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Molecular markers in ALL: Clinical implications

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Abstract

Acute lymphoblastic leukemia (ALL) is the most common childhood cancer and remains a main cause of death in children despite recent improvements in cure rates. In the past decade, development of massively parallel sequencing has enabled large scale genome profiling studies of ALL, which not only led to identification of new subtypes in both B-cell precursor ALL (BCP-ALL) and T-cell ALL (T-ALL), but has also identified potential new therapeutic approaches to target vulnerabilities of many subtypes. Several of these approaches have been validated in preclinical models and are now being formally evaluated in prospective clinical trials. In this review, we provide an overview of the recent advances in our knowledge of genomic bases of BCP-ALL, T-ALL, and relapsed ALL, and discuss their clinical implications.

Keywords

Acute lymphoblastic leukemia; ALL; B-ALL; T-ALL; *DUX4*; *ETV6-RUNX1*; Hyperdiploid; Hypodiploid; *KMT2A*; *TCF3*; *IKZF1*; *MEF2D*; *PAX5*; Ph-like; *ZNF384*; ETP; *TLX1/3*; *TAL1*; relapse

Introduction

Acute lymphoblastic leukemia (ALL) is a neoplasm of B- or T-lineage lymphoid progenitors, and comprises multiple distinct subtypes characterized by constellations of genetic alterations, including aneuploidy, chromosomal rearrangements, DNA copy number alterations, and sequence mutations [1–6]. Among them, chromosomal translocations are generally considered to be as initiating genetic events in leukemogenesis. The prevalence and prognosis of these alterations vary according to age, ethnicity, and inherited cancer susceptibility, and thus, accurate diagnosis and detection of these lesions are important for appropriate risk classification and precision medicine (Figure 1). Recent advances in massively parallel sequencing have revolutionized our understanding of genomic landscape of ALL by enabling comprehensive characterization of leukemia subtypes (Figure 2) [7–10].

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Herein, we will review the genomic landscape of B-cell precursor ALL (BCP-ALL), T-lineage ALL (T-ALL), and relapsed ALL with particular emphasis on newly described entities and targets during the past decade.

B-cell Precursor Acute Lymphoblastic Leukemia

Recurring chromosomal alterations

High hyperdiploidy (51–67 chromosomes) is one of the most common subtypes of ALL in children, comprising ~30% of pediatric BCP-ALL and is associated with a favorable prognosis (Table 1) [7,11]. Genetically, high hyperdiploidy is characterized by a nonrandom gain of chromosomes, typically +X, +4, +6, +10, +14, +17, +18, and +21 [11]. In particular, combined gain of chromosome 4, 10, and 17 is associated with favorable prognosis [12]. Alterations involving the Ras pathway (*KRAS, NRAS, FTL3, PTPN11*) and epigenetic modifiers (*CREBBP, WHSC1*) are frequent genetic events [11]. Importantly *CREBBP* mutations in the histone acetyl transferase (HAT) domain are generally selected for and preserved during disease evolution, and with Ras pathway mutations are common in aneuploid B-ALL cases, including those with hyperdiploidy and hypodiploidy [13].

Hypodiploid ALL is further subdivided into two principal subtypes with unfavorable prognosis according to the severity of aneuploidy: near haploidy (24–31 chromosomes) and low hypodiploidy (32–39 chromosomes) [14,15]. Accurate identification of hypodiploid ALL is important in view of the poor prognosis, and inherited genetic basis of low hypodiploid ALL in children. Duplication of the aneuploid genome, or masked hypodiploidy is common and may be mistaken for high hyperdiploidy [3]. This can be distinguished in that masked hypodiploidy typically has diploid and tetraploid chromosomes, whereas hyperdiploidy has a mixture of triploid and some tetraploid (e.g. 21, X), that is, the specific gained chromosomes differ. Although still associated with unfavorable prognosis, minimal residual disease (MRD) risk-stratified therapy has improved the outcome of hypodiploid ALL [16]. Near haploid ALL presents at a younger age and leukemic cells exhibit frequent gene alterations activating the Ras pathway (particularly *NF1*) and inactivating mutations/deletions of *IKZF3* (AIOLOS) [14]. Importantly, unlike all other chromosomes that may be aneuploid in hypodiploid ALL, loss of chromosome 21 is not observed even in near haploidy, nor in other forms of ALL [14]. Low hypodiploid ALL is rare but increases with age. The pattern of aneuploidy within low hypodiploidy is not random, and as with near haploidy, chromosome 21 is universally retained [14]. Importantly, almost all low hypodiploid ALL has biallelic alterations of *TP53* and 43% of pediatric (but not adult) cases harbor germline *TP53* alterations indicating that low hypodiploid ALL is a manifestation of Li-Fraumeni syndrome [17]. Frequent secondary alterations include *IKZF2* (HELIOS), *RBI*, and *CDKN2A/CDKN2B* [14]. Hypodiploid ALL cells have sensitivity to PI3K and BCL2 inhibitors, which might be a promising target [14,18].

Intrachromosomal amplification of chromosome 21 (iAMP21) is more common in older children and is characterized by gain of three or more extra copies of a region of chromosome 21 including *RUNX1* generated by breakage-fusion-bridge cycles and chromothripsis [19,20]. The germline Robertsonian translocation rob(15;21) or a germline ring chromosome 21 are associated with a markedly elevated risk of iAMP21 [21]. Patients

with iAMP21 usually lack other key cytogenetic alterations [3]. Clinically, this subtype showed a poor outcome with high rate of relapse when they treated as standard risk, however, intensive therapy can greatly improve the outcome [22,23].

ETV6-RUNX1 and ETV6-RUNX1-like ALL

The t(12:21)(p13:q22) translocation gives rise to *ETV6-RUNX1* fusion which is the most common fusion in BCP-ALL (20%−25% in children) with a favorable prognosis [7,24]. In general, this translocation is cryptic on cytogenetic analysis, and leukemic cells have a distinct immunophenotype (CD27 positive and CD44 low/negative) [25]. The *ETV6-RUNX1* fusion may be identified in umbilical cord blood, and thus, is considered to arise in utero as a leukemia-initiating alteration [24]. However, *ETV6-RUNX1* itself is insufficient to induce overt leukemia and requires the prolonged latency with additional genetic events including deletion of the non-rearranged *ETV6* allele, focal deletion of *PAX5* and *WHSC1* [4,24,26–28]. This is consistent with heterogeneity in the sub-clonal composition of *ETV6-RUNX1* ALL [24,29,30].

