

Chapter 2

Acute Leukemia

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Overview

Acute leukemia (AL) is the commonest malignancy in children less than 15 years of age [1]. Approximately 3,000 new cases of AL occur annually in the U.S.A., of which 80% are acute lymphoblastic leukemia (ALL). The 5-year survival rates for childhood AL, and especially ALL has dramatically improved from 61% in 1975–1978 to 89% in 1999–2002 [2–4]. The remarkable success story of pediatric ALL is attributed to the exponential increase in knowledge of the molecular mechanisms of the disease and the impact of well-designed clinical trials adapted to risk-stratified subgroups based on prognostic indicators, including evaluation of early response to the treatment (minimal residual disease detection). This has been accomplished by genomic studies employing a host of modern techniques, for example, conventional cytogenetics, fluorescent *in situ* hybridization (FISH), DNA and gene expression arrays, and proteomics. Many of these methodologies have moved from the research bench to clinical molecular diagnostics allowing their routine use in the diagnosis, classification, prognostication, and follow-up of acute leukemia. In this chapter we describe the clinical features and associated genetic abnormalities of AL and discuss their impact on clinical management of AL. The subsequent chapter on AL in this volume describes molecular techniques routinely used in the diagnosis and prognostication of acute leukemia.

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Clinical Features

Acute leukemia is the commonest childhood malignancy with an age-adjusted incidence of 44.3 per 1,000,000 persons (ALL 36.8; AML 7.5) [1, 5]. ALL is four times as common as acute myeloid leukemia (AML), and occurs more often in male than in female children [1, 5]. Since 80% of all childhood acute leukemia is ALL, the major discussion will be devoted to ALL, especially precursor B-ALL (80–85% of ALL). The applications of genomic studies to precursor B-ALL are similarly relevant to precursor T-ALL and AML; features specific to the latter will be highlighted separately. AL of infancy is biologically and clinically different from that in older children. Unlike older children, ALL and AML occur with nearly equal frequency in infancy, and there is a female rather than male preponderance [6]. Acute leukemia in infancy very often has high white blood cell counts and the presence of the translocation $t(4;11)(q21;q23)$, putting them in the clinically high-risk category with poor outcome.

Clinical symptoms of acute leukemia are related to replacement of normal hematopoietic cells in the bone marrow by the leukemic cells, and to organ infiltration by blasts. Fever, pallor, weakness, bleeding manifestations, and bone pains are the most common presenting symptoms [7]. Nearly 40% of affected children complain of joint aches, presumably from leukemic infiltration of the joint capsule [8]. Massive organomegaly and central nervous system (CNS) involvement at presentation are characteristic of acute leukemia in infancy [6]. Extramedullary solid tumor masses or chloromas are seen at presentation in a small number of patients, mostly AML with monocytic differentiation; orbits, paranasal sinuses, and skin are the usual sites for chloromas. Isolated testicular masses are more a feature of relapse than of primary presentation. Nearly 10% children, almost always with precursor T-lymphoblastic leukemia/lymphoma, present with a life-threatening tracheobronchial or cardiovascular compression syndrome due to leukemic infiltration of the thymus and other mediastinal structures, requiring prompt intervention by systemic corticosteroids or local radiation [9].

Diagnosis of Acute Leukemia

In addition to establishing the diagnosis of acute leukemia, the aim of physicians and laboratorians is to stratify patients according to the risk category for optimal management. Risk stratification is determined by (1) clinical features at presentation (age, white cell count, sex (in some protocols)), (2) cytogenetics (or molecular abnormalities), and (3) response to treatment (minimal residual disease). Laboratory methods are directed towards the latter two.

Morphology, immunophenotyping, and cytogenetic analyses form the cornerstone of diagnosis and risk stratification in acute leukemia. The white cell

count is usually high; counts exceeding 50,000/ μ L denote high risk and are seen in 20% of children at presentation, more often in infants. About 10% children present with hyperleukocytosis (leucocyte count > 100,000/ μ L) [10]. Circulating blasts are present in the peripheral blood in most patients, even those with normal white cell counts, although about 1%, may have an aleukemic or pancytopenic presentation. Anemia and thrombocytopenia may be mild or critically low.

Morphology

The diagnosis of acute leukemia based on cytomorphology and cytochemistry has undergone a drastic change in the last three decades. The initial French-American-British (FAB) classification of ALL [11] based on nuclear morphology (nuclear heterogeneity, contour, and nucleoli), and the FAB classification for AML [11] based on morphology, degree of maturation, and cytochemistry are insufficient for prognostication and risk stratification [12–14]. Still, morphological assessment is the first step towards diagnosis and guides subsequent investigation. In many instances, morphology provides a clue for the underlying genetic abnormality, for example, blasts with cytoplasmic and nuclear vacuoles (Burkitt-like; Fig. 2.1a, b) are consistent with mature B-ALL and presence of t(8;14), and abnormal heavily granulated promyelocytes suggest acute promyelocytic leukemia (APL) and presence of t(15;17) (Fig. 2.1c, d). Bone marrow evaluation is useful for diagnosis when the presentation is aleukemic or subleukemic, for the initial estimation of cellularity and leukemic burden, to obtain sample for karyotyping and molecular studies, to assess response to induction chemotherapy, and for predicting likelihood of relapse based on early response to chemotherapy. Extensive necrosis of the bone marrow at presentation is not uncommon [15, 16], and when present, it can make the morphological and immunophenotypic characterization very challenging.

Immunophenotype

The immunological classification of acute leukemia introduced in 1985 by the European Group on Immunological Classification of Leukemia (EGIL) was based on lineage determination by antigen panels and the hierarchical scheme of antigen expression by cells [17]. The 2008 WHO classification system has proposed a simpler algorithm that relies on fewer markers to determine lineage and categorize mixed phenotype acute leukemia (MPAL). Table 2.1 (adapted from the 2008 WHO classification) shows the critical antigens required for distinction of myeloid, precursor B, precursor T-lymphoid, or MPAL.

