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REVIEW ARTICLE

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Oncogenic lesions and molecular subtypes in adults with B-cell acute lymphoblastic leukemia

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Abstract

B-cell acute lymphoblastic leukemia (B-ALL), a genetically heterogeneous disease, is classified into different molecular subtypes that are defined by recurrent gene rearrangements, gross chromosomal abnormalities, or specific gene mutations. Cells with these genetic alterations acquire a leukemia-initiating ability and show unique expression profiles. The distribution of B-ALL molecular subtypes is greatly dependent on age, which also affects treatment responsiveness and long-term survival, partly accounting for the inferior outcome in adolescents and young adults (AYA) and (older) adults with B-ALL. Recent advances in sequencing technology, especially RNA sequencing and the application of these technologies in large B-ALL cohorts have uncovered B-ALL molecular subtypes prevalent in AYA and adults. These new insights supply more precise estimations of prognoses and targeted therapies informed by sequencing results, as well as a deeper understanding of the genetic basis of AYA/adult B-ALL. This article provides an account of these technological advances and an overview of the recent major findings of B-ALL molecular subtypes in adults.

KEYWORDS
acute lymphoblastic leukemia, adult, gene rearrangement, prognosis, RNA-seq

1 | INTRODUCTION

Therapeutic advances, including intensive combination chemotherapy, central nervous system-directed therapy, and risk stratification strategies, have enabled long-term survival of approximately 90% of pediatric patients with B-cell acute lymphoblastic leukemia (B-ALL).[1] However, a drastic reduction in the long-term survival of adolescents and young adults (AYA) group is seen, and the prognosis of B-ALL in adults is poor.[2] Although some overlaps in the spectrum of genetic alterations underlying pediatric and AYA/adult B-ALL are evident, the distribution of these alterations is substantially different.[3] Therefore, differences in prognostic outcomes between children and AYA/adults can be attributed, at least in part, to the distinct genetic basis underlying B-ALL in different age groups. However, the rarity of adult B-ALL renders the study on a large cohort of patients and generation of meaningful annotations for the underlying genetics challenging.

Recurrent gene rearrangements and gross chromosomal abnormalities are hallmarks of B-ALL. They play a central role in tumor progression and are also closely associated with patient characteristics, treatment responsiveness, and long-term outcome. Furthermore, these abnormalities have an almost completely exclusive relationship with one another, being regarded as key factors that define molecular subtypes. In 2016, World Health Organization classification established the common chromosomal abnormalities of B-ALL, including *BCR-ABL1*, *KMT2A*-rearranged, *ETV6-RUNX1*, hyperdiploidy (usually 51–65 chromosomes), hypodiploidy (usually 23–43 chromosomes), *IGH-IL3*, *TCF3-PBX1*, BCR-ABL1-like (expression profile

similar to *BCR-ABL1*-positive ALL), and iAMP21 (amplification of a portion of chromosome 21), as discrete molecular subtypes.[4]

B-ALL is a clonal disease derived from a single ancestral cell and positively selected owing to the acquisition of genetic alterations. Previous studies of concordant B-ALL in identical twins revealed that recurrent gene rearrangements or gross chromosomal abnormalities were shared between the twins, while copy number alterations and mutations were discordant. This suggests a two-hit model of childhood B-ALL[5–7] that can be briefly described as follows. In the initiation stage, gene rearrangements or gross chromosomal abnormalities generate a pre-leukemic clone in utero, then the acquisition of secondary genetic mutations after birth promotes the conversion to overt leukemia. Consistent with this model, the expanded clones involving gene rearrangements were identified in normal cord blood or neonatal blood cells.[8,9] In addition, in a mouse model, *ETV6-RUNX1* had the ability to inhibit B-cell differentiation without leukemogenic transformation, suggesting the existence of a pre-leukemic state driven by *ETV6-RUNX1*.[10,11] Based on these insights, the genetic events that define molecular subtypes have now been assumed to have leukemia-initiating potential and produce pre-leukemic clones that often precede B-ALL.

