Best Practice & Research Clinical Haematology 32 (2019) 101095

Contents lists available at ScienceDirect

Best Practice & Research Clinical Haematology

journal homepage: www.elsevier.com/locate/issn/15216926

How advanced are we in targeting novel subtypes of ALL?

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ARTICLE INFO

Keywords:
Acute lymphoblastic leukemia
ALL
ALL subtype
B-progenitor ALL
B-ALL
DUX4
ETV6-RUNX1
Genome profiling
Hypodiploid
IGH
IKZF1
MEF2D
NUTM1
PAX5
Ph-like
Taxonomy
ZNF384

ABSTRACT

Despite high cure rates in children, acute lymphoblastic leukemia (ALL) remains a leading cause of cancer death in the young, and the likelihood of treatment failure increases with age. With the exception of tyrosine kinase inhibitors, there have been few advances in repurposing or developing new therapeutic approaches tailored to vulnerabilities of ALL subtypes or individual cases. Large-scale genome profiling studies conducted over the last decade promise to improve ALL outcomes by refining risk stratification and modulation of therapeutic intensity, and by identifying new targets and pathways for immunotherapy. Many of these approaches have been validated in preclinical models and now merit testing in clinical trials. This review discusses the advances in our understanding of the genomic taxonomy and ontogeny of B-progenitor ALL, with an emphasis on those discoveries of clinical importance.

Introduction

Cure rates for B-precursor acute lymphoblastic leukemia (B-ALL) now exceed 90% in children treated with optimal, risk-adapted therapy [1]. This contrasts with the prognosis in older children and adults, where cure rates decline sharply to less than 50% in adults over the age of 40 [2]. Previously, genetic alterations identified by chromosomal karyotyping, fluorescence in situ hybridization, and identification of common fusion gene transcripts have been used to identify good-risk (eg, high hyperdiploidy and *ETV6-RUNX1* rearrangement) and poor-risk (*KMT2A* rearrangement, *BCR-ABL1,* and hypodiploidy) subsets of childhood ALL [3]. The repertoire of leukemia-driving and subtype-defining genetic alterations in adult ALL has been far less well understood, and treatment typically determined by the presence or absence of *BCR-ABL1*.

The last decade has witnessed major advances in the use of genomic approaches to identify leukemia subtypes and the constellations of inherited and somatic genomic alterations that characterize each, as well as the nature of genetic variegation that underlies clonal heterogeneity and disease progression in the setting of treatment failure and relapse [4–6]. These studies have been notable for (1) initial characterization of ALL in children, but with an increasing focus on the disease in adults; (2) progressively increased comprehensiveness of analysis, initially using single nucleotide polymorphism (SNP) microarrays to characterize DNA copy number alterations (CNAs) and copy-neutral loss of heterozygosity (LOH) [7,8] to whole exome (WES), transcriptome (WTS), and

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https://doi.org/10.1016/j.beha.2019.101095

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genome (WGS) sequencing approaches; and (3) analysis of large cohorts of cases across the age spectrum. These studies have been performed in both B-progenitor and T-lineage ALL [9], but it is in B-ALL, where a greater proportion of cases have previously lacked known drivers, that these studies have been most informative.

A revised taxonomy of B-ALL

Previously established subtypes of B-ALL include aneuploidy (high hyperdiploidy, low hypodiploidy, and near haploidy), complex chromosomal rearrangements (intrachromsomal amplification of chromosome 21, iAMP21), and rearrangements that deregulate oncogenes or result in the expression of chimeric fusion oncoproteins (eg, *ETV6-RUNX1, KMT2A* rearrangement, *TCF3-PBX1,* and *BCR-ABL1*) [3]. Conventional cytogenetic and molecular analyses fail to identify clonal driver alterations in approximately 25% of ALL in children and the majority of cases in adults. These clonal, subtype-defining chromosomal alterations are typically associated with distinct leukemic gene expression profiles upon microarray or WTS-based gene expression profiling. As WTS may characterize both gene rearrangements and gene expression, this platform has been increasingly used to profile ALL in children and adults to identify new subtypes and their drivers.

