Chapter 14
Acute Myeloid Neoplasms

Eric Y. Loo

Key Points

- While acute myeloid leukemia is identifiable by morphologic assessment alone, characterization of the underlying genetic abnormalities is needed for definitive subclassification in most cases.
- Current standard of care includes evaluating for selected gene sequence abnormalities (e.g., *FLT3*, *NPM1*, *CEBPA*, *KIT*, and others), in addition to traditional chromosome analysis and FISH studies.
- Karyotype still represents the single most important prognostic factor in predicting remission rates, relapse risks, and overall survival outcomes in acute myeloid leukemia.
- 40–50% of patients with de novo acute myeloid leukemia have a normal karyotype, and molecular profiling is quickly helping to better stratify this cohort with heterogeneous outcomes.

Key Online Resources

- National Comprehensive Cancer Network, Acute Myeloid Leukemia Guidelines: https://www.nccn.org/professionals/physician_gls/pdf/aml.pdf
- National Cancer Institute, Adult Acute Myeloid Leukemia Treatment: https://www.cancer.gove/types/leukemia/hp/adult-aml-treatment-pdq

E. Y. Loo (✉)
Geisel School of Medicine at Dartmouth, Hanover, NH, USA

Department of Pathology and Laboratory Medicine, Dartmouth-Hitchcock Medical Center, Lebanon, NH, USA
e-mail: Eric.Y.Loo@Hitchcock.org

L. J. Tafe, M. E. Arcila (eds.), *Genomic Medicine*,
https://doi.org/10.1007/978-3-030-22922-1_14

Introduction

Acute myeloid leukemia (AML) represents a heterogeneous group of disease, but all subtypes are characterized by clonal proliferations of immature myeloid hematopoietic precursor cells. The first widely accepted subclassification system for AML, developed by the French-American-British (FAB) working group in 1972, was based solely on morphologic findings. This classification system was unfortunately found to lack clinical utility as the proposed disease subtypes were largely unable to provide meaningful prognostic stratification. While the terminology from the FAB classification system still persists in present-day medical vernacular, this system is considered obsolete.

Janet Rowley described the t(8;21)(q22;q22.1) translocation in 1973; this was the first recurrent genetic aberrancy reported in association with AML. As the cytogenetic profile for this disease was slowly elucidated over subsequent decades, a diagnostic paradigm shift occurred in the 2001 third edition of the World Health Organization (WHO) classification with inclusion of genetic abnormalities into the diagnostic algorithms for AML diagnosis. The importance of underlying cytogenetic aberrancies was recognized, as was secondary-AML arising from lower-grade myeloid neoplasms, prior cytotoxic therapies for unrelated malignancy, or disease arising in a background of multilineage dysplasia. These categories were expanded and refined further in 2008 and 2016. The 2016 WHO classification system is the most current AML classification system, and the use of prior less-specific terminology is discouraged [1].

The ability to risk-stratify cases of primary-AML was somewhat limited in first iteration of the WHO classification system. Recognized genetic defects were limited to chromosomal translocations at the time, and conventional cytogenetic testing modalities fail to detect aberrancies in a significant subset of cases (a disease subgroup often referred to as “normal karyotype AML”). The 2008 WHO revision broadened the scope of genetics in AML diagnosis, accepting that multiple types of genetic lesions could cooperate to create a leukemic process. More recent molecular sequencing studies have further characterized the genetic landscape of AML and have helped to close the knowledge gap. It is now understood that numerous cooperating mutations occur in AML [2]. While molecular profiling analysis is initially focused on normal karyotype AML, somatic sequence mutations appear to demonstrate prognostic importance across other genetic AML subtypes, and sequencing analysis appears to be indicated in all cases of AML [2, 3]. At present, most cases of primary/de novo AML can be genetically categorized, and several specific subtypes of AML can be diagnosed on the basis of underlying genetics without regard to blast cell count. The subgroup of AML, not otherwise specified, which has no distinct clinical, immunophenotypic, or genetic features is expected to continue to shrink as knowledge of AML pathogenesis accumulates (Fig. 14.1).

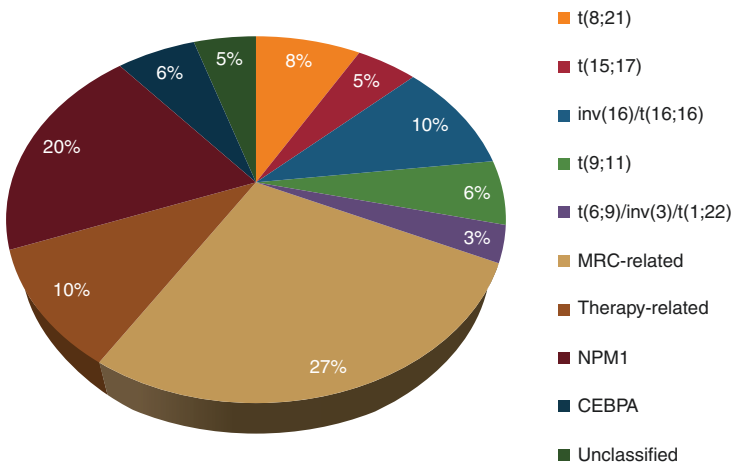

Fig. 14.1 General breakdown of AML subtypes by 2016 WHO classification

A Standard Genetic Workup

The specimen for evaluation (peripheral blood or bone marrow) should be obtained before initiation of any definitive therapy. At present, a standard workup for newly diagnosed AML should include:

- Complete karyotype and/or FISH analysis for subtype defining aberrancies
- *NPM1*, *CEBPA*, *RUNX1*, and *FLT3* somatic sequence mutation analysis
- *IDH1/2* mutation analysis for potential targeted therapy in relapsed/refractory disease
- *KIT* mutation analysis in all cases with t(8;21)(q22;q22.1) RUNX1-RUNX1T1 and AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22) CBFB-MYH11

Although detection of recurrent cytogenetic aberrancies generally provides the most significant prognostic information at diagnosis, nearly half of all adult AML cases will show no detectable abnormalities by karyotype. Molecular genetic analysis is quickly filling the knowledge gap. Many other gene mutations are also known to have prognostic significance or relevance for clinical trials in AML and may be readily evaluable by targeted next-generation sequencing gene mutation panels (a selection of these are found in Table 14.1).

AML with Recurrent Genetic Aberrancies

This category of acute myeloid leukemia includes entities that are defined by both by balanced chromosomal rearrangements and by specific gene sequence

Table 14.1 Genes with recurrent somatic sequence mutations in AML

Mutated gene	Prognostic significance in AML
ASXL1	Associated with worse DFS, EFS, RFS, and OS compared to cases with wt-*ASXL1*
CEBPA, biallelic	WHO AML subtype defining Better DFS, EFS, and OS compared to cases with single mutation or wt-CEBPA
DNMT3A	Conflicting reports regarding prognostic significance Concurrent mutation with mut-*NPM1* and *FLT3*-ITD appears to worsen EFS and OS
IDH1/2	Conflicting reports regarding prognostic significance Mut-*IDH1/2* qualifies for FDA-approved IDH inhibitor therapy in relapsed or refractory AML
FLT3-ITD	Significantly worse OS compared to those without *FLT3*-ITD, especially in persons <60 years old If present in the setting of AML with mutated *NPM1*, significantly worse EFS, RFS, DFS, and OS compared to cases with *NPM1* mutation alone 2017 ELN guidelines recognize increasingly worse RR and OS with increasing *FLT*-ITD mutant allele burden; ELN guidelines define *FLT3*-ITD^{low} as <0.5 and *FLT3*-ITD^{high} as ≥0.5 *FLT3*-ITD (and tyrosine kinase domain mutation) qualify for FDA-approved targeted therapy with Midostaurin
KIT	In t(8;21) AML, associated with shorter DFS, RFS, EFS, and OS compared to cases with wt-*KIT*
	In the setting of inv(16)/t(16;16) AML, no difference in EFS, RFS, PRS, or OS compared to cases with wt-*KIT* Some single studies report shorter RFS and OS compared to cases with wt-*KIT*
KMT2A-PTD	Among younger patients (<60 years), shorter OS than for patients without *KMT2A*-PTD
NPM1	WHO AML subtype defining In isolation, generally associated with good response to induction chemotherapy and a favorable prognosis If concurrent with *FLT3-ITD*, then EFS, RFS, DFS, and OS are significantly worsened
RUNX1	Potentially indicative of a WHO AML provisional subtype (in the absence of other subtype defining abnormalities) Shorter DFS, RFS, EFS, and OS compared to cases with wt-*RUNX1*
TET2	Conflicting reports regarding prognostic significance; some reports indicate no significant differences, whereas others report context-dependent worse EFS and OS
TP53, mut or loss	In the setting of a complex karyotype (≥3 abnormalities), associated with shorter RFS, EFS, and OS than for patients with wt-*TP53*
	In the setting of a complex karyotype (≥5 abnormalities), no significant difference in DFS or OS
	When associated with abnormalities of chromosomes, 5, 7, or 17, and/or a complex karyotype (≥5 abnormalities), associated with shorter OS than for patients with wt-*TP53*
WT1	Associated with worse RR and OS compared to cases with wt-*WT1* In the setting of pediatric AML, associated with worse treatment-resistant disease, EFS, and OS

DFS disease-free survival, *EFS* event-free survival, *ELN* European LeukemiaNet, *mut* mutant, *OS* overall survival, *PFS* progression-free survival, *PTD* partial tandem duplication, *RFS* relapse-free survival, *RR* risk of relapse, *wt* wild type

mutations. The 2016 WHO classification system recognizes eight subtypes defining balanced chromosomal gene fusions and three subtypes related to specific somatic gene sequence mutations, each with distinctive clinicopathologic features and prognostic associations. Many other balanced gene rearrangements are known to recur in AML [4], but these are very rare and are not currently recognized to represent distinct diagnostic entities.

