resolving tens of kilobases of sequence would be useful in the setting of indels and structural rearrangements, which are encountered in myeloid neoplasia. Additionally, the ability to resolve the phase of mutations, haplotypes, and discriminate between real and pseudogenes could potentially impact our understanding of myeloid neoplasia and diagnosis. Although there are methods that promise improved accuracy, the current techniques are hampered by high error rates. The possibility of using combinations of sequencing technologies to use strengths of one technique to offset the weaknesses of another is appealing but will require new software solutions to merge data from different platforms and produce a unified output. Currently, the main limitation of these techniques that limits their widespread use is the high error rates in sequenced DNA. Technological improvements may yield improvements in accuracy and strategies have been developed to increase the sequencing accuracy of individual molecules but such accuracy often comes at the expense of read length. Developments in these technologies and their successors warrant special attention and consideration for the potential scientific and diagnostic uses for these techniques in the setting of myeloid neoplasia should be considered in the future.

## *Other Technologies Present in the Literature*

Although there are several varieties of next-generation sequencing technologies that are in common use, multiple other technologies were commercialized and extensively used in the literature. While some technologies have faded from use or even been discontinued by the manufacturer, large amounts of data were generated using these techniques and studies using them may be encountered not only in older work [118, 119] but in recently published studies as well [120].

### Pyrosequencing Sequencing by Synthesis (454/Roche)

Key aspects of the 454 sequencing method include emulsion PCR and pyrosequencing. As part of the sequencing library preparation, target DNA is ligated to primers where one primer is linked to a biotin molecule. During library preparation, individual molecules of DNA are captured on streptavidin beads within picoliter emulsions of oil and reagents for PCR (emulsion PCR) [121]. After clonal amplification of the target DNA bound within a given droplet, the resulting clonal products are deposited on custom microtiter plate ("picotiterplate") with wells ~29 μm in diameter, with millions of clonal sequences deposited across a single plate. Sequence determination is done through the use of pyrosequencing technology, where individual nucleotides are sequentially added, and if a given nucleotide is incorporated into the nascent DNA strand, pyrophosphate (PPi) is released into the milieu of the microwell. Also present within the reaction mixture is the enzyme luciferase, which uses the PPi as a substrate to generate light for detection allowing inference of the incorporated nucleotide at a given position [122]. Similar to the semiconductor NGS methods used on the Ion Torrent platform, an increased signal is generated when homopolymer tracts are encountered because a chain-termination strategy is not employed. As such, uncertainty in homopolymer length is a common occurrence in this method, and computational methods have been developed to address this issue [80, 83, 84]. The pyrosequencing method is capable of producing large amounts of long, high-quality sequencing reads, and this technology has been described to produce read lengths that are longer than many other NGS technologies but less than the maximum sequence lengths generated using traditional Sanger techniques [61]. Further, this method had lower throughput than other systems [80, 123] and a higher cost-per-base [123]. The platform was purchased by Roche Diagnostics in 2007 and manufacturer support was scheduled to be discontinued in 2016.

### Sequencing by Oligonucleotide Ligation and Detection (SOLiD)

Similar to other NGS library preparation methods, SOLiD relies on single nucleic acid targets isolated for subsequent clonal enrichment. In SOLiD, magnetic beads are used to isolate a single target sequence per bead, and emulsion PCR is used to

amplify DNA to increase the number of copies of the unique target DNA bound to each bead as well as incorporate adapter sequences into the amplified DNA. Postamplification, PCR products are covalently bound to a glass slide for subsequent DNA sequencing. Using primers specific to the adapter sequences incorporated in PCR, the captured DNA is sequenced by using a set of four fluorescently labeled, two-base probes, which compete to be ligated to the sequencing primer. The fluorescence is measured to determine the incorporated probe, and the fluorescent molecule is released and the process is repeated. Following a series of ligation cycles, the extension product is removed and the template is reset with a primer complementary to the $n-1$ position for a second round of ligation cycles. This process is repeated multiple times for each adapter, allowing for each base to be interrogated in multiple independent ligation reactions by different primers [124]. This approach obtains specificity by interrogating every first and second base in each ligation reaction [125] and redundancy through interrogation by multiple ligation probes. The SOLiD method has been reported to have difficulty with palindromic sequences [126] and has been claimed to have decreased susceptibility to homopolymer tracts. This technology has been described to have intermediate throughput and costs compared to other NGS methods [123].

## *Scale of Sequencing*

Each of these sequencing-based methods is capable of being used on multiple scales. Briefly, the scales of next-generation sequencing are whole genome, exome, and targeted panels (Fig. 19.7). Applications used extensively by research groups include sequencing an entire genome for identification of alterations with potential clinical importance [127]. Whole-genome sequencing (WGS) has the advantage of relatively unbiased sequencing, the capability of detecting CNVs across the genome, and the ability to identify genetic changes that would be missed by more targeted methods. However, WGS generally has lower overall coverage of individual bases, requires additional instrumentation and bioinformatics resources compared to other

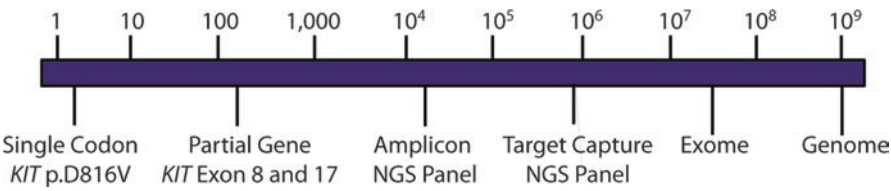


**Fig. 19.7** Linear scale approximating the number of base pairs analyzed in different scales of molecular diagnostic testing using single-site assays, limited exon targeting assays, amplicon-based NGS panels, comprehensive NGS panels using hybrid capture designs, whole-exome and whole-genome approaches. Note that typical target-capture NGS designs capture several orders of magnitude more base pairs than amplicon panels

methods, and thus, is generally more expensive to produce data. Multiple groups have used WGS to understand the evolution of individual neoplasms over time [128, 129]. While such studies may be useful to help identify individual mutations associated treatment response or failure, the breadth of data generated from such studies is unlikely to prove cost effective in routine clinical use at the present time.

Exome-level sequencing utilizes multiple techniques to isolate the coding regions of genes, most frequently utilizing hybrid-capture techniques where antisense oligonucleotides are used to enrich for the sequences of interest [130, 131]. Whole-exome sequencing (WES) offers the potential advantage of overall high-sequencing depth compared to WGS because the targeted DNA represents a much smaller fraction of the total genomic DNA, with decreased reagent and bioinformatics costs; however, the cost of the reagents needed to isolate the exonic DNA can be expensive and adds complexity to the sample library preparation. As a result of the relative decreased cost of sequencing and increased depth, many studies have been undertaken to evaluate exomes of hematopoietic neoplasms. Studies using whole-exome sequencing, like studies employing WGS, often identify known driver mutations, show evidence of clonal evolution through therapy, and identify new mutations that arise over time [132, 133]. Although the cost of exome sequencing and the ability to utilize the necessary bioinformatics pipelines are within the reach of many groups, routine exome sequencing may be in limited clinical use for the evaluation of neoplastic disease due to the more manageable data that are generated using targeted gene panels.

Utilizing techniques similar to WES, targeted gene sequencing panels can utilize hybrid capture techniques [65, 134] or a more limited coverage can be obtained using ligation-probe or PCR-based amplification techniques [135–138]. Such targeted techniques allow for greater sequencing depth in specific genes of interest, potentially allowing for greater sensitivity in detecting minor clonal components. Total assay costs may be less than WES and WGS because of the limited coverage allowing for the use of smaller scale sequencers, multiplexing of multiple samples, and less resource-intensive bioinformatics pipelines than those used in WES and WGS. One potential advantage of using a limited set of genes is the understanding that the significance of individual mutations may be more achievable than variants detected in more expansive platforms. When comparing hybrid capture methods against amplicon-based enrichment techniques, the ability to detect CNVs, large indels, and translocations may be greater in target-capture techniques due to the less-specific genomic enrichment using such techniques and the availability of numerous software packages that can be incorporated into the data analysis pipeline after sequence acquisition [87, 89, 139–141]. Numerous examples of custom-developed methods targeting specific genes related to myeloid neoplasia are described in the literature. Most methods describe the ability to detect mutations that are commonly encountered in myeloid neoplasia [142, 143], with hybridization-capture enrichment techniques often noting the ability to detect large structural rearrangements and translocations [144]. Although amplicon-based NGS panels typically are not designed to detect larger indels or CNVs, several bioinformatics

approaches have been developed that appear to glean such information from such assay designs [145, 146].

Assessment of the mutational spectrum of myeloid neoplasia has become a common tool in clinical care of patients and in the research setting. While techniques such as WGS and WES are of interest to researchers, their use in routine clinical care is not currently clear and limited. In contrast, the use of targeted gene panels offers the benefits of sequencing a more focused set of genomic targets, resulting in greater sequencing depth, lower cost, and potentially easier path to demonstrate clinical utility.