Recent advances in next-generation sequencing technologies have drastically improved our understanding of B-ALL at the molecular level. Recurrent rearrangements of *ZNF384*, *DUX4*, and *MEF2D* were recently identified by transcriptome sequencing (RNA-seq), most of which were unobtainable from classical karyotyping.[12–18] Initiating events (subtype defining events) deregulate downstream gene expression more strongly than secondary gene events, resulting in a unique expression profile that correlates with the initiating events. With the application of this feature to patients with B-ALL, several previously unknown subtypes were identified based on the integrated analysis of gene expression profiling and genetic alterations. These include ETV6-RUNX1-like (expression profile similar to *ETV6-RUNX1*-positive ALL), PAX5 P80R, PAX5alt (rearrangements or mutations), and BCL2/MYC.[14,19,20] More recently, two new high-risk subtypes involving *CDX2* ectopic expression (CDX2/UBTF) and *IDH1/2* mutations (IDH1/2-mut) were identified by some researchers, including us.[21–24] Notably, most of the recently identified subtypes were more prevalent in AYA/adults than in children. This review describes the recent developments in the molecular pathogenesis and clinical implication of BCR-ABL1-negative B-ALL molecular subtypes in AYA and adults, particularly focusing on three major subtypes, ZNF384-, DUX4-, and MEF2D-rearranged, and two novel subtypes, CDX2/UBTF and IDH1/2-mut.

2 | MOLECULAR PATHOGENESIS

2.1 | ZNF384-rearranged ALL

ZNF384 encodes a transcription factor that activates matrix metalloproteinases. The ability of *ZNF384* to fuse with *TAF15* or *EWSR1* in acute leukemia and the transformative properties of these fusions were first reported in 2002.[25] Subsequently, approximately 4% and over 20% of recurrent *ZNF384* rearrangements have been identified in Japanese pediatric B-ALL[12,26] and AYA/adults with BCR-ABL1-negative B-ALL,[17,24] respectively, indicating that rearranged ZNF384 is the most prevalent subtype in Japanese AYA and adults (Figure 1). Conversely, *ZNF384* rearrangements were seen only in 2%–3% of BCR-ABL1-negative B-ALL in a US cohort,[20] possibly reflecting ethnic differences (Figure 2). Recently, a multinational, multicenter genetic association study, which showed that East Asian ancestry positively correlated with the frequency of *ZNF384* rearrangements,[27] supported this possibility. Clinically, ZNF384-rearranged ALL has intermediate to favorable outcomes,[24,28] this depends on the fusion partners involved (Figure 3).[29]

ZNF384 fuses with various fusion partners (e.g., *EP300*, *TCF3*, and *CREBBP*) while retaining its entire coding region. The ZNF384 fusion protein has an oncogenic effect as it acquires an increased ability to bind to its canonical region or new sites with aberrant enhancer activation.[30,31] Mouse pro-B cell transplantation analysis showed that EP300-ZNF384-expressing pro-B cells proliferated in vivo and impaired their differentiation, leading to the development of B-ALL with a long latency.[17] Additionally, a genomic study of monozygotic twins with B-ALL showed that TCF3-ZNF384 fusion occurs before birth, and both twins shared the same breakpoint.[32] These results suggest that ZNF384 fusion has a leukemogenic initiation ability and potentially generates a pre-leukemic clone, but concomitant genomic alterations are required for leukemogenesis.

A unique feature of the immunophenotype of ZNF384-rearranged leukemia is the aberrant expression of CD13 and/or CD33, which are typical myeloid markers.[26] As expected from this lineage ambiguity, *ZNF384* rearrangements were also frequently observed in B/myeloid mixed phenotype acute leukemia (MPAL),[33] and human hematopoietic stem and progenitor cells that expressed ZNF384 fusion protein led to bi-phenotypic leukemia.[30] Although *ZNF384* rearrangements span the ALL–MPAL disease spectrum, genomic alterations and gene expression profiles were mostly similar between ZNF384-rearranged B-ALL and MPAL.[33] Furthermore, lineage switch was observed in some serial ZNF384-rearranged leukemic samples.[34–36] These results suggest a model where the ambiguous phenotype of ZNF384-rearranged leukemia results from the acquisition of gene rearrangements in immature hematopoietic progenitors and the decisive importance of the connection between the cell of origin and ZNF384 fusion protein (Table 1).[30,33]