Recent studies defined a new subtype, *ETV6-RI JNX*Hike ALL, showing similar gene expression profiles and immunophenotype to *ETV6-RUNX1* ALL despite the lack of *ETV6-RUNX1* fusion [7,8,25,31]. As with *ETV6-RUNX1* ALL, *ETV6-RJJNX*1-like ALL shows the highest prevalence in children and relatively favorable outcome [6,25,31]. This subtype includes several alternate rearrangements in *ETV6* (e.g. *ETV6-ELMO1*), *IKZF1* (e.g. *IKZF1-ETV6*), *TCF3* (e.g. *TCF3-FLII1*), and *FUS-ERG* as well as copy number alterations in *ETV6, IKZF1*, and *ARPP21*, suggesting that alteration of multiple ETS and other transcription factors are converging on the same mechanism of transformation (although not *ERG*, which is distinct in the DJX4-rearranged ALL) [7,25,31].

TCF3-PBX1/TCF3-HLF

The translocation t(1;19)(q23;p13) generating *TCF3-PBX1* fusion is present in 5%−6% of pediatric BCP-ALL and *TCF3-PBX1* ALL associates with a pre-B immunophenotype expressing cytoplasmic immunoglobulin heavy chain [3,4]. This subtype was previously considered as high risk due to higher central nervous system involvement and relapse, however with present intensive treatment, *TCF3-PBX1* ALL is classified as favorable or intermediate risk [3]. Conditional activation of *TCF3-PBX1* in B cell progenitors showed enhancement of self-renewal and led to leukemia with *PAX5* deletion and activation of JAK-STAT and Ras pathways after a pre-leukemia phase [32]. Although alterations of JAK-STAT and Ras pathways are not common in *TCF3-PBX1* ALL, targeting pre-BCR signaling by inhibition of the JAK/STAT pathway might be a good option [7,32,33]. Importantly, *TCF3-PBX1* ALL exhibits sensitivity to dasatinib and ponatinib, but not imatinib, which occurs as a result of inhibition of pre-BCR signaling by SRC kinases. Due to compensatory upregulation of *ROR1* expression, combination with ROR1 inhibition could enhance the sensitivity of dasatinib [34].

A variant of the t(1;19) translocation, t(17;19)(q22;p13), generates *TCF3-HLF* fusion, which defines a rare subtype of ALL associated with an extremely poor prognosis [3,7]. Comparison between *TCF3-PBX1* and *TCF3-HLF* ALL revealed distinct gene expression

profiles and mutational landscape [33]. *TCF3-HTF* ALL exhibited stem cell and myeloid features with enrichment of *PAX5* deletions and alterations of Ras pathway genes [7,33]. TCF-HLF fusion works as a pioneer transcription factor to recruit EP300 for driving MYC and shows vulnerability to EP300 inhibition [35]. Primary cells with *TCF3-HLF* show sensitivity to the BCL2 inhibitor venetoclax (ABT-199), representing a potential target therapeutic approach [33].

KMT2A-rearranged ALL

KMT2A (*MLL*) on chromosome 11q23 is rearranged to more than 80 different partner genes, and these rearrangements describe a distinct subtype of leukemia with both lymphoid and myeloid features and poor outcome [3,36]. This subtype is typically of the pro-B phenotype, lacking CD10 expression, with co-expression of myeloid markers. Approximately 80% of KMT2A-rearranged ALL is observed in infants, in whom *KMT2A* rearrangement is acquired in utero [4,36]. There is also a second peak in prevalence in adults and more than 75% of cases are fused to *AFF1* [36]. Less frequently exposure to topoisomerase II inhibitors induces therapy-related leukemia with *KMT2A* rearrangements [36]. Intriguingly *KMT2A* breakpoints in most infant ALL and therapy-related leukemia are similar, suggesting common mechanism of rearrangement [36]. In infant ALL, the most commonly perturbed pathways include PI3K and Ras pathways [37,38]. *KMT2A* rearrangement results in assembly of a large multi-protein complex that results in aberrant transcription and epigenetic dysregulation (e.g. high level of H3K79 methylation) through recruiting excessive DOT1L, H3K79 methyltransferase, which interacts with multiple *KMT2A* rearrangement partners [39]. Thus, targeted therapy against this complex is developing, which includes inhibition of DOT1L, bromodomain, Menin, and the polycomb repressive complex [36,39,40].

BCR-ABL1 (Ph+) ALL and Ph-like ALL

The Philadelphia chromosome (Ph), derivative chromosome 22, derives from the reciprocal t(9;22)(q34;q11) translocation, and encodes *BCR-ABL1* [7,41]. Although *BCR-ABL1* ALL is associated with poor prognosis, the addition of tyrosine kinase inhibitors (TKIs) to the conventional chemotherapy has greatly improved outcome [4,42,43]. However, the major secondary cooperative alterations, *IKZF1* deletions, are still predictive of an unfavorable outcome irrespective of TKI exposure [42]. Moreover, mutations in the kinase domain of *ABL1* (most frequently T315I) induces TKI resistance, which is more common in TKI monotherapy or in adults treated with less intensive chemotherapy, and less common in children treated with intensive chemotherapy [44]. In addition to ponatinib, targeting Y-catenin, a key component of BCR-ABL1 downstream, is promising irrespective of TKI resistance [4,45]. The deleterious effect of *IKZF1* mutations is in part due to loss of IKZF1 repression of stemness and cell-cell adhesion, phenotypes that may be reversed by rexinoids (via agonism of rexinoid X receptor alpha, which induces expression of wild type IKZF1) and focal adhesion kinase inhibitors (which inhibit downstream integrin signaling pathways) [46]. Before consensus guidelines for MRD assessment in *BCR-ABL1* ALL have been provided [47], several approaches have been tested for MRD monitoring (genome or transcriptome *BCR-ABL1* and Ig/TCR rearrangements) [48]. Importantly, some patients showed discrepancy of MRD results in Ig/TCR and *BCR-ABL1* transcript, which was

caused by the presence of *BCR-ABL1* fusion outside of the blast population [48]. This *BCR-ABL1* positive clonal hematopoiesis is suggestive of a CML-like disease exhibiting lymphoid blast crisis.