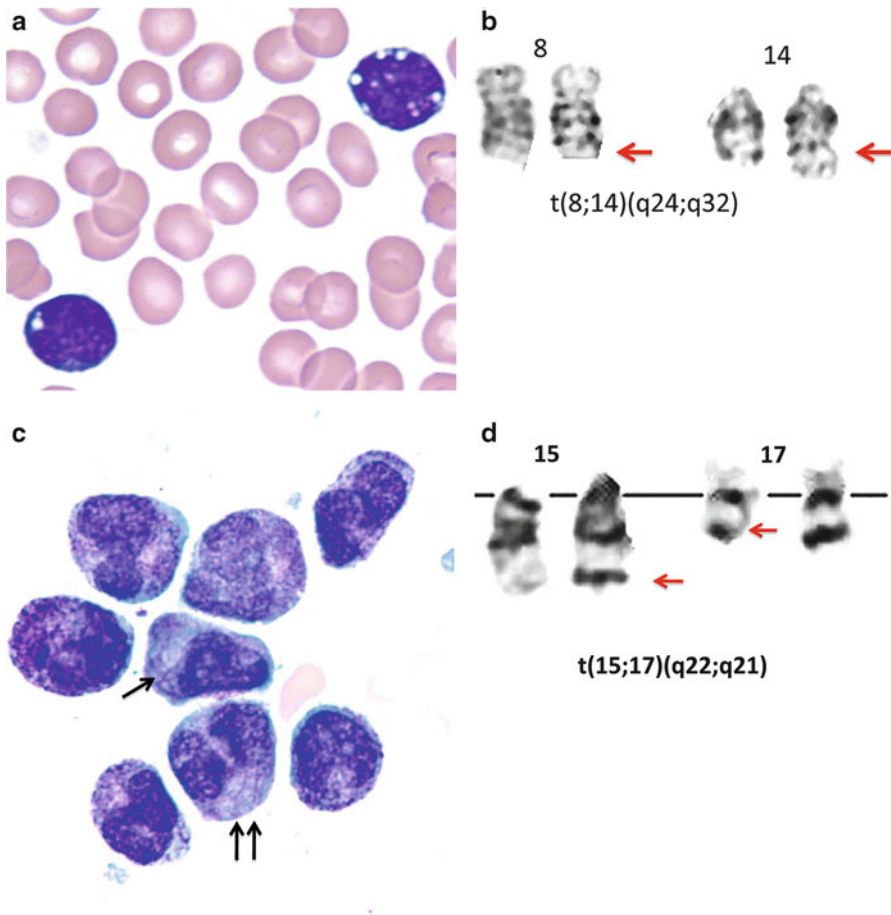


Fig. 2.1 (a) ALL with FAB-L3 morphology. Note the deep blue cytoplasm with cytoplasmic and nuclear vacuoles. (b) Partial karyogram showing balanced translocation t(8;14)(q24;q32). The translocation breakpoints are indicated by red arrows. (c) Acute promyelocytic leukemia{ (multiple Auer rods (*single black arrow*); cell with bilobed nucleus and hypergranular cytoplasm (*double black arrows*)). (d) partial karyogram showing balance translocation t(15;17)(q22;q21). The translocation breakpoints are denoted by red arrows

Table 2.1 Antigens for lineage determination and categorization of MPAL^a (WHO classification, 2008)

Lineage	Antigens
Myeloid	Myeloperoxidase, OR monocytic differentiation (at least two of the following: NSE, CD11c, CD14, CD64, lysozyme)
T-lineage	Cytoplasmic CD3 OR surface CD3
B-lineage	Strong CD19, AND strong expression of at least one of the following: CD79a, cytoplasmic CD22 or CD10; OR weak CD19, AND strong expression of at least two of the following: CD79a, cytoplasmic CD22 or CD10

^aMPAL mixed phenotype acute leukemia

B-Acute Lymphoblastic Leukemia

Based on the hierarchical scheme of antigen expression, precursor B-ALL has been subclassified as early or pro-B-ALL (TdT+, CD10-, CD19+, CD22±), intermediate stage or common ALL with expression of CD10 (CALLA), and pre-B-ALL (positive for cytoplasmic immunoglobulin (cIg)). Historically, immunophenotype has been used to predict the outcome in ALL, for example, expression of CALLA (CD10) is associated with better outcome compared with CALLA (-) ALL or cIg+ALL [18]. Many leukemia immunophenotypes are associated with specific chromosomal anomalies [19–22]. In fact the correlation of outcome with immunophenotypes is actually a function of the underlying chromosomal anomaly [23, 24]. For example, the observed favorable and unfavorable outcomes in the CD10 (CALLA)+pre-B ALL and in the CD10 (-) ALL are due to a higher representation of hyperdiploidy (>50 chromosomes) in the former and of t(4;11) in the latter, respectively [25, 26]. Likewise the presumed unfavorable prognosis associated with co-expression of myeloid antigens in newly diagnosed ALL is ascribed to the presence of the associated *MLL* (11q23) and *BCR-ABL1* [t(9;22)] translocations [25, 27–29]. Ninety to ninety-five percent of cIg+ALL [18] have the translocation t(1;19)(q23;p13) [23, 24]; although earlier reports documented these ALL to have an unfavorable outcome, several subsequent studies have reported that the negative impact of t(1;19) could be offset with intensive chemotherapy programs [30, 31]. Gene expression studies have validated the prediction of different leukemia subtypes by specific immunophenotypic profiles [15, 32, 33], such that the underlying genetic aberrations can be predicted by multiparametric flow cytometry to guide the selection of appropriate panels for FISH studies if conventional metaphase cytogenetics is noncontributory (Table 2.2).

Genetic Abnormalities

ALL is genetically heterogeneous. Multivariate analyses in several large clinical studies have clearly established that genetic abnormalities are the most important determinants of response to chemotherapy and outcome in precursor B-ALL [34–43]. Their relevance is likely to increase as targeted therapies are introduced. The genetic abnormalities, which currently have the most significant impact on treatment and management, are t(9;22)(q34;q11)/*BCR-ABL1*, t(4;11)(q21;q23)/*MLL-AFF1*, and near haploid/low hypodiploidy, all of which are poor prognostic markers, and to a lesser extent, t(12;21)(p13;q22)/*ETV6-RUNX1* and high hyperdiploidy that are favorable prognostic markers [39, 41, 44–46]. The genetic abnormalities that are considered mandatory in the evaluation of ALL, and for which clinical testing is available, are shown in Table 2.2 along with their prognostic implication and the various genetic techniques for their determination. These and other novel genetic aberrations are discussed below.

Table 2.2 Prognostically important genetic abnormalities in B-ALL

Chromosomal abnormality	Gene(s) affected/ fusion gene	Predicted outcome	Risk group	Frequency	Suggested detection method
t(9;22)(q34;q11)	<i>BCR-ABL1</i>	Poor	Very high	5 % of childhood ALL and 15 % in adolescents	Cytogenetics and FISH at diagnosis Qrt-PCR for baseline level; agarose gel electrophoresis of PCR product to distinguish between p190 and p210 Qrt-PCR for monitoring
t(4;11)(q21;q23)	<i>MLL-AFF1</i>	Poor	Very high	50–70 % of infant ALL and in ~5 % of pediatric and adult cases	rtPCR or cytogenetics at diagnosis (break-apart FISH probes cannot identify the specific translocation partner, which is prognostically important) rtPCR or FISH for follow-up
Near haploidy (24–29 chromosomes)	Numerical aberration with whole chromosome gains	Poor	Very high	Very rare, seen in 0.7–2.4 % of ALL	Cytogenetics at diagnosis Cytogenetics, ploidy analysis, or specific centromeric FISH probes for follow-up
t(8;14)(q24;q32) Or variants	<i>MYC-IGH</i>	Poor	High	Uncommon, represents only 1–2 % of ALL and is closer to a leukemic stage of a lymphoma than to other ALL types	FISH, cytogenetics at diagnosis FISH, cytogenetics for follow-up
Other <i>MLL</i> translocations: t(11;19); t(10–19); t(9;11), etc.	<i>MLL</i> fusion proteins	Intermediate	High	25 % of infant ALL, <10 % in adolescents	Cytogenetics or break-apart FISH probe for diagnostic screening; rtPCR for subsequent analysis FISH or cytogenetics for follow-up; rtPCR (for MRD)
t(12;21)(p13;q22)	<i>ETV6-RUNX1</i>	Good	Standard	Most frequent, present in 25 % of patients	FISH at diagnosis FISH for follow-up