The diagnosis of AML typically requires demonstrating a myeloblast population that represents at least 20% of the peripheral blood or bone marrow cellularity. However, the WHO permits assigning an AML diagnosis without regard to blast count for three entities, based on the strength of associated underlying cytogenetic aberrancies. These entities are the two core binding factor AMLs associated with t(8;21) and inv(16)/t(16;16) and acute promyelocytic leukemia with *PML-RARA* fusion. The minimum threshold of 20% myeloblasts is still required for an AML diagnosis with the remaining recurrent genetic aberrancies.

Core Binding Factor AML *(Tables 14.2 and 14.3)*

AML with t(8;21)(q22;q22.1) results in the fusion of *RUNX1* (also known as core binding factor-α) and *RUNX1T1*, often presenting with large myeloblasts that have abundant basophilic cytoplasm, azurophilic granules, few large pseudo-Chédiak-Higashi granules, and perinuclear hoffs. AML with inv(16)(p13q22) or t(16;16)(p13;q22) *CBFB-MYH11* disrupts the beta subunit of core binding factor, often presenting with myelomonocytic blasts and abnormal background eosinophils, usually with large basophilic colored granules. These translocations disrupt the function of core binding factor, a crucial heterodimeric transcription factor that helps control stem cell development and normal hematopoiesis. Together these represent about 12–15% of acute myeloid leukemia cases in adults and are commonly referred to as the core binding factor (CBF) leukemias.

Table 14.2 General features of AML with t(8;21)(q22;q22.1)

Defining aberrancy	t(8;21)(q22;q22.1)
Genes involved	*RUNX1-RUNX1T1*
Frequency in adult AML	1–5%
Myeloblast requirement	Does not require 20% blast threshold for diagnosis of AML
Prognostic implication	Generally favorable
Prognostic modifier(s)	*KIT* gene mutation (20–30% of cases): higher risk of relapse and worse overall survival
2° cytogenetic abnormalities	Found in >70% of cases; more frequent findings include loss of a sex chromosome or del(9q)
Diagnostic testing	Karyotype and FISH PCR testing is available, but typically not used for diagnosis

Table 14.3 General features of AML with inv(16)(p13q22) or t(16;16)(p13;q22)

Defining aberrancy	inv(16)(p13q22) or t(16;16)(p13;q22)
Genes involved	*CBFB-MYH11*
Frequency in adult AML	5–8%
Myeloblast requirement	Does not require 20% blast threshold for diagnosis of AML
Prognostic implication	Generally favorable
Prognostic modifier(s)	Unfavorable: Trisomy 8 *KIT* gene mutation (30–40% of cases): higher risk of relapse and worse overall survival, effect not as severe as in cases with t(8;21) [1] *FLT3* mutations Favorable: Trisomy 22
2° cytogenetic abnormalities	Found in ~40% of cases; more frequent findings include gains of chromosomes 8, 21, and 22 and losses of 7q[1]
Diagnostic testing	Karyotype and FISH inv(16) is often subtle and may be missed on chromosome analysis; thus FISH testing may be preferred PCR testing is available, but typically not used for diagnosis

Most of these cases will also carry other cytogenetic aberrancies. Presence of secondary cytogenetic aberrations or complex karyotypes do not appear to affect clinical outcomes for patients with t(8;21) AML [5]. In AML with inv(16) or t(16;16), trisomy 8 is associated with a worse prognosis, and trisomy 22 has been associated with an improved prognosis [6]. Somatic sequence mutations in *KIT* exons 8 and 17 are associated with a worse prognosis [1], and patients may benefit from hematopoietic stem cell transplant at first remission. Sequence mutations in genes activating tyrosine kinase signaling are frequent in both subtypes of CBF-AML; genes involving the RTK/RAS signaling pathways are affected in nearly 30% of cases and may suggest shorter event-free survival [7]. Genes involved in chromatin modification of the cohesin complex are seen at high frequencies in t(8;21) AML (42% and 18%, respectively), but are generally absent in inv(16)/t(16;16) AML [8]. Similarly *ASXL2* mutations are seen in 20–25% of patients with t(8;21) AML, but are uncommon in inv(16)/t(16;16) disease [9]. RT-PCR targeted against fusion transcripts have been used for minimal residual disease (MRD) assessment in CBF-AML which appears to allow for identification of patients at high risk of relapse [10, 11]. MRD monitoring early after transplant may be more predictive of relapse risk than presence of *KIT* mutations [12].

Acute Promyelocytic Leukemia (APL) (Table 14.4)

APL presents with a predominance of abnormal promyelocytes and arises in the setting of fusion of the *PML* (a nuclear regulatory factor) and *RARA* (retinoic acid

Table 14.4 General features of APL with *PML-RARA*

Defining aberrancy	t(15;17)(q24.1;q21.2) The WHO no longer includes the karyotype in the disease name as the disease is defined by the gene fusion, even when cryptic
Genes involved	*PML-RARA*
Frequency in adult AML	5–8%
Myeloblast requirement	Does not require 20% blast threshold for diagnosis of AML
Prognostic implication	Has most favorable long-term outcomes of all AML subtypes, though significant complications may arise at disease onset
Prognostic modifier(s)	Secondary genetic abnormalities are of unclear prognostic relevance in the context of current therapy FLT3 mutations found in 30–40% of cases, internal tandem duplications are more frequent Alternate *RARA* translocations with *ZBTB16* and *STAT5B* show resistance to ATRA differentiation therapy
2° cytogenetic abnormalities	Found in ~40% of cases; gains of chromosomes 8 in ~10–15% of cases
Diagnostic testing	Karyotype and FISH FISH testing is preferred due to the shorter time to result PCR testing is available; while typically used for disease monitoring, it may be helpful for diagnostic confirmation in rare FISH cryptic cases

receptor alpha) genes. This fusion protein acts as a constitutive transcriptional repressor of RARα target-genes, but this repression may be alleviated by pharmacologic doses of tretinoin [13]. The leukemic blasts are highly sensitive to differentiating agents, tretinoin (also referred to as ATRA, all-*trans*-retinoic acid) and arsenic trioxide [14], as well as to anthracycline-based chemotherapy. APL is classically associated with the t(15;17)(q24.1;q21.2) translocation, but may arise from cryptic or variant *PML-RARA* fusions.

Three breakpoint cluster regions (bcr) are described in the *PML* gene; fusions involving bcr1 and bcr2 are of similar size and are together referred to as long (L) isoform, and those involving bcr3 result in a short (S) isoform [15]. Hypergranular/typical APL represents ~70% of all cases and is often associated with the long isoform. The short isoform is more common in the microgranular (also called hypogranular) variant APL. Both variants are associated with a high risk of disseminated intravascular coagulation, increased fibrinolysis, and significant coagulopathy associated with early death [16].

Secondary cytogenetic abnormalities are found in about 40% of cases. *FLT3* mutations are found in 30–40% of cases, and *FLT3*-ITD is associated with a higher WBC count, microgranular morphology, and involvement of the bcr3 breakpoint [17]. Variant *RARA* translocations also occur with gene partners other than *PML*. Described variant fusion partners include *ZBTB16* at 11q23.2, *NUMA1* at 11q13.4, *NPM1* at 5q35.1, and *STAT5B* at 17q11.2 [18]. Such cases should be diagnosed as "APL with a variant *RARA* translocation." The *ZBTB16-RARA* and *STAT5B-RARA* translocations demonstrate resistance to ATRA differentiation therapy [19].

Minimal residual disease (MRD) monitoring for *PML-RARA* transcripts by PCR is currently the best predictor of relapse-free survival [20]. Detection of *PML-RARA* by RT-PCR in the immediate post-treatment period does not impact the clinical outcome, as abnormal promyelocytes may persist for several weeks after initiating therapy. However, detection of fusion transcripts after achieving complete remission is strongly predictive of relapse, and early pre-emptive therapy may prevent overt clinical relapse [20, 21].

