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Prognostic and Predictive Biomarkers in Precursor B-cell Acute Lymphoblastic Leukemia

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Abstract: Precursor B-cell acute lymphoblastic leukemia (B-ALL) is a hematologic malignancy characterized by clonal proliferation of abnormal B-cell precursors in the bone marrow. Most of the B-ALL cases are diagnosed in children, although it can present at any age. Thanks to the tremendous advances in our understanding of its biology, identification of more and more prognostic and predictive biomarkers, and application of individualized risk-adjusted treatment, B-ALL has become the most curable malignancy in children, with a long-term survival rate close to 90% in newly diagnosed patients. However, the prognosis of B-ALL remains dismal in adults and children with relapse. Relapsed B-ALL continues to be the leading cause of cancer-related death in children and young adults. Risk stratification is currently based on age, white blood cell count, early therapeutic response, and chromosomal abnormalities such as ploidy and translocations. Recent advances in molecular diagnostic technologies have led to a rapid expansion of the list of

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molecular biomarkers associated with B-ALL, which show promise to improve the accuracy of risk prediction, and eventually achieve better risk-adapted treatment and clinical outcome. In this chapter, we provide an overview of the prognostic and predictive biomarkers in B-ALL, including some recently identified genomic alterations with significant prognostic impact.

Keywords: genetic biomarkers for acute lymphoblastic leukemia; immunophenotypic biomarkers for acute lymphoblastic leukemia; molecular biomarkers for acute lymphoblastic leukemia; prognostic biomarkers in B-ALL; predictive biomarkers in B-ALL

INTRODUCTION

Precursor B-cell acute lymphoblastic leukemia (B-ALL) is a hematologic malignancy resulting from clonal proliferation of abnormal B-cell precursors (B-lymphoblasts) in bone marrow (BM). Most B-ALL cases are diagnosed in young children, although it can occur at any age. ALL, composed of approximately 85% B-ALL and 15% T-cell ALL (T-ALL), is the most common malignancy in children, accounting for approximately 30% of all pediatric cancer cases. The estimated number of new B-ALL cases in the USA is close to 5000 each year (1, 2). Thanks to the tremendous advances in our understanding of the biology of this disease, identification of more and more prognostic and predictive biomarkers, risk stratification and risk-adjusted treatment, B-ALL has become the most curable malignancy in children, with a long-term survival rate close to 90%. However, the prognosis of B-ALL remains dismal in adults and pediatric patients with relapse (1, 2). Relapse and chemotherapy related morbidity and mortality remain the big challenges to oncologists, and B-ALL continues to be the leading cause of cancer-related death in children and young adults. Therefore, new prognostic / predictive biomarkers and more accurate risk stratification are needed to further improve individualized treatment and achieve better clinical outcomes.

A biomarker is defined as a characteristic that is measured as an indicator of normal biological processes, pathological processes, or responses to an exposure or therapeutic intervention. Biomarkers could be molecular, morphologic, radiographic, physiological, or phenotypic. Molecular biomarkers are molecules that provide specific information about a given disease, or predisposition to a given disease. Molecular biomarkers are of many types, including characteristic chromosomal numerical or structural alterations, DNA sequence variants including indels, altered RNA, and altered/aberrant or new proteins. Molecular biomarkers play an increasingly important role in risk stratification and risk-adapted therapy of B-ALL.

Risk stratification of B-ALL is currently based on age, white blood cell count (WBC), early therapeutic response, chromosomal abnormalities such as ploidy, and translocations. Recent advances in molecular diagnostic technologies have led to a rapid expansion of the list of genetic biomarkers associated with B-ALL, which help identify new subtypes and show promise to improve the accuracy of risk stratification, and eventually achieve better personalized treatment and clinical outcomes. In this chapter, we provide an overview of the prognostic and

predictive biomarkers in B-ALL, including some recently identified genomic alterations with impact on clinical outcomes. The genetic biomarkers predicting toxicity and resistance to chemotherapy drugs are not included in this chapter; they are described in the chapter of *Novel Aspects of Leukemia Pharmacogenomics* (3).

CLINICAL CLASSIFICATION OF MOLECULAR BIOMARKERS

Based on their role in patient management, molecular biomarkers are classified into three groups: diagnostic biomarkers, prognostic biomarkers, and predictive biomarkers. Diagnostic biomarkers are molecules or molecular alterations that are pathognomonic for their associated diseases or are highly unique to the diseases. The presence of a diagnostic biomarker is essential for the diagnosis of the disease associated with it. Moreover, a diagnostic biomarker can be the initiator of the disease process, e.g., *BCR::ABL1* fusion in *BCR::ABL1* positive B-ALL. Some diagnostic biomarkers are indicators of an increased predisposition to their associated diseases, e.g., germline mutations in *ETV6*, *RUNX1*, *PAX5,* and *IKZF1* in B-ALL.

Prognostic biomarkers provide information about the biology or natural history of their associated diseases. They are essential for the clinical risk stratification of patients for different cancer treatment protocols. There may be many prognostic biomarkers for one malignancy, and one biomarker may be prognostic for more than one disease entity. In addition, a biomarker can be both diagnostic and prognostic. Like diagnostic biomarkers, a prognostic biomarker may also be the initiator of the disease or an indicator of increased predisposition to the disease.

Predictive biomarkers indicate the likelihood that a given cancer will or will not respond to a specific treatment. Positive predictive biomarkers are associated with positive or enhanced response to therapy, while negative predictive biomarkers predict resistance to therapy. Positive predictive biomarkers are often targets for targeted therapies. Some biomarkers are both prognostic and predictive. The common approaches for investigating molecular biomarkers are:

- *Proteins:* Flow cytometry (FCM), immunohistochemistry, and cytochemistry.
- *Chromosome abnormalities:* Karyotyping, and Fluorescence In-Situ Hybridization (FISH).
- *DNA:* PCR, Sanger sequencing, microarray, Multiplex Ligation-dependent Probe Amplification (MLPA), and NGS (single gene, targeted or panel, whole exome, and whole genome).
- *RNA:* RNASeq, Gene expression profiling, Reverse Transcription-PCR.

GENETIC BIOMARKERS

Genetic abnormalities are used as important diagnostic, prognostic, and predictive biomarkers to help early disease detection, risk stratification, and guide treatment. Cytogenetic abnormalities served as the basis for the listed entities in the 2022 5th edition of WHO B-ALL classification (4). However, technological

advances in molecular technology have greatly enhanced our ability to detect driver mutations in B-ALL. Applying technologies such as next generation sequencing (NGS) in the form of whole exome sequencing, whole genome sequencing, transcriptome sequencing (RNA-seq), and deletion-duplication analysis, has led to the description of 23 distinct genetic subtypes of B-ALL (5). Some of these subtypes are very rare. Significant abnormalities are described below and summarized in Figure 1 and Table 1.

Hyperdiploidy

More than 46 chromosomes represent the largest cytogenetic subgroup in childhood B-ALL. It can be subdivided into two groups, high hyperdiploidy (51–65 chromosomes or DNA index (DI) ≥ 1.16) and low hyperdiploidy (47–50 chromosomes or DI 1.0–1.16) with a different prognosis for each group.

High hyperdiploidy (HHyper) is a favorable prognostic factor, presenting in up to 30% of children and 10% of adults with B-ALL (6). Typically, children with HHyper B-ALL achieve negative minimal residual disease (MRD) after induction treatment and have excellent cure rates, with 5-year event-free survival (EFS) and overall survival (OS) rates of ~75%, and ~ 90%, respectively. The most commonly gained chromosomes are 4, 6, 8, 10, 14, 17, 18, 21 and X with the presence of double trisomy (+4, +10) or triple trisomy (+4, +10, +17) being a prognostic factor of a very low risk of relapse (7). Trisomy 18 also has been associated with a favorable prognosis, while gain of extra copies of chromosomes 5 and 20 has been associated with relatively poor prognosis compared with those lacking these trisomies (8).

