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Pediatric acute lymphoblastic leukemia

Hiroto Inaba[1,2] **and Charles G. Mullighan**[2,3]

[1]Department of Oncology; [2]Hematological Malignancies Program and [3]Department of Pathology, St. Jude Children's Research Hospital, Memphis, TN, USA

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ABSTRACT

The last decade has witnessed great advances in our understanding of the genetic and biological basis of childhood acute lymphoblastic leukemia (ALL), the development of experimental models to probe mechanisms and evaluate new therapies, and the development of more efficacious treatment stratification. Genomic analyses have revolutionized our understanding of the molecular taxonomy of ALL, and these advances have led the push to implement genome and transcriptome characterization in the clinical management of ALL to facilitate more accurate risk-stratification and, in some cases, targeted therapy. Although mutation- or pathway-directed targeted therapy (e.g., using tyrosine kinase inhibitors to treat Philadelphia chromosome [Ph]-positive and Ph-like B-cell-ALL) is currently available for only a minority of children with ALL, many of the newly identified molecular alterations have led to the exploration of approaches targeting deregulated cell pathways. The efficacy of cellular or humoral immunotherapy has been demonstrated with the success of chimeric antigen receptor T-cell therapy and the bispecific engager blinatumomab in treating advanced disease. This review describes key advances in our understanding of the biology of ALL and optimal approaches to risk-stratification and therapy, and it suggests key areas for basic and clinical research.

Correspondence:

HIROTO INABA
hiroto.inaba@stjude.org

CHARLES G. MULLIGHAN
charles.mullighan@stjude.org

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Introduction

Contemporary childhood ALL studies have shown improved 5-year overall survival (OS) rates exceeding 90% (Table 1).[1-9] However, OS for the St. Jude Total Therapy Study XVI (94.3%) was similar to that for the Total Therapy Study XV (93.5%) (Figure 1).[9] Therefore, with the conventional approach, the chemotherapy intensity has been raised to the limit of tolerance, and further improvements in outcomes and reduction of adverse effects will require novel therapeutic approaches. Historically, genetic factors identified by conventional karyotyping have been used to diagnose ALL and to risk-stratify children with the disease. However, the alterations thus identified, including hyper- and hypodiploidy and several chromosomal rearrangements, did not establish the basis of ALL in a substantial minority of children; nor did they satisfactorily reveal the nature of the genetic alterations driving leukemogenesis. Genomic studies have now clarified the subclassification of ALL and have demonstrated a close interplay between inherited and somatic genetic alterations in the biology of ALL. Many of these alterations have important implications for diagnosis and risk-stratification of ALL and for the use and development of novel and targeted approaches.

Heritable susceptibility to acute lymphoblastic leukemia

Several lines of evidence indicate that there is a genetic predisposition to acute lymphoblastic leukemia (ALL), at least in a subset of cases. This evidence includes the existence of: (i) rare constitutional syndromes with increased risk for ALL; (ii) familial cancer syndromes; (iii) non-coding DNA polymorphisms that subtly influence the risk of ALL; and (iv) genes harboring germline non-silent variants presumed to confer a risk of sporadic ALL. Constitutional syndromes such as Down syndrome and ataxia-telangiectasia are associated with increased risk of B-cell-ALL (with *CRLF2* rearrangement) and T-cell-ALL, respectively. Familial cancer syndromes such as Li-Fraumeni syndrome, constitutional mismatch repair deficiency syndrome, or DNA repair syndromes (e.g., Nijmegen breakage) have an increased

incidence of malignancy in general. Familial predisposition specific to leukemia is uncommon but has resulted in the identification of predisposing non-silent variants that are also observed in sporadic ALL cases, including *TP53* germline mutations and low hypodiploid B-ALL, *ETV6* variants and hyperdiploid ALL, and *PAX5* mutations and B-ALL with dicentric/isochromosome 9.[10-13] These susceptibility genes are targets of somatic mutation in ALL: *ETV6* and *PAX5* are rearranged, amplified/deleted, and mutated in B-ALL,[14,15] as is *TP53* in hypodiploid ALL.[10] Germline variants of *IKZF1* are observed in familial B-ALL and immunodeficiency,[16,17] and somatic *IKZF1* alterations are enriched in Philadelphia chromosome (Ph)-positive, Ph-like, and *DUX4*-rearranged B-ALL.[18-20] *RUNX1* germline

Table 1A. Treatment results for acute lymphoblastic leukemia in major pediatric clinical trials.

Study	Years of study	Subtype	Age (y)	Patients (n)	Steroid during induction (mg/m²/day)	MTX (g/m²/dose)	Cranial irradiation	Complete remission (%)	Cumulative incidence of relapse (5y, %) (SE or 95% CI)	Death in remission (5y, %) (SE or 95% CI)	Event-free survival (5y, %) (SE or 95% CI)	Overall survival (5y, %) (SE or 95% CI)
AIEOP/BFM ALL 2000	2000-2006	B and T	1-17	3720 (randomized pts)	Pred 60 (1867 pts) /Dex 10 (1853 pts) [R]	5	HR/T /CNS3	Pred: 97.8 Dex: 97.8 (*P*=1.00)	Pred: 15.6 (0.8) Dex: 10.8 (0.7) (*P*<0.001)	Pred: 1.7 Dex: 2.3 (*P*=0.24)	Pred: 80.8 (0.9) Dex: 83.9 (0.9) (*P*=0.024)	Pred: 90.5 (0.7) Dex: 90.3 (0.7) (*P*=0.61)
COG AALL0232	2004-2011	B, HR	1-30	2979	Pred 60 (427 pts)/ Dex 10 (424 pts) [R] (aged 1-9 y)	HD-MTX 5 (1282 pts)/ C-MTX (1291 pts) [R]	SER/ CNS3	NA	HD-MTX: 136 pts C-MTX: 183 pts	HD-MTX: 24 pts C-MTX: 25 pts (*P*=0.90)	75.3 (1.1) HD-MTX: 79.6 (1.6) C-MTX: 75.2 (1.7) (*P*=0.008)	85.0 (0.9) HD-MTX: 88.9 (1.2) C-MTX: 86.1 (1.4) (*P*=0.025)
COG AALL0331	2005-2010	B, SR	1-9	5377	Dex 6	0.1 (and escalating) with or without asparaginase	CNS3	98.0	124 of 3992 pts who continued post-induction	25 of 3992 pts who continued post-induction	88.96 (0.46) (6y)	95.54 (0.31) (6y)
COG AALL0434	2007-2014	T	1-30	1562	Pred 60	HD-MTX 5 (512 pts)/ C-MTX (519 pts) [R]	IR/HR	NA	HD-MTX: 59 pts C-MTX: 32 pts	HD-MTX: 11 pts C-MTX: 8 pts	83.8 (81.2-86.4) (5y DFS) HD-MTX: 85.3 (81.0-89.5) C-MTX: 91.5 (88.1-94.8) (*P*=0.005)	89.5 (87.4-91.7) HD-MTX: 89.4 (85.7-93.2) C-MTX: 93.7 (90.8-96.6) (*P*=0.036)
DFCI ALL Consortium Protocol 05-001	2005-2010	B and T	1-18	551	Pred 40	5	CNS3/T/B with WBC ≥100k/VHR	95.5	51 of 551 total 41 of 463 pts randomized PEG: 20 of 232 Native: 21 of 231	2 of 551 total	85 (82-88) (5y DFS) PEG: 90 (86-94) Native: 89 (85-93) (*P*=0.58)	91 (88-93) PEG: 96 (93-98) Native: 94 (89-96) (*P*=0.30)
DCOG ALL10	2004-2012	B and T	1-18	778	Pred 60	5	>3y and HR who do not receive HCT	98.0	8.3 (1.0)	2.6	87.0 (1.2)	91.9 (1.0)
MRC UK ALL 2003	2003-2011	B and T	1-24	3126	Dex 6	0.02 (SR/IR), C-MTX (HR)	CNS3 (until 2009)	98.9	8.8 (7.8-9.8)	2.7 (2.1-3.3)	87.3 (86.1-88.5)	91.6 (90.6-92.6)
NOPHO ALL2008	2008-2014	B and T	1-45	1509	Pred 60 or Dex 10 (T/WBC≥100k)	5	None	91.2	10 (1)	3 (0)	85 (1)	91 (1)
SJCRH Total XVI	2007-2017	B and T	0-18	598	Pred 40	2.5 (LR), 5 (SR/HR)	None	98.7	6.6 (4.4-8.7)	2.7 (1.4-4.0)	88.2 (84.9-91.5)	94.1 (91.7-96.5)