In an analysis of 1988 ALL cases, approximately two thirds from children (enrolled on St Jude Total Therapy and Children's Oncology Group studies) and one third from adults (treated on protocols of the Alliance-CALGB, ACRIN - Eastern Cooperative Oncology Group, MD Anderson Cancer Center, and Southwest Oncology Group), WTS was used in all cases to identify gene expression profiles, aneuploidy, sequence mutations and chromosomal rearrangements [10]. Multiple algorithms were used to cluster cases according to leukemia gene expression profile. This confirmed and expanded recent studies identifying new subtypes: 23 were identified with significant variation in prevalence according to age stratum (children < 16, adolescent and young adult (16–39), and older adult (> 39 years of age at diagnosis) (Table 1 and Fig. 1). These new subtypes were not identified by prior chromosomal analysis for several reasons: (1) rearrangements were cryptic on karyotyping; (2) some subtypes were defined by diverse rearrangements to a single partner gene (eg, *MEF2D*, *ZNF384,* and *NUTM1*-rearranged ALL); (3) several subtypes are driven by sequence mutations of transcription factors (eg, PAX5 P80R and IKZF1 N159Y), or (4) subtypes may have diverse alterations within a single group (eg, *PAX5*alt, or "*PAX5* altered" that comprises *PAX5* rearrangements, sequence mutations, and structural variants; and Ph-like ALL).

Although it is well established that several subtypes are associated with age (eg, high hyperdiploid and *ETV6-RUNX1* ALL in children, and *BCR-ABL1* and Ph-like ALL in the adolescent-young adult [AYA] and older adult population), this analysis also demonstrated additional associations with age: older adult ALL has a more restricted range of subtypes, the most common being low hypodiploid ALL, *KMT2A*-rearranged and kinase driven (*BCR-ABL1*+ and Ph-like ALL). In addition, many cases previously categorized as "B-other" harbored founding *PAX5* alterations, more commonly *PAX5*alt in children and older adults, and *PAX5* P80R in older adults. An additional group was identified with double or triple hit alterations in *BCL2*, *MYC,* and/or *BCL6*, lesions also observed in a subset of aggressive diffuse large cell lymphoma.

Many of these subtypes showed significant associations with event-free or overall survival in both children and adults. These associations were particularly striking in adults, where few alterations associated with favorable prognosis have previously been identified. Rearrangement of the double homeobox gene *DUX4* was associated with the most favorable outcome in both children and adults, *PAX5* P80R with intermediate outcome, *PAX5*alt with unfavorable outcome, and kinase-driven leukemia, *KMT2A*-rearrangemed and *BCL2/MYC*-rearranged leukemia with dismal outcome in adults. Thus, these data indicate the importance of accurate identification of ALL subtype at diagnosis to guide therapeutic intensification and, for good-risk subtypes, potential avoidance of intensive, toxic regimens such as allogeneic stem cell transplantation. A comprehensive review of the genetics and biology of each subtype is beyond the scope of this manuscript, but newly described entities with therapeutic implications are described below.

MEF2D-rearranged ALL

Recurrent rearrangements of *MEF2D* occur in approximately 4% of children and 7% of AYA patients, and is associated with older age of onset (median 14 years) and intermediate to poor outcome [11–13]. The rearrangements result in expression of chimeric fusion oncoproteins with *MEF2D* (myocyte enhancer factor 2D) at the N-terminus fused to *BCL9* (most commonly), *CSF1R*, *DAZAP1*, *FOXJ2*, *HNRNPUL1*, *HNRNPH1*, and *SS18* [11,13,14]. *MEF2D-CSF1R* is exceptional for being observed in Ph-like ALL rather than the other *MEF2D* fusions that have a distinct gene expression profile [15]. The immunophenotype of *MEF2D* ALL is notable for CD10 negativity and CD38 positivity. The fusions retain the DNA binding of *MEF2D*, and in vitro, result in transcriptional activation of known *MEF2D* targets such as HDAC9 and hematopoietic cell transformation [11]. The deregulation of HDAC9 results in a therapeutic vulnerability, as patient-derived leukemic cells are exquisitely sensitive to histone deacetylase inhibitors such as panobinostat [11].