High-hyperdiploidy (51–64 chromosomes) with trisomies 4, 10, 17
t(1;19)(q23;p13) Numerical aberration
Good Standard 30 % of childhood ALL Cytogenetics at diagnosis
Cytogenetics, ploidy analysis, or specific centromeric FISH probes for follow-up

TCF3-PBX1 Protocol dependent High/standard; protocol dependent 5–6 % of childhood ALL Cytogenetics

RECENTLY DESCRIBED STRUCTURAL ABNORMALITIES OF CLINICAL SIGNIFICANCE

iAMP 21	<i>RUNX1</i> overexpressed	Potentially high risk	2 % of childhood B-ALL	FISH
<i>PAX5</i> mutation	<i>PAX5-IGH</i> , <i>PAX5</i> deletion, point mutation	Potentially high risk	39 % of childhood B-ALL	Cytogenetics, FISH
<i>IGH-CRLF1</i>	<i>CRLF1</i> overexpressed	Potentially high risk	60 % of DS ALL and 10–15 % of <i>BCR-ABL1</i> negative ALL	FISH, gene-expression micro-array
<i>P2RY8-IGH</i>	<i>CRLF1</i> overexpressed	Potentially high risk		FISH
Translocation or deletion of <i>IKZF1</i>	<i>IKZF1</i> deletion	Potentially high risk	80 % of <i>BCR-ABL1</i> positive and 35 % of remaining B-ALL	Gene-expression microarray

Numerical Chromosomal Aberrations

The clinically significant numerical aberrations seen in ALL are high hyperdiploidy (HeH) (51–65 chromosomes), near haploidy (24–29 chromosomes), and near diploidy (31–39 chromosomes) [34, 39, 46, 47]. HeH, which is present in 23–42% of newly diagnosed precursor B-ALL, results from nonrandom gain of specific chromosomes (4, 6, 10, 14, 17, 18, 20, 21, and X) and is further sub grouped as those with 47–50, or more than 50 chromosomes [43, 48, 49]. HeH with 51–65 chromosomes is considered a favorable risk factor when associated with specific chromosomal gains, such as trisomies of chromosomes 4, 10, and 17 (Fig. 2.2a–c) [49]. HeH cells are more sensitive to apoptosis with certain drugs especially methotrexate, mercaptopurine, and L-asparaginase than nonhyperdiploid cells, which may account for their better response to chemotherapy [50]. Low hypodiploidy and near hypodiploidy have a worse outcome compared with near diploid chromosomes [51–53]. Good metaphase spreads are critical for accurate analysis. Low hypodiploidy often undergoes duplication and may be misdiagnosed as hyperdiploidy, thus precluding a distinction that is crucial for prognostication. Analysis of metaphase chromosomes by G-banding is still the gold standard and best method in the clinical laboratory to evaluate numerical abnormalities as it provides a global overview of the genome and a baseline to trace the evolution of the disease.

Common Structural Aberrations

t(9;22)(q34;q11)/*BCR-ABL1*/Philadelphia Chromosome

The Philadelphia (Ph) chromosome results from the balanced translocation t(9;22)(q34;q11) and is the hallmark of chronic myelogenous leukemia (CML). Three to five percent of childhood and 25% of adult ALL have the Ph chromosome [54]. Ph+ALL (Fig. 2.3a) have traditionally been considered as a very high risk group with a dismal outcome [55]. Addition of specific tyrosine kinase inhibitors, such as imatinib to the chemotherapy regimens, have changed this traditional outlook of Ph+ALL as a “hallmark high-risk disease” requiring stem cell transplantation to one with a good outcome [54, 56]. There are two major forms of *BCR-ABL1* translocations depending on the breakpoints on the *BCR* gene (Fig. 2.3b, c). The translocation consistently involves exon 2 of the *ABL* gene, but occurs in different exons of the *BCR* gene. The fusion involving the major breakpoint region (MBR) between exons 12 and 13 or 13 and 14 leads to expression of an 8.5 kb transcript coding for a 210 kD fusion protein (p210^{BCR-ABL1}) that characterizes adult CML, and about 30% of ALL. In 70% of Ph+ALL, the breakpoint in the *BCR* gene is between alternate exon 1 and exon 2 (minor breakpoint region/m-BR), resulting in a smaller 7.5 kb transcript coding for a 190 kD protein (p190^{BCR-ABL1}) [57, 58] (Fig. 2.3a, b). Other less common fusions [59], and co-expression of the p210^{BCR-ABL1} and p190^{BCR-ABL1}