Although HHyper is generally associated with a favorable prognosis, there are still approximately 20% of HHyper childhood B-ALL cases that relapse. Several co-existent or secondary genetic abnormalities are thought to have an impact on prognosis. The structural abnormality i(17q) and gain of 1q are correlated with poor outcome in some studies (9). A recent study shows evidence that duplication 1q is an independent adverse factor on the disease-free survival (DFS) of HHyper patients (10). The recurrent translocations, t(9;22)(q34;q11), t(12;21)(p13;q22), t(1;19)(q23;p13), and t(4;11)(q21;q23) have also been reported in 1–4% of HHyper B-ALL. In these cases, the prognostic impact of the translocation is believed to override the beneficial effect of the high hyperdiploidy (11). Previous data showed that patients with both t(9;22) and hyperdiploidy have better outcome compared to t(9;22) in a diploid background (10). Also, mutations targeting genes encoding histone modifiers (e.g., *CREBBP*) and the RTK-RAS pathway (e.g., *FLT3*) are common in patients with HHyper. Mutations in these genes as well as *IKZF1* deletion have been detected at a higher incidence in relapsed specimens in comparison with diagnostic B-ALL samples (12, 13).

Low hyperdiploidy (LHyper) is an unfavorable prognostic factor in B-ALL, presenting in 10–11% of pediatric and 10–15% of adult cases with increasing incidence with age (6). The gained chromosomes generally include chromosomes X, 21 and 14. Studies have shown that patients with LHyper have worse OS and relapse-free survival (RFS), a significantly shorter median time to first relapse and less frequently achieve second complete remission than the patients with normal karyotype and miscellaneous abnormalities (14).

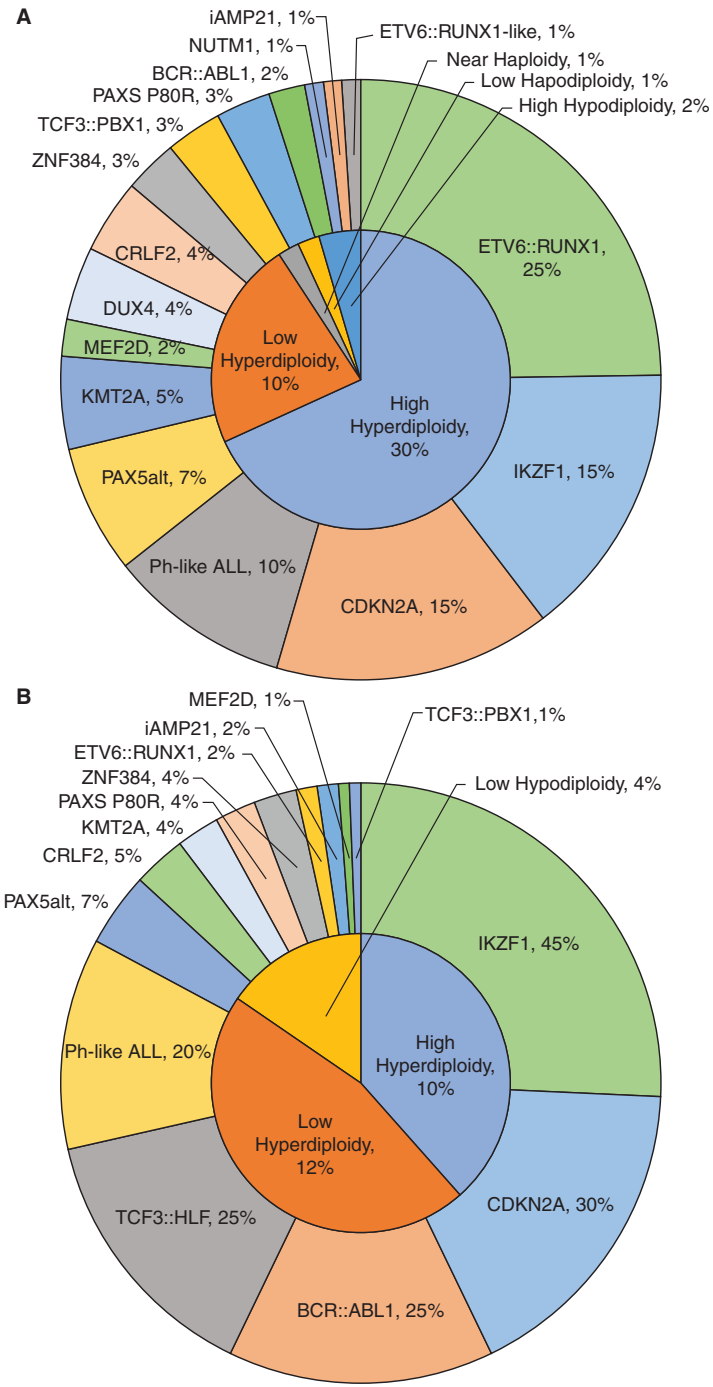

Figure 1. Pie chart of frequencies of the major subgroups of B-ALL. A, Pediatric B-ALL. **B,** Adult B-ALL.

TABLE 1 **Major genetic biomarkers in B-ALL**

Biomarker	Location	Function	Alteration	Prognostic	Predictive	Comment
High Hyperdiploidy	N/A	N/A	51–65 chromosomes	Excellent prognosis	N/A	Associated with mutations in genes encoding histone modifiers (e.g., *CREBBP*) and RTK-RAS pathway (e.g., *FLT3*)
Low Hyperdiploidy	N/A	N/A	47–50 chromosomes	Poor prognosis	N/A	-
Near Haploidy	N/A	N/A	24–29 chromosomes	Very Poor prognosis	Bcl2 inhibitors	May be associated with alterations in *NF1*, *NRAS*, *KRAS*, *FLT3*, etc.
Low Hypodiploidy	N/A	N/A	30–39 chromosomes	Very poor prognosis	Bcl2 inhibitors	Associated with *TP53* mutations (>90%)
High hypodiploidy	N/A	N/A	40–44 chromosomes	Poor prognosis	N/A	-
BCR::ABL1	*BCR*: 22q11; *ABL1*: 9q34.12	*ABL1*: Many diverse functions, including antigen receptor signaling in lymphocytes	Translocation	Poor prognosis; improved by TKIs	Positive; responds to ABL1 TKIs	High risk ALL.80% with *IKZF1* deletion
BCR::ABL1-like	N/A	N/A	Gene fusions (Table 2)	Poor prognosis	N/A	High risk ALL
CDKN2A	9p21.3	Cell cycle regulation, tumor suppression	Deletion/ hypermethylation	Poor prognosis	Negative	High risk ALL

(Continued)

TABLE 1 **Major genetic biomarkers in B-ALL (*Continued*)**

Biomarker	Location	Function	Alteration	Prognostic	Predictive	Comment
CRLF2	Xp22.33 and Yp11.2	B cell development through activation of the JAK-STAT pathway	Gene fusions/point mutation	Poor prognosis	Negative; trial of JAK TKIs	Standard and high risk ALL
DUX4	4q35.2	*DUX4*: Transcriptional activator	Gene fusions	Good prognosis	N/A	Most frequent partner is *IGH*. CD2 and CD371 positive.
ERG	21q22.2	*ERG*: Transcriptional regulator of hematopoiesis	Deletion	Good prognosis	N/A	Occurs exclusively in the DUX4r subgroup.
ETV6::RUNX1	*ETV6*: 12p13; *RUNX1*: 21q22	Transcriptional regulation	Translocation	Excellent prognosis	N/A	-
ETV6::RUNX1-like	N/A	N/A	Translocation	Poor prognosis	N/A	>80% occur in children. *ETV6* and *IKZF1* alterations
iAMP21	21q	N/A	Amplification	Poor prognosis	N/A	High risk ALL. Associated with RAS signaling pathway gene mutations,
IKZF1	7p12.2	B-cell development regulation	Deletion/point mutation/gene fusion	Poor prognosis	Resistance to ABL1 TKIs	High risk ALL. Older age at diagnosis. Emerging subtype IKZF1plus with very poor prognosis.