ZNF384-rearranged leukemia

Rearrangements of *ZNF384* (zinc finger 384) define a subtype of leukemia that may be diagnosed as pre-B ALL (with or without aberrant expression of myeloid antigens) or B/myeloid mixed phenotype acute leukemia [16]. The secondary genomic alterations and gene expression profiles of *ZNF384*-rearranged B-ALL and B/myeloid mixed phenotype acute leukemia (MPAL) are essentially indistinguishable, suggesting that the presence of the *ZNF384* rearrangement should be considered the hallmark, and diagnostic criterion, for this form of leukemia rather than initially stratifying patients by immunophenotype into B-ALL or MPAL. In B-ALL, *ZNF384*

Table 1
Prevalence and prognosis of subtypes in B-ALL.

ALL subtype	Category	Median age (yrs)	Peak prevalence	Genomic alterations	Clinical features	Therapy	References
Hyperdiploid (> 50 chromosomes)	Aneuploid	4	Children (25%)	Ras pathway, epigenetic modifiers	Excellent prognosis	Reduce intensity	[60]
Low hypodiploid (31–39 chromosomes)	Aneuploid	47	Adults (10–15%)	*IKZF2* deletion, *TP53* mutation (commonly inherited)	Poor prognosis	BCL2 inhibitors	[61,69]
Near haploid (24–30 chromosomes)	Aneuploid	5.4	< 3% in all ages	Ras pathway, *IKZF3* deletion	Intermediate prognosis	BCL2 inhibitors	[61,69]
iAMP21	Copy number gain	10	~3% in children and AYA	Complex structural alterations of chromosome 21	Good prognosis with intensive therapy, low WBC		[62]
ETV6-RUNX1 t (12; 21) (p13; q22)	TF rearrangement	4	Children (25%)	*PAX5* deletion, *WHSC1* mutation	Excellent prognosis	Reduce intensity	[7,63]
ETV6-RUNX1-like	TF rearrangement	3	Children (3%)	*ETV6* fusions and deletion, *IKZF1* fusions and deletion	Unknown	Reduce intensity	[42,43]
DUX4-rearranged	TF rearrangement	14.3	AYA (~8%)	*ERG* deletion, *IKZF1* deletion, Ras pathway	Excellent prognosis	Reduce intensity	[18,42,44]
KMT2A-rearranged	TF rearrangement	40	Infants (~90%) and adults (~15%)	Ras pathway (commonly subclonal)	Poor prognosis	Bortezomib, DOT1L inhibitors, Menin inhibition	[64]
TCF3-PBX1 t (1; 19) (q23; p13)	TF rearrangement	8	Children (5%)		Good prognosis, CNS relapse		[65,66]
ZNF384-rearranged	TF rearrangement	15	AYA (~5%)	Epigenetic modifiers, Ras pathway	Intermediate prognosis	FLT3 inhibition	[12,17,18]
MEF2D-rearranged	TF rearrangement	14	AYA (~7%)	Ras pathway	Intermediate prognosis,	HDAC inhibition	[11,13]
NUTM1-rearranged	TF rearrangement	3	Children (1%)	Unknown	Excellent prognosis	Bromodomain inhibitors	[10,22]
TCF3-HLF t (17; 19) (q22; p13)	TF rearrangement	15	Rare rare in all ages (< 1%)	*TCF3* mutation, *PAX5* deletion, Ras pathway	Very poor prognosis,	BCL2 inhibitors	[67]
PAX5alt	Other TF driven	10	Children (~11%)	*PAX5* fusion, mutation, amplification	Intermediate prognosis		[10,22]
PAX5 P80R	Other TF driven	22	Adults (~4%)	Ras pathway	Intermediate prognosis		[10,22]
IKZF1 N159Y	Other TF driven		Rare in all ages (< 1%)	Unknown	Unknown	FAK inhibitors, rexinoids	[10,22]
BCL2/MYC-rearranged	Other TF driven	48	AYA and adults (~3%)	Unknown	Poor prognosis		[10]
Ph-like	Kinase driven	21	AYA (25–30%)	Multiple kinase alterations, *IKZF1* deletion and mutation, *CDKN2A/B* deletion	Poor prognosis, amenable to TKI therapy	TKI, PI3Ki, BCL2 inhibitors	[15,26]
BCR-ABL1 t (9; 22) (q34; q11.2)	Kinase driven	40–45	Adults (40–50%)	*IKZF1* deletion and mutation, *CDKN2A/B* deletion	Prognosis improved with TKI	TKI, FAK inhibitors, rexinoids	[15,26,68]
Other		16	~5% children, ~10% AYA and adults	Unknown	Intermediate prognosis		

AYA, adolescent and young adult; amp, amplification; B-ALL, B-progenitor acute lymphoblastic leukemia; TF, transcription factor; TKI, tyrosine kinase inhibitor.