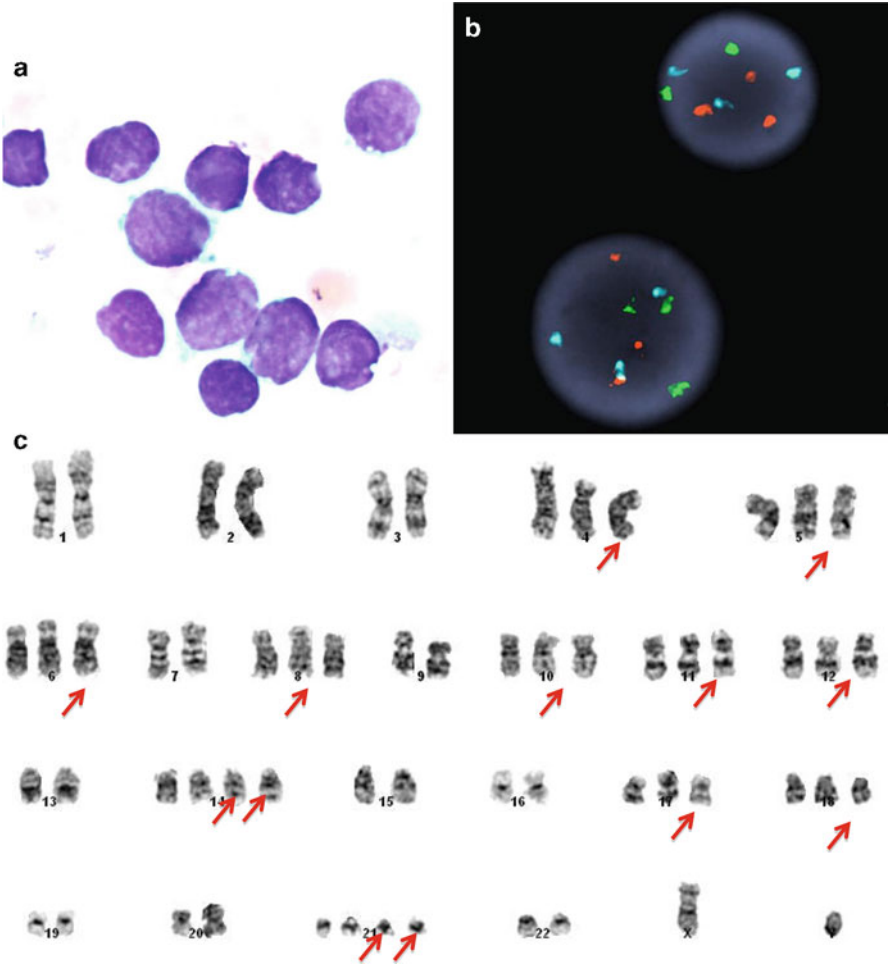


Fig. 2.2 ALL with triple trisomies. (a) blasts with “FAB-L2” morphology with variation in size and irregular nuclear contour; (b) fluorescence in situ hybridization using centromeric probes on interphase nuclei in an ALL patient with hyperdiploidy showing additional signals for chromosomes 4, 10, and 17 (triple trisomies); (c) karyogram of ALL blasts with HeH (*Photo courtesy Dr. Kaari Reichard. University of New Mexico.*)

encoding transcripts as a result of alternative splicing in the M-BR of *BCR*, have been reported [60–66]. rtPCR (reverse transcriptase polymerase chain reaction) and conventional cytogenetic analysis readily detect the *BCR-ABL1* fusion transcript/t(9;22)(q34;q11); in those cases with a failed, cryptic, or normal karyotype result, FISH provides a reliable alternative method (Fig. 2.3c) [51]. Dual-color, dual-fusion FISH probes (Fig. 2.3d) have high specificity and can additionally detect associated deletions of derivative chromosome 9, and complex or variant translocations [67, 68].

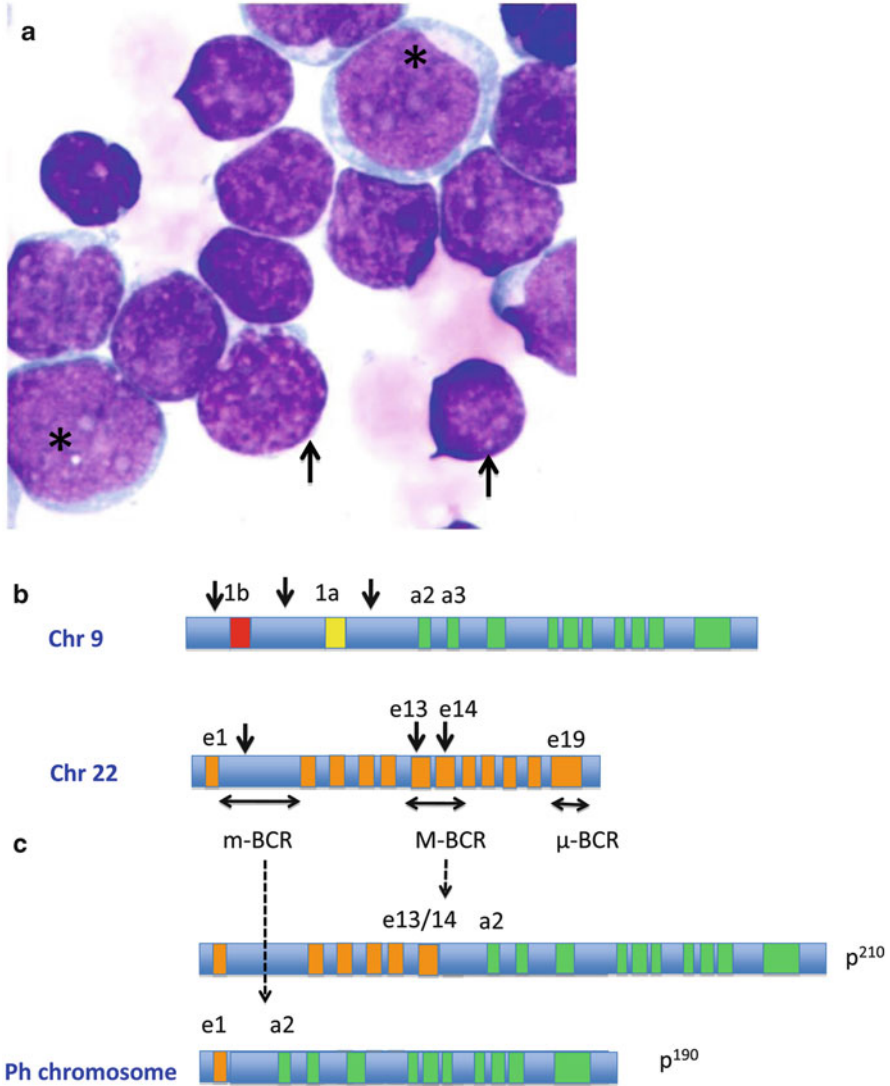


Fig. 2.3 Ph+ ALL. (a) Ph+ ALL. Note the variation in size of blasts; some with more cytoplasm and multiple nucleoli resemble myeloid blasts (*asterisk*), whereas others are small, lymphoblast-like (*arrow*). Immunophenotypically, Ph+ ALL often coexpress myeloid antigens. (b) t(9;22) (q34;q22) *arrows indicate* break-point regions on chromosomes 9 (*upper*) and chromosome 22 (*lower*). (c) Break at major breakpoint (M-BCR) resulting in BCR/ABL^{p210} and break at minor breakpoint (m-BCR) resulting in BCR/ABL^{p190} . ALL more often demonstrates the BCR/ABL^{p190} form of the translocation that translates into the p190 tyrosine kinase. (d) Interphase FISH showing the 1R1G2F pattern of the BCR/ABL translocation (*arrows point at fusion*). *Vysis dual-color, dual-fusion probe set*. Normal pattern: 2 Red (R), 2 Green (G). F=Fusion

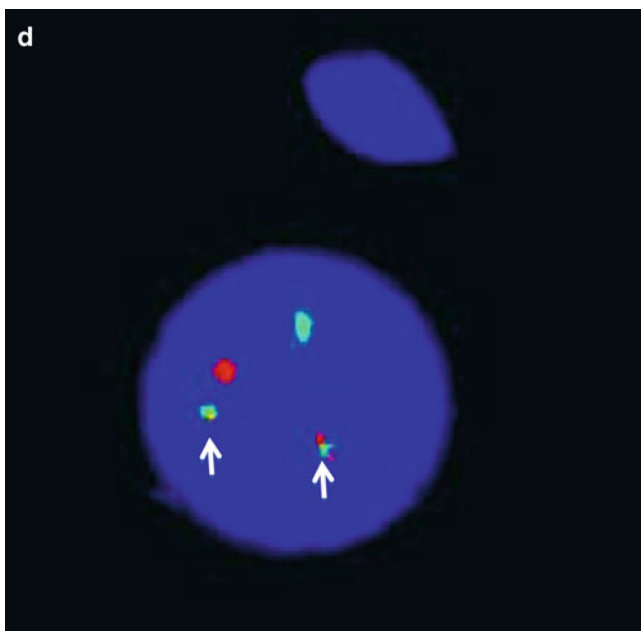


Fig. 2.3 (continued)

t(4;11)(q21;q23)/AFF1/MLL (AF4/MLL)