AML with t(9;11)(p21.3;q23.3), KMT2A-MLLT3 (Table 14.5)

This subtype accounts for about 2% of adult AML but represents 9–12% of pediatric cases. The leukemic blasts often show monocytic or myelomonocytic differentiation, and patients may present with disseminated intravascular coagulation, myeloid sarcoma, or soft tissue infiltration. *KMT2A* encodes a histone methyltransferase which participates in chromatin remodeling. While fusions involving *KMT2A* are seen in 5–10% of all AML, the WHO classification for this category is limited specifically to t(9;11)(p21.3;q23.3) [1].

Over 130 different translocations involving *KMT2A* have been described, including greater than 90 different gene fusion partners and at least 6 translocations with no obvious gene fusions [22]. Translocations with *MLLT3* are the most common of these (~30% of cases) and appear to define a more distinct pathologic entity [1, 22]. AML with other balanced translocations of 11q23.3 are classified as AML, not otherwise specified, though the translocation should also be stated in the diagnostic line (except in cases which meet criteria for therapy-related AML or AML with myelodysplasia-related changes).

Secondary cytogenetic aberrancies and complex karyotypes may be seen in t(9;11) AML, but do not appear to affect clinical outcomes for these patients [5]. Somatic sequence mutations of *NRAS* or *KRAS* are seen in 30–40% of cases, but

Table 14.5 General features of AML with t(9;11)(p21.3;q23.3)

Defining aberrancy	t(9;11)(p21.3;q23.3)
Genes involved	*KMT2A-MLLT3*
Frequency in adult AML	~2%
Myeloblast requirement	Requires ≥20% myeloblasts for AML diagnosis
Prognostic implication	Intermediate prognostic risk
Prognostic modifier(s)	Overexpression of *MECOM (EVI1)* associated with very poor prognosis
2° cytogenetic abnormalities	Varied, including trisomy 8 and complex karyotypes
Diagnostic testing	Karyotype and FISH

the incidence of *FLT3* mutations is low compared to other AML subtypes [23]. Overexpression of *MECOM* (previously known as *EVI1*) has been reported in about 40% of cases, and some reports suggest that t(9;11) AML positive for overexpression are biologically distinct from *MECOM*-negative cases [24]. Patients with de novo AML and t(9;11)(p21.3;q23.3) are at intermediate prognostic-risk but appear to have a relatively better survival than patients with other translocations at 11q23, who generally experience more adverse clinical outcomes [25]. Overexpression of *MECOM* in *KMT2A*-rearranged AML is associated with a very poor prognosis [24, 26].

AML with t(6;9)(p23;q34.1), DEK-NUP214 (Table 14.6)

This uncommon subtype accounts for 0.7–1.8% of all cases and often presents with basophilia (≥2% basophils), cytopenias, and multilineage dysplasia [1]. Despite the presence of multilineage dysplasia, the t(6;9) takes precedence over the less specific diagnosis of AML with myelodysplasia-related changes (AML-MRC). *NUP214* encodes the CAN nucleoporin. Fusion with the *DEK* oncogene results in abnormal transcription factor activity, most likely due to altered nuclear transport due to binding of soluble transport factors [27].

The *DEK-NUP214* fusion is the sole cytogenetic abnormality identified in nearly 90% of cases [28]. AML with t(6;9) has poor survival rates with conventional chemotherapy, and patients may benefit from allogeneic hematopoietic stem cell transplantation. Although the WHO requires ≥20% myeloblasts to diagnose this entity, this threshold requirement is controversial. *FLT3*-ITD mutation is found in 70–80% of cases, but the poor prognosis of this AML subtype is independent of *FLT3* mutation status [29, 30].

Table 14.6 General features of AML with t(6;9)(p23;q34.1)

Defining aberrancy	t(6;9)(p23;q34.1)
Genes involved	*DEK-NUP214*
Frequency in adult AML	0.7–1.8%
Myeloblast requirement	Requires ≥20% myeloblasts for AML diagnosis (controversial)
Prognostic implication	Poor prognostic risk
Prognostic modifier(s)	*FLT3* mutations found in 70–80% of cases, but finding does not appear to confer additional negative prognostic risk
2° cytogenetic abnormalities	Uncommon, though complex karyotypes have been described
Diagnostic testing	Karyotype and FISH

AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); GATA2, MECOM (*Table 14.7*)

This uncommon subtype accounts for 1–2% of AML and often presents with normal to increased platelet counts and multilineage dysplasia, typically with prominent uni- or bi-lobed dwarf megakaryocytes [1]. Despite the presence of multilineage dysplasia, the presence of inv(3) or t(3;3) takes diagnostic precedence over the less specific diagnosis of AML-MRC. This rearrangement pairs the oncogene *MECOM* with a *GATA2* enhancer. No abnormal fusion transcript is generated by this gene rearrangement, but it contributes to leukemogenesis by both stimulating *MECOM* expression and causing *GATA2* insufficiency [31, 32]. Inappropriate expression of *MECOM* (previously known as *EVI1*) is seen in a variety of AMLs, and high expression is a poor prognostic indicator independent of 3q26.2 translocations [33]. While other aberrancies involving chromosome 3q26.2 and variant fusion partners for *MECOM* have been described, these are currently excluded by WHO from the AML with recurrent genetic abnormalities disease category [1].

Secondary cytogenetic aberrancies are found in most cases of AML with inv(3) or t(3;3) and are of the variety that are typically associated with myelodysplasia. Monosomy 7 can be found in up to 66% cases, and chromosome 5q deletions and complex karyotypes are also commonly described [34]. Activating mutations in genes affecting the RAS/receptor tyrosine kinase signaling pathways are found in about 98% of cases, including *NRAS, PTPN11, FLT3, KRAS, NF1, CBL,* and *KIT* [35]. Other commonly mutated genes include *GATA2*, *RUNX1*, and *SF3B1* [35, 36].

This subtype of AML is typically associated with an aggressive disease course, therapy resistance, and short survival. Although the WHO requires ≥20% myeloblasts to diagnose this entity, this threshold requirement is controversial as disease associated with inv(3) or t(3;3) and <20% blasts have an equally poor outlook, similar clinicopathologic features, and identical mutational patterns at the molecular genomic level [1]. A complex karyotype or concomitant monosomy 7 worsens the already adverse prognosis associated with this subtype [34].

Table 14.7 General features of AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)

Defining aberrancy	inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)
Genes involved	*GATA2, MECOM*
Frequency in adult AML	1–2%
Myeloblast requirement	Requires ≥20% myeloblasts for AML diagnosis (controversial)
Prognostic implication	Poor prognostic risk
Prognostic modifier(s)	Monosomy 7 or complex karyotype associated with poorer prognosis
2° cytogenetic abnormalities	Seen in 75% of cases and are typically "MDS-related," including −5q, −7, and complex karyotypes
Diagnostic testing	FISH Chromosome 3q26.2 rearrangements may be cryptic to conventional chromosome analysis

Table 14.8 General features of AML with t(1;22)(p13.3;q13.1)

Defining aberrancy	t(1;22)(p13.3;q13.1)
Genes involved	*RBM15-MRTFA* (*MRTFA* previously known as *MKL1)*
Frequency in adult AML	<1%
Myeloblast requirement	Requires ≥20% myeloblasts for AML diagnosis
Prognostic implication	Intermediate prognostic risk
Prognostic modifier(s)	–
2° cytogenetic abnormalities	Uncommon, though additional abnormalities more frequently found in "older" patients (>6 months)
Diagnostic testing	Karyotype and FISH

AML (Megakaryoblastic) with t(1;22)(p13.3;q13.1), RBM15-MRTFA (Table 14.8)

This rare subtype with megakaryoblasts represents <1% of all AML cases. It presents almost exclusively in infants; 80% of diagnoses are made within the first year of life, and most occur within the first 6 months [1]. These children usually have marked hepatosplenomegaly, cytopenias, and a densely fibrotic marrow with bilateral periostitis or osteolytic lesions. The patient may also present with a soft tissue mass, mimicking other small round blue cell tumors. The translocation fuses *RBM15*, a RNA recognition motif-encoding gene, to *MRTFA* (previously known as *MLK1*), a protein with a DNA-binding motif involved in chromatin organization [37].

AML with t(1;22) represents only ~14% of the non-Down syndrome acute megakaryoblastic leukemias [38]. In most cases, the *RBM15-MRTFA* fusion is the sole cytogenetic aberrancy [1]. When compared to other de novo cases of non-Down syndrome acute megakaryoblastic leukemia, presence of the t(1;22) translocation appears to be associated with intermediate-risk disease and inferior event-free survival [38, 39].