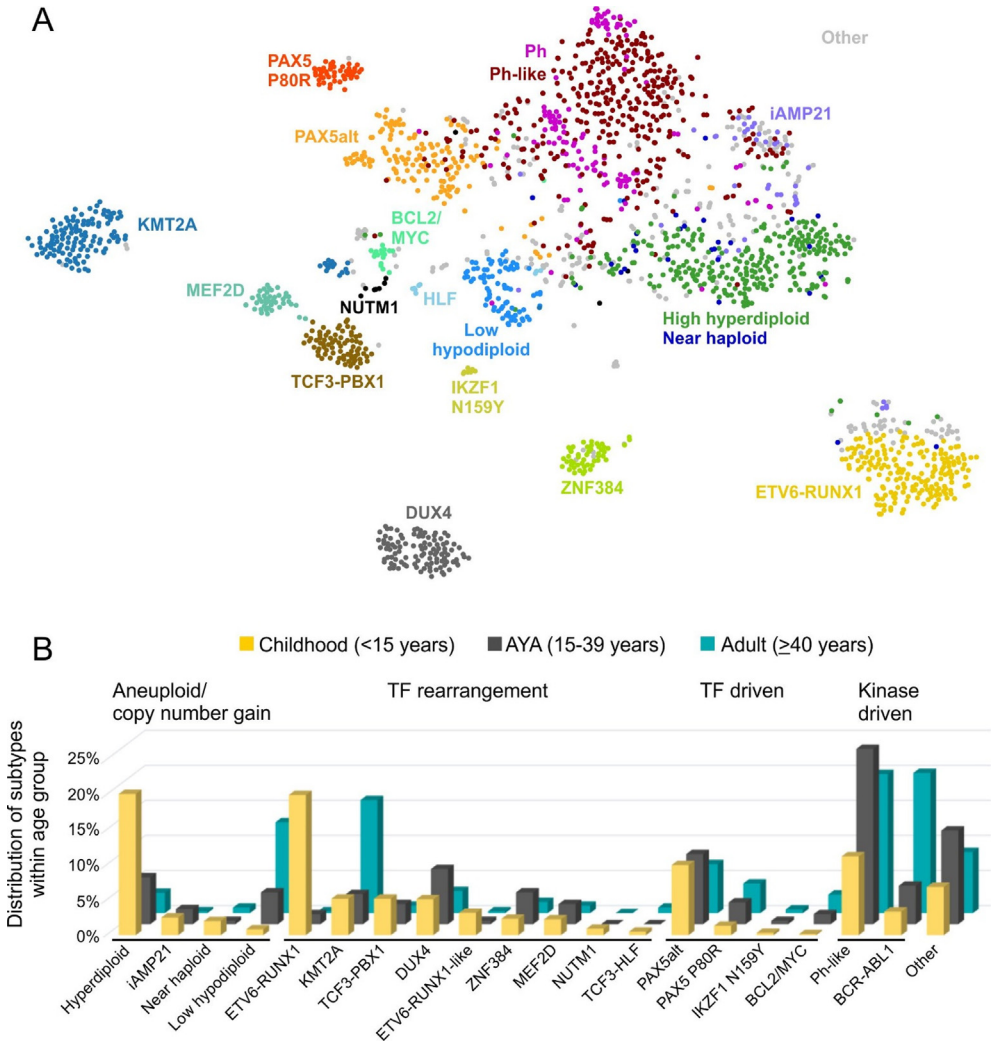

Fig. 1. (A) Major B-ALL subtypes based on gene expression profiling of 1988 cases. (B) Distribution of B-ALL subtypes within each age group. Subtypes are grouped as gross chromosomal abnormalities (aneuploidy or copy number gain), transcription factor (TF) rearrangement, other TF-driven, kinase-driven, and all others [10].