2.2 | DUX4-rearranged ALL

DUX4 is located within the D4Z4 repeats (11–150 copies) of chromosome 4q or 10q. *DUX4* encodes a transcription factor that activates cleavage-specific transcriptional program early in development,[37] while *DUX4* is considered to be transcriptionally repressed in most somatic tissues.[38] In 2016, recurrent *DUX4* rearrangements (mostly *DUX4-IGH*) were identified for the first time in 14% of patients with AYA B-ALL, which led to the discovery of aberrant *DUX4* expression

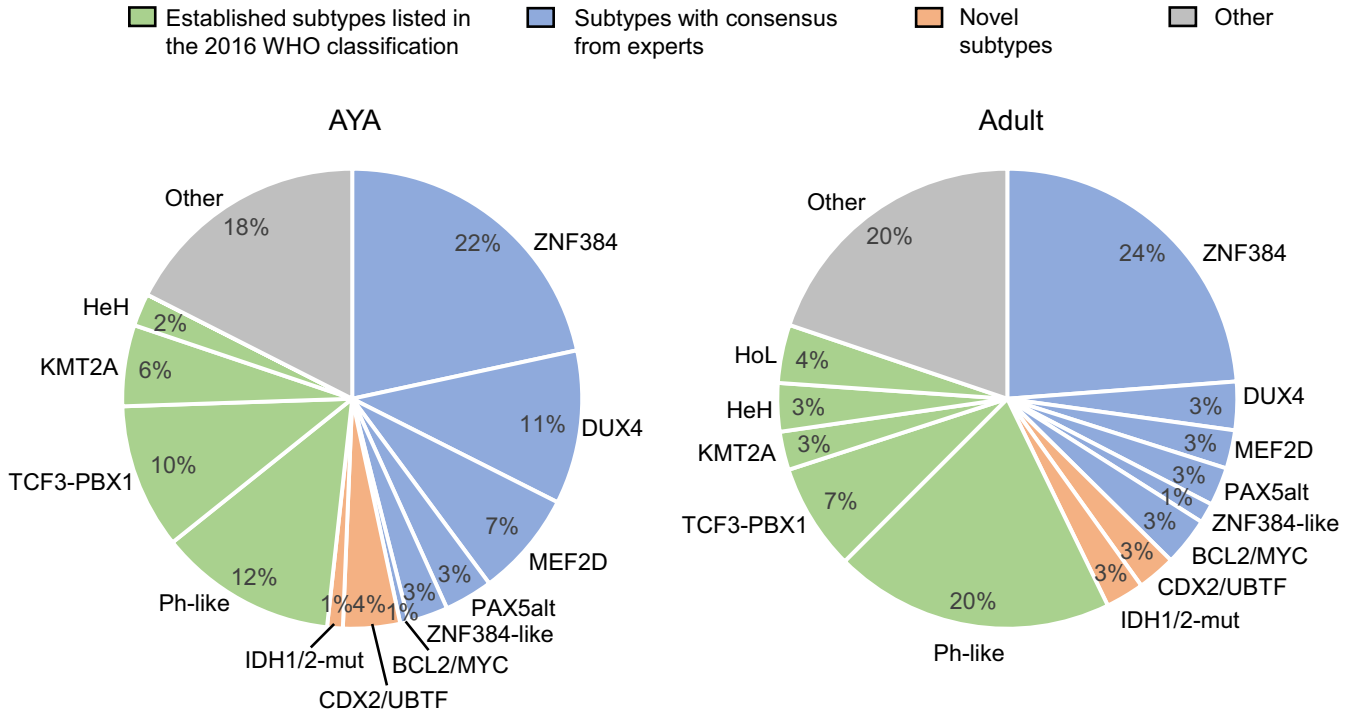

FIGURE 1 Distribution of *BCR-ABL1*-negative B-cell acute lymphoblastic leukemia (B-ALL) molecular subtypes in adolescents and young adults (AYA; left) and adults (right). The categories of molecular subtypes are color coded. CEBP/ZEB2, low-hyperdiploid, and PAX5 P80R subtypes were included in "Other" due to their low frequencies. HeH, high hyperdiploid; HoL, low hypodiploid. Data adapted from Yasuda et al.[24]