AML with Mutated NPM1 (Table 14.9)

Mutations in *NPM1* are among the most frequent acquired genetic abnormalities in AML, occurring in 2–8% of childhood cases, 27–35% of adult cases, and 45–64% of adult normal karyotype (NK) AML [1]. The *NPM1* gene encodes nucleophosmin, a multifunctional chaperone protein which localizes to the nucleus, participates in the biogenesis of ribosomes, and helps regulate the ARF-TP53 tumor suppressor pathway [40, 41]. Mutations typically involve exon 12 and lead to a frameshift in the C-terminal protein region, with subsequent cytoplasmic displacement of the protein [42]. *NPM1* mutations are also considered late aberrancies in

Table 14.9 General features of AML with mutated *NPM1*

Defining aberrancy	*NPM1* exon 12 mutation
Genes involved	*NPM1*
Frequency in adult AML	27–35%
Myeloblast requirement	Requires ≥20% myeloblasts for AML diagnosis
Prognostic implication	Generally favorable
Prognostic modifier(s)	MDS-related cytogenetic abnormalities take diagnostic priority Concurrent *FLT3-ITD* mutation is associated with an intermediate prognostic risk Concurrent FLT3-ITD and DNMT3A mutation have particularly adverse impact on overall and event-free survival
2° cytogenetic abnormalities	Typically normal karyotype; 5–15% of cases may have a diagnostically nonspecific abnormality
Diagnostic testing	Molecular testing (sequencing, fragment size analysis, etc.) Cytoplasmic nucleophosmin staining by immunohistochemistry has been used as a surrogate method for detection of the gene mutation

leukemogenesis, following earlier somatic mutations in genes involved in epigenetic regulatory processes such as DNA methylation, histone modification, and chromatin looping [43, 44].

The leukemic blasts often have a monocytic or myelomonocytic phenotype.

NPM1 mutations are usually mutually exclusive of other recurrent AML-defining cytogenetic aberrancies and are typically associated with normal karyotypes [45]. A minority of cases (5–15%) will carry nonspecific chromosomal alterations such as +4, +8, −Y, del(9q), and +21; however these findings do not appear to alter the disease profile or survival outcomes, when compared to "normal-karyotype disease" [45]. Cytogenetic aberrancies typically associated with myelodysplasia are uncommon in this setting of *NPM1*-mutated AML [46], but morphologic dysplasia may be seen in up to a quarter of cases. However, AML-MRC-related cytogenetic abnormalities should take diagnostic precedence if detected. Other acquired sequence mutations are common, and commutated genes often include *FLT3, TET2, DNMT3A, IDH1/2,* and *KRAS/NRAS* and cohesin complex genes [47].

In NK-AML, *NPM1* mutation confers a favorable prognosis, similar to that of core binding factor AMLs [48]. A significant minority (~25%) of *NPM1*-mutated NK-AML may have multilineage dysplasia, but the finding does not impact the good prognosis associated with *NPM1* mutation unless myelodysplasia-associated cytogenetic aberrancies are also detected [49]. About 40% of *NPM1*-mutated AML will have concurrent *FLT3*-ITD mutations, and this abnormality appears to negate the favorable prognostic effect [50]. The relative allelic ratio of *FLT3*-ITD appears to have prognostic significance in this setting, and *NPM1*-mutated patients with a low-allelic burden of *FLT3*-ITD (i.e., <0.5) seem to retain favorable outcomes [51]. Regardless, patients with *NPM1* mutation appear to have a better prognosis than

patients with *FLT3*-ITD and wild-type *NPM1*, especially in cases with a high *FLT3*-ITD allelic ratio (i.e., ≥0.5) [52, 53]. Concurrent mutations of *NPM1, FLT3*-ITD, and *DNMT3A* appear to have a particularly adverse impact on overall and event-free survival [54].

AML with Biallelic Mutations of CEBPA (Table 14.10)

Biallelic mutations of *CEBPA* may be seen in 4–9% of children with AML, at a lower frequency in adult disease, and are generally associated with a good prognosis similar to that seen in CBF-AML [1]. *CEBPA* encodes a protein called CCAAT enhancer-binding protein alpha, which serves multiple functions including as a hematopoiesis-associated transcription factor and also as a tumor suppressor gene. Biallelic gene mutation is required for diagnosis; the favorable prognostic association is linked to a specific gene expression profile that is not identified with single allele mutation [1, 55, 56]. Only sequence mutations of the *CEBPA* gene are taken into diagnostic consideration for this subtype, though there are many routes that can lead to *CEBPA* inactivation. This AML subtype does not have particularly distinctive morphologic features.

More than 70% of cases will be associated with a normal karyotype. Factors which might negatively impact the favorable prognostic risk include presence of cytogenetic aberrancies (i.e., an abnormal karyotype) and co-mutation with *FLT3*-ITD [57, 58], though this still requires additional clarification [1]. Concurrent *GATA1* and *WT1* mutations are relatively frequent in patients with biallelic *CEBPA* mutation, but *FLT3*-ITD*, NPM1, ASXL1,* and *RUNX1* mutations are uncommon and

Table 14.10 General features of AML with biallelic mutations of CEBPA

Defining aberrancy	Biallelic *CEBPA* mutations
Genes involved	*CEBPA*
Frequency in adult AML	~4–9% in children/young adults, likely less in adult disease
Myeloblast requirement	Requires ≥20% myeloblasts for AML diagnosis
Prognostic implication	Generally favorable
Prognostic modifier(s)	FLT3-ITD (5–9% of cases) and GATA2 mutations (~39% of cases) are described; currently have unclear significance Cytogenetic aberrancies; currently unclear significance Myelodysplasia-related abnormalities take diagnostic priority
2° cytogenetic abnormalities	Found in ~5–15% of cases; typically diagnostically nonspecific
Diagnostic testing	Molecular testing (gene sequencing) Sequencing CEBPA and confirmation of biallelic mutation is technically challenging due to high GC content and short sequence reads

Table 14.11 Cytogenetic abnormalities diagnostic[a] for AML-MRC

1. Complex karyotype (defined as three or more unrelated clonal abnormalities)	
2. Unbalanced abnormality	3. Balanced translocations
−7/del(7q)	t(11;16)(q23.3;p13.3)
del(5q)/t(5q)	t(3;21)(q26.2;q22.1)
i(17q)/t(17p)	t(1;3)(p26.3;q21.2)
−13/del(13q)	t(2;11)(p21;q23.3)
del(11q)	t(5;12)(q32;p13.2)
del(12p)/t(12p)	t(5;7)(q32;q11.2)
idic(x)(q13)	t(5;17)(q32;p13.2)
–	t(5;10)(q32;q21)
–	t(3;5)(q25.3;q35.1)

[a]Presence of any of these cytogenetic abnormalities is considered sufficiently specific to diagnose AML with MRC when there are ≥20% blood or marrow-based myeloblasts and prior therapy has been excluded

seen more frequently in *CEBPA* monoallelic cases [59]. Cytogenetic aberrancies typically associated with myelodysplasia are uncommon in this setting of biallelic *CEBPA* mutation, but morphologic dysplasia may be seen in about a quarter of cases [1, 60]. The finding of dysplasia alone does not influence the prognosis, but AML-MRC-related cytogenetic abnormalities should take diagnostic precedence if detected [1, 60] (see Table 14.11).

Germline mutation of *CEBPA* is also a described phenomenon and is well associated with predisposition to develop AML. Therefore, identification of biallelic *CEBPA* mutation in AML should prompt evaluation of possible germline inheritance, especially in patients presenting as children or young adults (see section "Myeloid Neoplasms with Germline Predisposition").

Provisional 2016 WHO AML with Recurrent Genetic Abnormality Subtypes

BCR-ABL1 fusion and *RUNX1* mutation define new provisional entities in the 2016 WHO AML classification system. AML with *BCR-ABL1* is a de novo AML with no evidence of chronic myeloid leukemia, both prior to and after therapy. This is a rare subtype, accounting for <1% of all cases of AML [1, 61]. Most cases demonstrate the p210 fusion, though a minority of reported cases had p190 transcripts. Most cases have additional cytogenetic abnormalities such as loss of chromosome 7, gain of chromosome 8, or complex karyotypes [61–63]. AML with *BCR-ABL1* is reported to be an aggressive disease with poor response to traditional AML therapy or tyrosine kinase inhibitor therapy alone.

RUNX1 mutations are reported to occur in 4–16% of AML, but can also be found in numerous other myeloid neoplasms. The diagnosis of AML with mutated *RUNX1*

should not be made for cases that fulfill criteria for *any* of the other specific AML subtypes, including AML with recurrent genetic abnormalities, AML with myelodysplasia-related changes, and therapy-related myeloid neoplasms [1]. *RUNX1* mutations found in the setting of myelodysplasia (MDS) frequently coincide with additional gene mutations including *SRSF2, EZH2, STAG2,* and *ASXL1*, and this profile appears similar in AML with mutated *RUNX1* [64, 65]. Some studies have associated *RUNX1* mutations with worse overall survival in AML. Germline mutation of *RUNX1* is also described and is associated with an autosomal dominant thrombocytopenia and also increased risk for MDS/AML. When identified in AML, *RUNX1* mutation should prompt evaluation of family history and possible consideration for germline sequence analysis (see section "Myeloid Neoplasms with Germline Predisposition").