rearrangements are observed in 5% of childhood and 10% of AYA cases and are associated with intermediate outcome. The fusions involve a range of N-terminal fusion partners, often transcriptional regulators or chromatin modifying genes fused to *ZNF384* (*ARIDIB*, *BMP2K*, *CLTC*, *CREBBP*, *EP300*, *EWSR1*, *NIPBL*, *SMARCA2*, *SYNRG*, *TAF15*, and *TCF3*) [12,17–19]. The majority of the fusions involve rearrangement of the entire coding region of *ZNF384*, resulting in the expression of both the wild-type protein and fusion chimera. Several observations suggest that the immunophenotypic aberrancy and frequent immunophenotypic multiclonality arises from inherent lineage plasticity: (1) the lack of variability of secondary mutations between immunophenotypic subclones; (2) the ability of flow-sorted immunophenotypic subpopulations to reconstitute the diversity of the primary tumor in immunocompromised mice; and (3) detection of founding lesions in MPAL such as *ZNF384* rearrangement or *WT1* mutation in a subset of hematopoietic stem cells [16]. Detection of this fusion has several clinical and therapeutic implications. There is a compelling genomic rationale that all *ZNF384*-rearranged cases should be managed in a uniform fashion, rather than using myeloid or lymphoid leukemia-directed therapy according to predominant lineage. *ZNF384*-rearranged leukemia is characterized by overexpression of (non-mutated) *FLT3*, and anecdotal reports have shown remarkable responsiveness of refractory *EP300-ZNF384* leukemia to the kinase inhibitor sunitinib [20]. Finally, the inherent lineage plasticity of *ZNF384*-rearranged leukemia is evident under selective pressure of conventional therapy, in which lymphoid disease may recur as myeloid disease and vice versa, with preservation of the founding fusions, and thus should not be mistaken for secondary leukemia. Similarly, chimeric antigen receptor (CAR) T-cell therapy may result in lineage drift or switch to myeloid disease [21].

Recent studies have identified ALL cases with alternate fusions that result in a similar gene expression profile: rearrangement of the zinc finger *ZNF362* to *SMARCA2* and *TAF15* [22]. It is not yet known if such cases also result in lineage aberrancy or potential vulnerability to *FLT3* inhibition.

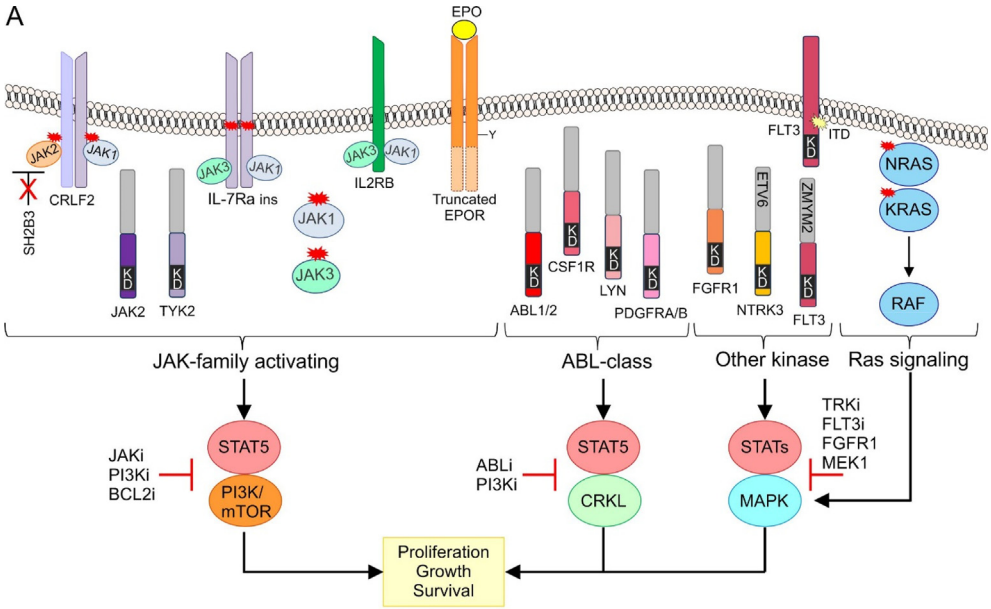

Fig. 2. Kinase alterations and signaling pathways dysregulated in Ph-like ALL. The majority of kinase and cytokine receptor alterations converge on two pathways that activate JAK-family member signaling or ABL-signaling. Alterations that activate JAK-STAT signaling can be targeted with JAK and PI3K inhibitors. ABL-class alterations can be targeted with ABL-inhibitors such as dasatinib. Other kinase alterations and those that activate Ras signaling can be targeted with specific inhibitors including those that inactivate TRK, FLT3, FGFR1, and MEK for the MAPK pathway.