Table 1B. Major findings in the study reports.

Study	Years of study	
AIEOP/BFM ALL 2000	2000-2006	Dexamethasone in induction resulted in less relapse but more treatment-related mortality than did prednisone. There was no survival benefit with dexamethasone except for T-ALL patients with good prednisone response.
COG AALL0232	2004-2011	5-y EFS and OS were better with HD-MTX than with C-MTX. Patients aged 1-9 y who received dexamethasone and HD-MTX had better outcomes than those in other groups.
COG AALL0331	2005-2010	SR patients had excellent outcomes. Adding intensified consolidation did not improve outcomes in patients with SR-average disease.
COG AALL0434	2007-2014	5-y DFS and OS were better with C-MTX than with HD-MTX.
DFCI ALL Consortium Protocol 05-001	2005-2010	IV PEG-asparaginase had similar toxicity and efficacy and resulted in less anxiety when compared with IM native *E. coli* asparaginase.
DCOG ALL10	2004-2012	MRD-based therapy reduction and intensification were successful.
MRC UK ALL 2003	2003-2011	MRD-based therapy reduction and intensification were successful.
NOPHO ALL2008	2008-2014	Pediatric-based protocol is tolerable and effective for young adults.
SJCRH Total XVI	2007-2017	Additional intrathecal therapy during early induction improved CNS control (any CNS relapse at 5-y: 1.5%).

AIEOP/BFM: Associazione Italiana di Ematologia e Oncologia Pediatrica/Berlin-Frankfurt-Münster; ALL: acute lymphoblastic leukemia; B: B-lineage; CI: confidence interval; CNS: central nervous system; C-MTX: Capizzi methotrexate; COG: Children's Oncology Group; DCOG: Dutch Childhood Oncology Group; Dex: dexamethasone; DFCI: Dana-Farber Cancer Institute; DFS: disease-free-survival; EFS: event-free survival; HD-MTX: high-dose methotrexate; HCT: hematopoietic cell transplantation; HR: high-risk; IM: intramuscular; IR: intermediate-risk; IV: intravenous; k.× 10^3/μL; LR: low-risk; MRC UK: Medical Research Council United Kingdom; MRD: minimal residual disease; MTX: methotrexate; n: number; NA: not available; NOPHO: Nordic Society of Pediatric Hematology and Oncology; OS: overall survival; PEG: polyethylene glycol; Pred: prednisolone; pts: patients; R: randomization; SE: standard error; SER: slow early response; SJCRH: St. Jude Children's Research Hospital; SR: standard-risk; T: T-lineage; WBC: white blood cell; y: year.

mutations can lead to both T-ALL and AML, and *ETV6* variants predispose carriers to B-ALL and myelodysplasia.[21,22]

Genome-wide association studies (GWAS) have identified non-coding variants in at least 13 loci associated with ALL. The relative risk associated with each variant is typically low (corresponding to an increase of up to 1.5- or 2-fold) but cumulatively, they may result in an increase of up to 10-fold in ALL risk. Risk variants are frequently at/near hematopoietic transcription factor or tumor suppressor genes, including *ARID5B*, *BAK1*, *CDKN2A/CDKN2B*, *BMI1-PIP4K2A*, *CEBPE*, *ELK3*, *ERG*, *GATA3*, *IGF2BP1*, *IKZF1*, *IKZF3*, *USP7*, and *LHPP*.[23-25] Several variants display ancestry and ALL subtype-specific associations, such as those of *GATA3* with Hispanics and Ph-like B-ALL, *ERG* with African Americans and *TCF3-PBX1* B-ALL, and *USP7* with African Americans and T-ALL with *TAL1* deregulation.[26-28]

Finally, germline genomic analysis has identified additional susceptibility variants in sporadic hyperdiploid B-ALL (*NBN*, *ETV6*, *FLT3*, *SH2B3*, and *CREBBP*), Down syndrome-associated B-ALL (*IKZF1*, *NBN*, *RTEL1*), and T-ALL (Fanconi-BRCA pathway mutations).[29-31]

Prenatal origin of leukemia

Several lines of investigation indicate that a subset of childhood leukemia cases arise before birth.[32,33] Chromosomal translocations, particularly *ETV6-RUNX1* (*TEL-AML1*) may be detected at birth in blood spots and cord blood years before the clinical onset of leukemia, providing support for a multi-step process of leukemogenesis. This is supported by genomic analyses of monozygotic, monochorionic twins concordant for leukemia, showing genetic identity of initiating lesions and discordance for secondary genetic alterations indicating inter-twin, intrauterine transmission of leukemia.[33,34] Evidence for *in utero* origin is strongest for *KMT2A*-rearranged and *ETV6-RUNX1* ALL. Anecdotal evidence supports *in utero* origin for other subtypes of B-ALL, including hyperdiploid and *ZNF384*-rearranged leukemia.[35]