Chromosomal translocation between the C-terminal of *MLL* gene at chromosome band 11q23 and *AFF1* on chromosome 4q21 results in the *MLL-AFF1* fusion (Fig. 2.4a, b). The *t(4;11)* demonstrates an age-dependent distribution, and is observed in 50–70% of infant ALL and roughly 5% of pediatric and adult cases. Clinically it is associated with high-risk ALL. Most patients have a CD10–, CD19+ (pro-B) profile with co-expression of myeloid antigens. Breakpoints on *MLL* gene are dispersed over a wide region, and clustered differently in infant vs. other patients. To date, 104 translocation partners are known of which 64 have been molecularly characterized [69]. The biological behavior and distribution of the different *MLL* translocations depend on the translocation partner, for example, *t(4;11) AFF1/MLL* is almost exclusively seen in infants and has the worst outcome; *t(11;19) MLL/MLLT1* (previously known as *MLL/ENL*) [70] occurs in both ALL and AML, and has a poor prognosis, whereas *t(9;11) MLLT3/MLL* (previously known as *AF9/MLL*) present in AML has a favorable prognosis. A systematic evaluation starting with a commercially available *MLL* split signal FISH probe is recommended for screening as a first step. Should an abnormality be detected, karyotype or genomic PCR-based molecular methods can be used to further delineate the recombination [71].

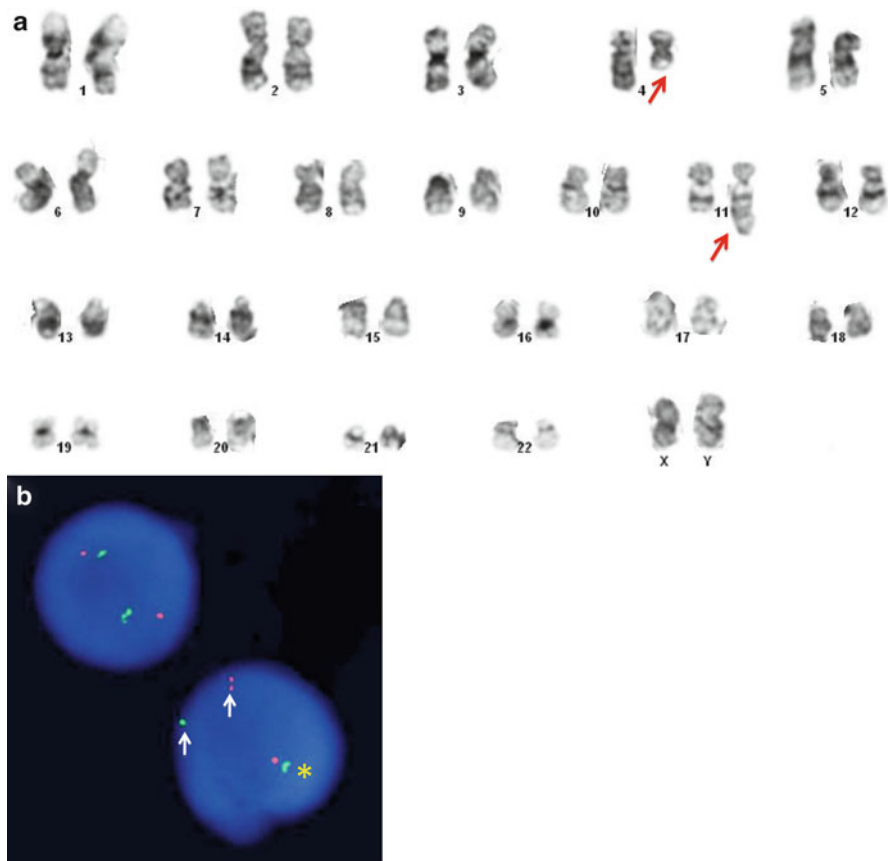


Fig. 2.4 (a) Karyogram showing $t(4;11)(q21;q23)$ (Photo courtesy Dr. Kaari Reichard, University of New Mexico.) (b) LSI *MLL*/11q23 dual-color, break-apart rearrangement probe (Vysis) in inter-phase nuclei from bone marrow blasts. Normally two fused signals would be seen. In *MLL* rearrangement, the abnormal pattern showing 1R1G1F is identified indicative of a rearranged *MLL* (the separated *green* and *red* signals) and the remaining normal allele (*yellow/fused*). When *red* and *green* signals are less than two signal width apart they are considered as single fused/overlapping signal (*asterisk*). (Photo courtesy Dr. Kaari Reichard, University of New Mexico.)

$t(1;19)(q23;p13)/TCF3-PBX1$ (*E2/PBX1*)

$t(1;19)$ is detected in 5–6% of childhood ALL and can occur as a balanced or an unbalanced translocation. It occurs almost exclusively in pre-B ALL expressing cytoplasmic immunoglobulin (CD10+, CD19+, cIg+) [23, 24]. Ninety to ninety-five percent of cIg + ALL [18] have the translocation $t(1;19)(q23;p13)$ [23, 24]. Although initially thought to connote an unfavorable outcome, several subsequent studies have reported that the negative impact of $t(1;19)$ could be offset with intensive chemotherapy programs [30, 31].

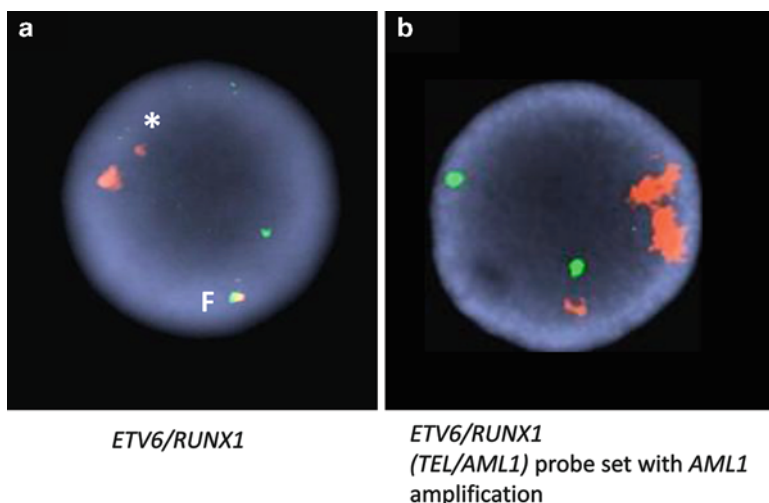


Fig. 2.5 (a) Interphase FISH with LSI *TEL/AML1* ES dual-color translocation probe showing 1 fusion (*TEL/AML1*), 2 red (normal *AML1* and asterisk showing smaller residual *AML1*), 1 green (*TEL*). Normal pattern: 2R2G *ETV6/12p13*; centromeric green, telomeric red; *ETV6/RUNX1* fusion: yellow. (b) same probe set showing *AML1* amplification (multiple red signals) (Photo courtesy Dr. Kaari Reichard, University of New Mexico.)