Ph-like ALL

Ph-like, or *BCR-ABL1*-like ALL is characterized by a leukemic cell transcriptional profile similar to Ph + ALL but lack the *BCR-ABL1* fusion gene [23,24], and is one of the few recently described subtypes of ALL now incorporated into the 2016 revision of the World Health Classification of acute leukemia [25]. The incidence of Ph-like ALL increases with age, from 10% to 15% of ALL in children, rising to 30% in young adults [15,26–31]. Ph-like ALL is consistently associated with unfavorable outcomes, including elevated measurable residual disease (MRD) levels and/or higher rates of treatment failure compared to non Ph-like ALL patients [15,26,32,33].

Ph-like ALL is extremely heterogeneous in the genes altered and the nature (rearrangements, mutations, or deletions) of alterations that result in the activated tyrosine kinase or cytokine receptor signaling characteristic of this subtype of ALL (Fig. 2). However, these fall into four main groups: (1) alterations driving JAK-STAT signaling, most commonly rearrangements of *CRLF2 (IGH-CRLF2, P2RY8-CRLF2,* CRLF2 F232C*),* and less commonly, rearrangements of *JAK2, EPOR,* or *TYK2,* and mutations/deletions of *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*) [15,26,27] (Fig. 2A). *IGH-CRLF2* is particularly common in adolescent/adult ALL but is less common in children. ABL-class fusions are most prevalent in children with high-risk ALL. A small subset of children harboring rearrangement of *CRLF2*, most commonly *P2RY8-CRLF2*, lack the Ph-like ALL gene expression signature [10].

Preclinical and anecdotal patient data have been reported to support evaluation of TKIs in trials of Ph-like ALL [34–37], but it is likely that responses will be variable and dependent on treatment regimen. For example, preclinical leukemia models driven by chimeric ABL-class or Janus kinase fusions are potently inhibited by imatinib/dasatinib or ruxolitinib, respectively, although with notable synergism with chemotherapy [38]. *ETV6-NTRK3* is a potent monogenic driver of Ph-like ALL and *ETV6-NTRK3* leukemic cells are eradicated by *NTRK* inhibitors such as larotrectinib [39]. In contrast, *CRLF2*-rearranged leukemias show variable JAK-STAT activation and response to type I JAK inhibitors such as ruxolitinib. Thus, it is critical that TKI efficacy is formally evaluated in clinical trials, and this is being pursued by St. Jude Children's Research Hospital (Total XVII, NCT03117751) [40] and Children's Oncology Group (COG) protocols (AALL1131, NCT01406756; AALL1521, NCT02723994) [32]. However, it is likely that alternative or adjunct treatment approaches will be required, particularly for *CRLF2*-rearranged ALL. Such approaches include combinatorial therapy with *PI3K* inhibitors [41] or *BCL2* inhibitors [38].

ETV6-RUNX1-like ALL

Transcriptome sequencing studies incorporating analysis of gene expression profiles have shown that the majority of subtypes of ALL are notable for “phenocopies,” in which cases with similar gene expression profile to a well characterized fusion are identified that are driven by alternative, or unknown drivers. The most striking of these is Ph-like ALL, in which the prevalence of phenocopies

(Ph-like) greatly outnumbers that of the index alteration (*BCR-ABL1*). The next most prevalent is *ETV6-RUNX1*-like ALL, which exhibits a gene expression profile and immunophenotype (CD27 positive, CD44 low to negative) similar to *ETV6-RUNX1* ALL [42,43]. As with *ETV6-RUNX1* ALL, *ETV6-RUNX1*-like ALL is almost exclusively identified in children (~3%) and confers a favorable prognosis, emphasizing the importance of detecting this subtype to potentially treat such cases with less intensive therapies. This subtype is associated with alternate lesions (gene fusions or copy number alterations) in *ETV6*, *IKZF1,* or *TCF3*, suggesting global deregulation of lymphoid development is a hallmark of this transcriptional signature [10].