(Continued)

TABLE 1 Major genetic biomarkers in B-ALL (*Continued*)

Biomarker	Location	Function	Alteration	Prognostic	Predictive	Comment
KMT2A	11q23	Transcription factor; regulates gene expression during hematopoiesis	Translocation/ inversion	Very poor prognosis	Resistance to glucocorticoids	High risk ALL. CD10 negative, CD15, CD33 and CD68 positive.
MEF2D	1q22	Control of cell growth, survival and apoptosis	Gene fusions	Poor prognosis	Responds to HDAC9 inhibitors	CD10 negative, CD38 bright. *IKZF1* and *CDKN2A/B* deletion
NUTM1	15q14	Role in regulating cell proliferation.	Gene fusions	Excellent prognosis	HDAC inhibitors	Rare; occurs mainly in infants
PAX5 P80R	9p13	*PAX5*: Transcription factor; regulates B-lineage specific genes	Hotspot mutation (PAX5 p.Pro80Arg mutation)	Intermediate prognosis; poor prognosis in children.	N/A	Accompanied by inactivation of the second *PAX5* allele in all cases.
PAX5alt	9p13	Transcription factor; regulates early stages of B cell development	Gene fusions/ deletion/ amplification	Intermediate prognosis; poor prognosis in adults.	N/A	High risk ALL. More than 20 fusion partners including *ETV6* etc.

(Continued)

TABLE 1 **Major genetic biomarkers in B-ALL (*Continued*)**

Biomarker	Location	Function	Alteration	Prognostic	Predictive	Comment
TCF3::HLF	*TCF3*: 19p13.3; *HLF*: 17q22	*TCF3*: Lymphopoiesis; required for B and T cell development; *HLF*: Transcriptional activator	Translocation	Very poor prognosis	N/A	High risk ALL. *PAX5* deletion and RAS pathway gene mutations.
TCF3::PBX1	*TCF3*: 19p13.3; *PBX1*: 1q23.3	*TCF3*: See above. *PBX1* regulates many embryonic processes including hematopoiesis.	Translocation	Intermediate to excellent prognosis	N/A	Intermediate risk ALL due to modern therapy. High risk of CNS leukemia and relapse. Expresses cytoplasmic immunoglobulin μ heavy chain
ZNF384	12p12	Transcription factor	Gene fusions	Intermediate prognosis; but varies with different partners	FLT3 inhibitors	CD13 and CD33 positive, negative or weak CD10.
IGH::IL3	*IGH*: 13q32; *IL3*: 5q31.1	*IL3*: cytokine controlling the production and differentiation of hematopoietic progenitor cells	Translocation	Poor prognosis	N/A	Present with reactive hypereosinophilia and lack of peripheral blasts.

Hypodiploidy

Hypodiploidy may be defined as the loss of one or more chromosomes and constitutes ~5% of B-ALL cases across all age groups (6). Hypodiploidy B-ALL is defined by most studies as ≤44 chromosomes and may be further divided into three groups: (i) high-hypodiploid (40–44 chromosomes), (ii) low-hypodiploid (30–39 chromosomes), and (iii) near-haploid (24–29 chromosomes) B-ALL. Near-haploid and low-hypodiploid B-ALL cases display significantly poorer clinical outcomes in comparison with high-hypodiploid pediatric B-ALL (6, 15, 16).

Near-haploid B-ALL presents in approximately 0.5% of pediatric B-ALL and has not been reported in adult ALL. It usually retains disomies for chromosomes 8, 10, 14, 18, 21, X and Y (17). In this subtype, up to 70.6% of patients may harbor mutations in receptor tyrosine kinase (RTK) and RAS pathway genes including *NF1*, *NRAS*, *KRAS*, *FLT3* and *PTPN1*. In addition, deletions of *IKZF3* and histone cluster at chromosome 6p22 are frequently observed. Other frequently detected abnormalities in this subtype include alterations in *CREBBP* and *PAG1*, deletions involving *CDKN2A/B, RB1*, and *PAX5*, and point mutations in *EP300* and *EZH2* (18).

Low hypodiploid B-ALL (LHypo) presents in 0.5% of pediatric patients and approximately 4% of adult patients and its frequency increases with age (19). Retained chromosomes generally include 1, 5, 6, 8, 10, 11, 14, 18, 19, 21, 22, X and Y. The characteristic molecular abnormality in LHypo B-ALL is *TP53* mutation, which is detected in >90% of both pediatric and adult patients in this subtype. Moreover, due to the frequent loss of chromosome 17, *TP53* mutations are found to be homozygous in virtually all LHypo B-ALL. In approximately 50% of pediatric LHypo B-ALL, *TP53* mutations are also found in non-tumor cells indicating germline *TP53* mutation associated with Li-Fraumeni syndrome. In contrast to pediatric cases, *TP53* mutations in LHypo adult B-ALL are somatic and are not found in non-tumor and remission samples (18, 20). In addition to *TP53* mutations, other cryptic cytogenetic or molecular genetic abnormalities frequently found in LHypo B-ALL include *IKZF2/Helios* loss, *RB1* alterations, *CDKN2A/B* alterations, and *CREBBP* mutations (18, 20).

Both near-haploid and low hypodiploid genomes can undergo endoreduplication resulting in doubling of the hypodiploid chromosome complement (Figure 2), which occurs in 60–65% of hypodiploid B-ALL. Often both hypodiploid and hyperdiploid (doubled) clones are present at the same time (16). A doubled clone has a modal chromosome number of 50–78, so-called “masked hypodiploidy”, which may be the only clone observed at diagnosis and may not be differentiated from a high-hyperdiploid or triploid clone cytogenetically (16). As hyperdiploidy with more than 50 chromosomes and hypodiploidy are associated with different prognoses, it is crucial to distinguish between true hyperdiploidy and masked hypodiploid B-ALL. A single nucleotide polymorphism (SNP) array should be performed to detect loss of heterozygosity (LOH) (Figure 2B), which is a very characteristic feature for masked hypodiploidy (19).

ETV6::RUNX1 Fusion – t(12;21)

The *ETV6::RUNX1* fusion (formerly called *TEL::AML1* fusion) is one of the most frequent genetic alterations that initiate B-cell lymphoblastic leukemogenesis. It results from the cytogenetically cryptic translocation t(12;21)(p13.2;q22.1), and

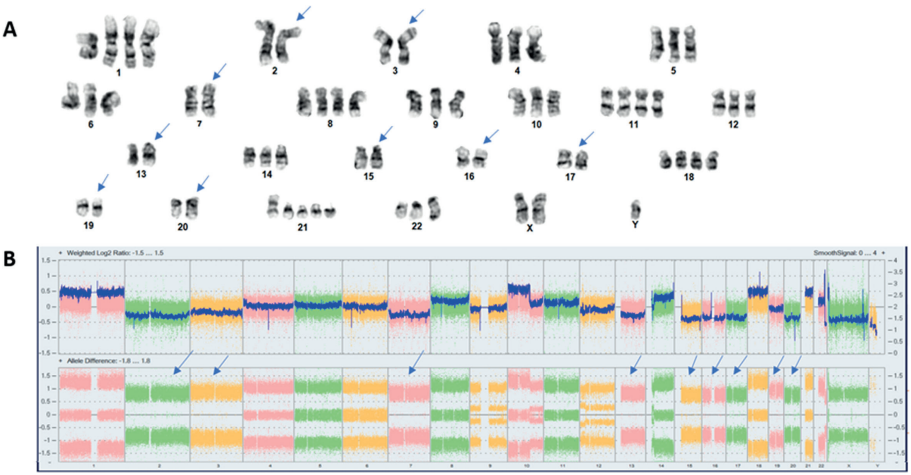

Figure 2. Doubled low hypodiploid B-ALL confirmed by microarray. A, A karyotype with 68 chromosomes which could be high hyperdiploid (good prognosis) or doubled low hypodiploid (poor prognosis). **B,** Microarray shows copy neutral loss of heterozygosity for the chromosomes with two copies (2, 3, 7, 13, 15, 16, 17, 19 and 20) (arrowed) confirming doubled low hypodiploid.

has been reported in 25 – 30% of pediatric B-ALL and 1 – 4% of adult B-ALL (2). *ETV6* is a transcriptional repressor which acts as a tumor suppressor. It is frequently involved in translocations, with at least 41 translocation partners discovered so far (21), with *RUNX1* as the most frequent partner in B-ALL. Despite the high prevalence of *ETV6-RUNX1* fusion in childhood B-ALL, the consensus now is that this fusion alone is unlikely to be responsible for causing overt leukemia, and a postnatal second-hit is required for completion of B-ALL leukemic transformation (22). Numerous secondary abnormalities have been reported in the *ETV6::RUNX1* subgroup with deletion of the non-translocated *ETV6* allele as the most common, followed by deletion of 6q and 9p, amongst others (23). *ETV6::RUNX1* fusion in B-ALL is associated with an excellent prognosis, with OS estimated at 94% at 5 years and 88% at 10 years (6). Recent larger studies have shown that the good prognosis associated with *ETV6::RUNX1* fusion remains even in the presence of additional genetic abnormalities (24).