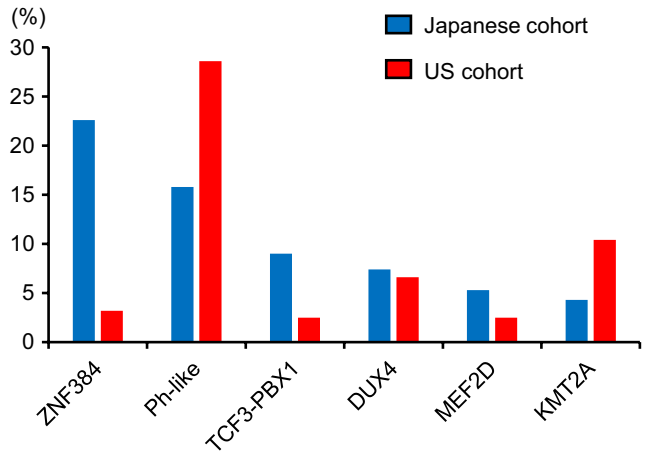

FIGURE 2 Comparison of frequencies of the major B-ALL subtypes in AYA and adults between the Japanese (blue) and US cohorts (red). Data adapted from Gu et al.[20] and Yasuda et al.[24]

in progenitor B cells (Figure 1).[17] Although detailed genomic analysis was challenging owing to the highly repeated sequence of D4Z4, three unique genomic structures of this rearrangement, which possibly give rise to *DUX4* deregulation and leukemogenic transformation, were detected.[17] First, the structural variation is not a gross chromosome translocation, but rather the insertion of a single or double *DUX4* unit into the *IGH* locus. *DUX4* de-repression may be triggered by the release of repeat-mediated epigenetic suppression of *DUX4*.[39] Second, the fusion event replaces the 3′ end of the *DUX4* coding region, which diminishes the proapoptotic ability of wild-type DUX4. Third, the polyadenylation signal, normally lacking in the D4Z4 region, is provided by partner genes, leading to the upregulation of stable DUX4 expression. In survival analyses, DUX4-rearranged ALL was generally associated with favorable prognosis (Figure 3),[20,24,28,40] but if *TP53* mutations coexist with this subtype, the outcome may be worse than with only *DUX4* rearrangement.[41]

A mouse pro-B cell transplantation assay demonstrated that DUX4-IGH (truncated DUX4 protein) expression in pro-B cells induced cell expansion, differential arrest, and development of pro-B cell leukemia with a long latency. Contrarily, mouse pro-B cells that express wild-type DUX4 cause cell death rather than cell proliferation. These different phenotypes probably originate from the distinct transcriptional activities between DUX4-IGH and wild-type DUX4.[42]

Microarray-based gene expression studies of childhood B-ALL demonstrated a group with specific expression profiles and frequent *ERG* deletions.[43,44] Following the discovery of *DUX4-IGH* rearrangements, the aberrantly spliced *ERG* transcript (ERGalt) and *ERG* deletion were frequently associated with DUX4-rearranged ALL,[14,18] therefore the groups with *ERG* deletions and *DUX4-IGH* rearrangements were determined to be identical.[14,18] Interestingly, DUX4 binds to the noncanonical first exon of ERGalt and induces the expression of this isoform, which has a dominant-negative effect on wild-type ERG function. These studies illustrated that *DUX4* rearrangement is an early leukemia-initiating event, and DUX4 deregulation by rearrangement results in a loss of ERG, due to either deletion

