

Targeting subtype in ALL - Section 18

Relevant subtypes in childhood ALL

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Take home messages

- Genetic lesions define B cell precursor (BCP) ALL subtypes with distinct biology and prognosis. Some of them define “Ph and Ph-like” subtypes that may be treated by tyrosine-kinase inhibitors.
- CNV signatures (including *IKZF1* deletion) have been validated to distinguish subgroups of BCP-ALL statistically defined according to event-free survival.
- Routine screening of relevant genetic subtypes is now a major tool in clinical practice and improves risk assessment, clinical decision, and implementation of precision medicine in BCP-ALL.

Mutually exclusive genetic lesions define ALL subtypes with distinct biology and prognosis

Acute lymphoblastic leukemia (ALL) is the most common malignant tumor in children. Despite major improvements in cure rates over the last decades, it remains one of the major causes of cancer-related death in children. ALL uncovers a constellation of different entities characterized by mutually exclusive recurrent genetic alterations establishing the biology of ALLs (Table 1). These entities, which prevalence varies with age and ethnicity, allow the classification of ALLs and often influence response to treatment.^{1,2} Thus, in B cell progenitor ALLs (BCP-ALL), the presence of hyperdiploidy or t(12;21)/ETV6-RUNX1, which are the most frequent alterations in young children, is associated with an excellent prognosis. On the other hand, t(9;22)/BCR-ABL1, *KMT2A* (*MLL*) rearrangements, chromosome 21 amplification (iAMP21), t(17/19)/TCF3-HLF and hypodiploidy, are associated with a high risk of relapse and are criteria for therapeutic intensification. Other ALL, including those with t(1;19)/TCF3-PBX1, are considered intermediate risk (Table 1). By allowing the assignment of patients to a risk group and the adjustment of treatment intensity, the identification of classifying genetic

abnormalities has led to significant progress in managing children’s ALLs and has been a key element in improving children’s ALL treatment. However, if increasing the intensity of chemotherapy has been effective in some subgroups (ie, iAMP21), others such as the rare subtype defined by the presence of a t(17;19)/TCF3-HLF remain highly resistant to conventional therapies and alternative therapeutic options based on their specific biology should be considered.³

Among the so-called “B-other ALL,” that is, the fraction of ALLs that still lacks a classifying genetic alteration, pan-genomic studies recently identified novel ALL subtypes (Table 1) defined by specific gene fusions involving several transcription factors such as *PAX5*, *ZNF384*, *MEF2D*, or *DUX4* (frequently associated with *ERG* deletions), each being possibly fused to a variety of partners.^{1,4,5,6,7,8} The prognostic value of these genetic lesions remains to be clarified and will require large international studies due to the rarity of each subtype.

“Ph and Ph-like” ALL subtypes: The promise of targeted therapies

Most precision medicine initiatives so far focus on genetically defined subtypes. Leukemias are no exception to the rule and recently such a type of relevant ALL subgroups has emerged with the development of therapies specifically targeting oncogenic alterations. The detection of t(9;22)/BCR-ABL1 now points to the use of tyrosine-kinase inhibitors (TKI), radically improving the prognosis of these leukemias that are highly resistant to conventional chemotherapy.^{9,10}

More recently, several teams have identified a subpopulation representing 10% to 15% of BCP-ALLs whose gene expression profile (transcriptome) is very similar to that of t(9;22)/BCR-ABL ALLs. This new group of ALLs, called “Ph-like” or “BCR-ABL-like,” has aroused great interest due to the poor prognosis associated with it, in line with the usual persistence of high levels of minimal

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Table 1**Classification of BCP-ALL and Subtype Relevance for Risk Assessment and Clinical Decision**