Ph-like or *BCR-ABL1-like* ALL exhibits a gene expression profile similar to *BCR-ABL1* ALL despite the lack of the *BCR-ABL1* fusion [49,50]. The prevalence and outcome of Ph-like ALL are similar to those of *BCL-ABL1* ALL, increasing in incidence with age and associated with elevated MRD levels and/or higher rates of treatment failure, although there is a difference in the prevalence of Ph-like ALL in AYA (higher than *BCR-ABL1* ALL) [41,51]. Similar to *BCR-ABL1* ALL, *IKZF1* alterations are characteristic, which result in acquisition of stem cell-like features and poor responsiveness to TKI. Although Ph-like ALL is genetically heterogeneous, this subtype can be fall into four main groups (Table 2): (1) alterations driving JAK-STAT signaling, including rearrangements and mutations/deletions of *CRLF2, JAK2, EPOR, TYK2, IL7R, SH2B3, JAK1, JAK3, TYK2, IL2RB*, (2) fusions involving ABL-class genes (*ABL1*, *ABL2, CSF1R, LYN, PDGFRA, PDGFRB*); (3) mutations activating Ras signaling (*NRAS, KRAS, PTPN11*); and (4) less common fusions (*FLT3, FGFR1, NTRK3*) [4,6,41,51]. Among them, *CRLF2* alterations are found in almost half of Ph-like ALL in AYAs and adults. *CRLF2* is located in the pseudoautosomal region of the sex chromosomes (PAR1) at Xp22.3/Yp11.3, and its alterations were incorporated into the criteria for "IKZF1^{plus}", a subtype with higher risk of relapse defined by co-occurrence of the *IKZF1* deletion with deletion of *CDKN2A, CDKN2B, PAX5*, and/or PAR1 region in the absence of *ERG* deletion [52]. Importantly, most alterations in Ph-like ALL can, theoretically be targeted by FDA-approved TKIs: JAK-STAT signaling (JAK inhibition); ABL-class fusions (ABL inhibitor); *FLT3* and *NTRK3* fusions (FLT3 and NTRK3 inhibitor) [6,41,53–55]. Combination of kinase inhibitors against multiple signaling shows synergistic effect in PDX model of *CRLF2/JAK* mutant (JAK and PI3K/mTOR inhibitors) and *ABL/PDGFR* mutant (dasatinib and PI3K/mTOR inhibitor) [56]. Some of these (ruxolitinib, imatinib, dasatinib, ponatinib) are being tested in frontline studies [57–59].

DUX4-rearranged ALL

Rearrangement and overexpression of the homeobox transcription factor gene *DUX4* constitutes a distinct subgroup, *DUX4-rearranged* ALL [7,31,60,61]. This subtype is also driven by deregulation of the ETS family transcription factor *ERG*, and comprises up to 5%–10% of BCP-ALL with a slight peak in AYAs. It has a distinct immunophenotype (CD2 and CD371 positive) and favorable outcome [7,31,60–62]. Deregulation of *DUX4* is induced by rearrangement to strong enhancer elements, most commonly the immunoglobulin heavy chain (IGH) enhancer, which results in expression of a C-terminal truncated DUX4 protein that is not normally expressed in B cells [60,61]. This truncated isoform of DUX4 then binds to an intragenic region of *ERG* resulting in transcriptional deregulation and expression of multiple aberrant coding and non-coding *ERG* isoforms, and deletion of *ERG* in up to 70% of *DUX4*-rearranged cases [60]. One isoform is ERGalt, a C-terminal fragment that retains the DNA-binding and transactivating domain of ERG, exerts dominant negative effect and is transforming [60]. The deletions of *ERG* are commonly polyclonal, and may encompass the region of *ERG* that encodes the N-terminus of ERGalt. These findings support a model in which an initiating rearrangement of *DUX4* results in gross transcriptional deregulation of

ERG and primes the locus for RAG-mediated deletion; and loss of ERG activity, either through deletion and/or expression of ERGalt, cooperates with DUX4 deregulation in leukemogenesis [60,63]. *DUX4*-rearrangement is associated with favorable outcome in children and adults, even with *IKZF1* deletion [60,61]. As clonal *ERG* deletions are not present in all *DUX4-rearranged* cases, the use of *ERG* deletion as a surrogate for this subtype, as is used in the definition of IKZF^{plus} is suboptimal and should be avoided. Accurate identification of this favorable subtype of ALL requires identification of *DUX4* rearrangement (either directly or through identification of elevated *DUX4* expression) [63]. In this regard, detection of strong CD371 cell surface expression by flow cytometry might serve as a promising surrogate marker for this subtype [62].

MEF2D-rearranged ALL

Rearrangement of *MEF2D* is associated with older age of onset and relatively inferior outcome due to early relapse [64–67]. MEF2D-rearranged ALL is characterized by an aberrant immunophenotype (low or absent expression of CD10, high expression of CD38 and cytoplasmic μ chain), mature B-ALL-like morphology, and distinct expression profiles [64,66,67]. The N-terminal of *MEF2D* is fused to several partner genes, retaining its DNA binding domain [64,66,67]. High expression of MEF2D fusion protein is induced by evasion from miRNA-mediated degradation [68], and results in transcriptional activation of MEF2D targets [66]. Dysregulated MEF2D targets includes overexpression of HDAC9, which confers therapeutic sensitivity to HDAC inhibitors such as panobinostat [66].

ZNF384-rearranged ALL

ZNF384-rearranged ALL is a unique subtype that can be diagnosed as BCP-ALL or B/myeloid mixed phenotype acute leukemia (MPAL) [69]. In BCP-ALL, peak age of onset and prognosis varies by fusion partners: *EP300-ZNF384* (median age 11, excellent outcome); *TCF3-ZNF384* (median age 5, frequent late relapse) [7,70,71]. In contrast, *ZNF384-rearranged* ALL shows uniformly distinct immunophenotype (weak CD10 and aberrant CD13 and/or CD33 expression) and gene expression profiles [69,70]. The secondary genomic alterations and gene expression profiles of *ZNF384-rearranged* BCP-ALL and MPAL cases are essentially indistinguishable, and both have lineage plasticity at diagnosis and relapse (lymphoid disease to myeloid disease and vice versa) [6,69]. Transplantation of sorted subpopulations of cells from *ZNF384-rearranged* cell line showed propagation of the immunophenotypic diversity [69]. Moreover, twin case of *ZNF384-rearranged* ALL indicated a fetal hematopoietic progenitor as the cell of origin of this case [72]. These are clinically important results which confer a compelling genomic rationale that *ZNF384-rearranged* cases should be treated in a uniform strategy rather than using lymphoid or lymphoid leukemia-directed therapy according to predominant lineage [6]. In this regard, *FLT3* overexpression is characteristic and can be targeted with the multi kinase inhibitor sunitinib [6].

