New biological and genetic classification and therapeutically relevant categories in childhood B-cell precursor acute lymphoblastic leukemia [version 1; peer review: 3 approved]

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Abstract
Traditionally, genetic abnormalities detected by conventional karyotyping, fluorescence in situ hybridization, and polymerase chain reaction divided childhood B-cell precursor acute lymphoblastic leukemia (BCP-ALL) into well-established genetic subtypes. This genetic classification has been prognostically relevant and thus used for the risk stratification of therapy. Recently, the introduction of genome-wide approaches, including massive parallel sequencing methods (whole-genome, -exome, and -transcriptome sequencing), enabled extensive genomic studies which, together with gene expression profiling, largely expanded our understanding of leukemia pathogenesis and its heterogeneity. Novel BCP-ALL subtypes have been described. Exact identification of recurrent genetic alterations and their combinations facilitates more precise risk stratification of patients. Discovery of targetable lesions in subsets of patients enables the introduction of new treatment modalities into clinical practice and stimulates the transfer of modern methods from research laboratories to routine practice.

Keywords
acute lymphoblastic leukemia, children, massive parallel sequencing, new BCP-ALL subtypes
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Introduction
Traditionally, B-cell precursor (BCP) acute lymphoblastic leukemia (ALL) has been classified into several distinct genetic subtypes defined by recurrent structural or numerical chromosomal alterations detected by cytogenetic methods (karyotyping and fluorescence in situ hybridization [FISH]), polymerase chain reaction (PCR), and flow cytometry (measurement of DNA content corresponding to ploidy)\(^1\). Six major genetic subtypes, altogether accounting for 70% to 75% of B-ALL, have been defined by high hyperdiploidy (51 to 67 chromosomes per leukemic cell), hypodiploidy (fewer than 45 chromosomes per leukemic cell), and \(\text{ETV6-RUNX1}\), \(\text{TCF3-PBX1}\), \(\text{BCR-ABL1}\), and \(\text{KMT2A}\) gene-involving fusions. These genetic aberrations have been characterized as first leukemogenetic hits which are present in all cells comprising the leukemic clone, defining its key biological features as well as impacting the clinical character of respective BCP-ALL subtypes. Their identification has remained important until today for diagnosis specification and, in some of them, for risk classification and targeted therapy\(^2\).

Rapid progress of modern genetic techniques yielded important discoveries in various human diseases, including BCP-ALL. Genome-wide profiling using array-based comparative genomic hybridization, single-nucleotide polymorphism arrays, and massive parallel sequencing (MPS) of whole genomes, and whole transcriptomes (RNA sequencing, or RNA-seq) resulted in the identification of novel recurrent genetic aberrations and patterns. Among them, several prognostically significant and druggable aberrations which have started to be implemented into risk-stratification algorithms and targeted therapy have been described\(^3\). Moreover, together with genome-wide gene expression profiling on microarrays and by RNA-seq, modern genomic studies led to the identification of novel biologically and clinically relevant BCP-ALL subtypes\(^4\). The so-called B-other ALL, a genetically and clinically heterogeneous subset of leukemias accounting for up to 30% of B-ALL (herein defined by negativity for all six above-mentioned classifying aberrations), was further dissected and better characterized. This review focuses on recent findings related to five novel BCP-ALL subtypes and selected clinically relevant genetic aberrations/patterns.

New BCP-ALL subtypes
Similar to the above-mentioned “classical” BCP-ALL subtypes, the five novel subtypes are distinguishable by their gene expression signatures; however, only three of them are also defined by the presence of a subtype-specific genetic aberration.