Chromosomal/Molecular Abnormalities	Estimated Frequency in Children and Adolescent	Prognostic and Theranostic Impact Specific Features
High hyperdiploidy [51–67] Chromosomes Specific pattern of chromosome gain: X, 4, 6, 10, 14, 17, 18, 21	30% Peak incidence: 2–10 years old	Excellent prognostic, correlated to the modal number of chromosomes with a better outcome in patients with 56–67 chromosomes
Hypodiploidy <44 Chromosomes Low hypodiploidy (31–39 chr.) Near haploidy (<30 chr.)	2–3%	Poor prognostic. Therapy intensification indicated Low hypodiploidy is associated in 90% of cases with a TP53 mutation, half of which are constitutional Hypodiploid blasts can undergo chromosomal doubling also known as “masked hypodiploidy”
iAMP21 (intrachromosomal amplification of chromosome 21)	2% Incidence increases in older children and adolescents	Poor prognostic. Therapy intensification indicated Rarely associated with other classifying lesions (eg, <i>BCR-ABL</i> , <i>ETV6-RUNX1</i>). Robertsonian translocation (rob15;21)(q10;q10)c predisposing to iAmp21 ALL
<i>ETV6-RUNX1 (TEL-AML1)</i> t(12;21)(p13;q22)	25% Peak incidence: 2–10 years old	Excellent prognostic
<i>TCF3-PBX1 (E2A-PBX1)</i> t(1;19)(q23;p13)	5–6%	Good prognostic (but poor prognosis at relapse) Absence of late relapses
<i>TCF3-HLF</i> t(17;19)(q22;p13)	<1%	Extremely poor prognostic. Intensifier and/or alternative therapy indicated
<i>MLL</i> (11q23) fusion Frequent partners t(4;11)(q21;q23)/ <i>MLL-AF4</i> t(9;11)/ <i>MLL-AF9</i> t(11;19)/ <i>ENL-MLL</i> Multiple other partners	3% Predominant (>80%) in infant BCP-ALL (<1 year old)	Associated with hypercalcemia and DIC at diagnosis Poor prognosis. Therapy intensification indicated Associated with high WBC at diagnosis
<i>NUTM1</i> fusions Multiple partners	<1% More frequent in infant BCP-ALL	Prognosis not precisely known Better outcome than <i>MLL</i> rearranged BCP-ALL in infant
<i>BCR-ABL</i> t(9;22)(q34;q11.2)	2–4% Incidence increases in older children and adolescents	Poor prognosis under conventional chemotherapy TKI in association with conventional chemotherapy indicated Associated (>60% cases) with <i>IKZF1</i> deletion or mutations
“Ph-like” or “BCR-ABL-like” Multiple partners ABL class (<i>ABL1</i> , <i>ABL2</i> , <i>CSFR</i> , <i>PDGFRB</i> , etc.) JAK/STAT class (<i>JAK2</i> , <i>EPOR</i> , etc.) <i>ETV6-NTRK3</i> <i>CRLF2</i> alterations (missense mutations, <i>P2RY8-CRLF2</i> , <i>IGH-CRLF2</i>)	10–15% (by gene expression profiling) CRLF2 alterations Variable incidence with age (increases in older children and adolescents) and ethnicity (higher in Hispanics) Frequently observed (40–50%) in Down syndrome patients	Poor prognosis under conventional chemotherapy Frequently associated with <i>IKZF1</i> deletion Confer in vitro sensitivity to TKI (ABL class), JAK inhibitors (ruxolitinib) (JAK class, CRLF2-r), ALK inhibitors (crizotinib) (<i>ETV6-NRTRK3</i>) In vivo sensitivity in association with conventional chemotherapy currently under evaluation Frequently associated of CRLF2 alterations with JAK point mutations
<i>MYC</i> rearrangement Fusion with IGH, K,L t(8;14)(q24;q32) t(2;8)(q12;q24) t(2;8)(q12;q24)	2%	Mature BCP-ALL, Burkitt Leukemia Rituximab in association with chemotherapy indicated
<i>IGH-DUX4</i> Associated with <i>ERG</i> intragenic deletions and/or alternative transcripts (<i>ERG^{alt}</i>)	5–7% Incidence increases in older children and adolescents	Good prognostic but delayed response to induction frequently observed <i>IKZF1</i> deletion frequently found without negative prognosis impact Technically difficult to identify at the genetic level. Typical CD371 ± CD2 expression
<i>ZNF384</i> rearrangement With multiple fusion partners	2%	Prognosis not precisely known and possibly dependent on fusion partners (<i>EP300-ZNF384</i> seems to have better outcome compared to <i>TCF3-ZNF384</i>) Delayed response to induction frequently observed Characteristic immuno-phenotype: weak CD10 and aberrant CD13 and/or CD33 expression. Can be found in MPAL
<i>MEF2D</i> rearrangement With multiple fusion partners	<1%	Prognosis not precisely known (but tend to be poor) and may dependent on fusion partners Distinct cytology and immunophenotype (weak or absent expression of CD10, high expression of CD38), with frequent oligoclonality of IG/TCR gene rearrangements Activation of HDAC9 expression potentially conferring sensitivity to HDAC inhibitor treatment
<i>PAX5</i> rearrangement <i>PAX5</i> fusions with multiple fusion partners (eg, dic(7;9), dic(9;12), and dic(9;20) at karyotype) <i>PAX5</i> intragenic amplification <i>PAX5</i> point mutations (eg, p.P80R)	2–5%	Prognosis not precisely known, currently described as average.