Genetics of B-cell acute lymphoblastic leukemia

B-cell acute lymphoblastic leukemia (B-ALL) is the most common form of ALL, comprising >20 subtypes of variable prevalence according to age that are associated with distinct gene expression profiles and are driven by three main types of initiating genetic alteration: chromosomal aneuploidy, rearrangements that deregulate oncogenes or encode chimeric transcription factors, and point mutations (Table 2 and Figure 2). Each subtype typically has co-occurring genetic alterations that perturb lymphoid development, cell-cycle regulation, and kinase signaling and chromatin regulation, and the genes involved and their frequency of involvement vary between subtypes.[36]

High hyperdiploidy (>50 chromosomes) is present in up to 30% of childhood ALL and is associated with mutations in the Ras pathway, chromatin modifiers such as CREBBP, and favorable outcomes.[37] Low hypodiploidy (31-39 chromosomes) is present in approximately 1% of children with ALL but in >10% of adults. It is characterized by the deletion of *IKZF2* and by near-universal *TP53* mutations, which are inherited in approximately half the cases.[10] Near haploidy (24-30 chromosomes) is present in approximately 2% of pediatric ALL and is associated with Ras mutations (particularly *NF1*) and deletions of *IKZF3*. Both low-hypodiploid and near-haploid ALL are associated with unfavorable outcomes. The prevalence of hypodiploidy may be underestimated because of the phenomenon of "masked" hypodiploidy, in which the hypodiploid genome is duplicated, leading to a hyperdiploid modal chromosome number.[10,38] Distinguishing masked-hypodiploid ALL from high-hyperdiploid ALL is important in view of the genetic (germline *TP53* alterations) and prognostic implications. Masked hypodiploidy may be suspected by the patterns of chromosomal gain (commonly diploid and tetrasomic chromosomes, rather than trisomies in high-hyperdiploid ALL) and may be formally confirmed by flow cytometric analysis of the DNA index, which commonly shows peaks for both non-masked and masked clones, and by techniques that assess loss of heterozygosity, such as SNP

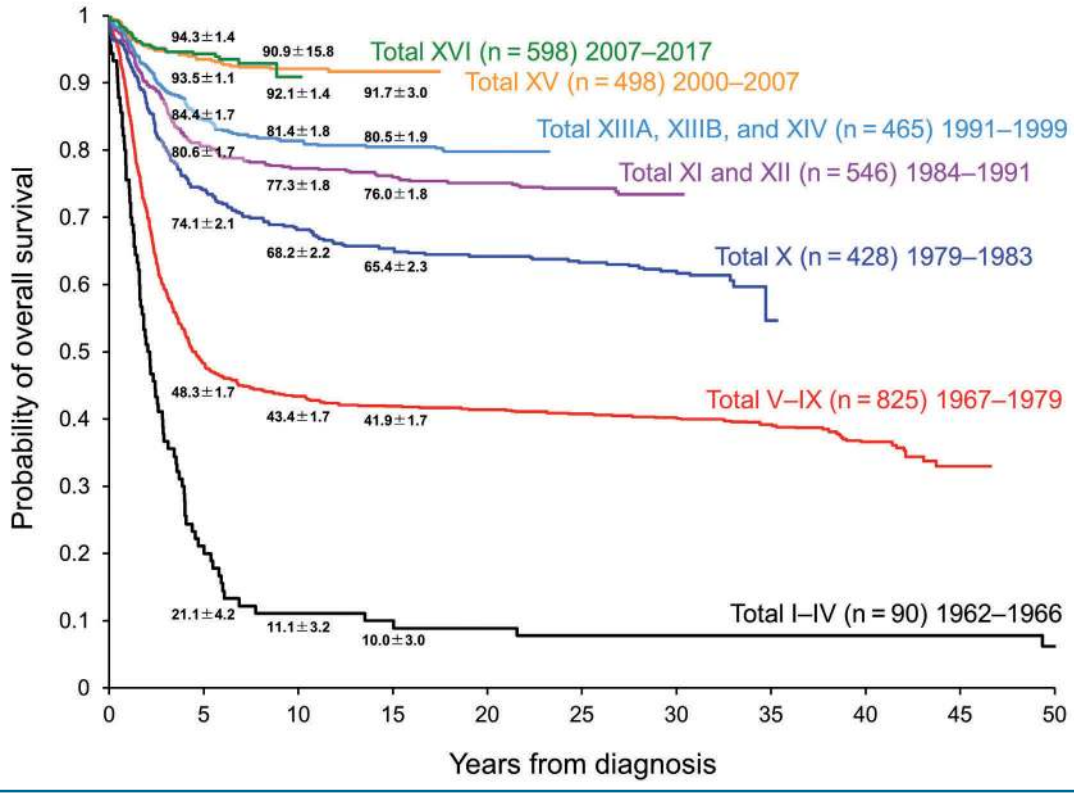

Figure 1. Change in overall survival of pediatric patients treated on the historical St. Jude Total Therapy studies.

arrays. In addition, the transcriptomic profiles and co-occurring genetic alterations (e.g., Ras pathway and *CREBBP* alterations) of near-haploid and high-hyperdiploid ALL are similar, suggesting a common origin for these entities.[15] ALL with intrachromosomal amplification of chromosome 21 (iAMP21) is most common in older children and is associated with poor prognosis, which has been improved with intensive treatment.[39]

Of the subtypes characterized by translocations, the most common in childhood B-ALL is t(12;21)(p13;q22) encoding *ETV6-RUNX1*, which is typically cryptic on cytogenetic analysis and is associated with favorable prognosis. The t(1;19)(q23;p13) translocation and variants encode *TCF3-PBX1*,[40] which is more common in African Americans and is associated with more frequent central nervous system (CNS) relapse and inferior outcomes with older,[41] but not contemporary, treatment regimens.[9] The t(9;22)(q34;q11.2) translocation results in the formation of the Philadelphia chromosome that encodes *BCR-ABL1* and is found in a subset of childhood ALL that was also associated with unfavorable outcomes, although the prognosis has now been improved with combined chemotherapy and tyrosine kinase inhibition.[42] Rearrangement of *KMT2A* (*MLL*) at 11q23 to >80 partners, most commonly t(4;11)(q21;q23) encoding *KMT2A-AFF1*, is common in infant ALL and is associated with a dismal prognosis.