The first subtype, \(\text{BCR-ABL1}\)-like/Philadelphia chromosome-like (Ph-like) ALL (hereafter \(\text{BCR-ABL1}\)-like ALL), was already described in the “pre-MPS” era thanks to gene expression profiling on microarrays\(^5\). It is defined by a gene expression signature similar to that of \(\text{BCR-ABL1}\)-positive ALL. This gene expression signature likely results from the activation of kinase signaling pathways, which, however, is not triggered by a single specific genetic aberration as in the case of \(\text{BCR-ABL1}\)-positive ALL. On the contrary, a very wide spectrum of different genetic aberrations likely inducing this signature has been described in \(\text{BCR-ABL1}\)-like ALL\(^6-11\). The aberrations affect genes encoding kinases (for example, \(\text{JAK1}\), \(\text{JAK2}\), \(\text{JAK3}\), \(\text{ABL1}\), \(\text{ABL2}\), and \(\text{TYK2}\)), cytokine and growth factor receptors (for example, \(\text{CRKL}\), \(\text{PDGFBR}\), \(\text{EPOR}\), \(\text{IL7R}\), \(\text{CSF1R}\), \(\text{NTRK3}\), and \(\text{FLT3}\)), and signaling mediators and regulators (\(\text{KRAS}\), \(\text{NRAS}\), \(\text{BRAF}\), \(\text{PTPN11}\), \(\text{NF1}\), and \(\text{SH2B3}\)). These aberrations can be of various types, including both small-scale (deletions, insertions, substitutions, and complex mutations) and large-scale (chromosomal translocations and deletions) mutations which may generate fusion genes; of note, the majority of kinase/cytokine-receptor genes (\(\text{JAK2}\), \(\text{ABL1}\), and so on) can be fused to several different fusion partners. Such “kinase-signaling aberrations” can be found in 39% to 91% of \(\text{BCR-ABL1}\)-like ALL\(^12,13\); however, some of them also occur in B-other ALL not classified as \(\text{BCR-ABL1}\)-like\(^14,15\).

This ALL subtype was discovered independently by two studies. Although \(\text{BCR-ABL1}\)-like and Ph-like ALL, described by Dutch and American investigators, respectively, are now considered a single subtype, their definition is not fully consistent\(^6,8\). Some patients can be classified discordantly on the basis of classification approaches used by these two studies\(^8\). Several factors may contribute to this discrepancy, including methodological differences in gene expression data analysis, different composition of discovery cohorts (with respect to representation of various BCP-ALL subtypes, risk groups, and ethnicities), and the limited specificity of \(\text{BCR-ABL1}\) gene expression signature.

\(\text{BCR-ABL1}\)-like ALL is the only novel subtype that was already added as a provisional entity into the 2016 update of World Health Organization (WHO) ALL classification\(^16\). It occurs in about 10% to 15% of pediatric BCP-ALL and accounts for about 15% to 35% of the B-other ALL group and its frequency increases with age. Both above-mentioned \(\text{BCR-ABL1}\)-like discovery studies reported its inferior outcome\(^5,6\). Although recent studies demonstrate that the overall survival of children with Ph-like ALL treated according to protocols employing minimal residual disease (MRD)-based risk stratification is not significantly inferior to that of non-\(\text{BCR-ABL1}\)-like cases, such patients commonly exhibit inferior early response to therapy requiring treatment intensification\(^15\). Of note, it has been shown that outcome depends on the genomic context: the type of “kinase aberration” or presence of the \(\text{IKZF1}\) gene deletion (which has been recognized as an unfavorable prognostic factor in BCP-ALL as detailed below)\(^16,17\).

The \(\text{ETV6-RUNX1}\)-like subtype was described within B-other ALL on the basis of tight co-clustering of some \(\text{ETV6-RUNX1}\)-negative cases with \(\text{ETV6-RUNX1}\)-positive ALL according to gene expression profiling\(^3\). All cases belonging to this ALL subtype which have been described so far have a common genetic denominator: aberrations of the \(\text{ETV6}\) gene\(^18,19\); however, these are not specific for the \(\text{ETV6-RUNX1}\)-like ALL and occur in other subtypes as well\(^15\). The \(\text{ETV6}\) aberrations are usually deletions, but other gene-disrupting structural aberrations that may result in fusion genes were also described. Various alterations of \(\text{IKZF1}\) are also frequently detected within
this subtype. Biological proximity to ETV6-RUNX1-positive ALL is further supported by a similar expression pattern of two cell surface markers—CD27 and CD44—which can be measured by flow cytometry. Unlike the vast majority of other BCP-ALL subtypes, both ETV6-RUNX1-like and ETV6-RUNX1-positive ALL express CD27 but are negative or only partially or weakly positive for CD44. Such genotype–phenotype correlation is rather rare in BCP-ALL. Two studies describing this subtype so far indicate that it represents 5% to 12% of B-others and 1% to 3% of all B-ALL cases. ETV6-RUNX1-like ALL does not appear to be associated with inferior prognosis; however, additional studies on larger cohorts are needed to reliably determine whether the prognosis of this novel subtype is as favorable as that of ETV6-RUNX1-positive ALL.