AML with Myelodysplasia-Related Changes

This diagnostic category represents 24–35% of AML and encompasses disease with ≥20% peripheral blood or bone marrow myeloblasts and (1) dysplasia in ≥50% of at least two cell lines, (2) a prior history of MDS or myelodysplastic/myeloproliferative neoplasm (MDS/MPN), or (3) underlying MDS-associated cytogenetic abnormalities [1]. Identifying an AML-associated recurrent cytogenetic aberrancy or history of cytotoxic/radiation therapy for unrelated disease would exclude this diagnostic category. The cytogenetic aberrancies associated with this category of AML are similar to those found in MDS and include complex karyotypes, unbalanced gains/losses of major chromosomal regions, and number of uncommon balanced translocations (Table 14.11). Some abnormalities that are common in MDS, such as trisomy 8, del(20q), and loss of chromosome Y, are not sufficiently specific in isolation to diagnose AML-MRC [1].

This category is generally associated with a poorer prognosis and lower rates of complete remission than other AML subtypes [66, 67]. There are generally no significant differences in survival between AMLs arising from myelodysplasia and de novo AMLs with multilineage dysplasia [67]. Some cases with a prior history of MDS, intermediate-risk cytogenetics, and relatively low blast counts (20–29%) may exhibit clinical behavior more similar to MDS [68], with response and survival benefit from hypomethylating agents. Cases with high-risk cytogenetics generally have no survival differences compared to AML cases with ≥30% blasts [68]. Of note, a significant minority of AML associated with *NPM1* or biallelic *CEBPA* mutations will show multilineage dysplasia. In the absence of MDS-specific cytogenetic aberrancies, these cases retain a good prognostic outlook, with similar behavior to cases without multilineage dysplasia [49, 60].

The 2016 WHO classification system does not recognize any somatic gene sequence mutations as being diagnostically specific for the AML-MRC category. However, acquired variants in some genes have frequent association with secondary AMLs arising from antecedent myeloid malignancy. Mutations in *SRSF2*, *SF3B1*,

U2AF1, *ZRSR2*, *ASXL1*, *EZH2*, *BCOR*, *STAG2, RUNX1,* and *TP53* are common in AML-MRC and occur at a higher frequency than in other forms of AML, NOS [69, 70]. Presence of *TP53* mutations is almost always associated with complex karyotypes and may suggest an even worse prognosis than other cases in this already poor prognostic group [69–72].

Therapy-Related Myeloid Neoplasms

Therapy-related myeloid neoplasms (t-MNs) arise as an uncommon late effect of chemotherapy and/or radiation therapy for an unrelated illness, usually another malignancy, solid organ transplant, or autoimmune disease. The morphologic presentation at diagnosis can be variable, and this category represents about 10–20% of all cases of AML, MDS, and MDS/MPN [1]. t-MNs are morphologically heterogeneous and can look like either MDS or AML, but the 2016 WHO classifies them collectively in a single category due to general behavioral similarities and extremely poor outcomes that are independent of blast counts [1]. The most common antecedent malignancies are breast, lung, and hematologic cancers, chiefly lymphomas and multiple myeloma [73, 74]. The leukemic blasts do not have diagnostically specific morphologic or immunophenotypic features.

The leukemic cells in t-MNs will demonstrate an abnormal karyotype in >90% of cases [75, 76]. Essentially all the balanced cytogenetic abnormalities associated with AML-MRC are also found in t-MNs; thus the clinical history is central to assigning a correct diagnosis. A positive history of cytotoxic therapy takes diagnostic precedence over morphologic dysplasia and MDS-associated cytogenetics. Two general subsets of t-MNs are clinically recognized, associated with either (1) alkylating agents and/or ionizing radiation therapy or (2) topoisomerase II inhibitor therapy (Table 14.12). However, as patients may undergo multiple therapeutic exposures, there can be overlap between the general archetypes [77]. The pathogenic effect of isolated limited-field radiation therapy is unclear, and the incidence of associated t-MNs associated with this form of therapy is uncertain [78].

The more common subtype arises after alkylating agent and/or radiation therapy (~70% of patients). There is usually latency period of 5–10 years, an MDS-like phase with dyspoiesis and cytopenias and rapid progression to overt AML with multilineage dysplasia. These cases are associated with unbalanced chromosomal losses (often involving chromosomes 5 and/or 7), complex karyotypes, and mutations or loss of *TP53.* Loss of 5q is often seen with additional chromosomal abnormalities in a complex karyotype, and up to 80% of patients with del(5q) will also have mutations or loss of *TP53* [1].

The second subtype arises after topoisomerase II inhibitor therapy, but may also be seen with radiation therapy alone. The latency period is shorter (1–5 years), patients usually do not have an MDS-phase, and overt leukemia is found on presentation. Balanced translocations are more frequent in this subgroup, often involving KMT2A at 11q23.3 or RUNX1 at 21q22.1. Category-specific balanced chromo-

Table 14.12 Major subtypes of therapy-related myeloid neoplasms

Class of prior therapy	Alkylating agent	Topoisomerase II inhibitor
Relative frequency	~70% of t-MNs	~30% of t-MNs
Latency to onset	5–10 years	1–5 years
MDS-phase preceding overt AML	Common	Uncommon
Common cytogenetic abnormalities	Unbalanced chromosomal losses; chromosomes 5 and/or 7 abnormalities, complex karyotypes, and mutations/loss of TP53	Balanced translocations, KMT2A at 11q23.3 or RUNX1 at 21q22.1 frequently involved
Implicated medications	Alkylating agents Platinum-based therapy Antimetabolites	Topoisomerase II inhibitors Anthracyclines

The incidence of t-MNs due to limited-field radiation therapy is unknown
Antitubulin agents (vincristine, vinblastine, docetaxel, etc.) have been implicated, but usually in combination with other agents
Topoisomerase II inhibitors may also be associated with therapy-associated lymphoblastic leukemia

somal rearrangements have been described, such as the t(15;17) *PML-RARA* fusion associated with APL or the inv(16) *CBFB-MYH11* fusion associated with CBF-AML. The clinical behavior of these cases is still unresolved; some groups have reported comparable outcomes to de novo disease, while others have indicated worse overall and event-free survival [79, 80].

In general, the prognosis of this disease category is exceptionally poor with overall 5-year survival rates that are often reported at <10%. Cases with abnormalities of chromosome 5 and/or 7, *TP53* mutations, or complex karyotypes have a median survival time of <1 year regardless of presentation as overt t-AML or as t-MDS [1]. Somatic sequence mutations are frequently reported in the *TET2, PTPN11, IDH1/2, NRAS,* and *FLT3* genes, but the clinical significance of these findings is still undetermined [81, 82].

Myeloid Neoplasms with Germline Predisposition and AML in Children

Myeloid Neoplasms with Germline Predisposition

A number of germline abnormalities have been linked with an inherited predisposition toward myeloid malignancies, but only a few are specifically predisposing to AML. These are rare disorders which represent <1% of AMLs, but the relative frequency of subtypes within this diagnostic category has not been well established [1]. Patients present more frequently in childhood, though few subtypes with late-onset have been described, and recognition is important for the screening of family

members. These disorders are quite rare but the few better-characterized entities fall into three groups within the 2016 WHO system, as summarized below:

- *Myeloid neoplasms with germline predisposition without a pre-existing disorder or organ dysfunction*
  - Acute myeloid leukemia with germline *CEBPA* mutation
  - Myeloid neoplasms with germline *DDX41* mutation
- *Myeloid neoplasms with germline predisposition and pre-existing platelet disorders*
  - Myeloid neoplasms with germline *RUNX1* mutation
  - Myeloid neoplasms with *ANKRD26* mutation
  - Myeloid neoplasms with *ETV6* mutation
- *Myeloid neoplasms with germline predisposition and other organ dysfunction*
  - Myeloid neoplasms with germline *GATA2* mutation
  - Myeloid neoplasms associated with bone marrow failure syndromes
  - Myeloid neoplasms associated with telomere biology disorders
  - Juvenile myelomonocytic leukemia associated with neurofibromatosis, Noonan syndrome, or Noonan syndrome-like disorders
  - Myeloid neoplasms associated with Down syndrome

AML may be seen associated with any of the germline predisposition entities, but a clinical picture dominated by either MDS or AML with no other significant organ dysfunction is primarily seen with the first group, including *CEBPA* and *DDX41* mutations. Disorders associated with germline *DDX41* mutation appear to have a longer latency period, with a median age of 62 years at malignancy onset [1]. An increased risk for lymphoid malignancies is also reported for the entities associated with *DDX41, RUNX1, ANKRD26,* and *ETV6* mutations and also with Down syndrome [1].