Genomic analyses, particularly transcriptome sequencing, have identified multiple new subtypes not evident on cytogenetic analysis because of cryptic and/or diverse rearrangements or sequence mutations acting as driver lesions. *ETV6-RUNX1*–like ALL is characterized by a gene expression profile and immunophenotype ($CD27^+$, CD44 low/negative) similar to that of *ETV6-RUNX1* ALL.[43,44] Such patients harbor alternate gene fusions or copy number alterations in ETS-family transcription factors (*ETV6*, *ERG*, *FLI1*), *IKZF1*, or *TCF3*. *ETV6-RUNX1*–like ALL occurs almost exclusively in children (representing ~3% of pediatric ALL) and is associated with relatively favorable prognosis.[15]

Translocation of *DUX4*, encoding a double-homeobox transcription factor, to the immunoglobulin heavy-chain locus (*IGH*) is also cytogenetically cryptic and is found in 5-10% of B-ALL. The translocation results in overexpression of DUX4 protein lacking the C-terminal domain. This truncated protein binds an intragenic region of the ETS-family transcription factor *ERG* (ETS-related gene), resulting in profound transcriptional deregulation of *ERG*. This in turn commonly results in expression of a C-terminal ERG protein fragment and/or *ERG* deletion. *DUX4*-rearranged B-ALL has a distinctive gene expression profile and immunophenotype ($CD2^{\pm}$, $CD371^+$), and despite the deletion of *IKZF1* (otherwise an adverse prognostic factor in ALL) in approximately 40% of cases, the outcome is typically excellent.[20,45]

ZNF384 rearrangement defines a distinct group of acute leukemias that may manifest as B-ALL (often with aberrant myeloid marker expression) or B/myeloid mixed-phenotype acute leukemia (MPAL; MPO-positive leukemia). *ZNF384* rearrangement is observed in 6% of childhood B-ALL and in 48% of childhood (but notably not adult) B/myeloid MPAL.[15,46-48] *ZNF384*-like cases, often with *ZNF362* rearrangements, are also observed. Both *ZNF384* and *ZNF362* encode C2H2-type zinc-finger tran-

scription factors and are rearranged with genes encoding N-terminal transcription factors (e.g., *TAF15* and *TCF3*) or chromatin modifiers (most commonly *EP300*, but also *CREBBP*, *SMARCA2*, and *ARID1B*).[47] *ZNF384*-rearranged leukemia is associated with elevated FLT3 expression, and there are anecdotal reports of profound responses to FLT3 inhibition.[49] The lineage-ambiguous phenotype of *ZNF384*-rearranged leukemia may shift during the disease course and may result in loss of CD19 expression and failure of chimeric antigen receptor T-cell therapy.[50]

MEF2D (myocyte enhancer factor 2D)-rearranged ALL (occurring in 4% of children and up to 10% of adults with ALL) has a distinct immunophenotype ($CD10^-$, $CD38^+$), an older age of diagnosis (median: 14-15 years), and a poor prognosis.[51-53] The rearrangements result in increased *HDAC9* expression and sensitivity to histone deacetylase inhibitor treatment.[51]

NUTM1 (nuclear protein in testis midline carcinoma family 1) rearrangements are observed in 1-2% of childhood B-ALL, with fusion to genes encoding various transcription factors and epigenetic regulators (e.g., *ACIN1*, *BRD9*, *CUX1*, *IKZF1*, *SLC12A6*, and *ZNF618*) that drive aberrant *NUTM1* expression.[15,47] In all fusions, the NUT domain is retained, and this is hypothesized to lead to global changes in chromatin acetylation and to sensitivity to histone deacetylase inhibitors or bromodomain inhibitors. ALL with *NUTM1* rearrangements has an excellent prognosis.

Other transcription factor-driven subtypes of B-cell acute lymphoblastic leukemia

Two B-ALL subtypes have distinct alterations of the lymphoid transcription factor PAX5. PAX5-altered (PAX5alt) B-ALL accounts for 10% of childhood B-ALL, with cases featuring diverse *PAX5* alterations, including rearrangements (most commonly with *ETV6* or *NOL4L*), sequence mutations or intragenic amplification,[54] and an intermediate prognosis.[15,47] *PAX5* P80R B-ALL accounts for approximately 2% of childhood B-ALL, with cases featuring universal P80R mutation and deletion/mutation of the remaining allele,[15,47,55] mutations in Ras and *JAK2* signaling genes, and an intermediate to favorable prognosis.[15,55] A single heterozygous mutation in *IKZF1* (N159Y) defines a novel subtype of ALL (representing <1% of cases) with IKZF1 nuclear mislocalization, enhanced intercellular adhesion,[56] and expression of genes involved in oncogenesis (*YAP1*), chromatin remodeling (*SALL1*), and JAK-STAT signaling.[15,47] The *IGH-CEBPE* fusion and ZEB2 H1038R mutation are common, but not universal, events in a transcriptionally distinct form of leukemia observed in approximately 1% of cases.

Kinase-driven subtypes

Of therapeutic relevance are the two kinase-driven subtypes: Philadelphia chromosome-positive (Ph^+ or *BCR-ABL1*$^+$) and Philadelphia chromosome-like (Ph-like or *BCR-ABL1*-like) ALL. Their frequency increases with age,[57]