ETV6::RUNX1 Fusion-Like

ETV6::RUNX1 fusion-like B-ALL shares similar gene expression and immunophenotype profiles (CD27 positive, CD44 low to negative) with *ETV6::RUNX1* fusion B-ALL but lacks the fusion gene (25). More than 80% of *ETV6::RUNX1*-like cases occur in children, accounting for 2–3% of pediatric B-ALL (6). Enriched genetic abnormalities in this subtype include *ETV6* deletion or rearrangement with *IKZF1* or deletion of *ELMO1*, *IKZF1* and *ARPP21*, deletions of histone gene cluster on 6p22.2, *BTG1* aberrations, as well as other chromosome rearrangements such as *TCF3::FLI1* and *FUS::ERG* fusion (5, 25). The prognosis of this subtype is yet to be determined. The average 5-year EFS is 66.7%, thus higher-intensity therapy may be considered for this subtype (26).

BCR::ABL1 Fusion – t(9;22)

BCR::ABL1 fusion presents in 1–3% of pediatric B-ALL and approximately 25% of adult B-ALL with increased frequency with age and is a poor prognostic biomarker (27). The majority of *BCR::ABL1* fusion results from t(9;22)(q34.1;q11.2) [Philadelphia chromosome positive (Ph+)] and gives rise to a constitutively active tyrosine kinase that can activate many pathways including RAS, RAC, PI3K/AKT/mTOR, NF-κB and JAK/STAT. Other genetic abnormalities frequently associated with Ph+ B-ALL include deletions of *IKZF1* (80%), *PAX5* (50%), *CDKN2A/B* (50%) and *EBF1* (14%) (28). Despite recent therapeutic advances, Ph+ ALL is still an adverse subtype with a 4-year EFS of 84% (29).

BCR::ABL1 Fusion-like (Ph-like)

Ph-like ALLs are leukemias characterized by gene expression profiles and phenotypic features similar to those of Ph+ ALL but lack the *BCR::ABL1* fusion. The prevalence varies from ~12% in children to 20 – 27% in adults. Ph-like ALL occurs in all age groups and is associated with poor prognosis, especially due to treatment failure, resulting in high rates of MRD positivity. The 5-year EFS is reported at 59.5%, compared to an average of 84.4% in other B-ALL cases (30).

A wide spectrum of genetic alterations has been described in Ph-like B-ALL cases including translocations, cryptic gene rearrangements, sequence mutations and copy number changes. The majority of these alterations lead to constitutively active kinase or cytokine receptor signaling, and many of them have been shown to be druggable with a variety of TKIs (Table 2) (31, 32). Founder mutations may be classified into four groups:

(i) JAK/STAT alterations including mutations activating cytokine receptors (e.g., *CRLF2*); rearrangements or gene fusions hijacking cytokine receptor expression (e.g., cryptic *EPOR* rearrangements, *IGH::CRLF2* and, *P2RY8::CRLF2*) (Figure 3); gene fusions and/or mutations activating kinases (e.g., *JAK1*) (33, 34). Approximately half of Ph-like ALL cases exhibit deregulated CRFL2 expression, with many of these cases showing alterations in the Janus kinases JAK1 or JAK2 (29).

TABLE 2 **Genetic alterations in Ph-like B-ALL**

Genetic Alterations	Genes Involved	Fusion Partner Genes	Incidence	Targeted Therapy
Deletions of B-cell developmental genes	*IKZF1*	N/A	70–80%	N/A
	PAX5	N/A	30%	N/A

(Continued)

TABLE 2 Genetic alterations in Ph-like B-ALL (*Continued*)

Genetic Alterations	Genes Involved	Fusion Partner Genes	Incidence	Targeted Therapy
Kinase Classes				
JAK-STAT	*CRLF2*	*IGH, P2RY8*	Children, 24.1% Adolescents (16–21 y), 32% Young adults (21–39 y), 14.6% Adults (40–86 y), 11.2%	JAK inhibitors PI3K/mTOR inhibitors
	JAK2	*ATF7IP, BCR, EBF1, ETV6, GOLGA5, HMBOX1, OFD1, PAX5, PCM1, PPFIBP1, RFX3, SMU1, SNX29, SSBP2, STRN3, TERF2, TPR, USP25, ZNF274, ZBTB46,*		JAK inhibitors PI3K/mTOR inhibitors
	EPOR	*IGH, IGK, LAIR1, THADA*		JAK inhibitors
	IL2RB	*MYH9*		JAK inhibitors
	TYK2	*MYB, SMARCA4, ZNF340*		TYK2 inhibitors
	JAK1, JAK3, IL7R, SH2B3	N/A		JAK inhibitors
ABL	*ABL1*	*CENPC, ETV6, FOXP1, LSM14, NUP214, NUP153, RCSD1, ANBP2, SNX2, SFPQ, SPTAN1, ZMIZ1*	Children, 16.7% Adolescents (16–21 y), 9% Young adults (21–39 y), 10.4% Adults (40–86 y), 9.2%	Dasatinib
	ABL2	*PAG1, RCSD1, ZC3HAV1*		Dasatinib
	CSF1R	*MEF2D, SSBP2, TBL1XR1*		Dasatinib
	LYN	NCOR1, GATAD2A		Dasatinib
	PDGFRA	FIP1L1		Dasatinib
	PDGFRB	ATF7IP, EBF1, ETV6, SSBP2, TNIP1, ZEB2, ZMYND8		Dasatinib
RAS pathway	*KRAS, NRAS, PTNP11, CBL1, NF1, BRAF*	N/A	4%	MEK Inhibitors
Rare fusions	*NTRK3*	*ETV6*	Children, 2.8% Adolescents (16–21 y), 3% Young adults (21–39 y), 5.2% Adults (40–86 y), 3.1%	TKI
	FLT3	*ZMYM2*		FLT3 inhibitors
	FGFR1	*BCR*		FGFR inhibitors
	BLNK	*DNIT*		unknown
	PT2KB	*KDM6A, STAG2, TMEM2*		FAK inhibitors

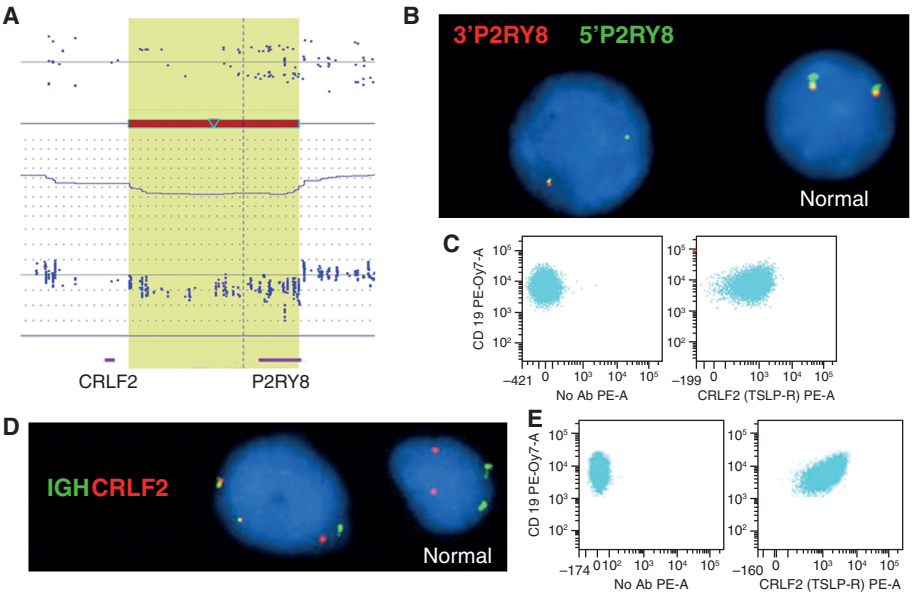

Figure 3. Cytogenetic and flow cytometry findings of B- ALL with *CRLF2* rearrangement. **A,** SNP-Array analysis shows *CRLF2-P2RY8* fusion. **B,** FISH analysis using *P2RY8* break-apart probes demonstrates 3'*P2RY8* deletion. **C,** Flow cytometry study shows CRLF2 expression. **D,** FISH analysis using *IGH* and *CRLF2* fusion probes demonstrates *IGH-CRLF2* fusion. **E,** Flow cytometry study shows CRLF2 expression. A, B and C from one case; D and E from another case.