DUX4-rearranged ALL

DUX4-rearranged ALL is particularly important in view of its favorable outcome in both children and adults. Such cases have a very distinctive gene expression profile and immunophenotype (CD2 and CD371 positive) [18,42,44–47]. This form of leukemia is driven by two transcription factor gene alterations. The initiating alteration is rearrangement of the homeobox transcription factor *DUX4* (double homeobox 4) that results in expression of a C-terminal truncated DUX4 protein. DUX4 then binds to an intragenic region of the ETS family transcription factor gene *ERG* (ETS-related gene). This results in multiple alterations of *ERG*: gross transcriptional deregulation with expression of multiple coding, non-coding, and anti-sense transcripts, and commonly, expression of a C-terminal truncated isoform of *ERG*, ERGalt, that is encoded by a non-canonical first exon induced by DUX4 binding. ERGalt retains the DNA-binding and transactivating domain of *ERG*, inhibits the transcriptional activity of wild-type *ERG*, and is transforming in mouse models of B-ALL [44]. Many *DUX4*-rearranged cases also exhibit *ERG* deletions induced by RAG-mediated recombination. This is widely, but inappropriately used as a biomarker of this subtype, as almost half of *DUX4* rearranged cases have no clonal *ERG* deletion. This is clinically important: *IKZF1* (Ikaros) alterations are common in *DUX4* rearranged ALL, but in contrast to *BCR-ABL1* and Ph-like ALL are not associated with poor outcome—in contrast, *DUX4*-rearranged ALL is associated with an excellent prognosis in both children and adults [10,44]. Thus, approaches to selectively genotype *ERG* and *IKZF1* alterations to refine treatment intensification are inadequate as many *IKZF1*-altered cases are *DUX4*-rearranged but *ERG* wild type, and without identification of *DUX4* rearrangement may be directed to inappropriately intensive therapy. Identification of *DUX4* rearrangements by genomic approaches, *DUX4* expression or flow cytometry is needed.

NUTM1 rearrangements

Fusion of almost all the coding region of *NUTM1* (nuclear protein in testis midline carcinoma family 1) to six different 5′ partners is observed in 1%–2% of childhood ALL: *ACIN1*, *BRD9*, *CUX1*, *IKZF1*, *SLC12A6,* and *ZNF618*, resulting in increased expression of *NUTM1* [10,22]. *NUTM1* is a chromatin modifier in the testis by recruiting EP300 to increase local histone acetylation [48]. *BRD4-NUTM1* is a hallmark of NUT midline carcinoma (NMC) [49]. *BRD4-NUTM1* acts to repress differentiation in NMC by recruiting histone acetyltransferases and other transcriptional cofactors to regions of chromatin that are actively transcribing pro-proliferative and anti-differentiation genes, including *MYC* [49]. The mechanistic basis of *NUTM1* rearrangements in leukemogenesis is unknown, but in view of the common involvement of *BRD9*, bromodomain or HDAC inhibitors merit investigation.

PAX5-driven subtypes

The B-cell transcription factor gene *PAX5* is commonly mutated in ALL, and with the exception of rearrangements, *PAX5* alterations were previously considered to be important, but secondary, cooperating lesions in leukemogenesis [7,8,50]. Large RNA-seq studies have now shown that *PAX5* alterations also define two distinct subtypes of ALL [10,22]. *PAX5*-altered (PAX5alt) ALL, comprises cases with diverse *PAX5* rearrangements (most commonly to *ETV6* or *NOL4L*), sequence mutations, or intragenic amplification [51], with the highest prevalence observed in children and AYA (10% each vs. 7% in adults) [10]. The second subtype is characterized by the presence of the PAX5 P80R mutation with inactivation of the wild-type *PAX5* allele from deletion of frameshift mutation, indicating loss/mutation of both *PAX5* alleles drives the unique gene expression profile of this subtype [10,22,52]. The prevalence of PAX5 P80R ALL increases with age, accounting for almost 5% of adults, and is associated with intermediate to favorable prognosis [10,52]. PAX5 P80R cases have a high frequency of signaling mutations, particularly in the Ras pathway. The notion that specific sequence mutations define B-ALL subtypes is provocative, but confirmed by the observation that mice harboring constitutive *PAX5* P80R mutations develop highly penetrant B-progenitor ALL, with inactivation of the wild-type *Pax5* allele as observed in human ALL [10]. The identification of *PAX5* subtypes as distinct entities highlights the importance of this gene in regulating B-cell differentiation and confirms *PAX5* alterations as founding lesions in B-lymphoid leukemogenesis as opposed to secondary cooperating events as previously thought.