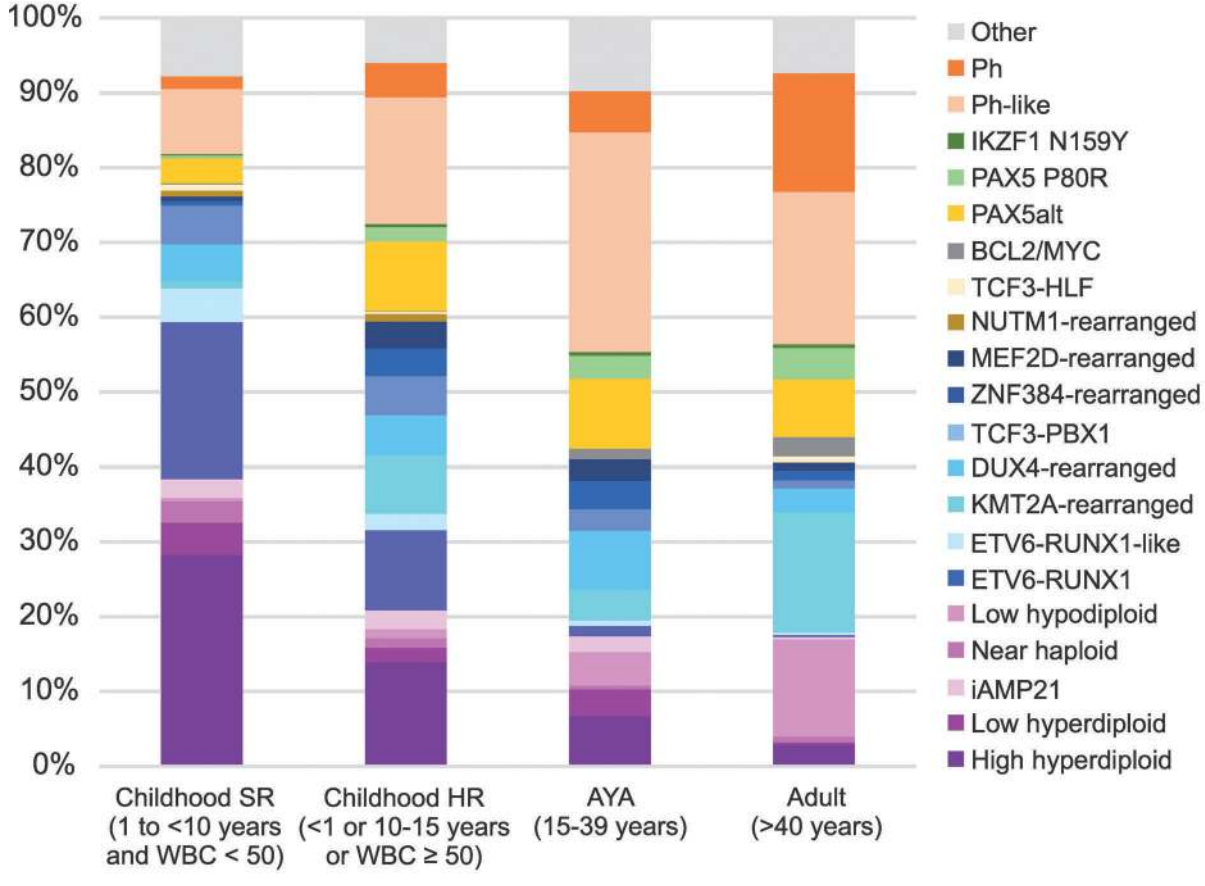

Figure 2. Distribution of B-cell acute lymphoblastic leukemia (B-ALL) subtypes within each age group. SR: standard risk; HR: high risk; WBC: white blood cell count; AYA: adolescent and young adult.

Table 2. Genetic alterations, age distribution, clinical features, and genetic-based therapy in pediatric B- and T-acute lymphoblastic leukemia.

Category	Age	Description	Potential therapeutic implications
B-cell precursor acute lymphoblastic leukemia			
Hyperdiploidy with more than 50 chromosomes	Children >> adults	Excellent prognosis; mutations in Ras signaling pathway and histone modifiers	Reduction of intensity
Near-haploid	Children-adults	24-31 chromosomes; poor prognosis; Ras-activating mutations; inactivation of *IKZF3*	BCL2 inhibitors
Low hypodiploid	Children < adults	32-39 chromosomes; poor prognosis; TP53 mutations (somatic and germline)	BCL2 inhibitors
iAMP21	Older children	Complex alterations of chromosome 21; requires high-risk therapy for good outcomes	Intensification of therapy
t(12;21)(p13;q22) encoding *ETV6-RUNX1*	Children >> adults	Excellent prognosis; cryptic rearrangement that is detectable by FISH	Reduction of intensity
ETV6-RUNX1–like	Children > adults	Absence of *ETV6-RUNX1* fusion; mutations in both *ETV6* and *IKZF1*	Reduction of intensity
t(1;19)(q23;p13) encoding *TCF3-PBX1*	Children-adults	Increased incidence in African Americans; favorable prognosis	
t(9;22)(q34;q11.2) encoding BCR-ABL1	Children << adults	Historically poor prognosis, improved with tyrosine kinase inhibitors; common deletions of IKZF1	ABL1 inhibitors, FAK inhibitors, rexinoids, BCL2 inhibitors
Ph-like	Children < adults	Kinase-activating lesions; poor outcome; potentially amenable to kinase inhibition	ABL1 inhibitors, JAK inhibitors, PI3K inhibitors, BCL2 inhibitors
CRLF2 rearranged (*IGH-CRLF2*; *P2RY8-CRLF2*)	Children < adults	Common in Down syndrome and Ph-like ALL; associated with *IKZF1* deletion and *JAK1/2* mutation	JAK inhibitors, BCL2 inhibitors
KMT2A (*MLL*) rearranged	Infants >> children-adults	Common in infant ALL; dismal prognosis; few co-operating mutations, commonly in RAS signaling pathway	DOT1L inhibitors, menin inhibitors, proteasome inhibitors, HDAC inhibitors, BCL2 inhibitors
DUX4 rearranged and *ERG* deregulated	Children-adults	Distinct gene expression profile; most have focal ERG deletions and favorable outcome despite *IKZF1* alterations	Reduction of intensity
MEF2D rearranged	Children-adults	Distinct gene expression profile; potential sensitivity to HDAC inhibition	HDAC inhibitors
ZNF384 rearranged	Children	Pro-B ALL phenotype; expression of myeloid markers; increased expression of *FLT3*	FLT3 inhibitors
PAX5alt	Children > adults	PAX5 fusions, mutation, or amplifications; intermediate prognosis	
PAX5 P80R	Children < adults	Frequent signaling pathway alterations	Kinase inhibitors
IKZF1 N159Y	Children-adults	Rare; unknown prognosis	FAK inhibitors, rexinoids
NUTM1 rearranged	Children	Exclusively in children; rare; excellent prognosis	HDAC inhibitors, bromodomain inhibitors
t(17;19)(q22;p13) encoding TCF3-HLF	Children-adults	Rare; dismal prognosis	BCL2 inhibitors
BCL2/MYC rearranged	Children << adults	Poor prognosis	
T-lineage acute lymphoblastic leukemia			
TAL1 deregulation	Children-adults	Enrichment of mutation in PI3K signaling pathway	PI3K inhibitors, nelarabine, BCL2 inhibitors
TLX3 deregulation	Children-adults	Poor prognosis; frequent co-operating mutation in ubiquitination and ribosomal genes	Nelarabine, BCL2 inhibitors
HOXA deregulation	Children-adults	Frequent mutations in JAK-STAT pathway, *KMT2A* rearrangements	JAK inhibitors, nelarabine, BCL2 inhibitors
TLX1 deregulation	Children > adults	Favorable prognosis	Nelarabine, BCL2 inhibitors
LMO2/LYL1 deregulation	Children-adults	Poor prognosis; enriched for ETP-ALL, frequent co-operating mutation in JAK-/STAT	JAK inhibitors, nelarabine, BCL2 inhibitors
NKX2-1 deregulation	Children-adults	Frequent co-operating mutation in ribosomal genes	Nelarabine, BCL2 inhibitors
NUP214-ABL1 with 9q34 amplification	Children-adults	Neutral prognosis, in contrast to kinase driven B-ALL; potentially amenable to tyrosine kinase inhibition	ABL1 inhibitors, nelarabine, BCL2 inhibitors
Early T-cell precursor ALL	Children-adults	Poor prognosis; genetically heterogeneous with mutations in hematopoietic regulators, cytokine and Ras signaling, and epigenetic modifiers	JAK inhibitors, BCL2 inhibitors