DIC = disseminated intravascular coagulation, HDAC = histone desacetylase, IG/TCR = immunoglobulin/T cell receptor, MPAL = mixed phenotype acute leukemia.

residual disease (MRD) during induction.^{*11,12} These ALLs are characterized by abnormal activation of signaling pathways. This activation is the result of a wide range of genomic translocations leading to gene fusions, some of which have the advantage of providing potential therapeutic targets.^{*1,*,11,12} Ph-like ALL can be separated in 2 major groups depending on the type of fusion. Alike BCR-ABL, fusions involving ABL, PDGFR, or CSF1R (ABL-class fusions) are potential targets for tyrosine-kinase inhibitor. Another group of fusions involving or JAK, EPOR, or CRLF2 (JAK-class fusions) are potential targets for JAK inhibitors. Although there is currently no unambiguous transcriptional signature adapted to a prospective identification of these ALLs in the context of care,¹³ the search for these translocations using techniques such as FISH or RNAseq makes it possible to identify patients for whom therapeutic alternatives can be proposed.^{*6,14,15} Several case reports have described the effectiveness of imatinib/dasatinib in the treatment of refractory Ph-like ALL with ABL-class fusions and may help to decrease the use of bone marrow transplant. However, although promising, the beneficial effects of ABL- or JAK-targeted therapies are still based on anecdotal reports. Optimal treatment design as well as potential toxicity in combination with conventional chemotherapy remain an issue but trials are now open that will soon bring more information.

CNV signatures distinguishing BCP-ALL with poor outcome

Although the so-called “classifying” abnormalities are considered to be the initiators of leukemia, these genetic lesions alone are not sufficient to induce leukemia. Indeed, pan-genomic approaches using DNA microarrays and, more recently, next-generation sequencing applied to the exome or the entire genome have made it possible to identify a number of recurrent mutations that coexist with classifying anomalies.¹⁶ These additional lesions frequently alter genes involved in classical oncogenic pathway (*RAS*, *P53*, *CDKN2A*, *RB1*, etc.) or B-lymphoid development genes (*IKZF1*, *PAX5*, *EBF1*, etc.). Among the latter, deletions of the IKAROS gene (*IKZF1*), found in 60% to 80% of positive BCR-ABL ALLs as well as in 15% of negative BCR-ABL ALLs, are of note. These deletions are associated with poor prognosis independently of known prognostic factors and therefore represent a promising marker for therapeutic stratification.¹⁷ The prognostic power of *IKZF1* deletions was shown to be in part context-dependent with an epistatic effect of copy number variations (CNV) of other genes such as *ERG*.¹⁸ Accordingly, several groups subsequently defined CNV-based classifiers, such as the IKZF1+ subgroup, consisting in deletion patterns of 6 to 8 loci including *IKZF1* and distinguishing high-risk ALL deserving treatment intensification.^{*19,20} Contrary to classifying lesions defining biologically characterized ALL subtypes, these CNV signatures, which are now implemented for risk assessment in ongoing therapeutic trials, can be considered as “pragmatic” prognostic factors, distinguishing statistically defined subgroups associated with low event-free survival and thus providing relevant and robust tools for risk assessment in clinical practice.

Future perspectives

In ALL, an increasing number of subtypes can be now considered relevant either for their interest in risk assessment in combination with MRD, or because their detection directs patients to protocols that include drugs targeted at these abnormalities. Their prospective screening is now implemented in most up-to-date clinical trials as part of the routine diagnosis workup.¹⁵ Targeted therapies are currently limited to the Ph- and Ph-like subtypes but it is likely that precision medicine

based on targeted therapies will soon be extended to other ALL subtypes emphasizing the need for strong diagnostic algorithms.