## Emerging Techniques and Applications

The previously described technologies and techniques have been applied to numerous scientific and clinical problems and have found acceptance in the diagnostic workup, prognostication, and therapy prediction of myeloid malignancies. Additional technologies and applications have been described and are currently undergoing evaluation for their use in clinical applications. It is unknown if any of the techniques or applications described will ultimately be found in routine use in the evaluation of myeloid neoplasia, but general understanding of the conceptual basis and early scientific data may be useful to practitioners as they encounter such techniques in the literature.

### *Minimal Residual Disease Detection*

It is increasingly being appreciated that minimal residual disease (MRD) detection by conventional means, such as flow cytometry, is useful for following the response to treatment and for identifying high-risk patients who are beginning to relapse. In some situations, treatment decisions that impact patient outcome may be made based on the MRD test results [147], making accurate and timely results very important in clinical care. Current clinical approaches for detecting MRD and supplementing morphologic examination and cytogenetic studies in myeloid neoplasms include flow cytometry [148], RT-PCR [48–51], and PCR assays [149]. While understanding of flow cytometry and PCR are common in many clinical laboratories, application of these methods to MRD detection requires some expertise to properly design these assays and interpret the results in order to ensure standardization for comparison of results. As such, such assays are often limited to larger commercial reference laboratory and academic institutions. The development of new laboratory approaches for minimal residual disease detection may result in improved techniques that may be less reliant on pathologist expertise to determine the MRD status.

Several promising approaches exist that may prove useful in adapting new techniques to MRD analysis, including deep mutation scanning of many different possible genetic mutations [150, 151] and deep sequencing of specific recurrent mutations that are observed in a subset of patients [152–154].

Many myeloid neoplasms have recurrent abnormalities and mutational profiles that show remarkable consistency between individuals [155]. Some studies have evaluated diagnostic methods that are specific to these unique genomic signatures of specific neoplasms, potentially allowing specific laboratory methods to be used to assess MRD in patients with neoplasms harboring these genetic lesions [155]. An example of using a recurrent mutation to monitor MRD with next-generation sequencing has been described in the setting of *NPM1*-mutated AML [152], which represents ~60% of AML cases with a normal karyotype. In this example, amplification and sequencing of exon 12 of *NPM1* allowed detection of low-burden AML disease that was shown to be capable of detecting mutant cells down to approximately 0.001% of a mixture, using next-generation sequencing. Compared to allele-specific PCR approaches, a priori knowledge of the patient's *NPM1* mutation allele is not needed. Next-generation sequencing of *NPM1* mutations also showed the potential of identifying subclonal heterogeneity within a given sample, offering the possibility of tracking clonal evolution of the neoplastic populations over time. Such an approach to MRD detection offers the potential for a more simplified approach to MRD monitoring that does not require the extensive expertise needed for flow cytometry assessment of MRD or the complexity of performing multiple allele-specific quantitative PCR approaches. It is, however, critical to recognize that with highly sensitive NGS methods, there is a possibility of sample-to-sample or amplicon contamination that must be addressed, and careful precautions, such as those used in viral molecular diagnostics, must be taken to limit the possibility of such events [156]. Clinical laboratories will also have to determine if maintaining and performing complex assays that are specific to only a subset of their patient population is a feasible operation from the standpoint of cost and turnaround time. Finally, the clinical significance of such low-level persistent or recurrent disease will need to be studied to determine if such methods are appropriate.

An alternative approach to neoplasm subtype specific assays is to identify the most common genes mutated across many myeloid malignancies and then design a method that is capable of identifying the majority of mutations in those genes. By expanding the pool of possible genetic targets, there is the possibility that the presence of multiple mutations will increase the specificity of the analysis, as well as potentially increase the sensitivity through the elimination of false-negative results. Such methods propose a more flexible approach, which could allow the MRD analysis to include novel mutations or to identify populations evolving from the original leukemic clone. Approaches have been described that are approximately tenfold more sensitive than Sanger-sequencing-based methods for detection of genetic variants, and was highly concordant multiple laboratories [150, 151]. While these results indicate that sensitive assays can be implemented across multiple laboratories and achieve comparable results, such methods are currently less sensitive than typical state-of-the-art multidimensional flow cytometric techniques used to mea-

sure MRD. Identifying patient-specific mutations early in disease and then using deep-sequencing of the mutated regions has been suggested to approach the sensitivity of flow cytometric methods [154], but such a strategy requires multistep, patient-specific evaluation methods and may lack the flexibility of the more generalized methods or may be relatively costly with limited sensitivity. Additionally, deep-sequencing methods using amplicon approaches run the risk of introducing PCR artifacts in early amplification cycles that could mimic, by chance, previously identified mutations, resulting in false positive results.

With all proposed methods for detecting MRD, the hope is that such approaches could shorten the window period between the recurrence of a neoplasm and the ability of laboratory techniques to detect disease relapse. Additional studies need to evaluate not only the analytical sensitivity and specificity of these methods, but whether clinical benefit is enhanced. While initial studies evaluating MRD in multiple laboratories have shown good concordance of results [150], additional work will need to demonstrate that such concordance continues, if such methods are brought into common practice, especially if other methods of MRD analysis are not available for confirmatory testing. With all approaches, it must be remembered that persistent, nonleukemic hematopoietic clones may present in any given patient and that such clones will share many mutations common with truly neoplastic leukemic populations, and that such populations may persist over time [157, 158], and even after initial chemotherapy treatment [159].

### Methods to Increase Accuracy of Deep Sequencing

Application of conventional NGS techniques to the ultrasensitive deep-sequencing techniques, such as identification of MRD, is hampered by the inability to resolve very small populations of mutant genetic changes from errors introduced by amplification or sequencing. One approach that has been suggested to remedy this problem is duplex sequencing, which proposes a method to identify the source of each individual amplicon in a sequencing reaction, allowing for the identification of artifacts that are present in only one strand of an amplification reaction [160]. A second comparable approach uses single molecular tagging to permit detection of rare variants beyond the traditional limits due to error-correction [160–162]. Through incorporation of additional random sequence "barcodes" to the primers for specific sequence targets, the providence of individual amplicons can be ascertained, such that artifacts can be excluded through the comparison of specific sequencing products. This method works by grouping individual sequences into sequence families that have the same barcodes identifier in common, which are then compared against other reads within the family to remove individual errors through creation of an error-corrected consensus sequence. Descriptions of this technique claim to allow a single mutation to be identified among $1 \times 10^7$ wild-type sequences, and a study using a similar approach in the setting of preleukemic clonal hematopoiesis in patients who later developed treatment-related myeloid neoplasms demonstrated the ability to identify 1 abnormal sequencing read in 10,000 [161]. These methods

offer the potential to detect mutations related to residual or incipient diseases with much greater sensitivity than currently available flow cytometric or nonerror-corrected NGS MRD methods.

Error-correction NGS methods will require adjustments in bioinformatics pipelines between the sequence demultiplexing and alignment steps, in addition to synthesis of primer sequences incorporating the random sequence barcodes. The depth of sequence at a given position will need to be high, so that there is adequate sampling of the available primers, which will require either more sequencing throughput or fewer targets so that sufficient sequencing depth can be achieved. Ultimately, the necessity for such error-correction methods in NGS MRD applications will be highly dependent on the clinical need and utility for such ultrasensitive monitoring of mutant populations and the lack of alternative methods to increase the fidelity of the sequence calls. Although there are ample data supporting the utility of clinical MRD assessment in the setting of myeloid neoplasia, there are many factors that may limit wider clinical adoption of current methods [163, 164]. The necessity for such error-correction methods in NGS MRD applications will be dependent on the clinical context and will be incumbent on the laboratory to demonstrate the enhanced analytic sensitivity will translate to enhanced clinical care. Nevertheless, the ability to probe and evaluate for ultrarare mutations beyond standard limits of detection will likely be important in the future [161, 165].

## *Single Cell Sequencing*

As NGS has become a common research tool, many groups have applied this toward the analysis of the clonal evolution of hematopoietic neoplasms. Some studies have demonstrated that the acquisition of mutations is likely to occur over the evolution of the neoplasm [166] and in response to treatment [159]. In many of these studies, the presence of unique or evolving mutations acquired by neoplastic clones within the larger leukemic population is inferred from the variant allele fraction (VAF), the measure of the proportion of sequencing reads with a mutation versus the total number of reads (mutation and wild-type) at a particular loci as determined by bulk sequencing of diseased tissue. However, this assumption may not be correct. Development of new approaches, including analysis of flow-cytometric cell sorted cells, has allowed a refinement of this understanding through the ability to sequence individual neoplastic cells in order to compare the genetic aberrations present in those cells compared to the bulk neoplasm.

Current single-cell sequencing techniques potentially require special preservation and handling of the sample material and currently require whole-genome amplification of the individual genomes [167]. Such an approach offers an unprecedented, granular assessment of the mutations within individual leukemic cells, but requires specialized expertise and is susceptible to incorporation of artifacts from whole-genome amplification [168, 169].

The evaluation of individual neoplastic cells has revealed that the mutation spectrum within leukemic subpopulations is more nuanced than previously appreciated [170]. In one study, individual AML cells were shown to possess *FLT3* and *NPM1* mutations in both heterozygous and homozygous states and were distributed across multiple, distinct clonal populations [170]. Although these observations give insight into the disease process, it is currently unknown if there is a clinical benefit to monitoring of this mutational heterogeneity in clinical samples, although it is readily conceivable that such evaluation at the single-cell level could identify clones that may be resistant to therapy and thereby could provide information to guide treatment decisions. This emerging technique, however, will require additional study and evaluation of clinical usefulness in order to determine the potential clinical benefits and establish feasibility of implementation in the clinical environment.