(ii) fusions involving ABL-class genes (e.g., *ABL1*)
(iii) mutations activating Ras signaling (*NRAS, KRAS, PTPN11*)
(iv) less common fusions (e.g., *FLT3*) with a growing number due to the application of sequencing techniques (33, 34).

Deletions involving B-cell developmental genes are common in Ph-like B-ALL. Similar to Ph+ ALL, *IKZF1* alterations are also a hallmark of Ph-like ALL, occurring in 70–80% of Ph-like B-ALL and conferring a poor prognostic outcome (34). *PAX5* is another gene frequently altered, occurring in ~30% of Ph-Like B-ALL cases. *IKZF1* and *PAX5* alterations often occur together (35).

KMT2A Rearrangements

KMT2A (formerly called *MLL*) gene has more than 90 translocation partners and is rearranged in greater than 80% of infant B-ALL (2) and 4 – 9% of adult B-ALL (29). In B-ALL, the most common partner is *AFF1* (formerly named *AF4*) at 4q21. The *KMT2A::AFF1* fusion [t(4;11)(q21;q23)] (Figure 4) is estimated to be present in 50% of infants with *KMT2A*-rearranged (*KMT2A*-r) B-ALL. The second most common fusion is *KMT2A::MLLT3* (*AF9*) resulting from t(9;11)(p22;q23) followed by *KMT2A::MLLT1* (*ENL*) originating from t(11;19)(q23;p13.3). Fusions with *KMT2A* breakpoint in intron 11 are reported to have a poorer outcome.

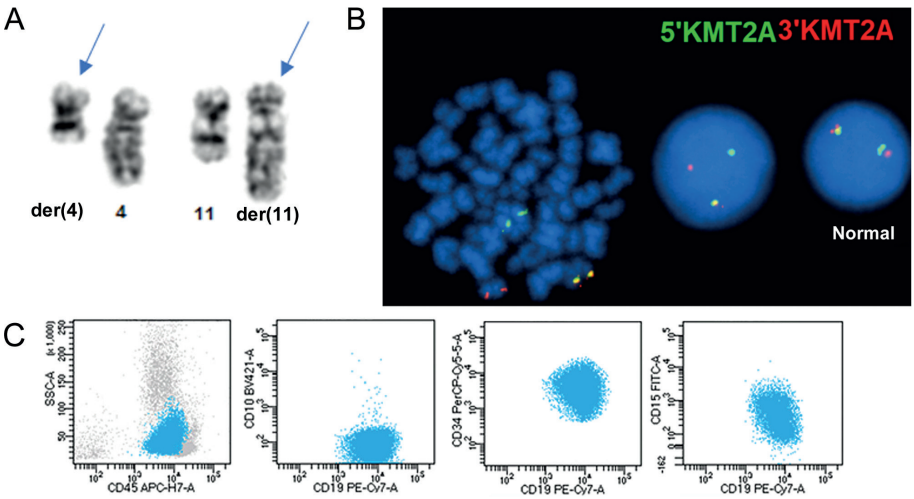

Figure 4. Cytogenetic and flow cytometry findings of B-ALL with *KMT2A* rearrangement. **A,** Conventional cytogenetic study shows t(4;11)(q21;q23). **B,** FISH analysis using *KMT2A* break-apart probes demonstrates *KMT2A* rearrangement. **C,** Flow cytometry study shows Pro-B-ALL immunophenotype and aberrant expression of CD15.

Other major partner genes in infant ALL patients include *MLLT10* (*AF10*), *MLLT6* (*AF17*), and *MLLT4* (*AF6*) (36). The immunophenotype associated with *KMT2A*-r B-ALL includes expression of CD19, lack of CD10, and co-expression of myeloid markers such as CD15, CD33, and CD68 (Figure 4C).

KMT2A-driven leukemias are aggressive and have a very poor prognosis in infants. The estimated 5-year EFS is 30 – 40% (37). Activating mutations in tyrosine kinase-PI3K-RAS signaling pathway components can be detected in 47% of cases *(38)*. The poor prognosis is associated, in some cases, with cooperating mutations in *FLT3, NF1* and *KRAS*. The prognosis is better for children older than 1 year.

TCF3 Rearrangement

TCF3 (also called *E2A*) has two major translocation partners, *PBX1* and *HLF*. *TCF3::PBX1* fusion results from t(1;19)(q23;p13); both the balanced and unbalanced variants of this translocation are found in 6% of pediatric B-ALL and 1 – 3% of adult B-ALL (29). B-ALL patients with *TCF3::PBX1* fusion usually have a pre-B immunophenotype that expresses cytoplasmic immunoglobulin μ heavy chain. It has an intermediate prognosis, reported at a 5-year EFS of 84% (29), the result of considerable improvement due to modern intensive, CNS-directed therapy.

TCF3::HLF fusion results from t(17;19)(q22;p13.3), occurring in less than 1% of childhood B-ALL and rarely in adults (39). *TCF3::HLF* is associated with a particularly poor prognosis (40). There are two types of *TCF3::HLF* fusion with same intron 3 breakpoint in *HLF* gene, but different breakpoints in *TCF3*. Type I rearrangement has breakpoint in intron 13 of *TCF3* associated with disseminated

intravascular coagulation (DIC) while type II has breakpoint in intron 12 of *TCF3* associated with hypercalcemia (41). Other genetic abnormalities, frequently associated with *TCF3::HLF* fusion, include *PAX5* and *VPREB1* deletions and aberrations in the RAS pathway genes (42).

IGH::IL3 Fusion - t(5;14)

IgH::IL3 fusion is seen in less than 1% of B-ALL and represents an aggressive subtype with poor outcomes (43). It results from t(5;14)(q31.1; q32.3) and leads to overexpression of *IL3* as a result of the juxtaposition of *IL3* gene to the potent *IGH* enhancer. These cases are rare and poorly characterized but are observed predominantly in males and the adolescent/young adult (AYA) age group. Patients with this translocation clinically present with reactive hypereosinophilia and lack of peripheral blasts.

Intrachromosomal amplification of chromosome 21 (iAMP21)

iAMP21 presents in approximately 2% of pediatric patients with B-ALL and is associated with worse prognosis when treated with a low-intensity National Cancer Institute (NCI) standard-risk (SR) regimen. It is extremely rare in adult B-ALL, and its prognostic effect in adults is unclear (44). Patients with iAMP21 are usually older children or adolescents with a common/pre-B immunophenotype, and generally have a low WBC. Individuals carrying constitutional Robertsonian translocation der(15;21)(q10;q10) and trisomy 21 have a 2700-fold and 10–12-fold increased risk, respectively, of developing B-ALL with iAMP21 compared to children without these genetic anomalies (6).