DUX4-rearranged ALL was originally described as a B-other ALL subset with specific gene expression profile and frequent deletions of the ETS transcription factor gene ERG. Only recently, these leukemias have been characterized by a unique genetic aberration: rearrangements of the gene encoding transcription factor DUX4 (DUX4). These rearrangements are most frequently insertions of DUX4 into the IGH gene, resulting in the IGH–DUX4 fusion. The expression of DUX4, which is physiologically silenced in somatic tissues, is activated in DUX4r-ALL by its juxtaposition under the control of an ectopic regulatory element. The deletions of ERG can be detected in about 50% to 63% of cases. Although the rearrangement of DUX4 is an early, leukemia-initiating event, it induces the expression of alternative ERG variant and perhaps also renders the ERG gene prone to deletions. Owing to the small size of the inserted chromosomal fragment, the IGH–DUX4 fusion (and other DUX4r) cannot be easily screened by FISH. Similarly, high variability of genomic breakpoints may provide a potential fusion screening by PCR challenging. Thus, so far, this ALL subtype has been determined on the basis of its unique expression signature or presence of DUX4 fusion transcripts detected by RNA-seq. DUX4r-ALL represents 4% to 8% of BCP-ALL and 15% to 30% of B-others. Before the DUX4r-ALL discovery, it had been demonstrated that DUX4 binds to and deregulates the transcription of ERG in DUX4r-ALL; it induces the expression of alternative ERG variant and perhaps also renders the ERG gene prone to deletions. The likely association of lineage switch with the DUX4r-ALL subtype and impact of the ERG deletions on this association remain to be elucidated by future studies.

ALL with the ZNF384 gene-involving fusions (ZNF384r) represents another novel subtype of B-ALL. The ZNF384 gene, encoding transcription factor zinc-finger protein 384, can be fused to at least nine different partners (most frequently TCF3, EP300, or TAF15) in B-ALL. ZNF384r-ALL represents 1% to 5% of B-ALL and 5% to 10% of B-others. The unique gene expression signature of this ALL subtype is enriched for hematopoietic stem cell and immature myeloid lineage features and reflects upregulation of the JAK-STAT signaling pathway. ZNF384r-ALL also frequently displays a distinct immunophenotype compared with the majority of other B-ALL subtypes; the immunophenotypically distinct ZNF384r-ALL cases do not express (or only weakly express) CD10 marker but express myeloid markers CD13 or CD33 (or both) and may be even classified as mixed-phenotype acute leukemias on the basis of EGIL (European Group for the Immunological Characterization of Leukaemias) or WHO criteria. The clinical features of ZNF384r-ALL may vary depending on ZNF384 fusion partner. However, based on published studies reporting relatively small numbers of patients so far, ZNF384r-ALL does not appear to be a high-risk subtype in children.

The last of the five novel ALL subtypes is defined by rearrangements of the MEF2D gene (MEF2Dr). So far, at least six distinct MEF2D-involving fusion genes (with TCL1 gene being the most frequent fusion partner) have been described in this ALL subtype. MEF2D encodes a transcription factor that plays a role in muscle and neuronal cell differentiation but is also expressed throughout B-cell differentiation. The specific gene expression signature suggests a later differentiation stage of MEF2Dr-ALL compared with other BCP-ALL subtypes. Immunophenotyping by flow cytometry may also reveal specific features pointing toward this ALL subtype; MEF2Dr-ALL cells typically have relatively weak expression of CD10 and high expression of CD38. MEF2Dr-ALL represents 1% to 3% of childhood BCP-ALL and 2% to 5% of B-others and is diagnosed more often in adolescents than in children. The outcome of MEF2Dr-ALL seems to be inferior to that of other ALL subtypes. Alternative therapy employing histone deacetylase (HDAC) inhibitors which target HDAC9 (one of the MEF2dr-ALL signature genes) and which were already successfully tested in vitro could potentially improve its outcome in the future.

About 40% of B-other ALL cannot be classified into any of the five novel BCP-ALL subtypes. Various recurrent genomic aberrations can be found in patients belonging to this subset; some of them were already identified decades ago and others more recently. These include, for example, intrachromosomal amplification of chromosome 21 (iAMP21), dic(9;20), IGH–MYC, and TCF3–HLF fusions, CRLF2, NUTM1, or PAX5 gene-involving fusions, and intragenic amplification of PAX5 (PAX5amp). Although some of them likely represent primary lesions and could be considered subtype-defining.
aberrations, others frequently represent secondary aberrations or co-occur with established primary lesions or across established BCP-ALL subtypes. Consequently, certain heterogeneity exists in the classification of BCP-ALL into genetic/biological subtypes throughout the literature as well as in the definition of B-other ALL, which thus should always be explicitly described when used. One example of a potentially confusing situation is the classification of ALL with iAMP21. This ALL subset was added provisionally into the WHO 2016 ALL classification together with BCR-ABL1-like ALL[10], although it has been shown that these two ALL subsets partly overlap[13]. Moreover, though it has been suggested to represent a primary lesion[41], iAMP21, in a minor proportion of patients, co-occurs with other primary aberrations such as BCR-ABL1 or ETV6–RUNX1 fusions. Similarly, CRLF2 rearrangement (CRLF2r) that is sometimes used to define the subset of patients within B-other ALL[45] is frequently a secondary aberration[46] and also occurs across several BCP-ALL subtypes (BCR-ABL1-positive ALL, BCR-ABL1-like ALL, and hyperdiploid and hypodiploid ALL)[14,47,48]. Nevertheless, identification of these aberrations is clinically relevant, as some of them were proven to have unfavorable prognostic impact and thus influence risk stratification and therapy (for example, iAMP21[49,50] and TCF3–HLF[51]) whereas in others the suggested prognostic impact needs further validation (for example, PAX5amp[52]). Importantly, various aberrations may qualify patients for a targeted therapy (for example, CRLF2r) as further discussed below.