PAX5-driven ALL (PAX5alt/PAX5 P80R)

The paired box DNA-binding transcription factor PAX5 is an essential regulator of the early stages of B cell development, and *PAX5*alterations are important in the pathogenesis of BCP-ALL as initiating or cooperation lesions. These include (1) disease initiating alterations

(PAX5 P80R, rearrangements/focal intragenic amplifications in P4X5-altered ALL [PAX5alt]); (2) secondary lesions (e.g. *PAX5* focal deletions in 30% of *ETV6-RUNX1* ALL, and *PAX5*mutations in multiple subtypes), and (3) germline alterations that predispose to ALL [5,7,8,26,74]. In mouse models, *Pax5* heterozygosity cooperates with constitutive activation of the JAK-STAT pathway in the development of BCP-ALL, supporting its role as a haploinsufficient tumor suppressor [73]. PAX5alt represents a group of cases with similar leukemic cell gene expression profiles, but diversity in the nature of underlying *PAX5* alterations. These include (1) cases with diverse (>20) *PAX5* rearrangements that typically preserve the N-terminal DNA-binding domain of PAX5, but with loss of the C-terminal transactivation domain; (2) cases with focal intragenic amplification of the *PAX5* DNA-binding paired domain (PAX5amp), and (3) cases with sequence mutations. Within this group, specific lesions are associated with variation in gene expression profile: for example, cases with *PAX5ETV6* rearrangement, or compound heterozygosity for p.Arg38 and p.Arg140 mutations in the DNA-binding paired domain have distinct gene expression profiles. PAX5alt is most common in children and the AYA population [7].

The PAX5 P80R subtype is characterized by the presence of the PAX5 P80R mutation with inactivation of the wild-type *PAX5* allele by deletion, loss-of-function mutation or copy-neutral loss of heterozygosity [7,75]. Notably, heterozygous $Pax5^{P80R/+}$ knock-in mice develop transplantable BCP-ALL, with genetic inactivation of the wildtype *Pax5* allele [7]. Thus, biallelic *PAX5* alterations are a hallmark of this subtype, and sequence mutations of lymphoid transcription factors such as PAX5 P80R and IKZF1 N159Y (see below) may be initiating events in leukemogenesis. The prevalence of PAX5 P80R increases with age and is associated with intermediate to favorable prognosis [7,74]. Additional important cooperating lesions include structural rearrangements of chromosomal arms 9p and 20q, which associate with the presence of dic(9:20) [74,75]. Moreover, mutations in the Ras and JAK-STAT pathway members are particularly enriched, highlighting the potential for targeted therapies [7,74].

Other subtypes of BCP-ALL.

BCP-ALL with *NUTM1* rearrangements is a rare distinct subtype observed exclusively in children [7,8]. NUTM1 is a chromatin modifier, recruiting EP300 to increase local histone acetylation [76]. While the common partner, *BRD9-NUTM1* is reported in BCP-ALL, *BRD4-NUTM1* is a hallmark of NUT midline carcinoma (NMC) and acts to repress differentiation in NMC by widespread repression of histone acetylation, indicating therapeutic approach with bromodomain and HDAC inhibitors [6,76].

IKZF1 alterations, like *PAX5*, are also common across the spectrum of B-ALL (particularly in *BCR-ABL1*-positive, Ph-like and DUX4 rearranged cases) but a specific mutation, IKZF1 pAsn159Tyr defines a subtype with gene expression profile cases [7,8]. In this subtype, the nonmutated wild-type allele of *IKZF1* is retained, and most cases have concurrent gain of chromosome 21 [7]. This mutation induces misregulation of IKZF1 transcriptional activation, in part through distinctive nuclear mislocalization and enhanced intercellular adhesion [77].

T-cell Acute Lymphoblastic Leukemia

Genetic heterogeneity in T-ALL

T-ALL is derived from thymic T-cell progenitors. T-ALL accounts for approximately 15% and 25% of pediatric and adult ALLs, respectively, and is twice as prevalent in males as in females [78–81]. Disruption of the normal differentiation, proliferation and survival during thymocyte development as a result of accumulation of genetic alterations results in the development of T-ALL [78,82]. *NOTCH1* activating mutations and loss of *CDKN2A* locus are a hallmark secondary events in T-ALL, found in over 70% of cases [78,80,81]. The majority of T-ALL cases harbor leukemia-initiating rearrangements or mutations that result in the aberrant expression of transcription factors and oncogenes, including basic helix-loop-helix factors (*TAL1, TAL2, LYL1*), LMO genes (*LMO1, LMO2*), homeobox genes (*TLX1* (*HOXH*), *TLX3* (*HOX11L2*), *NKX2.1, NKX2.2, NKX2.5, HOXA*), *MYB*, and *SPI1* [9,10,78,80,81]. These aberrant expression and dysregulated pathways confer unique gene expression signatures and classify T-ALL into several subtypes which reflect the point of differentiation arrest during T-cell development (Figure 3) [82]. T-ALL may also be classified by DNA methylation profiles which are associated with expression signatures, immunophenotypic profiles, and T-cell development stage [83]. In addition to fusion genes, somatic mutations and copy number alterations induce dysregulation in several pathways and genes, including JAK-STAT (*IL7R, JAK1, JAK3, DNM2*), Ras (*NRAS, KRAS*, and NF1), PI3K-AKT (*PTEN, AKT1, PIK3CA PIK3CD*), epigenetic regulators (*PHF6, SUZ12, EZH2, KDM6A*), transcription factors and regulators (*ETV6, GATA3, RUNX1, LEF1, WT1, BCL11B*), and translation regulators (*CNOT3, RPL5, RPL10*) [9,10,78,80,81].