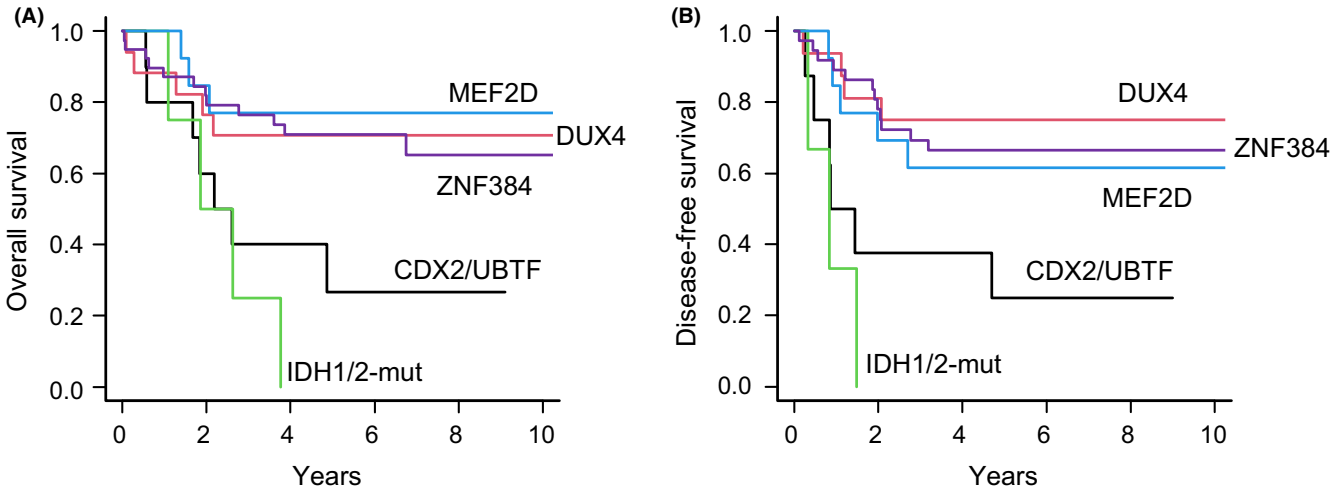

FIGURE 3 Clinical outcomes of molecular subtypes of B-ALL in AYA and adults. Kaplan–Meier survival curves were constructed for overall survival (left) and disease-free survival (right) for cases with indicated subtypes. Data adapted from Yasuda et al.[24]

or induced expression of the abnormal isoform, leading to B-ALL by coordinated deregulation of both transcription factors (Table 1).[18]

2.3 | MEF2D-rearranged ALL

MEF2D is a transcription factor that regulates muscle or neuronal cell differentiation. A *MEF2D–DAZAP1* fusion was first identified in TS-2 B-ALL cells, which had transformation activities in NIH 3T3 cells.[45,46] Following this, recurrent in-frame gene fusions between *MEF2D* and several partner genes, including *BCL9*, *HNRNPUL1*, *DAZAP1*, *CSF1R*, and *SS18*, were identified in childhood and AYA patients with B-ALL.[13,15–17] The most common *MEF2D* rearrangements were *MEF2D–BCL9* and *MEF2D–HNRNPUL1*, which were more frequently observed in older children or AYA, but rarely seen in younger children or adults (Figure 1).[13,15–17,20,24] *MEF2D* rearrangements have been associated with high-risk prognosis in childhood B-ALL,[13,16] whereas two studies recently showed that it had intermediate prognostic impact in adults (Figure 3).[24,28] The MEF2D fusion protein acts as an oncoprotein because its expression is significantly higher than that of the wild type, which is caused by the loss of miRNA target sites, resulting in enhanced transcriptional activity of the fusion gene.[13,17,47]

Based on gene expression profiling and immunophenotype data, MEF2D-rearranged ALL was assumed to be arrested at the pre-B cell stage.[15,48] Indeed, pre-B-cell receptor (pre-BCR) expression was confirmed on the cell surface in MEF2D-rearranged ALL cells by flow cytometric analysis.[49] ChIP sequencing and expression analysis using genome-edited *MEF2D*-rearranged cells that enabled fusion-specific ChIP and knockdown revealed that the MEF2D fusion protein was closely associated with pre-BCR expression. Furthermore, computerized algorithms identified MEF2D fusion as a component of the core regulatory circuitry (CRC), a transcription factor complex involved in cancer type-specific transcriptional regulation. The feed-forward regulatory loop between CRC involving the MEF2D fusion protein and pre-BCR signaling plays a central role in ALL maintenance (Table 1).[49]

2.4 | CDX2/UBTF ALL

We recently identified a novel high-risk subtype characterized by aberrant expression of *CDX2* and a frequent gain of 1q (>70%) by integrating analysis data, obtained using RNA-Seq and target Capture-Seq, of 354 AYA and adults with B-ALL (Figure 3).[24] This subtype was rare (approximately 3% in BCR-ABL1-negative B-ALL), but was more frequently observed in AYA and adults than in children (Figure 1). Soon, three other studies also reported the presence of the same subtype, which was associated with young adults, females, and a high-risk clinical course.[21–23] *CXCR4*-activating mutations and *PAX5* rearrangements were also characteristics of this subtype.[22,23]