New therapeutically relevant aberrations/categories

A variety of novel druggable lesions, especially of kinase-activating aberrations, have been described thanks to the introduction of MPS in recent years. As already mentioned above, the kinase-activating lesions are highly enriched in the BCR-ABL1-like ALL subtype[9,10,15]. A large proportion of these aberrations can be assigned into one of two functional classes according to the affected signaling pathway: JAK/STAT class and ABL class[84,17,53]. The more frequent JAK/STAT class aberrations comprise aberrations affecting CRLF2, JAK1, JAK2, JAK3, EPOR, IL7R, and SH2B3 genes and can be targeted by the JAK inhibitor ruxolitinib. The most frequently affected CRLF2 gene encodes one of two subunits of heterodimeric receptor for thymic stromal lymphopoietin. In addition to constitutively activating F232C mutation[4,54], two types of CRLF2-invoking fusion have been described: IGH–CRLF2, which results from interchromosomal translocation, and P2RY8–CRLF2, which is caused by deletion in the PAR1 region on genosomes[11,42]. Both fusions lead to an overexpression of intact CRLF2 protein on the cell surface, which can be reliably detected by flow cytometry[42,56]. Thus, with the use of anti-CRLF2 antibody, CRLF2r-positive patients (who represent up to 17% of B-other ALL[16] and who might be considered for targeted therapy by ruxolitinib) can be easily identified during diagnostic immunophenotyping. The second class of kinase-activating aberrations, the ABL class, comprise ABL1, ABL2, PDGFRB, PDGFRA, and CSF1R gene-invoking fusions. These aberrations can be inhibited by tyrosine-kinase inhibitors (TKIs) such as imatinib or dasatinib[55], both of which have already become an inherent component of the treatment of pediatric BCR-ABL1-positive ALL[57]. Aberration of additional receptor and non-receptor kinase genes (for example, NTRK3, TYK2, DGKH, and PTK2B) that do not belong to these two classes but that at least in some cases can be targeted by known inhibitors can be also detected, though rarely, in BCR-ABL1-like ALL[10]. In addition to these lesions associated with the BCR-ABL1-like phenotype, aberrations resulting in the activation of the Ras/Raf/MAPK pathway (hereafter, Ras pathway) occur frequently across various BCP-ALL subtypes[58–62]. Activating point mutations of the KRAS and NRAS genes are the most abundant, whereas mutations of the FLT3, PTPN11, NF1, and BRAF genes occur less frequently. It has been shown that leukemic cells with Ras pathway mutations are sensitive to MEK inhibitors in vitro[63,64].