Early T-cell precursor (ETP) ALL

ETP ALL is a distinct subtype of immature leukemia, that despite its name, exhibits genetic and transcriptomic features more similar to a hematopoietic stem cell than a T cell precursor, and should be considered a subgroup of immature, lineage ambiguous leukemias that are variably classified by immunophenotype, rather than genetic/biologic features. Other cases in this spectrum include undifferentiated leukemia and T/myeloid mixed phenotype acute leukemia (T/M MPAL). ETP ALL is defined by an immunophenotype similar to the earliest stages of T-cell development (cytoplasmic $CD3^{+}$, $CD7^{+}$; $CD8^{-}$, $CD1a^{-}$, $CD5^{weak}$) with aberrant expression of myeloid and/or stem-cell markers [84,85]. Previously, ETP-ALL has had an inferior outcome, although this is mitigated by contemporary risk-adapted therapy [86]. ETP-ALL is genetically characterized by somatic mutations in multiple signaling pathways (Ras, JAK-STAT, and epigenetic regulators) and transcription factors important for T-cell development [9,10,87]. Furthermore, ETP-ALL shows high-DNA methylation profiles at binding sites of polycomb repressive complex targets or components [83]. Importantly, ETP-ALL shares genomic and epigenomic features with T/M MPAL, in terms of frequent biallelic *WT1* alterations, and alterations in other several transcription factors (*ETV6, RUNX1, CEBPA*) and signaling pathways (JAK-STAT, *FLT3*, Ras) [69,87]. These data suggest that they are similar entities in the spectrum of immature leukemias and both might have sensitivity to FLT3 and/or JAK inhibition.

T-ALL in early stages of cortical thymocyte maturation

T-ALL that associates with aberrant activation of homeobox genes shows $CD1a^+$, $CD4^+$, and $CD8^+$ immunophenotype, reflecting a differentiation arrest in early stages of cortical thymocyte maturation, while some HOXA dysregulated cases show immature phenotype [80,84,88]. Clinically, this subtype of T-ALL represented favorable prognosis [10]. Genetically this subtype has the highest prevalence of both *NOTCH1* and *CDKN2A* alterations [9,10,78]. Dysregulated expression of homeobox genes is mostly induced by chromosomal translocations and inversions that relocate homeobox genes under the control of strong enhancers in the *TCR* and *BCL11B* regulatory regions [9]. *TLX1* and *TLX3* rearranged T-ALL is representative and has a similar gene expression signatures, DNA methylation profiles, and overlapping downstream targets [9,10,83]. Notably, *NUP214-ABL1* is found concurrently with *TLX1* and *TLX3* rearrangements and shows promising sensitivity to several TKIs [78]. Furthermore, due to frequent JAK-STAT pathway gene mutations in this subgroup, ruxolitinib, a JAK-STAT inhibitor, has been administered to patients with these alterations [58].

TAL1-driven T-ALL

T-ALL with aberrant expression of the *TAL1* oncogene has a late cortical thymocyte immunophenotype ($CD4^+$, $CD8^+$, $CD3^+$) and accounts for approximately 40% of T-ALL [9,10]. This subtype of T-ALL is further classified into two expression subtypes [10,88]. In normal T-cell development, TAL1 is transcriptionally silenced by double-positive stages [82]. Aberrant expression of *TAL1* is induced by chromosomal translocations, the sub-microscopic interstitial deletion (*STIL-TAL1*), disruption of insulated neighborhoods [89], and heterozygous somatic indels in a noncoding intergenic element upstream of the *TAL1* transcription start site [90]. Furthermore, TAL1 forms a positive auto-regulatory loop and complex with GATA3, RUNX1, and MYB [91]. Although TAL1 is such a major oncogene in T-ALL, only 30% of transgenic mice develop T-ALL after a latent period, and thus, additional abnormalities are required for leukemogenesis [79]. In addition, PI3K-AKT pathway gene mutations are frequently detected in TAL1-driven T-ALL [9,10], which associates with glucocorticoid resistance and can be reversed by the inhibition of this pathway [9,78].

NOTCH1 activating mutations in T-ALL

NOTCH1 acts as a ligand-dependent transcription factor and the NOTCH1 signaling pathway is essential for the commitment of T-cell lineage specification and for further T-cell development [82]. Aberrant activation of NOTCH1 pathway is mostly induced by somatic mutations that disrupt the negative regulatory region leading to ligand-independent activation, or impairment of the proteasomal degradation of intracellular domain of NOTCH1 which includes truncation of the PEST domain, *NOTCH1* mutations in 3' untranslated region, and *FBXW7* mutations [78,92–94]. These two types of *NOTCH1* activating mutations co-occur in more than 20% of T-ALL, which has synergistic effects [92]. However, the oncogenic activity of NOTCH1 is dose dependent and most mutations in human T-ALL do not generate sufficient signals to initiate leukemia development [95]. Moreover, most T-ALL harbor multiple subclonal *NOTCH1* activating mutations, and thus,

these mutations are acquired as a late secondary event, which has been confirmed by clonal analysis using single-cell sequencing [9,93,96,97]. One of the main direct targets of NOTCH1 is MYC which is regulated by T-cell-specific NOTCH1-controlled distal enhancer called “NMe” [94,98]. Furthermore, NOTCH1 and MYC control many overlapping target genes to promote cell growth [9,94].

Due to the importance of NOTCH1 signaling in T-ALL, several preclinical studies inhibiting NOTCH1 signaling with Y-secretase inhibitors (GSIs) have been initiated. However, lack of cytotoxic antitumor responses and dose-limiting gastrointestinal toxicities still limit their direct translation into patient benefit, although the combination of GSIs and glucocorticoids is promising [78,99].

Relapsed ALL

Although cure rate of primary ALL has greatly improved with risk-adjusted therapy, relapsed ALL is still a leading cause of death for children mainly due to therapy resistance. This resistance is frequently induced by acquired mutations but also influenced by the bone marrow microenvironment. Recent genomic analyses of paired primary and relapsed ALL samples have revealed that these secondary mutations have acquired during the disease progression with Darwinian selection, highly branched clonal architectures, especially in early relapse (9–36 months) [29,93,100–102]. Furthermore, chemotherapy of ALL has been postulated to induce *bona fide* drug resistance mutations including *NT5C2, PRPS1, NR3C1*, and *TP53* [100]. However, relapse-fated subclones present at diagnosis commonly exhibit drug resistance prior to the administration of any therapy [103].