t(12;21)(q21;q22)/*ETV6-RUNX1* (*TEL/AML1*)

This is the most frequent structural abnormality in pediatric ALL. It is present in 25% of patients and is associated with a favorable outcome. *ETV6/RUNX1* is a cryptic translocation and cannot be detected by conventional metaphase cytogenetics and requires FISH. Break-apart or dual-fusion extra signal probe strategies can be used for detection; the latter resulting in a fusion signal and a smaller residual *AML1* signal resulting from break within the *AML1* locus (Fig. 2.5a). The probe set can also demonstrate *AML1* amplification (Fig. 2.5b).

t(8;14)(q24;q32)/*MYC-IGH*

The translocation t(8;14)(q24;q32) (Fig. 2.1b) and the variant forms t(2;8)(p13;q24) and t(8;22)(q24;q11) occur in Burkitt lymphoma, some diffuse large B cell lymphomas, less commonly in precursor B-ALL, and rarely precursor T-ALL and some AMLs. Mature B-cell ALL or Burkitt-like ALL are rare, comprising 1–2% of pediatric ALL [72]. With a few exceptions, ALL blasts with translocations involving *MYC* have a FAB-L3 morphology (Fig. 2.1a) and demonstrate a mature B cell phenotype, with expression of surface immunoglobulin light chain [37, 72–77]. These translocations juxtapose the oncogene, *MYC*, located at 8q24 to the immunoglobulin

lin heavy chain gene (*IGH*) at 14q32, or the light chain genes kappa (*IGK*) or lambda (*IGL*) at 2p12 and 22q11, respectively. Patients with mature B-ALL respond poorly to conventional treatment for precursor B-ALL, but have an improved survival with very intensive chemotherapy [72]. Rapid diagnosis for quick intervention is easily achieved by FISH test using break-apart probe for the *MYC* locus.

Precursor T-Cell Leukemia/Lymphoma

About 10–15% of pediatric ALL have a T-cell immunophenotype. In comparison with precursor B-ALL, they are more frequent in the older children and adolescents, and present with a higher tumor burden [78]. Historically, T-ALL have been considered as high risk with increased incidence of relapses, although the outcome has greatly improved with more intensive protocols [78, 79].

Genetic Aberrations

In approximately 50% of T-ALL, structural chromosomal aberrations can be identified by conventional karyotyping [80–82]. Numerical changes except for tetraploidy are rare, seen in approximately 5% of cases, and are without prognostic significance [80]. Translocations involving the *TRA/TRD* (14q11) and *TRB* (7q34) genes are seen in 35% of patients [80, 83–86] and result in upregulation of oncogenic transcription factors involved in T-cell differentiation. Other rearrangements include fusion translocations not involving the T-cell receptor loci, for example, the cryptic interstitial deletion of *TAL1* at 1p32 resulting in the *SIL/TAL1* chimeric gene (~30% T-ALL), t(10;11)(p13;q14)/*CALM/MLL10* (*CALM/AFF10*) [87], and t(5;14)(q34;q32) [88] both of which are cryptic, and translocations involving the *MLL* gene [89]. Specific signatures obtained by GEP analyses can segregate the different genetic lesions according to the major oncogenic pathways. The major oncogene clusters are *NOTCH1* [90–92]; *HOXA* [89, 93–97]; *TLX1* (*HOX11*) AND *TLX3* (*HOX11L2*) [98–104]; beta-helix-loop-helix (*SCL/TAL1*) [105, 106], *LYL1* [107]; *ABL1* [108, 109]; *JAK1* [110]; and *JAK2* [111].

The genetic aberrations within these oncogenic clusters predict outcome in precursor T-ALL [103, 104]. T-ALL with translocations in the *SCL/TAL1* cluster—t(1;14) (p32;q11), or del(1p32) and in the *HOX11* cluster—t(10;14)(q24;q11) or t(7;10)(q35;q24) have a better outcome, whereas those involving *HOX11L2*—t(5;14)(q35;q32) and t(10;11)(p14;q14)/*CALM-MLL10*, *JAK1*, and *ABL1* have a poor outcome independently, or in combination [99, 101–106, 110, 112, 113].

Unlike precursor B-ALL, the clinical significance of immunophenotypic subtyping based on surface antigen expression in T-ALL is not well established [114]; some exceptions are a correlation of expression of certain developmental stage specific antigens with some recurrent chromosomal aberrations, for example, *TAL1* translocations

commonly have a surface CD3+, TCR+, mature T-cell phenotype; translocations involving *HOX11* are frequently CD1a+, representing a cortical or intermediate stage of development, and early precursor T-ALL (ETP-ALL), a recently described distinct biological subtype with high risk of remission induction failure or relapse, has a distinctive immunophenotype—CD1a-, CD8-, CD5 (weak), and presence of stem-cell or myeloid markers, consistent with its origin from early T-cell precursors [115].

About 50% of the abnormalities in T-ALL can be detected by conventional metaphase cytogenetics [80, 113, 116]. A high percentage of cryptic abnormalities, such as cryptic deletions of 9p21 and 1p32, and translocations with breakpoints near terminal regions of chromosomes, for example, t(5;14)(q35;q32), 9q34 breakpoints, and rearrangements of *TRB* at 7q34, are often disclosed only with appropriate FISH probes [80]. A recent study has established a novel Q-rPCR assay for the detection of *NUP214-ABL1* fusion [117]. Although risk-stratified protocols have not been employed commonly in T-ALL, the discoveries of involvement of *NOTCH1*, *ABL1*, and *JAK* kinases in T-ALL have the potential for translation into novel targeted therapies [91, 92, 109, 110, 117], and may require a precise molecular stratification in future.

Acute Myeloid Leukemia

Compared with ALL, AML is less common in the pediatric age group, accounting for 16% of AL in children less than 15 years of age, and 36% in adolescents and young adults in the 15–20 years age group. A bimodal distribution is observed, with increased frequency of AML in children less than 2 years compared to older children.

Genetic Aberrations

Cytogenetics is the most important predictor of outcome in childhood AML [44, 78, 118–120]. Cytogenetic abnormalities are seen in 70–85% of pediatric AML [44, 119], and form the basis of classification [121] and risk stratification of AML [44, 122, 123]. The cytogenetic and molecular subtypes of AML carry the same prognostic significance in children as in adults, although there is a difference in their distribution. For example, the frequency of *MLL* (11q23) translocations decreases from nearly 40% of AML in infants to 10% in older children [119], and the translocation t(1;22)(p13;q13) resulting in the aberrant expression of *RBM15/MKLI(OTT-MAL)* fusion gene occurs exclusively in non-Down syndrome-associated acute megakaryoblastic leukemia in infants (Fig. 2.6a, b) [124, 125].