### *Expression Profiling*

Expression profiling of AML has provided scientific insight into the implications of the altered genetic makeup of leukemic cells [171]. Such profiling has revealed that some neoplasms may have different subtypes that are distinguished by expression profiling [172]. Transcriptional profiling has even been done at the single-cell level for myeloid cells, demonstrating that individual cells have expression profiles indicating a distinct lineage without overlap [173]. Some recent studies have suggested that alterations in expression may have prognostic significance [174–177] but these analyses have not yet been widely translated into routine clinical practice.

## RNA-SEQ

RNA-seq is a modification of NGS in which the starting template is RNA from the patient sample and not DNA. RNA-seq has been proposed as a useful complementary technology to conventional diagnostic techniques because of the ability to detect complex genomic events and splice-site alterations that may not be as readily identifiable by other techniques [178]. RNA-Seq may be useful at identifying novel or unexpected translocations that may be clinically or diagnostically relevant but not readily identifiable by standard techniques [179, 180]. RNA-seq has demonstrated that differential expression of genes can be detected in different cell types related to disease entities [181] or discerning between closely related clones that have different characteristics [182]. RNA-seq may be useful in identifying splice-site changes or variants that may not be detectable by other means [183–185]. Research uses for RNA-seq include identification of mechanisms of drug resistance in the setting of myeloid neoplasia [186], but its utility in a routine clinical environment is yet unproven. While there are potential advantages of using RNA-seq, such as more efficient detection of the impact of epigenetic changes, splice-site changes

and structural rearrangements, the ability to analyze these parameters requires specialized informatics tools [187, 188]. Some limitations of RNA-seq include the starting substrate is more labile than DNA and thus is technically more difficult with which to work, and, at the present time, offers few advantages over complimentary methods. As such, it currently is uncertain if RNA-seq will prove a useful additional to the clinical armamentarium but will most certainly be useful in research. New approaches for RNA-seq include the ability to profile transcriptomes of tens of thousands of single cells [189, 190]. This technology provides unprecedented granularity and insight into the different cell populations that may be present in normal and disease [173, 191] and is likely to provide insight into the complexity of clonal competition and evolution in hematologic neoplasms.

### *Epigenetics*

Epigenetic modifications are reversible alterations to DNA or histones of a cell that impact gene expression without altering the underlying genetic sequence of the cell. Many genes involved in the epigenetic modification of the genome have been identified to be mutated in myeloid disorders and have been the subject of intense study [192–195]. Further work has suggested that genes involved in epigenetic modification may be useful as prognostic markers in the setting of myeloid neoplasia [196, 197] and studies investigating the use of therapies targeting aberrant epigenetic modification have been published [198, 199]. The relationship between individual disease entities, genomic mutations in genes related to epigenetics, and the consequences of the mutations are under active investigation [200]. Tools for the evaluation of epigenetic alterations in myeloid neoplasia in clinical settings are available [201] but it is unknown if the information provided by such methods will add information to what is already captured through the extensive analysis that is currently applied to many individuals with myeloid malignancies [202]. As with many other laboratory techniques, the clinical utility of the approach will need to be evaluated to determine if there is meaningful added clinical benefit.

## Confounding

As next-generation sequencing was applied in different clinical arenas, it became unexpectedly apparent that many of the mutations commonly associated with myeloid neoplasia can be detected in individuals without evidence or involvement of a myeloid malignancy. The detection of these mutations confounds routine clinical laboratory testing as the presence of such mutations does not necessarily indicate the presence of disease as currently defined or understood. In such situations, additional clinical testing could be unnecessary and may result in misdiagnosis. Studies examining the prevalence of hematopoietic mutations in otherwise healthy

populations have shown that the incidence of mutations in genes commonly associated with myeloid neoplasms appears to increase with age [157, 158, 203]. In these individuals, a small percentage of the individuals with age-related hematopoietic clones appear to develop hematological neoplasia, with individuals with a larger proportion of the cells in the peripheral circulation harboring such mutations having an increased risk. Other studies have demonstrated that some mutations in hematopoietic cells arise in patients with other types of cancer, often after the selective pressure of chemotherapy [204]. While the emergence of hematopoietic clones is not uncommon, the incidence of hematopoietic neoplasia in such settings appears to be uncommon based on retrospective studies [205, 206]. Some authors have termed this phenomena, "clonal hematopoiesis of indeterminate potential," or as abbreviated, CHIP [206]. Additional studies evaluating the future risk for neoplastic disease and the recommendations for reporting, screening, and monitoring of such clonal populations will need to be developed.

As another example, new approaches to prenatal diagnosis evaluating genomic CNVs in cell-free DNA (cfDNA) have also reported several instances of inadvertent detection of maternal neoplasia in pregnant women undergoing routine screening [207]. It is conceivable that assays evaluating either fetal or neoplastic cfDNA may be capable of detecting CNVs and mutations that are originating in hematopoietic cells. Awareness of the possibility may be sufficient to identify ancillary methods that may be useful in resolving the observed data, but additional tools may be useful in the future to help resolve these situations. For example, recent work has demonstrated that the cell-of-origin of cfDNA fragments can be ascertained based upon nucleosome signatures of the detected DNA molecules [208], potentially allowing for the discernment not only of the variants associated with neoplasia, but also the cell of origin (i.e., hematopoietic or nonhematopoietic in nature). Incorporation of such methods into cfDNA screening and monitoring methods will help to ensure that detected mutations are appropriately classified and understood.

These examples together highlight the importance of open communication between clinicians and laboratory pathologists. Indeed, awareness of these potential confounders of sequencing data obtained from advanced diagnostic tests will help physicians and scientists appropriate counsel providers and patients.

## Conclusions

The determination of the diagnosis and prognosis of myeloid neoplasia is greatly facilitated by advanced laboratory techniques that have been employed to dissect the underlying molecular lesions related to the malignancy. The classification of myeloid neoplasia has become more granular, specific, and detailed as the available laboratory techniques and biological understanding of the diseases have advanced. Laboratory techniques such as karyotype, FISH, PCR, Sanger sequencing, and microarray remain the mainstay of current clinical diagnostic laboratories, but are now often complemented by massively parallel sequencing. Due to the

comprehensive nature of NGS, it is conceivable that this technology may replace some of the more traditional methods for molecular cytogenetic analysis. As NGS has proliferated in scientific and clinical applications, new permutations have been proposed that may aid in improvements in clinical care, if analyses of clinical utility bear out. As these new technologies and tools continue to mature, new opportunities will arise to allow greater scientific understanding and potential for improved clinical care. Through knowledge of the laboratory methods that are currently in clinical use, the strengths and limitations of the different approaches will be important to consider.

## References

1. Smith HO, Wilcox KW. A restriction enzyme from Hemophilus influenzae. I Purification and general properties. J Mol Biol. 1970;51(2):379–91.
2. Price PM, Hirschhorn K. In situ hybridization of chromosome loci. Fed Proc. 1975;34(13):2227–32.
3. Pinkel D, Straume T, Gray JW. Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. Proc Natl Acad Sci U S A. 1986;83(9):2934–8.
4. Sanger F, Coulson AR. A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. J Mol Biol. 1975;94(3):441–8.
5. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci U S A. 1977;74(12):5463–7.
6. Maxam AM, Gilbert W. A new method for sequencing DNA. Proc Natl Acad Sci U S A. 1977;74(2):560–4.
7. Bartlett JM, Stirling D. A short history of the polymerase chain reaction. Methods Mol Biol. 2003;226:3–6. doi:10.1385/1-59259-384-4:3.
8. Saiki RK, Bugawan TL, Horn GT, Mullis KB, Erlich HA. Analysis of enzymatically amplified beta-globin and HLA-DQ alpha DNA with allele-specific oligonucleotide probes. Nature. 1986;324(6093):163–6. doi:10.1038/324163a0.
9. Lawyer FC, Stoffel S, Saiki RK, Myambo K, Drummond R, Gelfand DH. Isolation, characterization, and expression in Escherichia coli of the DNA polymerase gene from Thermus aquaticus. J Biol Chem. 1989;264(11):6427–37.
10. Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science. 1988;239(4839):487–91.
11. Dryja TP, Rapaport JM, Joyce JM, Petersen RA. Molecular detection of deletions involving band q14 of chromosome 13 in retinoblastomas. Proc Natl Acad Sci U S A. 1986;83(19):7391–4.
12. Lübbert M, Mirro J, Miller CW, Kahan J, Isaac G, Kitchingman G, Mertelsmann R, Herrmann F, McCormick F, Koeffler HP. N-ras gene point mutations in childhood acute lymphocytic leukemia correlate with a poor prognosis. Blood. 1990;75(5):1163–9.
13. Iggo R, Gatter K, Bartek J, Lane D, Harris AL. Increased expression of mutant forms of p53 oncogene in primary lung cancer. Lancet. 1990;335(8691):675–9.
14. Komminoth P, Kunz EK, Matias-Guiu X, Hiort O, Christiansen G, Colomer A, Roth J, Heitz PU. Analysis of RET protooncogene point mutations distinguishes heritable from nonheritable medullary thyroid carcinomas. Cancer. 1995;76(3):479–89.
15. Claas EC, Melchers WJ, van der Linden HC, Lindeman J, Quint WG. Human papillomavirus detection in paraffin-embedded cervical carcinomas and metastases of the carcinomas by the polymerase chain reaction. Am J Pathol. 1989;135(4):703–9.