One of the representative relapse specific somatic alterations is *CREBBP* alterations which occur in up to 20% of relapsed B-ALL and impair glucocorticoid sensitivity [13]. Most early relapse is associated with 6-MP resistance, as a result of *NT5C2* gain-of function mutations, *PRPS1* mutations, and loss of *MSH6*. *NT5C2* mutations confer resistance to purine analogs at the cost of impaired tumor cell growth and reduced leukemia-initiating cell activity [104]. NT5C2 inhibitors might be promising, however, several problems lie ahead such as development of mutant specific inhibitor [105]. Importantly, *NT5C2* and *PRPS1* mutations are not detectable at primary samples even in a minor clone [100,104,106]. Other recurrent somatic alterations in relapsed ALL include mutations in *SETD2, KDM6*, and *KMT2D* (*MLL2*) [29,100,102]. Tracking of these mutations as MRD may offer the opportunity to identify the relapse-fated clone early in disease evolution and modulate therapy accordingly to circumvent relapse.

Summary

Recent development of sequence technology and integrated large-scale analyses have greatly improved our knowledge of the genetic basis of ALL in terms of identification of new subtypes and dysregulated pathways associated with therapeutic targets. Many clinically important alterations are not evident using conventional cytogenetic and molecular approaches, and optimal ALL diagnostics requires next-generation sequencing, with RNA-seq capturing most clinically relevant information [58]. These alterations have several major

implications for clinical management including appropriate risk stratification, monitoring of treatment response, and most importantly development of precision medicine. Indeed, the use of TKIs has brought a great impact on the outcome of newly diagnosed *BCR-ABL1* ALL, however, most targeted therapies have been tested as single agents in relapsed or refractory patients. In this point, several ongoing studies using targeted therapies in frontline treatment are underway. However, although the majority of ALL subtypes are found to be driven by alterations of transcription factor, they are not intuitively directly druggable. Newly developed antibody and cellular immunotherapy are efficacious in a proportion of patients, however many these approaches fail in a substantial proportion of patients. It is thus now critical to take these genomic discoveries forward to characterize mechanisms of leukemogenesis, and explore novel therapeutic approaches to find subtype specific vulnerabilities for the improvement of treatment strategy in future.

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Declaration of competing interest

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Practice Points

Comprehensive genetic analysis has provided major implications for clinical management.

Several genomically-informed targeted therapies have been incorporated into frontline studies for the evaluation of the efficacy.

Exploring genetic basis to find subtype specific vulnerabilities is important for new therapeutic approaches.

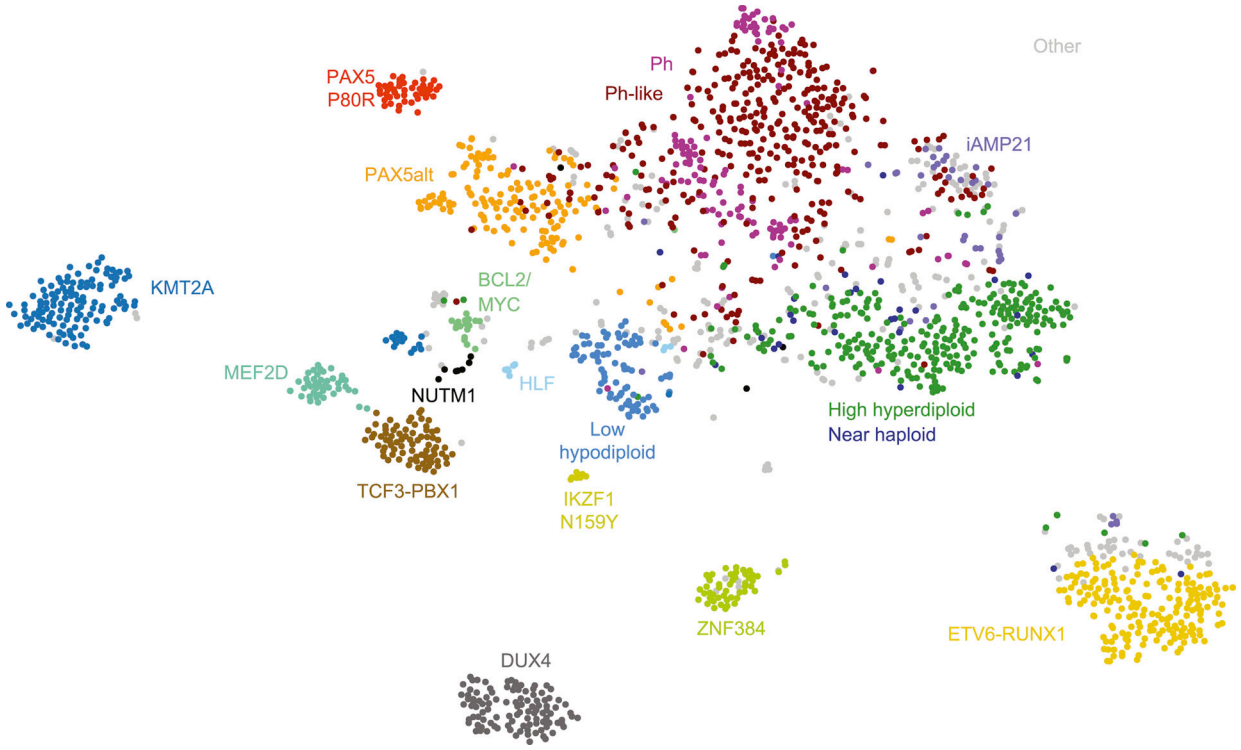

Figure 1.

t-SNE plot showing major B-cell precursor acute lymphoblastic leukemia (BCP-ALL) subtypes based on gene expression profiling of 1988 cases [7].