IKZF1 N159Y

A second example of mutation-initiated ALL is a subtype with heterozygous IKZF1 N159Y mutations [10,22]. Like *PAX5*, *IKZF1* mutations are common in ALL, however IKZF1 N159Y defines this subtype. The mutation is located at a critical DNA-binding residue of the N-terminal zinc fingers of IKZF1 and results in misregulation of *IKZF1* transcriptional activation, in part through subcellular mislocalization [53]. As with *PAX5* P80R, the reason why this specific mutation, but not similarly deleterious mutations, drives a distinct subtype is unclear, but is important as *IKZF1* alterations result in aberrant leukemic cell adhesion and drug resistance that is reversible with rexinoids and focal adhesion kinase inhibitors [53,54].

IGH rearrangements

The *IGH* locus is rearranged to multiple partner genes in ALL across the age spectrum including *CRLF2*, *CEBP* family members (CCAAT/enhancer binding protein) and *ID4* and generally confer a poor prognosis [55,56]. Transcriptomic sequencing has identified a subset of cases with pre-B immunophenotype and a unique transcriptional signature characterized by rearrangement of *IGH* to *BCL2*, *MYC,* and/or *BCL6* (*BCL2/MYC*) [10] that resemble those observed in "double-hit" lymphoma, and are rarely identified in ALL [55,57–59]. This subtype is predominantly identified in adults (median age 48.5 years) and is associated with an extremely unfavorable outcome.

Hypodiploid ALL

Hypodiploid ALL comprises two main subtypes of generally unfavorable prognosis: near haploid ALL with a modal chromosome number of 24–31, and low hypodiploid ALL with 32–39 chromosomes. These subtypes have distinct gene expression profiles and genetic alterations; high hypodiploid cases (40–44 chromosomes) are heterogeneous and not a distinct entity. Near haploid ALL is characterized by a high frequency of Ras pathway alterations (particularly *NF1*) and *IKZF2* (Helios) alterations, and low hypodiploid ALL *IKZF3* (Aiolos) and *TP53* mutations. Near hypodiploid ALL is mostly observed in children, while low hypodiploid ALL is more common in adults. In children, many low hypodiploid ALL cases harbor germline *TP53* alterations, whereas in adults, these alterations are somatic. Hypodiploid ALL cells are exquisitely sensitive to *BCL2* inhibitors such as venetoclax [69], and these agents (venetoclax and navitoclax) are now being tested in clinical trials.

Conclusions

A decade of intensive effort has now resolved the genetic drivers of the majority of B-ALL cases across the age spectrum. This has had several major implications for clinical management. First, in view of the striking associations with outcome in both children and adults, accurate identification of both classical subtype-defining lesions, drivers of phenocopies, and submicroscopic lesions (*PAX5* P80R, *IZKF* N159Y) is required. This required careful genomic analysis, ideally with sequencing-based approaches, but such approaches are increasingly widely utilized. Secondly, while there are attractive therapeutic options for several subtypes, much work is needed. The majority of subtypes are driven by transcription factor alterations that are not intuitively directly druggable; and some subtypes with dismal prognosis have no immediately attractive therapy. Thus, while the ALL field has enthusiastically pursued genotype-agnostic approaches such as antibody and cellular immunotherapy, much work is needed to define the tumor-intrinsic and -extrinsic determinants of response, as well as systematically investigating the vulnerabilities of each subtype to new therapeutic approaches. Ideally, each new agent will be tested in clinical trials of newly diagnosed ALL rather than at relapse, but this will pose major challenges for trial design and accrual. However, these challenges have been met for Ph+ ALL.

Declaration of competing interest

Consulting fees: Amgen, Pfizer; Speakers Bureau: Amgen, Pfizer; Contracted research:Loxo Oncology.

Acknowledgements

The author thanks colleagues at St. Jude, the Children's Oncology Group, and the multiple centers and leukemia cooperative study groups that have contributed samples and expertise to many of the studies described in this review. The author is supported by a National Cancer Institute R35 CA197695 Outstanding Investigator Award, a St Baldrick's Foundation Robert J. Arceci Innovation Award, and the Henry Schueler 41&9 Foundation.

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