The iAMP21 chromosome is a single abnormal chromosome 21 resulting from Breakage–Fusion–Bridge (BFB) cycles followed by chromothripsis, thus containing multiple regions of gain, amplification, inversion, and deletion (Figure 5). It is defined as the amplification of the 5.1-Mb common region containing genes mapping to the Down Syndrome Critical Region (DSCR), *RUNX1* and *miR-802*, with the presence of three or more extra copies of *RUNX1* on a single abnormal chromosome 21 (a total of five or more *RUNX1* signals per cell) by FISH (37). However, a recent study has shown that approximately 9% of iAMP21 B-ALL failed to meet the FISH definition, but amplifications were confirmed by microarray, indicating the importance of incorporation of microarray into testing strategy (45). Common secondary abnormalities in B-ALL with iAMP21 include gain of chromosomes X, 10, or 14; monosomy 7/deletion of 7q; deletions of 11q including the *ATM* and *KMT2A* genes; as well as abnormalities affecting *IKZF1, CDKN2A, PAX5, ETV6*, and *RB1*. More than 60% of iAMP21-ALL patients have a mutation in genes related to the RAS signaling pathway, and 20% of patients have a *P2RY8/CRLF2* gene fusion (46).

Dic(9;20)

The dic(9;20) is a rare aberration seen in 2% of children and 1% of adults with B-ALL. Both favorable and poor prognoses associated with dic(9;20) have been reported. Relapses in these patients are fairly common; however, treatment after

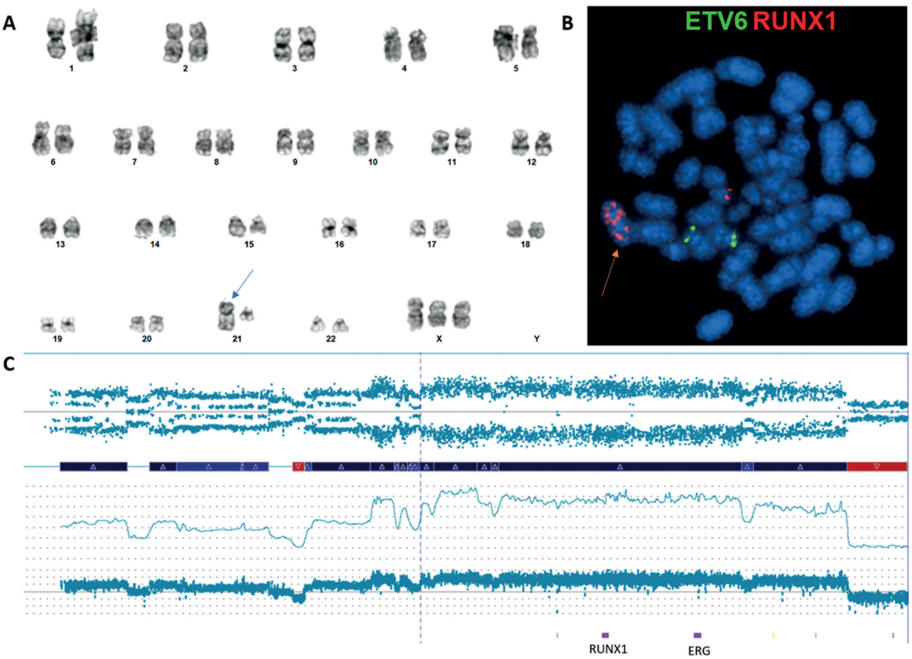

Figure 5. Intrachromosomal amplification of chromosome 21 (iAMP21). A, A big marker chromosome in the position of chromosome 21 with absence of a normal chromosome 21. **B,** Metaphase FISH shows the marker chromosome contains multiple copies of *RUNX1* probes (red) consistent with *RUNX1* amplification. **C,** Microarray shows complex structural abnormalities including amplification, gains, and deletions (across an approximately 11 Mb region).

relapse is often successful. Additional studies are needed to accurately define the prognostic value of dic(9;20) (6). The dic(9;20) arises from the fusion of chromosomes 9p and 20q resulting in the loss of 9p and 20q material, which masquerades as monosomy 20. The breakpoints on 9p target *PAX5*, and the breakpoints on 20q target *ASXL1*.

Other significant gene alterations

CRLF2 deregulation rearrangements, resulting in overexpression of its gene product, thymic stromal lymphopoietin receptor (TSLPR), are seen in ~5% of pediatric B-ALL, ~5% of adult B-ALL, ~50% of Down syndrome-associated B-ALL, and ~50% of Ph-like B-ALL cases (29). *CRLF2* has two main translocation partners, *IGH* and *P2RY8* (Figure 3), and both translocations are associated with poor prognosis. Rarely, activating mutations can also result in *CRLF2* overexpression. The *CRLF2::P2RY8* fusion is caused by interstitial deletions within the pseudoautosomal region (PAR1) located at Xp22.3 or Yp11.3 which bring *CRLF2* to the *P2RY8* promoter. *CRLF2* rearrangements have been associated with *IKZF1* deletion and activation of the JAK-STAT, ERK and mTOR/PI3K pathways with 50% of cases harboring mutations in *JAK* family genes. The concomitance of *CRLF2*

overexpression and *JAK2* mutations is associated with inferior outcomes (47). Although the rearrangements of *CRLF2* are the most common alterations in Ph-like ALL., approximately 5–10% of *CRLF2*-rearranged B-ALL cases are not Ph-like ALL. *P2RY8::CRLF2* fusion is also often a secondary lesion in leukemias with iAMP21, hyperdiploidy, or dic(9;20) (48).

DUX4 rearrangement is a newly identified subtype seen in 4–7% of pediatric B-ALL cases with a slightly higher incidence in AYA patients and rarely seen in adult B-ALL (6). *DUX4*-rearranged B-ALL has a unique immunophenotype (CD2 and CD371 positive) with CD371 expression being pathognomonic of this leukemia (49). The most frequent translocation partner is *IgH*. The fusion results in truncation of the highly conserved C terminus of *DUX4*. This truncated form binds *ERG* (ETS-related gene), coding for a C-terminal ERG protein fragment that is a dominant-negative inhibitor of wild-type ERG function, thus contributing to leukemogenesis (49). Other partners, such as *ERG* and *ZNF384*, have also been reported (25). Interestingly, more than 50% of patients within this group harbor intragenic deletions of *ERG*, and *ERG* deletions occur exclusively in this subgroup.

DUX4 rearrangement is associated with excellent prognosis, with an 8-year EFS and 8-year OS of 86.4% and 95.6%, respectively (50). The presence of *ERG* deletion in these rearrangements is reported to neutralize the bad prognostic effect of *IKZF1* alterations (51).

ZNF384 rearrangement is a new subtype present in 3–5% of pediatric and 3–8% of adult patients with B-ALL. Overall, its prognostic impact is intermediate, but varies with different partner genes. The *EP300::ZNF384* fusion is reported to be associated with a better outcome than *TCF3::ZNF384* fusion. Patients with *ZNF384* fusions share a characteristic immunophenotype of negative or weak CD10 expression and aberrant expression of myeloid antigens CD13/33 (52). More than 10 partner genes have been identified with *EP300, TCF3, TAF15*, and *CREBBP* being the most common. Alterations in *NRAS* and *FLT3* occur in 60% of cases (53). In addition, deletions in lymphoid regulator genes including *LEF1, EBF1, CDKN2A, FBXW7*, and *ETV6* have also been detected in *ZNF384*-rearranged B-ALL.

MEF2D rearrangement is present in 1–4% of pediatric B-ALL (usually older children and adolescents) and 1% of adult patients. It is associated with high WBC and classified as an intermediate to high risk factor. Patients with the *MEF2D* fusion gene have an immunophenotype of low or no CD10 expression and high CD38 expression. The most commonly associated fusion partners are *BCL9* and *HNRNPUL1*. Additional genetic alterations observed in this group include deletions in *IKZF1* and a significantly higher prevalence of *CDKN2A/CDKN2B* deletions (6, 42).

NUTM1 rearrangement is a rare subtype present in 5–7% of all infants B-ALL (21.7% of non-*KMT2A*-rearranged infant cases) and 1% in children, with no report in adults. The current limited data suggests *NUTM1* rearrangement is a favorable prognostic factor in B-ALL. Reported partner genes include *ACIN1, CUX1, BRD9*, and *ZNF618* (54).