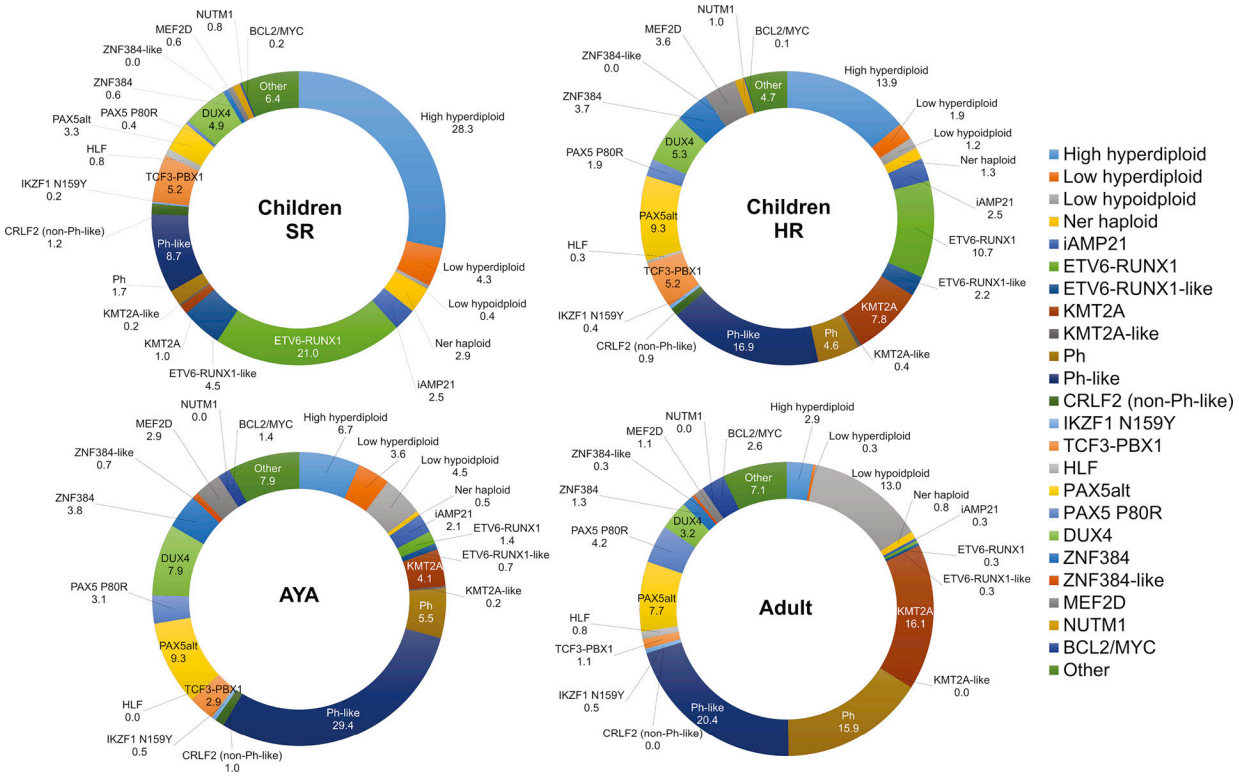

Figure 2.

Prevalence of each major subtype in B-cell precursor acute lymphoblastic leukemia (BCP-ALL) across age or risk. SR, standard risk; HR, high risk [7].

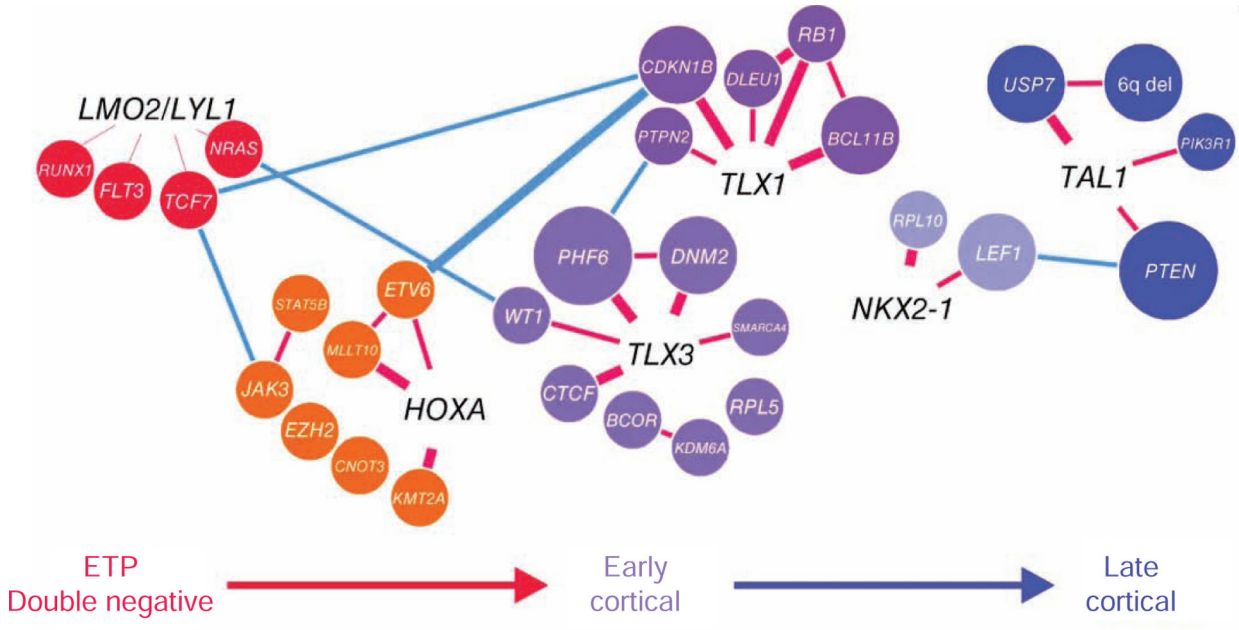

Figure 3.

Associations between genetic alterations, each T-cell acute lymphoblastic leukemia (T-ALL) subgroups, and T-cell differentiation stages [9].

Table 1.

Characteristics of each BCP-ALL subtype.