16. Feinmesser R, Miyazaki I, Cheung R, Freeman JL, Noyek AM, Dosch HM. Diagnosis of nasopharyngeal carcinoma by DNA amplification of tissue obtained by fine-needle aspiration. N Engl J Med. 1992;326(1):17–21.
17. Melo JV, Gordon DE, Cross NC, Goldman JM. The ABL-BCR fusion gene is expressed in chronic myeloid leukemia. Blood. 1993;81(1):158–65.
18. Dobrovic A, Trainor KJ, Morley AA. Detection of the molecular abnormality in chronic myeloid leukemia by use of the polymerase chain reaction. Blood. 1988;72(6):2063–5.
19. Thompson JD, Brodsky I, Yunis JJ. Molecular quantification of residual disease in chronic myelogenous leukemia after bone marrow transplantation. Blood. 1992;79(6):1629–35.
20. Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. Blood. 2002;100(7):2292–302.
21. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood. 2009;114(5):937–51.
22. Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood. 2016;127(20): 2375–90.
23. Creighton HB, McClintock B. A correlation of cytological and Genetical crossing-over in Zea Mays. Proc Natl Acad Sci U S A. 1931;17(8):492–7.
24. Coe E, Kass LB. Proof of physical exchange of genes on the chromosomes. Proc Natl Acad Sci U S A. 2005;102(19):6641–6.
25. Martin CL, Warburton D. Detection of chromosomal aberrations in clinical practice: from Karyotype to genome sequence. Annu Rev Genomics Hum Genet. 2015;16:309–26.
26. Marcucci G, Mrozek K, Bloomfield CD. Molecular heterogeneity and prognostic biomarkers in adults with acute myeloid leukemia and normal cytogenetics. Curr Opin Hematol. 2005;12(1):68–75.
27. Van Den Berghe H, Louwagie A, Broeckaert-Van Orshoven A, David G, Verwilghen R, Michaux JL, Sokal G. Chromosome abnormalities in acute promyelocytic leukemia (APL). Cancer. 1979;43(2):558–62.
28. Berger R, Bernheim A, Daniel MT, Valensi F, Flandrin G. Karyotype and cell phenotypes in primary acute leukemias. Blood Cells. 1981;7(2):287–92.
29. Sakurai M, Hayata I, Sandberg AA. Prognostic value of chromosomal findings in Ph1-positive chronic myelocytic leukemia. Cancer Res. 1976;36(2 Pt 1):313–8.
30. Meisner L, Inhorn SL, Nielsen P. Karyotype evolution of cells with the Philadelphia chromosome. Acta Cytol. 1970;14(4):192–9.
31. Grimwade D, Walker H, Oliver F, Wheatley K, Clack R, Burnett A, Goldstone A. What happens subsequently in AML when cytogenetic abnormalities persist at bone marrow harvest? Results of the 10th UK MRC AML trial. Medical Research Council Leukaemia Working Parties. Bone Marrow Transpl. 1997;19(11):1117–23.
32. Langer-Safer PR, Levine M, Ward DC. Immunological method for mapping genes on drosophila polytene chromosomes. Proc Natl Acad Sci U S A. 1982;79(14):4381–5.
33. Landstrom AP, Tefferi A. Fluorescent in situ hybridization in the diagnosis, prognosis, and treatment monitoring of chronic myeloid leukemia. Leuk Lymphoma. 2006;47(3):397–402.
34. Welch JS, Westervelt P, Ding L, Larson DE, Klco JM, Kulkarni S, et al. Use of whole-genome sequencing to diagnose a cryptic fusion oncogene. JAMA. 2011;305(15):1577–84.
35. Huang XC, Quesada MA, Mathies RA. DNA sequencing using capillary array electrophoresis. Anal Chem. 1992;64(18):2149–54.
36. Heather JM, Chain B. The sequence of sequencers: the history of sequencing DNA. Genomics. 2016;107(1):1–8.
37. Hood LE, Hunkapiller MW, Smith LM. Automated DNA sequencing and analysis of the human genome. Genomics. 1987;1(3):201–12.
38. Zimmermann J, Voss H, Schwager C, Stegemann J, Ansorge W. Automated Sanger dideoxy sequencing reaction protocol. FEBS Lett. 1988;233(2):432–6.

39. Hood L, Rowen L. The human genome project: big science transforms biology and medicine. Genome Med. 2013;5(9):79.
40. Fleischmann RD, Adams MD, White O, Clayton RA, Kirkness EF, Kerlavage AR, et al. Whole-genome random sequencing and assembly of Haemophilus influenzae rd. Science. 1995;269(5223):496–512.
41. Venter JC, Adams MD, Sutton GG, Kerlavage AR, Smith HO, Hunkapiller M. Shotgun sequencing of the human genome. Science. 1998;280(5369):1540–2.
42. Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, et al. The sequence of the human genome. Science. 2001;291(5507):1304–51.
43. Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, et al.; Consortium IHGS (2001) Initial sequencing and analysis of the human genome. Nature 409(6822):860–921.
44. Schnittger S, Bacher U, Kern W, Alpermann T, Haferlach C, Haferlach T. Prognostic impact of FLT3-ITD load in NPM1 mutated acute myeloid leukemia. Leukemia. 2011;25(8):1297–304. doi:10.1038/leu.2011.97.
45. Kern W, Haferlach C, Haferlach T, Schnittger S. Monitoring of minimal residual disease in acute myeloid leukemia. Cancer. 2008;112(1):4–16.
46. Jobbagy Z, van Atta R, Murphy KM, Eshleman JR, Gocke CD. Evaluation of the Cepheid GeneXpert BCR-ABL assay. J Mol Diagn. 2007;9(2):220–7.
47. Yin JA, O'Brien MA, Hills RK, Daly SB, Wheatley K, Burnett AK. Minimal residual disease monitoring by quantitative RT-PCR in core binding factor AML allows risk stratification and predicts relapse: results of the United Kingdom MRC AML-15 trial. Blood. 2012;120(14):2826–35.
48. van Dongen JJ, Macintyre EA, Gabert JA, Delabesse E, Rossi V, Saglio G, et al. Standardized RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia for detection of minimal residual disease. Report of the BIOMED-1 concerted action: investigation of minimal residual disease in acute leukemia. Leukemia. 1999;13(12):1901–28.
49. van der Velden VH, Hochhaus A, Cazzaniga G, Szczepanski T, Gabert J, van Dongen JJ. Detection of minimal residual disease in hematologic malignancies by real-time quantitative PCR: principles, approaches, and laboratory aspects. Leukemia. 2003;17(6):1013–34.
50. Santamaria C, Chillon MC, Fernandez C, Martin-Jimenez P, Balanzategui A, Garcia Sanz R, San Miguel JF, Gonzalez MG. Using quantification of the PML-RARalpha transcript to stratify the risk of relapse in patients with acute promyelocytic leukemia. Haematologica. 2007;92(3):315–22.
51. Gabert J, Beillard E, van der Velden VH, Bi W, Grimwade D, Pallisgaard N, et al. Standardization and quality control studies of 'real-time' quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia - a Europe against cancer program. Leukemia. 2003;17(12):2318–57.
52. Kafatos FC, Jones CW, Efstratiadis A. Determination of nucleic acid sequence homologies and relative concentrations by a dot hybridization procedure. Nucleic Acids Res. 1979;7(6):1541–52.
53. Southern EM. DNA microarrays. History and overview. Methods Mol Biol. 2001;170:1–15.
54. Hoheisel JD. (1994) application of hybridization techniques to genome mapping and sequencing. Trends Genet. 1994 Mar;10(3):79–83.
55. Iafrate AJ, Feuk L, Rivera MN, Listewnik ML, Donahoe PK, Qi Y, Scherer SW, Lee C. Detection of large-scale variation in the human genome. Nat Genet. 2004;36(9):949–51.
56. Hemmat M, Chen W, Anguiano A, Naggar ME, Racke FK, Jones D, et al. Submicroscopic deletion of 5q involving tumor suppressor genes (CTNNA1, HSPA9) and copy neutral loss of heterozygosity associated with TET2 and EZH2 mutations in a case of MDS with normal chromosome and FISH results. Mol Cytogenet. 2014;7:35.
57. Cheung KJ, Rogic S, Ben-Neriah S, Boyle M, Connors JM, Gascoyne RD, Horsman DE. SNP analysis of minimally evolved t(14;18)(q32;q21)-positive follicular lymphomas reveals a common copy-neutral loss of heterozygosity pattern. Cytogenet Genome Res. 2012;136(1):38–43.