IGH rearrangements, although individually rare, are present in approximately 5% of B-ALL cases, forming part of the B-other-ALL subgroup. *IGH* rearrangements are detected in all age groups with peak incidence in AYA. Collectively, they have been associated with an adverse outcome in adults, although they did not

represent an independent prognostic factor in children and adolescents (51, 55). In addition to the previously mentioned *IL3, CRLF2,* and *DUX4*, there are other reported partners, for example, *MYC* and *CEBPA* (56). Most karyotypes are relatively simple and near-diploid, with many having cooperating deletions of *CDKN2A* and/or *PAX5* (51).

IKZF1 alterations are present in approximately 15% of pediatric and 40–50% of adult patients with B-ALL and are highly prevalent in *Ph*+ (~85%) and *Ph*-like (~70%) subtypes (47). It is also over- represented in Down syndrome-ALL and patients with other features of high-risk disease, but rarely detected in cases with *TCF3*-rearrangements (3%) and *ETV6::RUNX1* fusion (3%). It ranges from 15-20% among other subtypes such as hyperdiploid and B-ALL, NOS (6). Various studies have found *IKZF1* aberrations to have a negative prognostic impact, manifesting as resistance to TKI therapy, high level of MRD, poor survival and increased frequency of relapse (57, 58). *IKZF1* plays a key role in hematopoiesis, differentiation, and proliferation of all lymphoid lineages, especially in the activation and development of B cells. Deletions are responsible for up to 90% of cases with *IKZF1* alterations, with the rest being point mutations (59). Deletions of the *IKZF1* gene which result in haploinsufficiency constitute up to 55% of B-ALL with *IKZF1* deletions. Focal exons 4–7 deletions affecting the DNA-binding domain comprise 33% of *IKZF1* deletions, and exert a dominant–negative effect over the unaffected allele, resulting in loss of the tumor suppressor function attributed to wildtype *IKZF1* (60). Exons 4–7 deletions lead to more severe phenotype than haploinsufficiency in B-ALL patients. The adverse prognosis normally associated with *IKZF1* deletion is abrogated by the presence of *ERG* deletions (61). Point mutations resulting in loss-of-function of *IKZF1* are described in up to 10% of *Ph*+ and ~1% of non-*Ph*+ cases without *IKZF1* deletions, with a similar impact on outcome (57). Specifically, *IKZF1* p.Asn159Tyr (N159Y) mutation has been considered as a distinct subtype in B-ALL with unique expression profile. In addition, an increasing number of cases with fusion transcripts involving *IKZF1* have been described (*IKZF1::PRDM16, IKZF1::NUMT1, IKZF1::ETV6, IKZF1::CDK2, IKZF1::ZEB2, IKZF1::SETD5, IKZF1::STIM2*) (5). A new group of B-ALL, called *IKZF1*plus, has been defined recently. *IKZF1*plus is characterized by co-existence of *IKZF1* deletions with deletions of *CDKN2A, CDKN2B* or *PAX5* or the PAR1 region in the absence of *ERG* deletion. This group confers the most unfavorable outcome in MRD-positive patients with childhood B-ALL (62).

PAX5 alterations are present in ~30% of B-ALL cases (63). Deletion and mutations have been considered to be secondary events because they are present in many subtypes of B-ALL. However, two categories of distinct alterations in the *PAX5* gene have been identified as drivers: the *PAX5* p.Pro80Arg (PAX5 P80R) point mutation and the *PAX5*-altered (PAX5alt), which have different gene expression profiles and are now considered distinct genetic subtypes (5). The PAX5 P80R subtype presents in 3–4% of pediatric and 4% of adults B-ALL and is associated with intermediate prognosis. The PAX5 P80R mutation is accompanied by inactivation of the second PAX5 allele (biallelic events) through deletion or a second mutation (homozygous or compound heterozygous) or loss of heterozygosity, in all reported cases. This mutation also frequently co-occurs with biallelic *CDKN2A/B* deletion and mutations in the RAS or JAK-STAT pathways as well as *FLT3, BRAF* and *PIK3CA* (5, 64). The PAX5alt subtype is present in 7.4% of B-ALL and confers an intermediate prognosis in children treated with intensive

chemotherapy, but poor prognosis in adults (5). This subtype contains diverse *PAX5* alterations, including rearrangements with partner genes, sequence mutations and focal/intragenic amplifications. More than 20 fusion partners have been reported with *ETV6* being the most common. The PAX5alt subtype commonly has codeletion of the *IKZF1* and *CDKN2A/B* genes, giving rise to the poorer outcome of *IKZF1*plus subtype (62).

CDKN2A deletions are found in 15–35% of children and 30–45% of adults with B-ALL, and commonly involve both *CDKN2A* and *CDKN2B* as well as *PAX5* due to their co-location on chromosome 9p. The deletions are more frequently found in *Ph+* and *Ph*-like ALL than in *ETV6::RUNX1* and hyperdiploid ALL (48). *CDKN2A/B* losses have been reported to be associated with an inferior outcome in adults, but do not appear to affect the outcome in pediatric B-ALL (65).

MYC, BCL2 and/or *BCL6* rearrangements are known to be associated with B-cell non-Hodgkin's lymphomas. In B-ALL, translocations of *MYC, BCL2* and/or *BCL6* with immunoglobulin genes (*IGH/ IGL/IGK*) form a rare subgroup, which is seen predominantly in adults and is associated with a poor prognosis (66).

RB1, BTG1, EBF1 and other genes involved in cell cycle control, lymphoid development, signaling, or tumor suppressor genes, are frequently altered in B-ALL. *RB1* gene deletions are present in 2–4% of children with B-ALL. It is frequently deleted (39%) in children with iAMP21 and low hypodiploidy. *BTG1* gene deletions are seen in up to 10% of children with B-ALL and are known to be clustered with *ETV6::RUNX1* fusion (15%). *EBF1* deletions were present in 6% of B-ALL and were enriched in *Ph*-like cases (15%), but absent in *KMT2A*-rearranged and *TCF3::PBX1* fusion cases. Patients carrying *EBF1* deletion tend to have higher MRD levels compared with the cases without *EBF1* deletion. Other genes, which have been reported to be deleted in B-ALL, include *ABL1, CASP8AP2, CD200/ BTLA, MLLT3, IKZF2, NF1, PHF6, PTEN, PTPN2, TBL1XR1, TP53*, and *VPREB1*. Deletions in *VPREB1, RB1, IKZF2,* and *TBL1XR1* can be biallelic (60).

IMMUNOPHENOTYPIC BIOMARKERS

As above-mentioned, genetic biomarkers are critically important for prognostic prediction and WHO classification of B-ALL. The prognostic value provided by immunophenotyping is most likely attributed to its prediction of certain cytogenetic/molecular subtypes. B-ALL can be subclassified as pro-B-ALL (CD10–), common ALL (CD10+) and pre-B-ALL (cytoplasmic IgM+) based on the developmental stage demonstrated by immunophenotyping (67). Pro-B-ALL is commonly seen in B-ALL with *KMT2A* rearrangement, which is also commonly positive for CD15 or other myeloid markers (Figure 4 C). Pre-B-ALL is commonly seen in B-ALLs with *TCF3::PBX1* and *MEF2D* fusions (68). Surface CD371 expression is specifically associated with *DUX4* rearrangement. CD25 expression is commonly seen in Ph+ B-ALL. Expression of *CRLF2* gene product (TSLPR) assessed by FCM (Figure 3, C and E) can serve as a screening test for *CRLF2* gene rearrangement and the identification of Ph-like B-ALL (69). Approximately 80% of our CRLF2+ cases identified by FCM had *CRLF2* gene rearrangements identified by cytogenetic study and/or next generation sequencing (data not published). Besides these

associations, some antigens have been studied and showed data indicating their roles as independent prognostic biomarkers. In addition, some leukemia cell surface antigens are the targets of recently developed immunotherapies and are important predictors of the response to these treatments.