Molecular subtype	Category	Median age (yrs)	Peak prevalence	Genomic alterations	Characteristics	Ref
High hyperdiploid (51–67 chromosomes)	Aneuploid	4	Children (25%)	Ras pathway, epigenetic modifiers	• Excellent prognosis • Association of *CREBBP* mutations with relapse	[11]
Low hypodiploid (32–39 chromosomes)	Aneuploid	47	Adults (10–15%)	*IKZF2* deletion, biallelic *TP53* alterations	• Poor prognosis • *TP53* alterations are commonly inherited in children, but not in adult	[14]
Near haploid (24–31 chromosomes)	Aneuploid	5.4	< 3% in all ages	Ras pathway (*NF1*), *IKZF3* deletions	• Intermediate prognosis • No loss of chromosome 21 is observed	[14]
iAMP21	Copy number gain	10	< 3% in children and AYA	Gain of three or more extra copies of a region of chromosome 21 including *RUNX1*	• Good prognosis with intensive therapy • Low WBC at diagnosis • The germline Robertsonian translocation rob(15;21) or a germline ring chromosome 21 are reported to increase the risk of iAMP21	[21]
ETV6-RUNX1	TF rearrangement	4	Children (25%)	deletion of the non-rearranged *ETV6* allele, *PAX5* deletions, *WHSC1* mutations	• Excellent prognosis • Expression of CD27 and low/negative expression of CD44 • Acquired in utero	[26,28]
ETV6-RUNXHike ALL	TF rearrangement	3	Children (3%)	Alterations (fusions/deletions) in *ETV6, IKZF1, TCF3*	• Intermediate to favorable prognosis • Similar gene expression profile to *ETV6-RUNX1* ALL • Expression of CD27 and low/negative expression of CD44	[7,25,31]
TCF3-PBX1	TF rearrangement	8	Children (8%)		• Good prognosis • pre-B immunophenotype	[7,33]
TCF3-HLF	TF rearrangement	15	< 1% in all ages	*PAX5* deletions, Ras pathway	• Very poor prognosis	[7,33]
KMT2A-rearranged	TF rearrangement	40	Infants (80%) and adults (15%)	Ras pathway (subclonal), PI3K pathway	• Poor prognosis	[37]

Molecular subtype	Category	Median age (yrs)	Peak prevalence	Genomic alterations	Characteristics	Ref
					• Relation to therapy-related leukemia (topoisomerase II inhibitors) • Acquired in utero (infant ALL)	
BCR-ABL1 (Ph+)	Kinase driven	40–45	Adults (40–50%)	*IKZF1* alterations, *CDKN2AIB* deletions	• Prognosis improved with TKI	[41,51,10]
Ph-like	Kinase driven	21	AYA (25–30%)	Multiple kinase alterations, *IKZF1* alterations, *CDKN2AIB* deletions	• Poor prognosis, amenable to TKI therapy • Similar gene expression profile to *BCR-ABL1* ALL	[41,51]
DUX4-rearranged	TF rearrangement	14.3	AYA (8%)	*ERG* deletions (polyclonal), *IKZF1* deletions, Ras pathway	• Excellent prognosis • Distinct immunophenotype (CD2 and CD371 positive)	[60–62]
MEF2D-rearranged	TF rearrangement	14	AYA (7%)	Ras pathway	• Intermediate to unfavorable prognosis • Low or absent expression of CD10 and high expression of CD38	[64,66]
ZNF384-rearranged	TF rearrangement	15	AYA (5%)	Ras pathway, epigenetic modifiers	• Peak age of onset and prognosis varies by fusion partners • Low CD 10 expression and aberrant CD13 and/or CD33 expression • Frequently found in B/M MPAL	[61,69,70]
PAX5alt	Other TF driven	10	Children (10%)	*PAX5* rearrangements	• Intermediate prognosis • Loss of heterozygosity or acquisition of compound heterozygosity of PAX5	[7]
PAX5 P80R	Other TF driven	22	Adults (4%)	Ras pathway, JAK-STAT pathway	• Intermediate to favorable prognosis • Association with dic(9:20)	[7]
NUTM1-rearranged	TF rearrangement	3	Children (l%)		• Excellent prognosis	[7]
IKZF1 N159Y	Other TF driven		< 1% in all ages	Gain of whole chromosome 21	• Retain non- mutated wild type allele of *IKZF1*	[7]
BCL2/MYC-rearranged	Other TF driven	48	AYA and adults (3%)		• Poor prognosis	[7]

Table 2.

Four main groups in Ph-like ALL

Category	Kinase gene	Representative alterations	Target therapy
JAK-STAT signaling	*CRLF2*	mutations (F232C), fusions (*CSF2RA, IGH, P2RYS*)	JAK inhibitor
	EPOR	fusions (*IGH, IGK, LAIR1, THADA*)	JAK inhibitor
	TYK2	fusions (*MYB, SMARCA4, ZNF340*)	TYK2 inhibitor
	TSLP	fusions (*IQGAP2*)	JAK inhibitor
	SH2B3	Deletion/mutations	JAK inhibitor
	IL7RA	mutations, indels	JAK inhibitor
	JAK1	mutations (V658F)	JAK inhibitor
	JAK2	mutations (R683G), fusions (*ATF7IP, BCR, EBF1, ETV6, PAX5, SNX29, SSBP2, ZNF340*)	JAK inhibitor
	JAK3	mutations	JAK inhibitor
	IL2RB	fusions (*MYH9*)	JAK inhibitor
ABL signaling	*ABL1*	fusions (*ETV6, FOXP1, NUP214, RANBP2*)	Imatinib/dasatinib
	ABL2	fusions (*PAG1, RCSD1, ZC3HAV1*)	Imatinib/dasatinib
	CSF1R	fusions (*MEF2D, SSBP2, TBL1XR1*)	Imatinib/dasatinib
	LYN	fusions (*GATAD2A, NCOR1*)	Imatinib/dasatinib
	PDGFRA	fusions (*FIP1L1*)	Imatinib/dasatinib
	PDGFRB	fusions (*ATF7IP, ETV6, SNX29, SSBP2, ZMYND8*)	Imatinib/dasatinib
Ras signaling	*NRAS*	mutations	
	KRAS	mutations	
	PTPN11	mutations	
	NF1	Mutations/deletions	
	BRAF	mutations	
	CBL	fusions (*KANK1*)	
Other signaling	*FLT3*	*FLT3-ITB*, fusions (*AMYM2*)	FLT3 inhibitor
	NTRK3	fusions (*ETV6*)	NTRK3 inhibitor
	FGFR1	fusions (*BCR*)	Ponatinib
	PTK2B	fusions (*KDM6A, STAG2, TMEM2*)	FAK inhibitor
	DGKH	fusions (*ZFAND3*)	
	BLNK	fusions (*DNTT*)	