58. Greisman HA, Hoffman NG, Yi HS. Rapid high-resolution mapping of balanced chromosomal rearrangements on tiling CGH arrays. J Mol Diagn. 2011;13(6):621–33.
59. Shendure J, Porreca GJ, Reppas NB, Lin X, McCutcheon JP, Rosenbaum AM, Wang MD, Zhang K, Mitra RD, Church GM. Accurate multiplex polony sequencing of an evolved bacterial genome. Science. 2005;309(5741):1728–32.
60. Brenner S, Johnson M, Bridgham J, Golda G, Lloyd DH, Johnson D, et al. Gene expression analysis by massively parallel signature sequencing (MPSS) on microbead arrays. Nat Biotechnol. 2000;18(6):630–4.
61. Mardis ER. Next-generation DNA sequencing methods. Annu Rev Genomics Hum Genet. 2008;9:387–402.
62. Pareek CS, Smoczynski R, Tretyn A. Sequencing technologies and genome sequencing. J Appl Genet. 2011;52(4):413–35.
63. Gullapalli RR, Lyons-Weiler M, Petrosko P, Dhir R, Becich MJ, LaFramboise WA. Clinical integration of next-generation sequencing technology. Clin Lab Med. 2012;32(4):585–99.
64. Vosberg S, Herold T, Hartmann L, Neumann M, Opatz S, Metzeler KH, et al. Close correlation of copy number aberrations detected by next-generation sequencing with results from routine cytogenetics in acute myeloid leukemia. Genes Chromosomes Cancer. 2016;55(7):553–67.
65. Pritchard CC, Salipante SJ, Koehler K, Smith C, Scroggins S, Wood B, et al. Validation and implementation of targeted capture and sequencing for the detection of actionable mutation, copy number variation, and Gene rearrangement in clinical cancer specimens. J Mol Diagn 2014 Jan. 2013;16(1):56–67.
66. Richter BG, Sexton DP. Managing and analyzing next-generation sequence data. PLoS Comput Biol. 2009;5(6):e1000369.
67. Batley J, Edwards D. Genome sequence data: management, storage, and visualization. BioTechniques. 2009;46(5):333–334, 336.
68. Sinard JH, Powell SZ, Karcher DS. Pathology training in informatics: evolving to meet a growing need. Arch Pathol Lab Med. 2014;138(4):505–11.
69. Haspel RL, Olsen RJ, Berry A, Hill CE, Pfeifer JD, Schrijver I, Kaul KL. Progress and potential: training in genomic pathology. Arch Pathol Lab Med. 2014;138(4):498–504.
70. Overby CL, Kohane I, Kannry JL, Williams MS, Starren J, Bottinger E, Gottesman O, Denny JC, Weng C, Tarczy-Hornoch P, Hripcsak G. Opportunities for genomic clinical decision support interventions. Genet Med. 2013;15(10):817–23.
71. Gray SW, Hicks-Courant K, Cronin A, Rollins BJ. Weeks JC (2014) Physicians' attitudes about multiplex tumor genomic testing. J Clin Oncol. 2014 May 1;32(13):1317–23.
72. Starren J, Williams MS, Bottinger EP. Crossing the omic chasm: a time for omic ancillary systems. JAMA. 2013;309(12):1237–8.
73. Guo J, Xu N, Li Z, Zhang S, Wu J, Kim DH, et al. Four-color DNA sequencing with 3′-O-modified nucleotide reversible terminators and chemically cleavable fluorescent dideoxynucleotides. Proc Natl Acad Sci U S A. 2008;105(27):9145–50.
74. Ju J, Kim DH, Bi L, Meng Q, Bai X, Li Z, et al. Four-color DNA sequencing by synthesis using cleavable fluorescent nucleotide reversible terminators. Proc Natl Acad Sci U S A. 2006;103(52):19635–40.
75. Bentley DR, Balasubramanian S, Swerdlow HP, Smith GP, Milton J, Brown CG, et al. Accurate whole human genome sequencing using reversible terminator chemistry. Nature. 2008;456(7218):53–9.
76. Dohm JC, Lottaz C, Borodina T, Himmelbauer H. Substantial biases in ultra-short read data sets from high-throughput DNA sequencing. Nucleic Acids Res. 2008;36(16):e105.
77. Benjamini Y, Speed TP. Summarizing and correcting the GC content bias in high-throughput sequencing. Nucleic Acids Res. 2012;40(10):e72.
78. Ross MG, Russ C, Costello M, Hollinger A, Lennon NJ, Hegarty R, Nusbaum C, Jaffe DB. Characterizing and measuring bias in sequence data. Genome Biol. 2013;14(5):R51.
79. Aird D, Ross MG, Chen WS, Danielsson M, Fennell T, Russ C, Jaffe DB, Nusbaum C, Gnirke A. Analyzing and minimizing PCR amplification bias in Illumina sequencing libraries. Genome Biol. 2011;12(2):R18.

80. Loman NJ, Misra RV, Dallman TJ, Constantinidou C, Gharbia SE, Wain J, Pallen MJ. Performance comparison of benchtop high-throughput sequencing platforms. Nat Biotechnol. 2012;30(5):434–9.
81. Kircher M, Heyn P, Kelso J. Addressing challenges in the production and analysis of illumina sequencing data. BMC Genomics. 2011;12:382.
82. Rothberg JM, Hinz W, Rearick TM, Schultz J, Mileski W, Davey M, et al. An integrated semiconductor device enabling non-optical genome sequencing. Nature. 2011;475(7356):348–52.
83. Yeo ZX, Chan M, Yap YS, Ang P, Rozen S, Lee AS. Improving indel detection specificity of the ion torrent PGM benchtop sequencer. PLoS One. 2012;7(9):e45798.
84. Zeng F, Jiang R, Chen T. PyroHMMsnp: an SNP caller for ion torrent and 454 sequencing data. Nucleic Acids Res. 2013;41(13):e136.
85. Loman NJ, Quinlan AR. Poretools: a toolkit for analyzing nanopore sequence data. Bioinformatics. 2014;30(23):3399–401.
86. Merriman B, Ion Torrent R, Team D, Rothberg JM. Progress in ion torrent semiconductor chip based sequencing. Electrophoresis. 2012;33(23):3397–417.
87. Chen K, Wallis JW, McLellan MD, Larson DE, Kalicki JM, Pohl CS, et al. BreakDancer: an algorithm for high-resolution mapping of genomic structural variation. Nat Methods. 2009;6(9):677–81.
88. Abel HJ, Al-Kateb H, Cottrell CE, Bredemeyer AJ, Pritchard CC, Grossmann AH, Wallander ML, Pfeifer JD, Lockwood CM, Duncavage EJ. Detection of gene rearrangements in targeted clinical next-generation sequencing. J Mol Diagn. 2014;16(4):405–17.
89. Wang J, Mullighan CG, Easton J, Roberts S, Heatley SL, Ma J, et al. CREST maps somatic structural variation in cancer genomes with base-pair resolution. Nat Methods. 2011;8(8):652–4.
90. Gnerre S, Maccallum I, Przybylski D, Ribeiro FJ, Burton JN, Walker BJ, et al. High-quality draft assemblies of mammalian genomes from massively parallel sequence data. Proc Natl Acad Sci U S A. 2011;108(4):1513–8.
91. Schatz MC, Delcher AL, Salzberg SL. Assembly of large genomes using second-generation sequencing. Genome Res. 2010;20(9):1165–73.
92. Wang Y, Yang Q, Wang Z. The evolution of nanopore sequencing. Front Genet. 2014;5:449.
93. Shin SC, Ahn do H, Kim SJ, Lee H, Oh TJ, Lee JE, Park H. Advantages of single-molecule real-time sequencing in high-GC content genomes. PLoS One. 2013;8(7):e68824.
94. Vaughn CP, Robles J, Swensen JJ, Miller CE, Lyon E, Mao R, Bayrak-Toydemir P, Samowitz WS. Clinical analysis of PMS2: mutation detection and avoidance of pseudogenes. Hum Mutat. 2010;31(5):588–93.
95. Hansen MF, Neckmann U, Lavik LA, Vold T, Gilde B, Toft RK, Sjursen W. A massive parallel sequencing workflow for diagnostic genetic testing of mismatch repair genes. Mol Genet Genomic Med. 2014;2(2):186–200.
96. KF A, Underwood JG, Lee L, Wong WH. Improving PacBio long read accuracy by short read alignment. PLoS One. 2012;7(10):e46679.
97. Eid J, Fehr A, Gray J, Luong K, Lyle J, Otto G, et al. Real-time DNA sequencing from single polymerase molecules. Science. 2009;323(5910):133–8.
98. Schadt EE, Turner S, Kasarskis A. A window into third-generation sequencing. Hum Mol Genet. 2010;19(R2):R227–40.
99. Rasko DA, Webster DR, Sahl JW, Bashir A, Boisen N, Scheutz F, et al. Origins of the E. coli strain causing an outbreak of hemolytic-uremic syndrome in Germany. N Engl J Med. 2011;365(8):709–17.
100. Chin CS, Sorenson J, Harris JB, Robins WP, Charles RC, Jean-Charles RR, Bullard J, Webster DR, Kasarskis A, Peluso P, Paxinos EE, Yamaichi Y, Calderwood SB, Mekalanos JJ, Schadt EE, Waldor MK. The origin of the Haitian cholera outbreak strain. N Engl J Med. 2011;364(1):33–42.
101. Huddleston J, Ranade S, Malig M, Antonacci F, Chaisson M, Hon L, et al. Reconstructing complex regions of genomes using long-read sequencing technology. Genome Res. 2014;24(4):688–96.