Transient Abnormal Myelopoiesis and Myeloid Leukemia Associated with Down Syndrome

Persons with Down syndrome have a 10- to 100-fold increased risk of developing acute leukemia than unaffected persons. About 70% of these cases have a megakaryoblastic phenotype, which is rare in non-Down syndrome associated AML [1]. Additionally, a significant minority of infants with Down syndrome may also present with a temporary clonal myeloid proliferation whose features can mimic and even meet criteria for AML. This unusual condition is referred to as transient abnormal myelopoiesis (TAM) associated with Down syndrome. The blasts found in the vast majority of TAM also exhibit a megakaryoblastic immunophenotype [1]. The unique clinical characteristics of both these myeloid proliferations were recognized by the WHO, resulting in a separate categorization in 2008, which has persisted into the 2016 update.

Trisomy 21 itself causes perturbation of fetal hematopoiesis with abnormal production in the liver, increases in the number of megakaryocyte-erythroid progenitors, and increases in the hematopoietic stem cell compartment [83]. These abnormalities are congenital and precede the acquisition of disease-associated somatic mutations [84]. Essentially all cases of Down syndrome-associated TAM and AML will acquire a subsequent mutation of *GATA1*, a hematopoietic transcription factor that regulates normal megakaryocyte and erythrocyte differentiation [83, 85, 86]. More than 95% of the pathologically significant variants are in exon 2 with the remainder in exon 3, with resultant N-terminal protein truncation [87]. Additionally, up to 25–30% of all neonates with Down syndrome may be found to carry these mutations, though the reason for the high frequency in this setting is unclear [88].

However, *GATA1* mutations are insufficient in isolation to cause myeloid leukemia associated with Down syndrome; 80–90% of patients with TAM will show spontaneous regression of the process within the first 3 months of life [1, 89, 90]. Patients with TAM who do progress to acute leukemia usually do so within the first 5 years of life, and acquisition of additional oncogenic mutations can usually be demonstrated. Trisomy 8 is common in this setting (13–44% of cases), but monosomy 7 is very rare [1, 91]. Whole genome or exome sequencing studies at progression to acute leukemia have shown about 50% of cases acquire mutations in cohesin complex genes (*RAD21*, *SMC1A*, *SMC3,* and *STAG2*), 45% will involve epigenetic regulators such as *EZH2* and *KANSL1*, and 20% will involve the transcription factor *CTCF* [89, 90]. Other signaling pathways such as JAK kinases, *MPL*, and RAS pathway genes (*NRAS, KRAS, CBL, PTPN11,* and *NF1*) were implicated in a smaller subset of cases [89, 90]. However, no specific genetic abnormalities can consistently predict transformation of TAM to acute leukemia at present.

Childhood AML

AML accounts for only 20% of pediatric acute leukemias, but is overtaking acute lymphoblastic leukemia as the leading cause of childhood leukemia-related mortality [92]. Both adult and childhood AML have a low overall mutation burden compared to other human cancers, with a broad spectrum of recurrently impacted but relatively infrequently affected genes [92]. However, the landscape of structural and sequence-related genomic aberrancies in pediatric AML shows significant differences from the adult cohort.

While there is some overlap of recurrent cytogenetic abnormalities seen in adult and childhood AML, the general types of balanced and unbalanced chromosomal abnormalities are different. Structural variants are disproportionately prevalent in younger patients, with a variety of uncommon recurrent balanced translocations and inversions beyond the specifically named entities in WHO classification system. A selection of these rare balanced rearrangements with higher prevalence in pediatric AML may be found in Table 14.13. Rearrangements involving *KMT2A* are the most common, seen in ~10–20% of children but in nearly half of affected infants [92, 93]. Similar to adult patients, AML associated with t(8;21), inv(16), and t(15;17) are

Table 14.13 Chromosomal translocations with a higher prevalence in pediatric AML

Translocation	Associated genes	Frequency in children	Frequency in adults	Age cohort bias	Prognosis
11q23 fusion family					
11q23.3	*KMT2A* translocated	25%	5–10%	Infants	Dependent on partner gene
t(9;11) (p21.3;q23.3)	*KMT2A-MLLT3*	9.5%	2%	Children	Intermediate
t(10;11) (p12;q23.3)	*KMT2A-MLLT10*	3.5%	1%	Children	Adverse
t(6;11) (q27;q23.3)	*KMT2A-AFDN*	ND	ND	Children	Adverse [97]
t(11;19) (q23.3;p13.11)	*KMT2A-ELL*	ND	ND	Infants, children	Adverse [98]
t(6;11) (q27;q23.3)	*KMT2A-AFDN*	2%	<0.5%	Children	Adverse
t(1;11) (q21;q23.3)	*KMT2A-MLLT11*	1%	<0.5%	Children	Favorable
NUP fusion family					
t(6;9) (p23;q34.1)	*DEK-NUP214*	1.7%	0.7–1.8%	Older children, rare in infants	Adverse
t(5;11) (q35.3;p15.5)[a]	*NUP98-NSD1*	7%	3%	Older children and young adults	Adverse
t(11;12) (p15.5;p13.5)[a]	*NUP98-KDM5A*	3%	0%	Children <5 years	Intermediate
ETS fusion family					
t(7;12) (q36.3;p13.2)	*MNX1-ETV6*	0.8%	<0.5%	Infants	Adverse
t(2;12) (q33.3;p13.2)	*ETV6-INO80D*	ND	ND	Infants	ND
t(16;21) (p11.2;q22.2)	*FUS-ERG*	ND	ND	Infants, children	ND
GLIS2 fusion family					
inv(16) (p13.3q24.3)[a]	*CBFA2T3-GLIS2*	3%	0%	Infants	Adverse
Others					
t(1;22) (p13.3;q13.1)	*RBM15-MRTFA*	0.8%	<0.5%	Infants, 95% of cases <2 years old	Intermediate
t(8;16) (p11.2;q13.3)	*KAT6A-CREBBP*	0.5%	<0.5%	Infants and children	Spontaneous regression reported in some infant cases, Intermediate risk in later childhood

ND not defined
[a]*Described as a cryptic translocation*

associated with superior outcomes, while complex karyotypes and monosomy 7 are associated with poor outcomes [92–94]. Monosomal karyotypes have also been described as an indicator of poor outcome [95, 96]. Recurrent focal deletions are other characteristic findings in pediatric AML. Copy number loss are more common in children, and the *ZEB2, MBNL1,* and *ELF1* genes are often affected; *ZEB2* and *MBNL1* co-deletion is a relatively frequent finding, and half of these are found accompanying *KMT2A-MLLT3* fusions [92]. *KMT2A* fusions were also commonly associated with RAS-related mutations (*KRAS, NRAS, PTPN1,* or *NF1*), and a subset of *KMT2A* fusions also showed recurrent mutation in post-transcriptional splicing genes (i.e., *SETD2, U2AF1,* and *DICER1*) as the sole additional abnormality [92].

Mutations of *WT1* appear to be mutually exclusive with those in *ASXL1* and *EZH2,* but *WT1* or *EZH2* variants are seen in about one-quarter of pediatric AML cases and may represent early clonal or near-clonal origin [92]. Widespread gene silencing by aberrant promoter methylation is enriched in younger patients with *WT1* mutations, and mutations of *WT1, ASXL1,* or *EZH2* are associated with induction failure [92]. Other recurrently mutated genes in pediatric AML include variants in *GATA2*, *CBL*, *MYC*-ITD, *NRAS*, and *KRAS*. *NRAS* and *WT1* are mutated more often in younger patients than adults; conversely, mutations in *DNMT3A, IDH1/2, RUNX1, NPM1,* and *TP53*, which are common in adults, are seen more often in older patients [92]. Given the ongoing discovery clarifying the genetics of pediatric AML, more robust classification systems for diagnosis and treatment of childhood AML will likely be forthcoming.