NPM1 and *FLT3* mutations are less commonly observed in childhood AML. *NPM1* mutations occur in 8–10% of childhood AML and in approximately 25% of those with a normal karyotype [126, 127] as opposed to the overall frequency of 35% adult

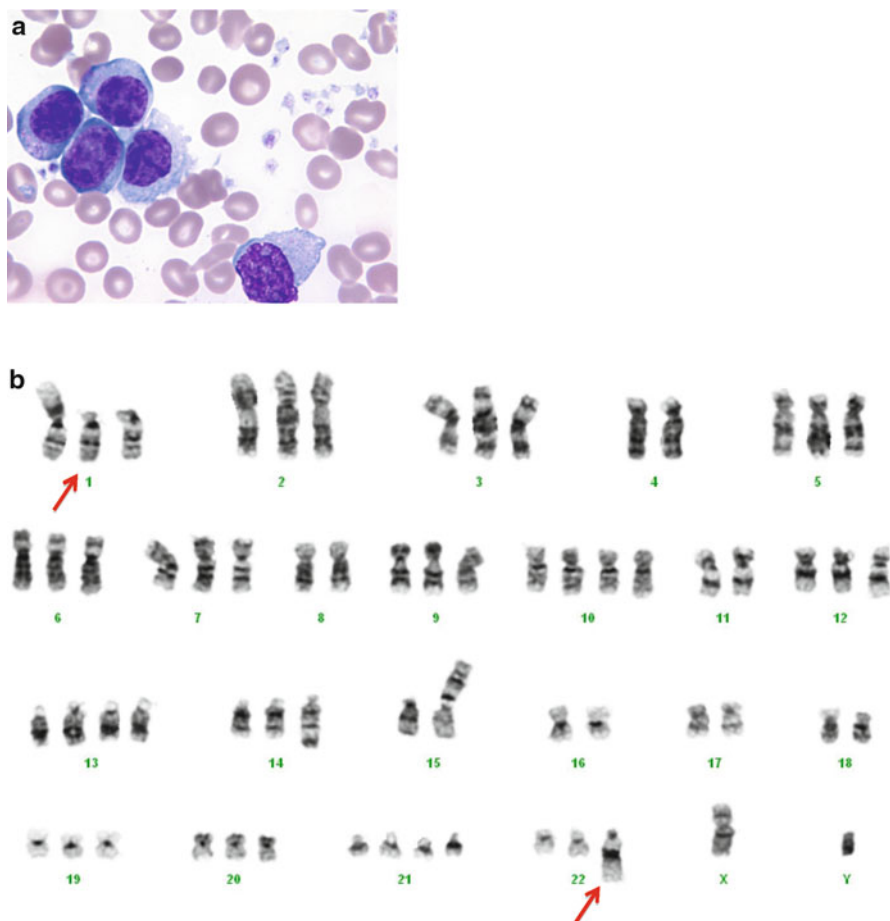


Fig. 2.6 (a) Acute megakaryoblastic leukemia. Note the cytoplasmic blebs in the blasts. (b) Karyogram showing hyperdiploidy with multiple numerical and structural abnormalities including t(1;22)(p13;q13)/*RBM15/MKLI* translocation (arrows) (Photo courtesy Dr. Kaari Reichard, University of New Mexico.)

AML and 60% of those with normal karyotype [128, 129]. The frequency of *FLT3* internal tandem duplication (*FLT3* ITD) is 10–15% in children vs. 20–30% in adults [130–132]. The prognostic implications of *NPM1* and *FLT3* mutations are similar in children as in adult patients [130, 132–134], i.e., the presence of *NPM1* mutation in the absence of *FLT3*-ITD is associated with a favorable outcome; the coexistence of *NPM1* mutations and *FLT3*-ITD has an intermediate prognosis; and *FLT3*-ITD alone predicts a poor outcome for the patient [126, 130, 135]. *NPM1* mutations are stable during disease evolution and represent a possible marker for minimal residual disease detection. Table 2.3 lists the cytogenetic and molecular genetic abnormalities that are common and prognostically important in pediatric AML.

Table 2.3 Prognostically important genetic abnormalities in childhood AML

Chromosomal abnormality	Gene(s) affected/ fusion gene	Outcome predictor	Description	Suggested detection method
t(1;22)(p13;q13)	<i>RBM15/MKLI</i>	Poor	Rare; seen in 0–3 % of childhood AML, and is restricted to the infant AMKL in non-Down syndrome children. Very rarely reported in Down syndrome patients. Results may be false negative if marrow fibrosis in AMKL results in insufficient sample for cytogenetic analysis	Cytogenetics and FISH at diagnosis and follow-up
Chromosome 3 abnormalities: inv(3)(q21;q26), t(3;3)(q21;q26), t(3;21), gain and loss of 3q	Overexpression of <i>EVII</i>	Poor	3q21–26 abnormalities are usually associated with AML–M7 (AMKL) and myelodysplasia. Gains of 3q portend evolution to AML in Fanconi anemia patients	Cytogenetics and FISH; FISH preferred
t(6;11)(q24.1;p155)	<i>NUP98-C6orf80</i>	Indeterminate	Occurs in 2–4 % of childhood AML and has been detected in AMKL	Cytogenetics
t(6;17)(q23;q11.2)		Poor	Rare recurrent chromosomal abnormality in congenital AML (within the first 4 weeks of birth)	Cytogenetics
-5/del(5q)		Poor	0–2.5 % of childhood leukemia, more in therapy-related AML following alkylating agents	Cytogenetics, FISH FISH for follow-up
t(5;11)(q35;p15.5)	<i>NSD1/NUP98</i>	Poor	Rare; cytogenetically cryptic	FISH

(continued)

Table 2.3 (continued)

Chromosomal abnormality	Gene(s) affected/ fusion gene	Outcome predictor	Description	Suggested detection method
t(7;12)(q36;p13)	HLXB9/ETV6	Poor	Rare abnormality occurring almost exclusively in infant AML (30 % of infant AML);	FISH
-7/del(7q)		Poor	Monosomy 7/del(7q) are seen in 2–7 % of childhood AML. It is particularly common in t-MN following chemotherapy with alkylating agents, and is the most commonly acquired abnormality in children with Fanconi anemia predisposed to AML	
Trisomy 8		Poor	Seen in 5–10 % of childhood AML, and ~30% when associated with Down syndrome.	Cytogenetics, FISH
t(8;16)(p11;p13)	MOZ-CBP	Poor	Rare abnormality; associated with hemophagocytosis.	FISH for follow-up Cytogenetics
t(8;21)(q22;q22)	RUNX1/TL/RUNX1	Favorable	Seen in 7–16 % of childhood AML. Because of a favorable outcome, its detection is mandatory in most trials. Fifty percent relapse (these are often associated with <i>KIT</i> mutations)	Cytogenetics, FISH FISH for follow-up
t(11q23): t(11;19); t(10–19); t(9;11), etc.	X- <i>MLL</i> fusion proteins	Biological behavior varies with translocation. t(9;11) favorable t(10;11) and t(11;19) poor	Most frequent abnormality in childhood AML (14–22 % overall; ~60 % infant AML). Frequently present in t-AML following therapy with topoisomerase-II inhibitors	Cytogenetics or break-apart FISH probe at diagnosis; rtPCR FISH, cytogenetics for follow-up; rtPCR (for MRD)