FISH: fluorescence *in situ* hybridization; ALL: acute lymphoblastic leukemia; HDAC: histone deacetylase.

and they account for 25% and 20%, respectively, of adult ALL. The prevalence of *BCR-ABL1* ALL rises progressively from <20% of ALL in adults younger than 25 years to more than half of adults aged 50-60 years, whereas the prevalence of Ph-like ALL peaks in young adulthood, and this subtype is observed in up to 25% of adults. Alterations of B-lineage transcription factor genes, particularly *IKZF1*, are a hallmark of *BCR-ABL1* ALL[18] and are a key determinant of lymphoid lineage and resistance to therapy.[56] *IKZF1* alterations are associated with poor outcome in ALL overall,[19] particularly because of the high prevalence in *BCR-ABL1* and Ph-like ALL; however, they are not associated with poor outcome in *DUX4*-rearranged ALL. This has led to the definition of "IKZF1-plus" as a marker of poor outcome in ALL, being defined by the presence of alterations in *IKZF1* and *CDKN2A/B*, *PAX5*, or pseudoautosomal region 1 (PAR1, as a surrogate for *CRLF2* rearrangement), but not *ERG* (as a surrogate for *DUX4*-rearranged ALL), commonly detected by multiplex ligation-dependent probe amplification (MLPA).[58] Although used for risk-stratification in several clinical trials, the utility of this approach is limited by the inability of MLPA to identify all cases with key high-risk (*CRLF2* rearrangement) and favorable-risk (*DUX4* rearrangements) that co-occur with *IKZF1* alterations.

Ph-like ALL has a similar transcriptional profile to Ph-positive ALL but is *BCR-ABL1* negative.[19,59] It is genetically heterogeneous with multiple rearrangements (e.g., of *CRLF2*, *ABL*-class genes, *JAK-STAT* signaling genes, *FGFR1*, and/or *NTRK3*), copy number alterations, and sequence mutations that activate tyrosine kinase or cytokine receptor signaling (Figure 3). Ph-like ALL is associated with elevated minimal residual disease (MRD) levels and/or high rates of treatment failure. The diverse genetic alterations characteristic of Ph-like ALL and its responsiveness to tyrosine kinase inhibitors (at least for *ABL*-class and *NTRK3*-rearranged ALL) have spurred the use of RNA-sequencing approaches to identify such alterations at diagnosis and direct patients to targeted therapy.[36]

Genetic basis of T-cell acute lymphoblastic leukemia

Childhood T-cell acute lymphoblastic leukemia is characterized by recurrent alterations in ten pathways, but in most cases, three pathways are deregulated: expression of T-lineage transcription factors, NOTCH1/MYC signaling, and cell-cycle control. Gene expression profiling enables classification of >90% of T-ALL into core subgroups defined by deregulation of T-ALL transcription factors as a result of rearrangement with T-cell receptor enhancers, structural variants, or enhancer mutations of *TAL1*, *TAL2*, *TLX1*, *TLX*, *HOXA*, *LMO1/LMO2*, *LMO2/LYL1*, or *NKX2-1* (Table 2).[60-62] A more recently described mechanism of deregulation is through small insertion/deletion mutations upstream of *TAL1*, which lead to a new binding motif for MYB or TCF1/TCF2 and subsequent changes in *TAL1* expression.[62,63] A similar mechanism has been described for other oncogenes in T-ALL, including *LMO2*.[64] Additional transcription factor genes, including *ETV6*, *RUNX1*, and *GATA3*, are altered by deletion or sequence mutation but are not subtype-defining.[65-67] The second core transcriptional pathway mutation found in most T-ALL cases is aberrant activation of *NOTCH1*, a critical transcription factor for T-cell development.[68] Constitutive *NOTCH1* activity, caused by activating *NOTCH1* mutations (in >75% of cases) and/or inhibitor mutations in the negative regulator *FBXW7* (in 25% of cases), promotes uncontrolled cell growth, partly through increased *MYC* expression.[69-71] The third core alteration observed in pediatric T-ALL is deletion of tumor suppressor loci, primarily *CDKN2A/CDKN2B* (in 80% of cases) and, less commonly, *CDKN1B*, *RB1*, or *CCND3*.[62,72]

In addition to the aforementioned core alterations, T-ALL frequently involves derangement of additional transcriptional regulators (*MYB*, *LEF1*, and *BCL11B*), ribosomal function, ubiquitination through loss-of-function *USP7* mutations, RNA processing, signaling pathways, and epigenetic modifiers such as *PHF6*, *KDM6A*, and genes of polycomb repressive complex 2 (*EED*, *SUZ12*, and *EZH2*).[62] The signaling pathway most commonly activated is PI3K-AKT, through loss of negative regulation by PTEN.[73] JAK-STAT pathway activation can occur through gain-of-function mutations in *IL7R*, *JAK1*, *JAK3*, or *STAT5B* or through loss-of-function alterations in the JAK-STAT regulators *PTPN2* and *SH2B3*,[74,75] whereas mutations in RAS-MAPK signaling are less common, except in early T-cell precursor (ETP) ALL. Kinase rearrangements are observed in a minority of cases, particularly the *NUP214-ABL1* rearrangement.[76]

Genetics of relapse

The subclonal complexity of ALL is now well established, and the clonal dynamics during therapy and at relapse have been examined through genomic sequencing and single-cell analysis.[77,78] Chimeric fusions, when present, are often clonal leukemia-initiating lesions that are typically retained throughout disease progression. Alterations of signaling pathway lesions (*FLT3*, *KRAS*, *NRAS*) are often subclonal and are frequently lost or gained between diagnosis and relapse.[79]

In B-ALL, mutations in genes such as the histone acetyl transferase gene *CREBBP*, the histone methyltransferase gene *SETD2*, and the steroid receptor genes *NR3C1* and *NR3C2* are enriched at relapse.[80-83] At diagnosis, minor relapse-initiating subclones can exhibit inherent resistance to chemotherapy, even before secondary mutation acquisition.[84] Other relapse-specific mutations in *PRPS1*, *PRSP2*, *NT5C2*, or *MSH6*, each influencing thiopurine metabolism, may emerge only during therapy, being driven by selective therapeutic pressure.[81,83,85,86] These mutations confer chemotherapy resistance and might have implications for disease monitoring and therapeutic decisions.[85,86] Inherited genomic variants in specific ethnic/racial groups also contribute to relapse risk as a result of differential drug metabolism or acquisition of distinct somatic mutations.[87-89] Monitoring the dynamics of mutation clearance during induction therapy or monitoring for the emergence of relapse-associated mutations might identify patients who will benefit from early modification of therapy.