102. Okoniewski MJ, Meienberg J, Patrignani A, Szabelska A, Matyas G, Schlapbach R. Precise breakpoint localization of large genomic deletions using PacBio and Illumina next-generation sequencers. BioTechniques. 2013;54(2):98–100.
103. Ritz A, Bashir A, Sindi S, Hsu D, Hajirasouliha I, Raphael BJ. Characterization of structural variants with single molecule and hybrid sequencing approaches. Bioinformatics. 2014;30(24):3458–66.
104. Koren S, Schatz MC, Walenz BP, Martin J, Howard JT, Ganapathy G, Wang Z, Rasko DA, McCombie WR, Jarvis ED, Phillippy AM. Hybrid error correction and de novo assembly of single-molecule sequencing reads. Nat Biotechnol. 2012;30(7):693–700. doi:10.1038/nbt.2280.
105. Archer J, Weber J, Henry K, Winner D, Gibson R, Lee L, et al. Use of four next-generation sequencing platforms to determine HIV-1 coreceptor tropism. PLoS One. 2012;7(11):e49602.
106. Jiao X, Zheng X, Ma L, Kutty G, Gogineni E, Sun Q, et al. A benchmark study on error assessment and quality control of CCS reads derived from the PacBio RS. J Data Mining Genomics Proteomics. 2013;4(3):1–5.
107. Stoddart D, Heron AJ, Klingelhoefer J, Mikhailova E, Maglia G, Bayley H. Nucleobase recognition in ssDNA at the central constriction of the alpha-hemolysin pore. Nano Lett. 2010;10(9):3633–7.
108. Manrao EA, Derrington IM, Laszlo AH, Langford KW, Hopper MK, Gillgren N, Pavlenok M, Niederweis M, Gundlach JH. Reading DNA at single-nucleotide resolution with a mutant MspA nanopore and phi29 DNA polymerase. Nat Biotechnol. 2012;30(4):349–53.
109. Tsutsui M, He Y, Furuhashi M, Rahong S, Taniguchi M, Kawai T. Transverse electric field dragging of DNA in a nanochannel. Sci Rep. 2012;2:394.
110. Singer A, McNally B, Torre RD, Meller A. DNA sequencing by nanopore-induced photon emission. Methods Mol Biol. 2012;870:99–114.
111. Derrington IM, Butler TZ, Collins MD, Manrao E, Pavlenok M, Niederweis M, Gundlach JH. Nanopore DNA sequencing with MspA. Proc Natl Acad Sci U S A. 2010;107(37):16060–5.
112. Laszlo AH, Derrington IM, Ross BC, Brinkerhoff H, Adey A, Nova IC, Craig JM, Langford KW, Samson JM, Daza R, Doering K, Shendure J, Gundlach JH. Decoding long nanopore sequencing reads of natural DNA. Nat Biotechnol. 2014;32(8):829–33.
113. Ashton PM, Nair S, Dallman T, Rubino S, Rabsch W, Mwaigwisya S, Wain J. O'Grady J (2014) MinION nanopore sequencing identifies the position and structure of a bacterial antibiotic resistance island. Nat Biotechnol. 2015 Mar;33(3):296–300.
114. Jain M, Fiddes IT, Miga KH, Olsen HE, Paten B, Akeson M. Improved data analysis for the MinION nanopore sequencer. Nat Methods. 2015;12:351–6.
115. Mikheyev AS, Tin MM. A first look at the Oxford Nanopore MinION sequencer. Mol Ecol Resour. 2014;14(6):1097–102.
116. O'Donnell CR, Wang H, Dunbar WB. Error analysis of idealized nanopore sequencing. Electrophoresis. 2013;34(15):2137–44.
117. Cavelier L, Ameur A, Haggqvist S, Hoijer I, Cahill N, Olsson-Stromberg U, Hermanson M. Clonal distribution of BCR-ABL1 mutations and splice isoforms by single-molecule long-read RNA sequencing. BMC Cancer. 2015;15:45.
118. Kastner R, Zopf A, Preuner S, Proll J, Niklas N, Foskett P, Valent P, Lion T, Gabriel C. Rapid identification of compound mutations in patients with Philadelphia-positive leukaemias by long-range next generation sequencing. Eur J Cancer. 2014;50(4):793–800.
119. Grossmann V, Kohlmann A, Klein HU, Schindela S, Schnittger S, Dicker F, et al. Targeted next-generation sequencing detects point mutations, insertions, deletions and balanced chromosomal rearrangements as well as identifies novel leukemia-specific fusion genes in a single procedure. Leukemia. 2011;25(4):671–80.
120. Baer C, Kern W, Koch S, Nadarajah N, Schindela S, Meggendorfer M, Haferlach C, Haferlach T. Ultra-deep sequencing leads to earlier and more sensitive detection of the tyrosine kinase inhibitor resistance mutation T315I in chronic myeloid leukemia. Haematologica. 2016;101(7):830–8.

121. Dressman D, Yan H, Traverso G, Kinzler KW, Vogelstein B. Transforming single DNA molecules into fluorescent magnetic particles for detection and enumeration of genetic variations. Proc Natl Acad Sci U S A. 2003;100(15):8817–22.
122. Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, Bemben LA, et al. Genome sequencing in microfabricated high-density picolitre reactors. Nature. 2005;437(7057):376–80.
123. Liu L, Li Y, Li S, Hu N, He Y, Pong R, Lin D, Lu L, Law M. Comparison of next-generation sequencing systems. J Biomed Biotechnol. 2012;2012:251364.
124. Thiede C, Koch S, Creutzig E, Steudel C, Illmer T, Schaich M, Ehninger G. Prevalence and prognostic impact of NPM1 mutations in 1485 adult patients with acute myeloid leukemia (AML). Blood. 2006;107(10):4011–20.
125. Valouev A, Ichikawa J, Tonthat T, Stuart J, Ranade S, Peckham H, et al. A high-resolution, nucleosome position map of C. elegans reveals a lack of universal sequence-dictated positioning. Genome Res. 2008;18(7):1051–63.
126. Huang YF, Chen SC, Chiang YS, Chen TH, Chiu KP. Palindromic sequence impedes sequencing-by-ligation mechanism. BMC Syst Biol. 2012;6(Suppl 2):S10.
127. Tuna M, Amos CI. Genomic sequencing in cancer. Cancer Lett. 2013;340(2):161–70.
128. Kim T, Yoshida K, Kim YK, Tyndel MS, Park HJ, Choi SH, et al. Clonal dynamics in a single AML case tracked for 9 years reveals the complexity of leukemia progression. Leukemia. 2016;30(2):295–302.
129. Farrar JE, Schuback HL, Ries RE, Wai D, Hampton OA, Trevino LR, et al. Genomic profiling of pediatric acute myeloid leukemia reveals a changing mutational landscape from disease diagnosis to relapse. Cancer Res. 2016;76(8):2197–205.
130. Clark MJ, Chen R, Lam HY, Karczewski KJ, Euskirchen G, Butte AJ, Snyder M. Performance comparison of exome DNA sequencing technologies. Nat Biotechnol. 2011;29(10):908–14.
131. Wooderchak-Donahue WL, O'Fallon B, Furtado LV, Durtschi JD, Plant P, Ridge PG, et al. A direct comparison of next generation sequencing enrichment methods using an aortopathy gene panel- clinical diagnostics perspective. BMC Med Genet. 2012;5:50.
132. Shiba N, Yoshida K, Shiraishi Y, Okuno Y, Yamato G, Hara Y, et al. Whole-exome sequencing reveals the spectrum of gene mutations and the clonal evolution patterns in paediatric acute myeloid leukaemia. Br J Haematol. 2016;175(3):476–89.
133. Masetti R, Castelli I, Astolfi A, Bertuccio SN, Indio V, Togni M, Belotti T, Serravalle S, Tarantino G, Zecca M, Pigazzi M, Basso G, Pession A. Locatelli F (2016) genomic complexity and dynamics of clonal evolution in childhood acute myeloid leukemia studied with whole-exome sequencing. Oncotarget. 2016 Aug 30;7(35):56746–57.
134. Cottrell CE, Al-Kateb H, Bredemeyer AJ, Duncavage EJ, Spencer DH, et al. Validation of a next-generation sequencing assay for clinical molecular oncology. J Mol Diagn. 2014;16(1):89–105.
135. Tsongalis GJ, Peterson JD, de Abreu FB, Tunkey CD, Gallagher TL, Strausbaugh LD, et al. Routine use of the ion torrent AmpliSeq™ cancer hotspot panel for identification of clinically actionable somatic mutations. Clin Chem Lab Med. 2014;52(5):707–14.
136. Singh RR, Patel KP, Routbort MJ, Reddy NG, Barkoh BA, Handal B, et al. Clinical validation of a next-generation sequencing screen for mutational hotspots in 46 cancer-related genes. J Mol Diagn. 2013;15(5):607–22.
137. Beadling C, Neff TL, Heinrich MC, Rhodes K, Thornton M, Leamon J, Andersen M, Corless CL. Combining highly multiplexed PCR with semiconductor-based sequencing for rapid cancer genotyping. J Mol Diagn. 2013;15(2):171–6.
138. Hadd AG, Houghton J, Choudhary A, Sah S, Chen L, Marko AC, et al. Targeted, high-depth, next-generation sequencing of cancer genes in formalin-fixed, paraffin-embedded and fine-needle aspiration tumor specimens. J Mol Diagn. 2013;15(2):234–47.
139. Escaramís G, Tornador C, Bassaganyas L, Rabionet R, Tubio JM, Martínez-Fundichely A, et al. PeSV-fisher: identification of somatic and non-somatic structural variants using next generation sequencing data. PLoS One. 2013;8(5):e63377.
140. Ye K, Schulz MH, Long Q, Apweiler R, Ning Z. Pindel: a pattern growth approach to detect break points of large deletions and medium sized insertions from paired-end short reads. Bioinformatics. 2009;25(21):2865–71.