t(15;17)(q22;q21)	PML-RARA	Favorable	Acute promyelocytic leukemia (APL), seen in 2–10 % childhood AML, mostly in older children. The outcome is excellent (cure rate 70–90 %) with all trans retinoic acid therapy. A high proportion (~35 %) of childhood APL are associated with FLT3-ITD	FISH at diagnosis (quick diagnosis required) FISH for follow-up
inv(16)(p13q22)/ t(16;16)(p13;q22)	MYH11-CBFβ	Favorable	Comprise 3–8 % of childhood AML. These abnormalities are very often cytogenetically cryptic and require FISH for diagnosis. Trisomy 22 is frequently present as an additional abnormality.	Cytogenetics for diagnosis Cytogenetics, ploidy analysis, or specific centromeric FISH probes for follow-up

Therapy-Related Myeloid Neoplasms (t-MN; previously called t-MDS/AML)

Use of intensive treatment protocols for childhood cancers has improved outcome for the primary tumors at the cost of increase in t-MN. It has been estimated that t-MN affects at least 1% of childhood cancer patients [136], with a cumulative incidence of 2% at 15 years. The frequency of t-MN in children depends on the nature of the primary tumor, nature of therapy [25, 137, 138], duration of therapy, and the underlying genetic predisposition [139]. A higher incidence is seen in children treated for Hodgkin lymphoma Ewing's sarcoma/PNET, and precursor B-ALL patients who received topoisomerase-II inhibitors [25, 137, 138, 140–143]. A higher incidence is also observed for children with neurofibromatosis 1 (NF1) and other germline DNA repair disorders [138], those with polymorphisms in drug metabolizing enzymes [144–145], or in children who received granulocyte colony-stimulating factor (G-CSF) following ALL induction chemotherapy [146].

Most of the therapy-related leukemias are t-AML. Therapy-related ALL comprise 5% of secondary leukemias. Molecular detection of *IGH@* and *TCR@* gene rearrangements can facilitate the identification of therapy-related ALL from the primary ALL. Abnormalities of chromosome 5, 7, or 21q22 translocation are commonly observed in t-MN; the frequency of classical recurrent nonrandom translocations t(8;21), t(15;17), 11q23, and inv 16 are common in pediatric t-MN due to the frequent use of topoisomerase II inhibitors, especially in ALL. The outcome for t-MN is very poor. Developing individualized treatment protocols based on the genetic framework of the patient and targeted to a specific antigen or genetic pathway in the specific tumor is the goal for molecular medicine to check the increase in therapy-related neoplasms.

Acute Leukemia Associated with Down Syndrome

Children with Down Syndrome (DS) have a 150 times greater risk of developing AML [147, 148] than those without DS. Acute megakaryoblastic leukemia (AMkL) is the commonest subtype of AML in DS. DS children have a 500-fold increased risk of developing AMkL [149] in DS patients. DS-AML has an excellent cure rate of nearly 80% compared with the non-DS-AML. Virtually all children with DS-AMkL have somatic mutations in the *GATA1* gene [150]. The mutations result in a truncated GATA1 protein (also referred to as GATA1s) that contributes to uncontrolled megakaryoblastic proliferation. Somatic *GATA1* mutations are specific to AMkL occurring in trisomy 21 patients [150]; the underlying mechanism of this association is as yet unresolved.

DS children also have a 20-fold increased risk for developing ALL [148, 151]. In contrast to DS-AML, DS-ALL is of very high risk and has a worse outcome compared to non-DS ALL [152–154]. *GATA1* mutations are not present in DS-ALL [155]. Recent studies have shown the presence of a R683 somatic mutation in *JAK2* in 18–28% of DS-ALL patients [156, 157]. Aberrantly increased expression of

cytokine receptor *CRLF2* is present in the majority of DS-ALL with R683-mutated *JAK2* [158, 159], suggesting a synergistic effect between the two mutations. Activation of the downstream *CRLF2*-*JAK*-*STAT* signaling pathway in a majority of this DS-ALL holds therapeutic potential for use of *JAK* inhibitors.

In AMkL, conventional cytogenetics and FISH are important in the detection of trisomy 21, the latter is especially useful in DS mosaics [160]. Testing for the *GATA1* mutation can stratify AMkL into chemosensitive (*GATA-1* mutation positive) or chemoresistant (mutation negative) groups [161]. *GATA1* mutations can be detected by sequencing and this testing can be employed in MRD evaluation. DS-related myeloid proliferations and their link to AL are discussed in greater detail in the chapter on pediatric myelodysplasia and myeloid proliferation.

Blastic Plasmacytoid Dendritic Cell Tumor

Blastic plasmacytoid dendritic cell tumor (BPDCN), formerly known as blastic natural killer (NK)-cell lymphoma or CD4+/CD56+ hematodermic neoplasm [162], is a rare hematological neoplasm with a lymphoblast-like morphology. It is typically seen in adults, rare cases have been described in children [162, 163]. Although usually presenting as skin nodules, a fatal leukemic phase may develop rapidly, more so in children, which can be difficult to distinguish from ALL, hence the inclusion in this section. Based upon the phenotypic (CD4+, CD56+, CD123+ BDCA-4 +, CD303/BDCA-2+, TCL1+, CD68–), functional, and genetic features, the cell of origin is believed to be a hematopoietic precursor committed to a plasmacytoid dendritic cell (PDC) lineage [162, 164]. Two-thirds of BPDCN have complex, nonrandom cytogenetic abnormalities [162]. GEP studies have identified a set of genes expressed in BPDCN not described in other hematopoietic cells, but highly expressed in neuronal cells and implicated in neurogenesis [165].

Hereditary Disorders with Predisposition to AL

An increased predisposition for development of AML has been observed in children with inherited disorders that include disorders of DNA repair and increased chromosomal fragility, for example, Fanconi anemia and Bloom syndrome; bone marrow failure syndromes, such as Kostmann syndrome, Diamond-Blackfan anemia, familial platelet disorder, and Shwachmann-Diamond syndrome; and in Neurofibromatosis type I [162, 166–170]. The development of leukemia in these disorders often occurs in adulthood. Highly sensitive and specific diagnostic tests based on the mutations identified for many of the inherited disorders are available [166] for confirmation of the underlying germline genetic disorder and in investi-

gation of family members of the affected individual. These tests and the different laboratories offering them are available at <http://www.genetests.org>.

The next section deals with the different molecular genetic techniques helpful in the evaluation of acute leukemia in the pediatric population.

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