Mixed-phenotype acute leukemia

Mixed-phenotype acute leukemia (MPAL) is uncommon, representing only 2-5% of pediatric acute leukemia.[48] The 2016 World Health Organization (WHO) classification defines MPAL as acute leukemia expressing a combination of antigens not restricted to a single lineage with the following categories: B/myeloid, not otherwise specified (NOS) and T/myeloid, NOS, in addition to two genetic subgroups of MPAL: that with t(9;22)(q34.1;q11.2), *BCR-ABL1;* and that with t(v;11q23.3), *KMT2A*-rearranged.[90] Genetic characteriza-

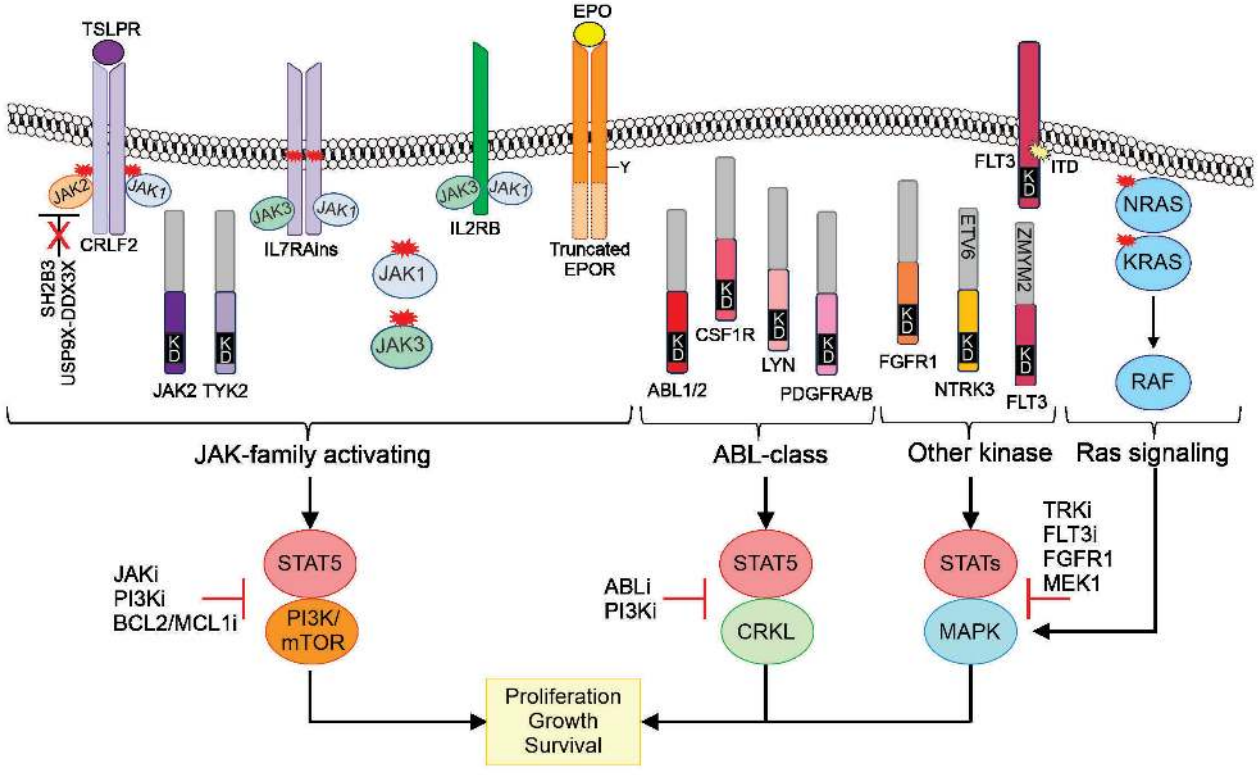

Figure 3. Kinase pathways deregulated in Philadelphia chromosome (Ph)-like acute lymphoblastic leukemia (ALL). The diverse signaling alterations observed in Ph-like ALL are grouped into JAK-STAT activating lesions (most commonly *CRLF2* rearrangement, but also *JAK* mutation and rearrangement, *IL7R* mutation, truncating rearrangements of *EPOR*, and *SH2B3* deletion/mutation), rearrangements involving ABL-class tyrosine kinases; rearrangements of genes encoding other kinases (*FGFR1*, *NTRK3*, *FLT3*), and Ras pathway mutations. Ras pathway mutations are not restricted to Ph-like ALL and are observed in other subtypes of leukemia (e.g., hyperdiploid ALL, *PAX5 P80R* ALL). They are also observed as co-mutations in a proportion of cases with *CRLF2* rearrangements. These alterations typically activate the logical downstream signaling pathway, as well as other pathways that serve as additional avenues for therapeutic intervention (e.g., PI3K, BCL2).

tion of pediatric MPAL revealed that rearrangement of *ZNF384* is common in B/myeloid MPAL and biallelic *WT1* alterations are common in T/myeloid MPAL, which shares genomic features with ETP ALL.[48] Such genetic alterations are consistent with the results of a retrospective multinational study showing that ALL-type therapy is more effective than AML- or combined-type treatment in patients with MPAL.[91] Furthermore, the immunophenotypic heterogeneity within MPAL populations is not driven by distinct genomic subclones. Such a phenotypic fate results from the acquisition of mutations in early hematopoietic progenitors with preserved myeloid and lymphoid potentials, and individual phenotypic subpopulations can reconstitute the immunophenotypic diversity.[48]

Risk assignment for treatment

Age (infant or ≥10 years), white blood cell (WBC) count at diagnosis (≥50x10^9/L), central nervous system (CNS) involvement, T-cell immunophenotype, race (Hispanic or black), and male sex have been considered clinical adverse prognostic factors (Table 3). Furthermore, certain somatic genetic alterations are significantly associated with outcome and can partly explain the clinical factors.[15] For example, patients with hyperdiploidy (>50 chromosomes or DNA index≥1.16) and *ETV6-RUNX1* have a better prognosis and are commonly young children with low WBC counts. Conversely, patients with hypodiploidy (<44 chromosomes), Ph-positive or Ph-like ALL, *KMT2A*, *MEF2D*, or *BCL2/MYC* rearrangements, or *TCF3-HLF* have worse prognoses and are more commonly adolescents or adults with higher WBC counts and/or CNS involvement.[36,92] Hispanic patients have greater incidences of Ph-like ALL with *CRLF2* fusions. Infant leukemia is strongly associated with *KMT2A* rearrangements.