141. Krumm N, Sudmant PH, Ko A, O'Roak BJ, Malig M, Coe BP, Quinlan AR, Nickerson DA, Eichler EE, Project NES. Copy number variation detection and genotyping from exome sequence data. Genome Res. 2012;22(8):1525–32.
142. Metzeler KH, Herold T, Rothenberg-Thurley M, Amler S, Sauerland MC, Gorlich D, et al. Spectrum and prognostic relevance of driver gene mutations in acute myeloid leukemia. Blood. 2016;128(5):686–98.
143. Kluk MJ, Lindsley RC, Aster JC, Lindeman NI, Szeto D, Hall D, Kuo FC. Validation and implementation of a custom next-generation sequencing clinical assay for hematologic malignancies. J Mol Diagn. 2016;18(4):507–15.
144. McKerrell T, Moreno T, Ponstingl H, Bolli N, Dias JM, Tischler G, et al. Development and validation of a comprehensive genomic diagnostic tool for myeloid malignancies. Blood. 2016;128(1):e1–9.
145. Grasso C, Butler T, Rhodes K, Quist M, Neff TL, Moore S, Tomlins SA, Reinig E, Beadling C, Andersen M. Corless CL (2015) assessing copy number alterations in targeted, amplicon-based next-generation sequencing data. J Mol Diagn. 2015 Jan;17(1):53–63.
146. Kadri S, Zhen CJ, Wurst MN, Long BC, Jiang ZF, Wang YL, Furtado LV, Segal JP. Amplicon Indel hunter is a novel bioinformatics tool to detect large somatic insertion/deletion mutations in amplicon-based next-generation sequencing data. J Mol Diagn. 2015;17(6):635–43.
147. Vidriales MB, Perez-Lopez E, Pegenaute C, Castellanos M, Perez JJ, Chandia M, et al. Minimal residual disease evaluation by flow cytometry is a complementary tool to cytogenetics for treatment decisions in acute myeloid leukaemia. Leuk Res. 2016;40:1–9.
148. Zhou Y, Othus M, Araki D, Wood BL, Radich JP, Halpern AB, et al. Pre- and post-transplant quantification of measurable ('minimal') residual disease via multiparameter flow cytometry in adult acute myeloid leukemia. Leukemia. 2016;30(7):1456–64.
149. Duployez N, Nibourel O, Marceau-Renaut A, Willekens C, Helevaut N, Caillault A, et al. Minimal residual disease monitoring in t(8;21) acute myeloid leukemia based on RUNX1-RUNX1T1 fusion quantification on genomic DNA. Am J Hematol. 2014;89(6):610–5.
150. Kohlmann A, Klein HU, Weissmann S, Bresolin S, Chaplin T, Cuppens H, et al. The Interlaboratory RObustness of next-generation sequencing (IRON) study: a deep sequencing investigation of TET2, CBL and KRAS mutations by an international consortium involving 10 laboratories. Leukemia. 2011;25(12):1840–8.
151. Grossmann V, Roller A, Klein HU, Weissmann S, Kern W, Haferlach C, Dugas M, Haferlach T, Schnittger S, Kohlmann A. Robustness of amplicon deep sequencing underlines its utility in clinical applications. J Mol Diagn. 2013;15(4):473–84.
152. Salipante SJ, Fromm JR, Shendure J, Wood BL, Wu D. Detection of minimal residual disease in NPM1-mutated acute myeloid leukemia by next-generation sequencing. Mod Pathol. 2014;27(11):1438–46.
153. Kohlmann A, Nadarajah N, Alpermann T, Grossmann V, Schindela S, Dicker F, et al. Monitoring of residual disease by next-generation deep-sequencing of RUNX1 mutations can identify acute myeloid leukemia patients with resistant disease. Leukemia. 2014;28(1):129–37.
154. Malmberg EB, Stahlman S, Rehammar A, Samuelsson T, Alm SJ, Kristiansson E, et al. Patient-tailored analysis of minimal residual disease in acute myeloid leukemia using next generation sequencing. Eur J Haematol. 2017;98(1):26–37.
155. Ivey A, Hills RK, Simpson MA, Jovanovic JV, Gilkes A, Grech A, et al. Group UKNCRIAW. Assessment of minimal residual disease in standard-risk AML. N Engl J Med. 2016; 374(5):422–433.
156. Bartram J, Mountjoy E, Brooks T, Hancock J, Williamson H, Wright G, Moppett J, Goulden N, Hubank M. Accurate sample assignment in a multiplexed, ultrasensitive, high-throughput sequencing assay for minimal residual disease. J Mol Diagn. 2016;18(4):494–506.
157. Genovese G, Kähler AK, Handsaker RE, Lindberg J, Rose SA, Bakhoum SF, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N Engl J Med. 2014;371(26):2477–87.
158. Jaiswal S, Fontanillas P, Flannick J, Manning A, Grauman PV, Mar BG, et al. Age-related clonal hematopoiesis associated with adverse outcomes. N Engl J Med. 2014;371(26):2488–98.

159. Wong TN, Miller CA, Klco JM, Petti A, Demeter R, Helton NM, et al. Rapid expansion of preexisting nonleukemic hematopoietic clones frequently follows induction therapy for de novo AML. Blood. 2016;127(7):893–7.
160. Schmitt MW, Fox EJ, Prindle MJ, Reid-Bayliss KS, True LD, Radich JP, Loeb LA. Sequencing small genomic targets with high efficiency and extreme accuracy. Nat Methods. 2015;12(5):423–5.
161. Young AL, Wong TN, Hughes AE, Heath SE, Ley TJ, Link DC, Druley TE. Quantifying ultra-rare pre-leukemic clones via targeted error-corrected sequencing. Leukemia. 2015;29(7): 1608–11.
162. Hiatt JB, Pritchard CC, Salipante SJ, O'Roak BJ, Shendure J. Single molecule molecular inversion probes for targeted, high-accuracy detection of low-frequency variation. Genome Res. 2013;23(5):843–54.
163. Hokland P, Ommen HB, Mule MP, Hourigan CS. Advancing the minimal residual disease concept in acute myeloid leukemia. Semin Hematol. 2015;52(3):184–92.
164. Hourigan CS, Karp JE. Minimal residual disease in acute myeloid leukaemia. Nat Rev Clin Oncol. 2013;10(8):460–71.
165. Young AL, Challen GA, Birmann BM, Druley TE. Clonal haematopoiesis harbouring AML-associated mutations is ubiquitous in healthy adults. Nat Commun. 2016;7:12484.
166. Xie M, Lu C, Wang J, McLellan MD, Johnson KJ, Wendl MC, et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. Nat med. 014 Dec. 2014;20(12):1472–8.
167. Babayan A, Alawi M, Gormley M, Muller V, Wikman H, McMullin RP, et al. Comparative study of whole genome amplification and next generation sequencing performance of single cancer cells. Oncotarget. 2016; doi:10.18632/oncotarget.10701. [Epub ahead of print].
168. Sabina J, Leamon JH. Bias in whole genome amplification: causes and considerations. Methods Mol Biol. 2015;1347:15–41.
169. Pugh TJ, Delaney AD, Farnoud N, Flibotte S, Griffith M, Li HI, Qian H, Farinha P, Gascoyne RD, Marra MA. Impact of whole genome amplification on analysis of copy number variants. Nucleic Acids Res. 2008;36(13):e80.
170. Paguirigan AL, Smith J, Meshinchi S, Carroll M, Maley C, Radich JP. Single-cell genotyping demonstrates complex clonal diversity in acute myeloid leukemia. Sci Transl Med. 2015;7(281):281re282.
171. Silva FP, Swagemakers SM, Erpelinck-Verschueren C, Wouters BJ, Delwel R, Vrieling H, van der Spek P, Valk PJ, Giphart-Gassler M. Gene expression profiling of minimally differentiated acute myeloid leukemia: M0 is a distinct entity subdivided by RUNX1 mutation status. Blood. 2009;114(14):3001–7.
172. Bullinger L, Rucker FG, Kurz S, Du J, Scholl C, Sander S, Corbacioglu A, Lottaz C, Krauter J, Frohling S, Ganser A, Schlenk RF, Dohner K, Pollack JR, Dohner H. Gene-expression profiling identifies distinct subclasses of core binding factor acute myeloid leukemia. Blood. 2007;110(4):1291–300.
173. Paul F, Arkin Y, Giladi A, Jaitin DA, Kenigsberg E, Keren-Shaul H, et al. Transcriptional heterogeneity and lineage commitment in myeloid progenitors. Cell. 2015;163(7):1663–77.
174. Yan P, Frankhouser D, Murphy M, Tam HH, Rodriguez B, Curfman J, et al. Genome-wide methylation profiling in decitabine-treated patients with acute myeloid leukemia. Blood. 2012;120(12):2466–74.
175. Metzeler KH, Heilmeier B, Edmaier KE, Rawat VP, Dufour A, Dohner K, et al. High expression of lymphoid enhancer-binding factor-1 (LEF1) is a novel favorable prognostic factor in cytogenetically normal acute myeloid leukemia. Blood. 2012;120(10):2118–26.
176. Kuhnl A, Valk PJ, Sanders MA, Ivey A, Hills RK, Mills KI, et al. Downregulation of the Wnt inhibitor CXXC5 predicts a better prognosis in acute myeloid leukemia. Blood. 2015;125(19):2985–94.
177. de Jonge HJ, Valk PJ, Veeger NJ, ter Elst A, den Boer ML, Cloos J, et al. High VEGFC expression is associated with unique gene expression profiles and predicts adverse prognosis in pediatric and adult acute myeloid leukemia. Blood. 2010;116(10):1747–54.