CDX2 regulates *HOX* genes during embryonic hematopoiesis, but it is not expressed in normal adult hematopoietic cells. Aberrant expression of *CDX2* is one of the most prominent features of this subtype. However, apart from a mild change in *HOX* gene expression,[24] little is known about its downstream effects. *CDX2* deregulation was closely associated with 13q12.2 deletion, which was probably caused by recombinase activating gene (RAG)-mediated inappropriate recombination events. Unlike the deletion of the 13q12.2 region that has been previously reported in other types of B-ALL,[50] which leads to *FLT3* expression by deletion of the *PAN3* promoter region, both *FLT3* and *PAN3* promoter regions are deleted in this subtype. These specific genomic alterations in the 13q12.2 region results in monoallelic *CDX2* deregulation by *PAN3* enhancer hijacking.[22,23]

Another interesting finding is that the novel in-frame fusion transcript, *UBTF-ATXN7L3*, was identified in almost all the cases of this subtype.[21–23] The fusion involving *UBTF-ATXN7L3* results from a 17q21.31 microdeletion between exon 17 of *UBTF* and exon 1 of *ATXN7L3*. UBTF is a nuclear protein that epigenetically regulates rDNA and rRNA transcription. A recent study showed that *UBTF*

TABLE 1 Characteristics of B-ALL molecular subtypes of AYA and adults

Subtype	Peak prevalence	Key genetic alterations	Key molecular pathogenesis	Prognosis	Therapeutics
ZNF384-rearranged	AYA = Adults	*ZNF384* rearrangement, *FLT3* mutation, *ETV6* loss/mutation	Lineage aberrancy driven by ZNF384 fusion protein in immature cells	Intermediate or favorable	FLT3 inhibitor
DUX4-rearranged	AYA > Adults	*DUX4* rearrangement (*DUX4-IGH*), *ERG* deletion	Upregulation of C-terminal truncated DUX4 and loss of function of ERG	Favorable	-
MEF2D-rearranged	AYA > Adults	*MEF2D* rearrangement, *CDKN2A/2B* loss	Self-enforcing regulatory loop between MEF2D fusion complex and pre-BCR signaling	Inferior in children and AYA, intermediate in adults	Pre-BCR inhibitor
CDX2/UBTF	AYA > Adults	13q12.2 deletion, 17q21.31 deletion (*UBTF-ATXN7L3*)	Upregulation of CDX2 and UBTF-ATXN7L3 fusion protein expression	Inferior	-
IDH1/2-mut	AYA < Adults	*IDH1* R132C/*IDH2* R140Q mutations	Global DNA hypermethylation	Inferior	Potentially sensitive to IDH1/2 inhibitor

Abbreviation: AYA, adolescents and young adults.

tandem duplication was recurrently identified in pediatric AML, and this alteration may represent a novel subtype-defining lesion.[51] Although the molecular mechanism for B-ALL development of this subtype is poorly understood, defining a close relationship between this group and two universal genomic deletions (13q and 17q deletion) provides us with a hint about a leukemogenic process that may be driven by cooperative effects of the UBTF-ATXN7L3 fusion protein and CDX2 deregulation (Table 1).

2.5 | IDH1/2-mut ALL

IDH1 and *IDH2* are key genes in cellular metabolism and epigenetic regulation. Mutations in these genes occur in various malignancies, including low grade glioma, chondrosarcoma, and AML, and are known as initiating or early genetic events. *IDH1/2* mutations have been previously reported in B-ALL,[52,53] but their frequencies, biological significance, and clinical impacts are poorly understood. We recently identified recurrent *IDH1* R132C and *IDH2* R140Q mutations in Japanese AYA and adult B-ALL cohorts at a frequency of 1%–2%.[24] Conversely, these mutations were rarely detected in the Japanese pediatric cohort.[41] Specific expression profiling and mutually exclusive relationships between *IDH1/2* mutations and other subtype-defining lesions suggest that both mutations are probably initiating or early genetic events.[24] Methylation analysis supported this finding because this subtype showed hypermethylated profiles, which were clearly distinguishable from other established subtypes (Table 1).[24] Importantly, survival analysis showed that this subtype was associated with extremely inferior outcomes (Figure 3).[24]