References

- *1. Iacobucci I, Mullighan CG. Genetic basis of acute lymphoblastic leukemia. *J Clin Oncol*. 2017;35:975–983.
- Comprehensive and nicely presented review of BCP-ALL novel genetic subgroups.
2. Moorman AV. The clinical relevance of chromosomal and genomic abnormalities in B-cell precursor acute lymphoblastic leukaemia. *Blood Rev*. 2012;26:123–135.
3. Frimantas V, Dobay MP, Rinaldi A, et al. Ex vivo drug response profiling detects recurrent sensitivity patterns in drug-resistant acute lymphoblastic leukemia. *Blood*. 2017;129:e26–e37.
4. Gu Z, Churchman M, Roberts K, et al. Genomic analyses identify recurrent MEF2D fusions in acute lymphoblastic leukaemia. *Nat Commun*. 2016;7:13331.
5. Alexander TB, Gu Z, Iacobucci I, et al. The genetic basis and cell of origin of mixed phenotype acute leukaemia. *Nature*. 2018;562:373–379.
- *6. Zaliouva M, Stuchly J, Winkowska L, et al. Genomic landscape of pediatric B-other acute lymphoblastic leukemia in a consecutive European cohort. *Haematologica*. 2019;pii: haematol.2018.204974.
- First prospective screening of novel BCP-ALL subgroups in an European cohort of patients.
- *7. Gu Z, Churchman ML, Roberts KG, et al. PAX5-driven subtypes of B-progenitor acute lymphoblastic leukemia. *Nat Genet*. 2019;51:296–307.
- Inspiring representation of a very large number of BCP-ALL clustering according to their expression profile and genetic subgroup
8. Li JF, Dai YT, Lilljebjörn H, et al. Transcriptional landscape of B cell precursor acute lymphoblastic leukemia based on an international study of 1,223 cases. *Proc Natl Acad Sci USA*. 2018;115:E11711–E11720.
9. Biondi A, Gandemer V, De Lorenzo P, et al. Imatinib treatment of paediatric Philadelphia chromosome-positive acute lymphoblastic leukaemia (EsPhALL2010): a prospective, intergroup, open-label, single-arm clinical trial. *Lancet Haematol*. 2018;5:e641–e652.
10. Slayton WB, Schultz KR, Kairalla JA, et al. Dasatinib plus intensive chemotherapy in children, adolescents, and young adults with Philadelphia chromosome-positive acute lymphoblastic leukemia: results of Children’s Oncology Group Trial AALL0622. *J Clin Oncol*. 2018;36:2306–2314.
- *11. Roberts KG, Li Y, Payne-Turner D, et al. Targetable kinase-activating lesions in Ph-like acute lymphoblastic leukemia. *N Engl J Med*. 2014;371:1005–1015.
- First large-scale demonstration of functional effects of fusion proteins and of the efficacy of tyrosine kinase inhibitors in mouse pre-B cells and xenografts of human Ph-like ALL
12. Roberts KG, Janke LJ, Zhao Y, et al. ETV6-NTRK3 induces aggressive acute lymphoblastic leukemia highly sensitive to selective TRK inhibition. *Blood*. 2018;132:861–865.
13. Boer JM, de Boer ML. BCR-ABL1-like acute lymphoblastic leukaemia: from bench to bedside. *Eur J Cancer*. 2017;82:203–218.
14. Schwab C, Ryan SL, Chilton L, et al. EBF1-PDGFRB fusion in pediatric B-cell precursor acute lymphoblastic leukemia (BCP-ALL): genetic profile and clinical implications. *Blood*. 2016;127:2214–2218.
15. Inaba H, Azzato EM, Mullighan CG. Integration of next-generation sequencing to treat acute lymphoblastic leukemia with targetable lesions: the St. Jude Children’s Research Hospital approach. *Front Pediatr*. 2017;5:258.
16. Mullighan CG, Goorha S, Radtke I, et al. Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. *Nature*. 2007;446:758–764.

17. Clappier E, Gardel N, Bakkus M, et al. IKZF1 deletion is an independent prognostic marker in childhood B-cell precursor acute lymphoblastic leukemia, and distinguishes patients benefiting from pulses during maintenance therapy: results of the EORTC Children's Leukemia Group study 58951. *Leukemia*. 2015;29:2154–2161.
 18. Clappier E, Auclerc MF, Rapion J, et al. An intragenic ERG deletion is a marker of an oncogenic subtype of B-cell precursor acute lymphoblastic leukemia with a favorable outcome despite frequent IKZF1 deletions. *Leukemia*. 2014;28:70–77.
 - *19. Stanulla M, Dagdan E, Zaliova M, et al. IKZF1(plus) defines a new minimal residual disease-dependent very-poor prognostic profile in pediatric B-cell precursor acute lymphoblastic leukemia. *J Clin Oncol*. 2018;36:1240–1249.
- Validation on 2 cohorts of homogeneously treated patients of the prognostic value of the IKZF1+ CNV signature**
20. Moorman AV, Enshaei A, Schwab C, et al. A novel integrated cytogenetic and genomic classification refines risk stratification in pediatric acute lymphoblastic leukemia. *Blood*. 2014;124:1434–1444.