References

1. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, editors. WHO classification of tumours of haematopoietic and lymphoid tissues (Revised 4th edition). Lyon: IARC; 2017.
2. Cancer Genome Atlas Research Network. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J Med. 2013;368:2059–74.
3. Devillier R, Gelsi-Boyer V, Brecqueville M, Carbuccia N, Murati A, Vey N, et al. Acute myeloid leukemia with myelodysplasia-related changes are characterized by a specific molecular pattern with high frequency of ASXL1 mutations. Am J Hematol. 2012;87:659–62.
4. 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.
5. 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.
6. Paschka P, Du J, Schlenk RF, Gaidzik VI, Bullinger L, Corbacioglu A, 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.
7. Itzykson R, Duployez N, Fasan A, Decool G, Marceau-Renaut A, Meggendorfer M, et al. Clonal interference of signaling mutations holds prognostic relevance in core binding factor acute myeloid leukemia. Blood. 2018;132:187–96. Epub ahead of print. PMID: 29692343

8. 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):2451–9.
9. Micol JB, Duployez N, Boissel N, Petit A, Geffroy S, Nibourel O, et al. Frequent ASXL2 mutations in acute myeloid leukemia patients with t(8;21)/RUNX1-RUNX1T1 chromosomal translocations. Blood. 2014;124(9):1445–9.
10. 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.
11. Zhu HH, Zhang XH, Qin YZ, Liu DH, Jiang H, Chen H, et al. MRD-directed risk stratification treatment may improve outcomes of t(8;21) AML in the first complete remission: results from the AML05 multicenter trial. Blood. 2013;121(20):4056–62.
12. Wang Y, Wu DP, Liu QF, Qin YZ, Wang JB, Xu LP, et al. In adults with t(8;21)AML, post-transplant RUNX1/RUNX1T1-based MRD monitoring, rather than c-KIT mutations, allows further risk stratification. Blood. 2014;124(12):1880–6.
13. Pandolfi PP. In vivo analysis of the molecular genetics of acute promyelocytic leukemia. Oncogene. 2001;20:5726–35.
14. Shen ZX, Shi ZZ, Fang J, Gu W, Li JM, Zhu YM, et al. All-trans retinoic acid/As2O3 combination yields a high quality remission and survival in newly diagnosed acute promyelocytic leukemia. Proc Natl Acad Sci U S A. 2004;101(15):5328–35.
15. Lo-Coco F, Ammatuna E. The biology of acute promyelocytic leukemia and its impact on diagnosis and treatment. Hematology Am Soc Hematol Educ Program. 2006:156–61, 514. https://www.ncbi.nlm.nih.gov/pubmed/17124055.
16. Rahmé R, Thomas X, Recher C, Vey N, Delaunay J, Deconinck E, et al. Early death in acute promyelocytic leukemia (APL) in French centers: a multicenter study in 399 patients. Leukemia. 2014;28(12):2422–4.
17. Breccia M, Loglisci G, Loglisci MG, Ricci R, Diverio D, Latagliata R, et al. FLT3-ITD confers poor prognosis in patients with acute promyelocytic leukemia treated with AIDA protocols: long-term follow-up analysis. Haematologica. 2013;98(12):e161–3.
18. Zelent A, Guidez F, Melnick A, Waxman S, Licht JD. Translocations of the RARalpha gene in acute promyelocytic leukemia. Oncogene. 2001;20:7186–203.
19. Melnick A, Licht JD. Deconstructing a disease: RARalpha, its fusion partners, and their roles in the pathogenesis of acute promyelocytic leukemia. Blood. 1999;93:3167–215.
20. Grimwade D, Jovanovic JV, Hills RK, Nugent EA, Patel Y, Flora R, et al. Prospective minimal residual disease monitoring to predict relapse of acute promyelocytic leukemia and to direct pre-emptive arsenic trioxide therapy. J Clin Oncol. 2009;27:3650–8.
21. Esteve J, Escoda L, Martin G, Rubio V, Díaz-Mediavilla J, González M, et al. Outcome of patients with acute promyelocytic leukemia failing to front-line treatment with all-trans retinoic acid and anthracycline-based chemotherapy: benefit of an early intervention. Leukemia. 2007;21:446–52.
22. Meyer C, Burmeister T, Gröger D, Tsaur G, Fechina L, Renneville A, et al. The MLL recombinome of acute leukemias in 2017. Leukemia. 2018;32(2):273–84.
23. Chandra P, Luthra R, Zuo Z, Yao H, Ravandi F, Reddy N, et al. Acute myeloid leukemia with t(9;11)(p21-22;q23): common properties of dysregulated ras pathway signaling and genomic progression characterize de novo and therapy-related cases. Am J Clin Pathol. 2010;133(5):686–93.
24. Bindels EM, Havermans M, Lugthart S, Erpelinck C, Wocjtowicz E, Krivtsov AV, et al. EVI1 is critical for the pathogenesis of a subset of MLL-AF9-rearranged AMLs. Blood. 2012;119(24):5838–49.
25. Mrózek K, Heinonen K, Lawrence D, Carroll AJ, Koduru PR, Rao KW, et al. Adult patients with de novo acute myeloid leukemia and t(9;11)(p22;q23) have a superior outcome to patients with other translocations involving band 11q23: a Cancer and Leukemia Group B study. Blood. 1997;90:4532–8.
26. Gröschel S, Schlenk RF, Engelmann J, Rockova V, Teleanu V, Kühn MW, et al. Deregulated expression of EVI1 defines a poor prognostic subset of MLL-rearranged acute myeloid leuke-

mias: a study of the German-Austrian Acute Myeloid Leukemia Study Group and the Dutch-Belgian-Swiss HOVON/SAKK Cooperative Group. J Clin Oncol. 2013;31(1):95–103.
27. Scandura JM, Boccuni P, Cammenga J, Nimer SD. Transcription factor fusions in acute leukemia: variations on a theme. Oncogene. 2002;21(21):3422–44.
28. Slovak ML, Gundacker H, Bloomfield CD, Dewald G, Appelbaum FR, Larson RA, et al. A retrospective study of 69 patients with t(6;9)(p23;q34) AML emphasizes the need for a prospective, multicenter initiative for rare 'poor prognosis' myeloid malignancies. Leukemia. 2006;20(7):1295–7.
29. Oyarzo MP, Lin P, Glassman A, Bueso-Ramos CE, Luthra R, Medeiros LJ. Acute myeloid leukemia with t(6;9)(p23;q34) is associated with dysplasia and a high frequency of flt3 gene mutations. Am J Clin Pathol. 2004;122(3):348–58.
30. Tarlock K, Alonzo TA, Moraleda PP, Gerbing RB, Raimondi SC, Hirsch BA, et al. Acute myeloid leukaemia (AML) with t(6;9)(p23;q34) is associated with poor outcome in childhood AML regardless of FLT3-ITD status: a report from the Children's Oncology Group. Br J Haematol. 2014;166(2):254–9.
31. Gröschel S, Sanders MA, Hoogenboezem R, de Wit E, Bouwman BAM, Erpelinck C, et al. A single oncogenic enhancer rearrangement causes concomitant EVI1 and GATA2 deregulation in leukemia. Cell. 2014;157(2):369–81.
32. 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.
33. Nucifora G, Laricchia-Robbio L, Senyuk V. EVI1 and hematopoietic disorders: history and perspectives. Gene. 2006;368:1–11.
34. Lugthart S, Gröschel S, Beverloo HB, Kayser S, Valk PJ, van Zelderen-Bhola SL, et al. Clinical, molecular, and prognostic significance of WHO type inv(3)(q21q26.2)/t(3;3)(q21;q26.2) and various other 3q abnormalities in acute myeloid leukemia. J Clin Oncol. 2010;28(24):3890–8.
35. Gröschel S, Sanders MA, Hoogenboezem R, Zeilemaker A, Havermans M, Erpelinck C, et al. 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.
36. Haferlach C, Bacher U, Haferlach T, Dicker F, Alpermann T, Kern W, et al. 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.
37. 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.
38. 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.
39. Schweitzer J, Zimmermann M, Rasche M, von Neuhoff C, Creutzig U, Dworzak M, et al. Improved outcome of pediatric patients with acute megakaryoblastic leukemia in the AML-BFM 04 trial. Ann Hematol. 2015;94(8):1327–36.
40. Lindström MS. NPM1/B23: a multifunctional chaperone in ribosome biogenesis and chromatin remodeling. Biochem Res Int. 2011;2011:195209.
41. Colombo E, Marine JC, Danovi D, Falini B, Pelicci PG. Nucleophosmin regulates the stability and transcriptional activity of p53. Nat Cell Biol. 2002;4:529–33.
42. 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.
43. Corces-Zimmerman MR, Hong WJ, Weissman IL, Medeiros BC, Majeti R. Preleukemic mutations in human acute myeloid leukemia affect epigenetic regulators and persist in remission. Proc Natl Acad Sci U S A. 2014;111(7):2548–53.
44. Shlush LI, Zandi S, Mitchell A, Chen WC, Brandwein JM, Gupta V, et al. Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. Nature. 2014;506(7488):328–33.