3 | CLINICAL IMPLICATIONS

Accurate molecular diagnosis of B-ALL is necessary to appropriately manage patients and may lead to better treatment outcomes. The revised taxonomy that classifies heterogeneous B-ALL into more precise subtypes has been recently defined.[20,24] The classification criteria include three major categories: gene rearrangements, copy number abnormalities, and genetic alterations combined with gene expression. RNA-Seq is a powerful sequencing tool that simultaneously identifies gene rearrangements, copy number variations,[54] sequence mutations,[55] *IGH* rearrangements,[56] and gene expression profiles in a single platform. However, in many cases, molecular diagnosis using RNA-Seq is still challenging in a clinical setting owing to the lack of accurate and rigorous clinical assay platforms. To overcome these barriers, the feasibility of RNA-Seq for clinical application is being evaluated and is intended to be incorporated into prospective clinical trials.[40,57,58]

Classifying B-ALL into molecular subtypes has strong clinical significance in predicting prognosis and stratifying treatment, especially in classifying high-risk subtypes such as BCR-ABL1-like, CDX2/UBTF, or IDH1/2-mut. This enables patients to be appropriately guided to undergo intensified chemotherapy, allogeneic stem cell

transplantation, or novel immunotherapies (Figure 3). Minimal residual disease (MRD) testing is also an accepted and powerful prognostic indicator of B-ALL in adults. To stratify patients with B-ALL into more precise risk categories and tailor their therapy accordingly, it may be more useful to classify patients based on integrated analysis of both comprehensive molecular subtypes and MRD measurement. Therefore, evaluation of response kinetics, optimal timing, and clinically relevant cutoff levels of MRD testing in each molecular subtype is essential.[28,40,59]

Patients with the recently identified subtypes may benefit from molecularly targeted therapy. First, the ZNF384 fusion protein directly regulates *FLT3* expression,[30] and *FLT* expression and mutations (including *FLT3*-ITD) are associated with ZNF384-rearranged ALL.[24,60] The *ZNF384*-rearranged xenograft and clinical specimen showed sensitivity to *FLT3* inhibition.[30,60] Second, while the MEF2D fusion protein itself seemingly cannot be targeted, disruption of the positive feed loop between CRC and pre-BCR signaling, a central mechanism for MEF2D-rearranged ALL maintenance, may be a potential target for therapeutic treatment.[49] We demonstrated that the pre-BCR signaling inhibitor or *SREBF1* (a component of CRC) inhibitor showed therapeutic efficacies in MEF2D-rearranged ALL. Finally, the *IDH1* inhibitor (ivosidenib) or *IDH2* inhibitor (enasidenib) is a promising drug for the IDH1/2-mut subtype.

4 | CONCLUDING REMARKS

Advances in sequencing technology have undoubtedly revolutionized our understanding of the genetic basis of AYA and adults with B-ALL, especially with the identification of novel molecular subtypes. Implementation of precision medicine to treat B-ALL based on these novel findings will promote efficient risk stratification and targeted therapy. However, two major drawbacks of B-ALL need to be addressed. First, approximately 5% of children and 15% of AYA/adult B-ALL cases are genetically uncharacterized and present challenges for classification into molecular subtypes. Second, little is known about the cell of origin in adult B-ALL: when the founder clone emerges, the molecular mechanisms that are critically involved and the influences of the microenvironment on those mechanisms are unknown. This is in contrast to pediatric B-ALL where initiation events and pre-leukemic clones are proposed to occur in utero, and dysregulated immune response to infection may trigger secondary genetic events postnataly.[5] Although identification of early events in B-ALL may be difficult owing to its apparent lack of a pre-clinical period, recent studies on the clonal expansion in several normal tissues have provided an opportunity to understand early carcinogenesis.[61] Further studies are warranted to fully identify the very early events of leukemic initiation, as well as the repertoire of genomic alterations observed in advanced B-ALL. These studies contribute to finding a potential cure for AYA/adult B-ALL with a high success rate and may even be able to prevent this high-risk disease.

CONFLICTS OF INTEREST

The authors have no conflict of interest.

ETHICAL APPROVAL

Approval of the research protocol by an institutional review board: N/A.

Informed consent: N/A.

Registry and the registration no. of the study/trial: N/A.

Animal studies: N/A.

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