Sensitivity tests performed in vitro and in patient-derived xenografts provided a solid rationale for prospective clinical testing of TKIs in BCP-ALL with novel kinase-activating aberrations[17,53]. However, a relatively limited number of such children already treated by TKIs have been reported in the literature so far[10,14,65–72]. Although several case reports described good response to ABL class inhibitors in patients with ABL1/ABL2 or PDGFRB gene-invoking fusions[66,67,70,72,75] and overall results are generally encouraging, in some patients the TKI-invailing treatment failed to induce long-term remission. This can be at least partially due to the fact that TKIs were added to treatment only upon diagnosis of disease resistance/relapse. Additionally, cases of secondary TKI resistance caused by mutations in targeted kinase (that is, by the most common mechanism of resistance known in BCR-ABL1-positive leukemias) have already been described[73,74]. The first clinical trials have been initiated recently which use frontline TKIs added to chemotherapy backbone for selected groups of patients with kinase-activating lesions (ClinicalTrials.gov Identifier: NCT03117751 sponsored by St. Jude Children’s Research Hospital, Memphis, TN, USA; ClinicalTrials.gov Identifier: NCT02420717 sponsored by MD Anderson Cancer Center, Houston, TX, USA; ClinicalTrials.gov Identifier: NCT02883049 sponsored by the National Cancer Institute, Rockville, MD, USA; and ClinicalTrials.gov Identifier: NCT03020030 sponsored by the Dana-Farber Cancer Institute, Boston, MA, USA). Importantly, several clinical and biological aspects should be considered in the strategies for prospective TKI testing in newly diagnosed BCP-ALL. It should be discussed carefully whether it is justified to use TKIs in all children with any targetable kinase-activating aberration, whether they should be used only in children harboring a lesion unambiguously associated with unfavorable outcome (similar to BCR–ABL1 fusion), or whether the TKIs should be reserved for patients with worse early response to treatment or with resistant disease, where the potential benefits most likely outweigh an increase of treatment toxicity[77]. Moreover, it is important to consider that while some of the kinase and cytokine-receptor gene alterations are supposed to represent founding lesions present in all leukemic cells and essential for their survival[58–60], some aberrations may be either founding or secondary lesions (for example, CRLF2r) and others are typically secondary subclonal lesions (for example, JAK/RAF gene mutations), possibly without a
resistance- or relapse-driving role, thus probably representing less-suitable therapeutic targets.

Deletions of the IKZF1 gene (IKZF1del) encoding lymphoid transcription factor IkARos occur in 9% to 15% of BCP-ALL and more frequently in B-other, BCR-ABL1-positive, and hypodiploid ALL compared with remaining subtypes. In 2009, the IKZF1del was associated with poor outcome; subsequently, multiple studies confirmed its negative prognostic impact in BCP-ALL. These findings stimulated the incorporation of IKZF1del into risk-stratification algorithms as a factor qualifying for treatment intensification in some ALL trials (ClinicalTrials.gov Identifier: NCT02716233 sponsored by Assistance Publique – Hôpitaux de Paris, France). However, partially discrepant findings of individual studies showed that the prognostic impact of IKZF1del (its strength and independence on other known risk factors) depends on, for example, risk-stratification algorithms, applied therapy, and early treatment response. Moreover, it was revealed that it is modified by the presence of other genetic lesions. First, it was shown that deletions in the ERG gene (ERGdel) attenuate the negative prognostic impact of IKZF1del; a subsequent study demonstrated that other recurrent deletions may have the opposite effect. These findings led to the establishment of the IKZF1plus category, defined as IKZF1del with concurrent deletion of PAX5 or CDKN2A or CDKN2B genes or deletion of PAR1 region (resulting in P2RY8–CRLF2 fusion gene) or with a combination of these, in the absence of ERGdel. The IKZF1plus genomic pattern occurs in 6% of BCP-ALL, is associated with inferior outcome in patients with detectable minimal residual disease at the end of induction treatment, and will be used for risk stratification in the upcoming AIEOP-BFM (Associazione Italiana di Ematologia ed Oncologia Pediatrica–Berlin-Frankfurt-Münster) ALL trial. IKZF1plus occurs predominantly in B-other ALL; although we can assume its enrichment in subtypes with higher frequency of IKZF1del, such as BCR-ABL1-like ALL, its distribution and prognostic role across novel BCP-ALL subtypes remain to be elucidated by future studies. These studies could also help to clarify the strong association between IKZF1plus prognostic value and the early therapy response.

Conclusions

Herein, we briefly reviewed some of the most important recent findings in pediatric BCP-ALL, demonstrating that the use of modern high-throughput technologies not only advanced our insight into the genetics and biology of BCP-ALL but also paved the way for novel treatment options. However, with growing knowledge, we have begun to recognize more extensively that the significance (for example, clinical relevance) of individual factors may vary substantially depending on additional genetic/biological contexts. Enormous effort will still be needed to further improve our understanding of the considerably complex relationships between genetic/biological and clinical aspects of BCP-ALL and to better translate this knowledge into further improvements in patient outcome.

Abbreviations

ALL, acute lymphoblastic leukemia; BCP, B-cell precursor; FISH, fluorescence in situ hybridization; MPS, massive parallel sequencing; PCR, polymerase chain reaction; Ph-like, Philadelphia chromosome-like; RNA-seq, RNA sequencing; TKI, tyrosine-kinase inhibitor; WHO, World Health Organization

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References


inhibitors in a child with precursor B-cell acute lymphoblastic leukemia with EBF1-PDGFRB therapy in a refractory B-cell precursor acute lymphoblastic leukemia.


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