45. 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.
46. 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.
47. 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.
48. Döhner K, Schlenk RF, Habdank M, Scholl C, Rücker FG, Corbacioglu A, et al. Mutant nucleophosmin (NPM1) predicts favorable prognosis in younger adults with acute myeloid leukemia and normal cytogenetics: interaction with other gene mutations. Blood. 2005;106(12):3740–6.
49. 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.
50. Schlenk RF, Döhner K, Krauter J, Fröhling 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.
51. Pratcorona M, Brunet S, Nomdedéu J, Ribera JM, Tormo M, Duarte R, et al. Favorable outcome of patients with acute myeloid leukemia harboring a low-allelic burden FLT3-ITD mutation and concomitant NPM1 mutation: relevance to post-remission therapy. Blood. 2013;121(14):2734–8.
52. Gale RE, Green C, Allen C, Mead AJ, Burnett AK, Hills RK, et al. The impact of FLT3 internal tandem duplication mutant level, number, size, and interaction with NPM1 mutations in a large cohort of young adult patients with acute myeloid leukemia. Blood. 2008;111(5):2776–84.
53. Schlenk RF, Kayser S, Bullinger L, Kobbe G, Casper J, Ringhoffer M, et al. Differential impact of allelic ratio and insertion site in FLT3-ITD-positive AML with respect to allogeneic transplantation. Blood. 2014;124(23):3441–9.
54. Loghavi S, Zuo Z, Ravandi F, Kantarjian HM, Bueso-Ramos C, Zhang L, et al. Clinical features of de novo acute myeloid leukemia with concurrent DNMT3A, FLT3 and NPM1 mutations. J Hematol Oncol. 2014;7:74.
55. 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.
56. 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.
57. Renneville A, Boissel N, Gachard N, Naguib D, Bastard C, de Botton S, et al. The favorable impact of CEBPA mutations in patients with acute myeloid leukemia is only observed in the absence of associated cytogenetic abnormalities and FLT3 internal duplication. Blood. 2009;113(21):5090–3.
58. 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.
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. Paietta E, Racevskis J, Bennett JM, Neuberg D, Cassileth PA, Rowe JM, et al. Biologic heterogeneity in Philadelphia chromosome-positive acute leukemia with myeloid morphology: the Eastern Cooperative Oncology Group experience. Leukemia. 1998;12(12):1881–5.
62. 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.
63. 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.
64. Haferlach T, Nagata Y, Grossmann V, Okuno Y, Bacher U, Nagae G, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. Leukemia. 2014;28(2):241–7.
65. Papaemmanuil E, Gerstung M, Malcovati L, Tauro S, Gundem G, Van Loo P, et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. Blood. 2013;122(22):3616–27.
66. 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.
67. 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.
68. Hasserjian RP, Campigotto F, Klepeis V, Fu B, Wang SA, Bueso-Ramos C, et al. De novo acute myeloid leukemia with 20–29% blasts is less aggressive than acute myeloid leukemia with ≥30% blasts in older adults: a Bone Marrow Pathology Group study. Am J Hematol. 2014;89(11):E193–9.
69. 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.
70. 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.
71. Devillier R, Mansat-De Mas V, Gelsi-Boyer V, Demur C, Murati A, Corre J, et al. Role of ASXL1 and TP53 mutations in the molecular classification and prognosis of acute myeloid leukemias with myelodysplasia-related changes. Oncotarget. 2015;6(10):8388–96.
72. Ohgami RS, Ma L, Merker JD, Gotlib JR, Schrijver I, Zehnder JL, et al. Next-generation sequencing of acute myeloid leukemia identifies the significance of TP53, U2AF1, ASXL1, and TET2 mutations. Mod Pathol. 2015;28(5):706–14.
73. Morton LM, Dores GM, Tucker MA, Kim CJ, Onel K, Gilbert ES, et al. Evolving risk of therapy-related acute myeloid leukemia following cancer chemotherapy among adults in the United States, 1975–2008. Blood. 2013;121(15):2996–3004.
74. Radivoyevitch T, Sachs RK, Gale RP, Molenaar RJ, Brenner DJ, Hill BT, et al. Defining AML and MDS second cancer risk dynamics after diagnoses of first cancers treated or not with radiation. Leukemia. 2016;30(2):285–94.
75. Mauritzson N, Albin M, Rylander L, Billström R, Ahlgren T, Mikoczy Z, et al. Pooled analysis of clinical and cytogenetic features in treatment-related and de novo adult acute myeloid leukemia and myelodysplastic syndromes based on a consecutive series of 761 patients analyzed 1976–1993 and on 5098 unselected cases reported in the literature 1974–2001. Leukemia. 2002;16(12):2366–78.
76. Rowley JD, Olney HJ. International workshop on the relationship of prior therapy to balanced chromosome aberrations in therapy-related myelodysplastic syndromes and acute leukemia: overview report. Genes Chromosomes Cancer. 2002;33(4):331–45.
77. Kayser S, Döhner K, Krauter J, Köhne CH, Horst HA, Held G, et al. The impact of therapy-related acute myeloid leukemia (AML) on outcome in 2853 adult patients with newly diagnosed AML. Blood. 2011;117(7):2137–45.

78. Nardi V, Winkfield KM, Ok CY, Niemierko A, Kluk MJ, Attar EC, et al. Acute myeloid leukemia and myelodysplastic syndromes after radiation therapy are similar to de novo disease and differ from other therapy-related myeloid neoplasms. J Clin Oncol. 2012;30(19):2340–7.
79. 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.
80. 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.
81. 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.
82. 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.
83. Roy A, Cowan G, Mead AJ, Filippi S, Bohn G, Chaidos A, et al. Perturbation of fetal liver hematopoietic stem and progenitor cell development by trisomy 21. Proc Natl Acad Sci U S A. 2012;109(43):17579–84.
84. Tunstall-Pedoe O, Roy A, Karadimitris A, de la Fuente J, Fisk NM, Bennett P, et al. Abnormalities in the myeloid progenitor compartment in Down syndrome fetal liver precede acquisition of GATA1 mutations. Blood. 2008;112(12):4507–11.
85. Chou ST, Opalinska JB, Yao Y, Fernandes MA, Kalota A, Brooks JS, et al. Trisomy 21 enhances human fetal erythro-megakaryocytic development. Blood. 2008;112(12):4503–6.
86. Wechsler J, Greene M, McDevitt MA, Anastasi J, Karp JE, Le Beau MM, et al. Acquired mutations in GATA1 in the megakaryoblastic leukemia of Down syndrome. Nat Genet. 2002;32(1):148–52.
87. Alford KA, Reinhardt K, Garnett C, Norton A, Böhmer K, von Neuhoff C, et al. Analysis of GATA1 mutations in Down syndrome transient myeloproliferative disorder and myeloid leukemia. Blood. 2011;118(8):2222–38.
88. Roberts I, Alford K, Hall G, Juban G, Richmond H, Norton A, 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(24):3908–17.
89. Yoshida K, Toki T, Okuno Y, Kanezaki R, Shiraishi Y, Sato-Otsubo A, et al. The landscape of somatic mutations in Down syndrome-related myeloid disorders. Nat Genet. 2013;45(11):1293–9.
90. 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.
91. 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.
92. Bolouri H, Farrar JE, Triche T Jr, Ries RE, Lim EL, Alonzo TA, et al. The molecular landscape of pediatric acute myeloid leukemia reveals recurrent structural alterations and age-specific mutational interactions. Nat Med. 2018;24(1):103–12.
93. Harrison CJ, Hills RK, Moorman AV, Grimwade DJ, Hann I, Webb DK, et al. Cytogenetics of childhood acute myeloid leukemia: United Kingdom Medical Research Council Treatment trials AML 10 and 12. J Clin Oncol. 2010;28(16):2674–81.
94. von Neuhoff C, Reinhardt D, Sander A, Zimmermann M, Bradtke J, Betts DR, et al. Prognostic impact of specific chromosomal aberrations in a large group of pediatric patients with acute myeloid leukemia treated uniformly according to trial AML-BFM 98. J Clin Oncol. 2010;28(16):2682–9.

95. Sandahl JD, Kjeldsen E, Abrahamsson J, Ha SY, Heldrup J, Jahnukainen K, et al. Ploidy and clinical characteristics of childhood acute myeloid leukemia: a NOPHO-AML study. Genes Chromosomes Cancer. 2014;53(8):667–75.
96. Rasche M, von Neuhoff C, Dworzak M, Bourquin JP, Bradtke J, Göhring G, et al. Genotype-outcome correlations in pediatric AML: the impact of a monosomal karyotype in trial AML-BFM 2004. Leukemia. 2017;31(12):2807–14.
97. Balgobind BV, Raimondi SC, Harbott J, Zimmermann M, Alonzo TA, Auvrignon A, et al. Novel prognostic subgroups in childhood 11q23/MLL-rearranged acute myeloid leukemia: results of an international retrospective study. Blood. 2009;114(12):2489–96.
98. Huret JL, Brizard A, Slater R, Charrin C, Bertheas MF, Guilgot F, et al. Cytogenetic heterogeneity in t(11;19) acute leukemia: clinical, hematological and cytogenetic analyses of 48 patients—updated published cases and 16 new observations. Leukemia. 1993;7(2):152–60.