178. Piazza R, Cecchetti C, Pirola A, Donandoni C, Fontana D, Mezzatesta C, et al. RNA-seq is a valuable complement of conventional diagnostic tools in newly diagnosed AML patients. Am J Hematol. 2015;90(12):E227–8.
179. Panagopoulos I, Gorunova L, Zeller B, Tierens A, Heim S. Cryptic FUS-ERG fusion identified by RNA-sequencing in childhood acute myeloid leukemia. Oncol Rep. 2013;30(6):2587–92.
180. Zhou JB, Zhang T, Wang BF, Gao HZ, Xu X. Identification of a novel gene fusion RNF213SLC26A11 in chronic myeloid leukemia by RNA-Seq. Mol Med Rep. 2013;7(2): 591–7.
181. Guglielmelli P, Bisognin A, Saccoman C, Mannarelli C, Coppe A, Vannucchi AM, Bortoluzzi S. Small RNA sequencing uncovers new miRNAs and moRNAs differentially expressed in normal and primary Myelofibrosis CD34+ cells. PLoS One. 2015;10(10):e0140445.
182. Wilhelm BT, Briau M, Austin P, Faubert A, Boucher G, Chagnon P, Hope K, Girard S, Mayotte N, Landry JR, Hebert J, Sauvageau G. RNA-seq analysis of 2 closely related leukemia clones that differ in their self-renewal capacity. Blood. 2011;117(2):e27–38.
183. Spinelli R, Pirola A, Redaelli S, Sharma N, Raman H, Valletta S, et al. Identification of novel point mutations in splicing sites integrating whole-exome and RNA-seq data in myeloproliferative diseases. Mol Genet Genomic Med. 2013;1(4):246–59.
184. Brooks AN, Choi PS, de Waal L, Sharifnia T, Imielinski M, Saksena G, et al. A pan-cancer analysis of transcriptome changes associated with somatic mutations in U2AF1 reveals commonly altered splicing events. PLoS One. 2014;9(1):e87361.
185. Li XY, Yao X, Li SN, Suo AL, Ruan ZP, Liang X, Kong Y, Zhang WG, Yao Y. RNA-Seq profiling reveals aberrant RNA splicing in patient with adult acute myeloid leukemia during treatment. Eur Rev Med Pharmacol Sci. 2014;18(9):1426–33.
186. Rathe SK, Moriarity BS, Stoltenberg CB, Kurata M, Aumann NK, Rahrmann EP, et al. Using RNA-seq and targeted nucleases to identify mechanisms of drug resistance in acute myeloid leukemia. Sci Rep. 2014;4:6048.
187. Swanson L, Robertson G, Mungall KL, Butterfield YS, Chiu R, Corbett RD, et al. Barnacle: detecting and characterizing tandem duplications and fusions in transcriptome assemblies. BMC Genomics. 2013;14:550.
188. Francis RW, Thompson-Wicking K, Carter KW, Anderson D, Kees UR, Beesley AH. FusionFinder: a software tool to identify expressed gene fusion candidates from RNA-Seq data. PLoS One. 2012;7(6):e39987.
189. Macosko EZ, Basu A, Satija R, Nemesh J, Shekhar K, Goldman M, et al. Highly parallel genome-wide expression profiling of individual cells using Nanoliter droplets. Cell. 2015;161(5):1202–14.
190. Klein AM, Mazutis L, Akartuna I, Tallapragada N, Veres A, Li V, et al. Droplet barcoding for single-cell transcriptomics applied to embryonic stem cells. Cell. 2015;161(5):1187–201.
191. Nestorowa S, Hamey FK, Pijuan Sala B, Diamanti E, Shepherd M, Laurenti E, Wilson NK, Kent DG, Gottgens B. A single-cell resolution map of mouse hematopoietic stem and progenitor cell differentiation. Blood. 2016;128(8):e20–31.
192. Spencer DH, Young MA, Lamprecht TL, Helton NM, Fulton R, O'Laughlin M, et al. Epigenomic analysis of the HOX gene loci reveals mechanisms that may control canonical expression patterns in AML and normal hematopoietic cells. Leukemia. 2015;29(6):1279–89.
193. Haladyna JN, Yamauchi T, Neff T, Bernt KM. Epigenetic modifiers in normal and malignant hematopoiesis. Epigenomics. 2015;7(2):301–20.
194. Fong CY, Morison J, Dawson MA. Epigenetics in the hematologic malignancies. Haematologica. 2014;99(12):1772–83.
195. Network CGAR. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J Med. 2013;368(22):2059–74.
196. Wakita S, Yamaguchi H, Omori I, Terada K, Ueda T, Manabe E, et al. Mutations of the epigenetics-modifying gene (DNMT3a, TET2, IDH1/2) at diagnosis may induce FLT3-ITD at relapse in de novo acute myeloid leukemia. Leukemia. 2013;27(5):1044–52.
197. Ahn JS, Kim HJ, Kim YK, Jung SH, Yang DH, Lee JJ, et al. Adverse prognostic effect of homozygous TET2 mutation on the relapse risk of acute myeloid leukemia in patients of normal karyotype. Haematologica. 2015;100(9):e351–3.

198. Voso MT, Lo-Coco F, Fianchi L. Epigenetic therapy of myelodysplastic syndromes and acute myeloid leukemia. Curr Opin Oncol. 2015;27(6):532–9.
199. Gill H, Leung AY, Kwong YL. Molecularly targeted therapy in acute myeloid leukemia. Future Oncol. 2016;12(6):827–38.
200. Bravo GM, Lee E, Merchan B, Kantarjian HM, Garcia-Manero G. Integrating genetics and epigenetics in myelodysplastic syndromes: advances in pathogenesis and disease evolution. Br J Haematol. 2014;166(5):646–59.
201. Gronbaek K, Muller-Tidow C, Perini G, Lehmann S, Bach Treppendahl M, et al.; Epigenomics Study on MDS, Aml CABM (2012) A critical appraisal of tools available for monitoring epigenetic changes in clinical samples from patients with myeloid malignancies. Haematologica 97(9):1380–8.
202. McDevitt MA. Clinical applications of epigenetic markers and epigenetic profiling in myeloid malignancies. Semin Oncol. 2012;39(1):109–22.
203. Holstege H, Pfeiffer W, Sie D, Hulsman M, Nicholas TJ, Lee CC, et al. Somatic mutations found in the healthy blood compartment of a 115-yr-old woman demonstrate oligoclonal hematopoiesis. Genome Res. 2014;24(5):733–42.
204. Swisher EM, Harrell MI, Norquist BM, Walsh T, Brady M, Lee M, et al. Somatic mosaic mutations in PPM1D and TP53 in the blood of women with ovarian carcinoma. JAMA Oncol. 2016;2(3):370–2.
205. Tang G, Goswami RS, Liang CS, Bueso-Ramos CE, Hu S, DiNardo C, Medeiros LJ. Isolated del(5q) in patients following therapies for various malignancies may not all be clinically significant. Am J Clin Pathol. 2015;144(1):78–86.
206. Steensma DP, Bejar R, Jaiswal S, Lindsley RC, Sekeres MA, Hasserjian RP, Ebert BL. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. Blood. 2015;126(1):9–16.
207. Bianchi DW, Chudova D, Sehnert AJ, Bhatt S, Murray K, Prosen TL, Garber JE, Wilkins-Haug L, Vora NL, Warsof S, Goldberg J, Ziainia T, Halks-Miller M. Noninvasive prenatal testing and incidental detection of occult maternal malignancies. JAMA. 2015;314(2):162–9.
208. Snyder MW, Kircher M, Hill AJ, Daza RM, Shendure J. Cell-free DNA comprises an in vivo Nucleosome footprint that informs its tissues-of-origin. Cell. 2016;164(1–2):57–68.

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