CD45

CD45, also known as leukocyte common antigen, is a receptor type protein tyrosine phosphatase expressed in all leukocytes and most hematopoietic precursors. Most B-ALL cases express low level CD45. There have been a few studies demonstrating the adverse prognostic impact of high CD45 expression on pediatric ALL (70–72). The early Pediatric Oncology Group (POG) study on 1231 pediatric B-ALL cases showed that high CD45 intensity (>75th percentile) was associated with a worse EFS, and the association was not related to other known poor prognostic factors such as NCI risk group, ploidy, and unfavorable translocations (72). A Japanese study conducted several years later confirmed the negative impact of high CD45 intensity on EFS only in the NCI HR pediatric B-ALL patients (70). Later, a German team also found a shorter EFS in high-CD45 group due to a higher cumulative incidence of relapse (CIR). And they also found that high CD45 expression was associated with NCI HR, presence of unfavorable genetic biomarkers, and poor prednisone response (71).

CD20

CD20 is a B-lymphocyte-specific membrane protein, which plays a role in B-cell development, differentiation, and activation. It is expressed by most B lymphocytes including intermediate and late-stage B-cell precursors, naïve B cells and memory B cells. Approximately 40–50% of B-ALL cases show CD20 expression, which is often dim and variable. The prognostic impact of CD20 on pediatric B-ALL is contradictory among different studies (72, 73), which questions the role of CD20 as a prognostic biomarker in pediatric B-ALL. In contrast, the studies in adult B-ALL patients consistently demonstrated that CD20 positivity was generally associated with an inferior outcome (74, 75). As a commonly used target by immunotherapy for B-cell malignancy, the expression of CD20 in adult B-ALL provides a therapeutic option for using Rituximab (anti-CD20 monoclonal antibody). A randomized clinical study has demonstrated that the addition of Rituximab to the B-ALL-chemotherapy protocol can improve the outcome for younger adults with CD20+ Ph-negative B-ALL (76).

Antigens targeted by immunotherapy

Novel therapies such as monoclonal antibodies or chimeric antigen receptor transfused T cells (CART) have shown significant promise in the management of relapsed/refractory B- ALL (1, 2). The most common targeted surface antigens for B-ALL treatment are CD19 and CD22. The expression of these antigens in leukemic cells is a prerequisite for the success of these treatments. Moreover, the expression level or intensity of these antigens may also predict the therapeutic response.

CD19 is a diagnostic biomarker for B-ALL and is present in greater than 90% of all cases. Blinatumomab is a bispecific T cell engager (BiTE) monoclonal antibody directed against CD19 and CD3. By binding to CD19+ leukemic cells and CD3+ T cells simultaneously, it induces antibody-dependent cellular cytotoxicity against the leukemic cells, thereby eliminating the CD19+ blasts. The efficacy of blinatumomab in relapsed/refractory B-ALL is well established, with superiority over high dose chemotherapy in children and young adults. Tisagenlecleucel (Kymriah), a CD19 CART cell treatment, is the most common CART cell therapy in B-ALL. It has shown remarkable efficacy for relapsed/refractory B-ALL in children and adults, including relapsed CNS disease. Antigen density was demonstrated as a major factor influencing the activity of CART cells, and low surface CD19 density pre-CART-19 treatment was associated with poor response (77, 78).

CD22 is an inhibitory B-cell co-receptor, which is positive in more than 90% of B-ALL cases. Inotuzumab ozogamicin (IO) is a monoclonal anti-CD22 antibody attached to calicheamicin. Initial phase 2 and phase 3 studies have demonstrated the superiority of IO over standard chemotherapy in relapsed/refractory B-ALL patients (79). CD22 CART therapy and dual CD19/CD22 CART therapy are also at different stages of clinical development.

Others

CD36 is a membrane glycoprotein present on monocytes, macrophages, platelets, erythroblasts, adipocytes, and some epithelia. It is a scavenger receptor involved in many physiologic functions. A one-center retrospective study has shown the negative impact of CD36 expression on the outcome of pediatric B-ALL cases. In this study, 5-year EFS and OS of the NCI-SR patients were significantly worse in CD36+ group compared with CD36- group (80). CD34 is a transmembrane glycoprotein expressed on lymphohematopoietic progenitor cells. A large proportion of B-ALL cases demonstrate CD34 expression, which is commonly partial or variable. A recently published study (81) demonstrated high CD34 expression as a predictor of poor induction therapy response. The CD34+ cases were approximately 6.5 times more likely to have a positive MRD result at the end of induction compared with CD34- cases. Further studies with increased number of patients are needed to confirm these results.

RESPONSE TO CHEMOTHERAPY AND MEASURABLE RESIDUAL DISEASE

Early response to therapy is an independent prognostic factor in pediatric B-ALL, and patients with a slower early response are more likely to have an adverse event than patients with a more rapid early response. It has been traditionally assessed by morphologic evaluation of BM and peripheral blood (PB). The response to induction treatment has been categorized based on lymphoblast count in BM: M1, <5%; M2, 5 to <25%; and M3, ≥25%. Complete remission (CR) is defined as M1 BM at the end of induction, absence of leukemic blasts in PB and cerebrospinal

fluid (CSF), and no evidence of local disease. Although the vast majority of pediatric B-ALL cases achieve CR based on current treatment protocols, a significant proportion of the cases will relapse. Clearly, it is not enough to use therapeutic response for risk prediction based on morphology alone.

Measurable residual disease (MRD), also known as minimal residual disease, refers to the presence of a small number of malignant cells in leukemia patients during or after treatment. MRD is under the detection limit of morphology, and is usually detected by FCM and/or molecular methods. MRD is an in-depth measure of the therapeutic response, and it has been demonstrated as the strongest independent predictor of relapse and survival outcome. The patients with undetectable MRD or good MRD response consistently demonstrate a lower risk of relapse and better survival outcomes compared with similarly treated patients with positive MRD or poor MRD response. Please see the MRD chapter for more information (82).

OTHER BIOMARKERS

Initial WBC, a measure of extramedullary leukemia burden, has been one of the historical strongest risk factors. It has been used for NCI risk stratification (83) in pediatric B-ALL. The two groups of patients with NCI-SR (WBC <50,00/uL and age 1–9 years) or NCI-HR (all others) had significantly different 4-year EFS (80% vs. 60%) (83). NCI risk stratification is currently still in use to guide risk-adapted treatment for pediatric B-ALL. A study involving 2666 ALL patients from five Nordic countries showed that WBC was not associated with the risk of an event for B-ALL or T-ALL in patients with day 29 MRD (MRDd29) <0.1%. In contrast, for patients with MRDd29 ≥ 0.1% and <5%, the 5-year EFS for patients with WBC ≥ 100,000/uL was significantly shorter than that of patients with WBC <100,000/uL (84). Other blood cell counts such as platelet and neutrophil counts may also have some prognostic value in certain patient groups, however, their independent prognostic values are in question and should be further studied.

Initial CSF finding (CNS involvement) has also been found to have an impact on the outcome of pediatric B-ALL patients. A COG study published in 2017 demonstrated significantly lower 5-year EFS and OS rates in the CNS2 group (76% and 86.8%) and CNS3 group (76% and 82.1%) than those in the CNS1 (no blasts) group (85% and 92.7%), regardless of NCI risks (85). These findings indicate that the presence of blasts in CSF, regardless of CSF cell count, is an independent predictor of adverse outcome in pediatric B-ALL patients.

CONCLUSION

There have been many prognostic and predictive biomarkers identified in B-ALL. With these biomarkers, B-ALL cases can be categorized into a risk group and treated accordingly. This risk-adapted treatment has led to the current very high curable rate in de novo pediatric ALL cases. However, a significant proportion of B-ALL cases will relapse, and the prognosis of relapsed cases is dismal.

Recent advances in sequencing technology and integrated analyses of large-scale data have allowed the discovery of many new genetic biomarkers, which show promise to improve the accuracy of risk stratification, identify new genetic/molecular defects associated with potential therapeutic targets, and eventually improve the overall clinical outcome.

Conflict of Interest: The authors declare no potential conflicts of interest with respect to research, authorship and/or publication of this manuscript.

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