Early response to chemotherapy in terms of MRD is another important prognostic factor.[93,94] MRD can be measured by flow cytometry for leukemia-specific aberrant immunophenotypes or by polymerase chain reaction (PCR) for unique immunoglobulin and T-cell receptor genes or fusion transcripts. Next-generation sequencing is more sensitive than flow cytometry or PCR for MRD detection,[95] but the superiority of this methodology for clinical application needs to be confirmed in larger studies. The relapse risk at a given MRD level differs between genetic subtypes.[93,94] Patients with favorable genetic subtypes clear MRD faster than those with high-risk genetics and T-ALL. Although patients with high-risk genetic features remain at risk for relapse even with undetectable or very low level (e.g., <0.01%) MRD at the end of induction, low-level MRD can be overcome in patients with low-risk features by subsequent treatment. Current treatment protocols incorporate clinical factors, leukemia genetics, and MRD for risk-stratification.

Treatment

Treatment of ALL comprises three phases: remission induction, consolidation (or intensification), and mainte-

Table 3. Risk factors in pediatric acute lymphoblastic leukemia

Factor	Better	Worse
Patient and clinical characteristics		
Age at diagnosis	1 to <10 years	<1 year or ≥10 years
Sex	Female	Male
Race	Caucasian, Asian	African American, Hispanic
Down syndrome	No	Yes
WBC counts at diagnosis	$<50 \times 10^9$/L	$\geq 50 \times 10^9$/L
CNS involvement at diagnosis	CNS 1	CNS 2 and CNS 3, traumatic tap with blasts
Testicular involvement	No	Yes
Immunophenotype	B-ALL	T-ALL
Cytogenetic and genetics		
	High hyperdiploidy (51-65 chromosomes)	Hypodiploidy (<44 chromosomes)
	ETV6-RUNX1: t(12;21)(p13.2;q22.1)	*KMT2A* rearrangement: t(v;11q23.3)
	NUMT1 rearrangement	*BCR-ABL1*: t(9;22)(q34.1;q11.2) (Ph+)
		BCR-ABL1-like (Ph-like)
		TCF3-HLF: t(17;19)(q22;p13)
		MEF2D rearrangement
		Intrachromosomal amplification of chromosome 21 (iAMP21)
		BCL2 or *MYC* rearrangements
Minimal residual disease		
	Negative	Positive
	Continuously decreasing and becoming negative	Increasing and/or persistently positive while monitored

WBC: white blood cell; CNS: central nervous system; ALL: acute lymphoblastic leukemia; Ph: Philadelphia chromosome.

nance (or continuation), and lasts for 2-2.5 years. Most conventional chemotherapeutic agents were developed before 1970, and the optimal dosages and schedules for combination chemotherapy were developed with dose adjustments based on tolerability, response evaluation with MRD, and individualized pharmacodynamic and pharmacogenomic studies, but with limited use of the biological features of ALL cells obtained through genomic analyses. Allogeneic hematopoietic cell transplantation (HCT) has been used for patients at very high risk. In the last decade, molecularly targeted agents and immunotherapy have emerged as novel therapeutic strategies.

Survivors treated before 1990 experienced late effects in multiple organ systems (e.g., reproductive, neurological, or gastrointestinal effects or infections), but those treated on more recent protocols have experienced predominantly musculoskeletal effects, possibly due to more intensive use of dexamethasone and asparaginase.[96] In addition, cranial radiotherapy-induced hypothalamic dysfunction has given way to impaired glucose metabolism and obesity as the use of radiotherapy has been reduced. The recent pattern of late effects could be managed by prevention or intervention as well as by rational reduction of conventional chemotherapy combined with molecularly targeted therapy and immunotherapy.

Remission-induction therapy

Remission-induction therapy consists of three drugs (glucocorticoid [prednisone or dexamethasone], vincristine, and asparaginase) or four drugs (the 3 aforementioned drugs plus anthracycline) administered over 4-6 weeks and induces complete remission (CR) in approximately 98% of pediatric patients.

Compared to prednisone, dexamethasone has a longer half-life and better CNS penetration, which improves CNS disease control.[97] In randomized studies comparing prednisone and dexamethasone, patients who received dexamethasone had better event-free survival (EFS) than did those who received prednisone at a prednisone-to-dexamethasone dose ratio of <7 (Table 1).[1,97] However, OS was similar in both arms, except in one study that found dexamethasone beneficial in patients with T-ALL who had a good prednisone-prophase response.[1] Furthermore, dexamethasone is associated with more frequent adverse effects, such as infection, bone fracture, osteonecrosis, mood/behavior problems, and myopathy. When the dose ratio was >7, there was no difference in outcomes with the two glucocorticoids.[97] Therefore, the dose, schedule, and type of glucocorticoid are determined based on the patient's age, relapse risk, and treatment phase. Bacterial infection can be reduced with prophylactic antibiotics, such as levofloxacin during neutropenia.[98,99] Alternate-week dexamethasone administration, as opposed to a continuous schedule, can reduce the risk of osteonecrosis.[100] Adding hydrocortisone to dexamethasone can reduce neuropsychological adverse effects, possibly by reducing the cortisol depletion in the cerebral mineralocorticoid receptors.[101]

Vincristine does not typically cause significant myelosuppression and is given weekly during induction therapy and as monthly or tri-monthly pulses with glucocorticoids during continuation at doses of 1.5-2.0 mg/m^2. However, its adverse effects include peripheral sensory and motor neuropathy, and the dose is typically capped at 2.0 mg. A GWAS in children with ALL revealed that a polymorphism within the promoter region of *CEP72* was associated with increased incidence and severity of vincristine-related peripheral neuropathy.[102]

In many countries, polyethylene glycol (PEG)-*Escherichia coli* L-asparaginase (pegaspargase) has replaced native *E. coli* L-asparaginase, compared with which pegaspargase has a longer half-life and a lower incidence of hypersensitivity. Compared with intramuscular native *E. coli* L-asparaginase, intravenous pegaspargase was similar-

ly efficacious, no more toxic, and associated with decreased patient anxiety at administration (Table 1).[5] Although pegaspargase is typically used at 1000 IU/m^2 to 2500 IU/m^2, a therapeutic drug monitoring study showed trough levels of asparaginase activity of >100 IU/L even with administration at 450 IU/m^2 every two weeks.[103] Interestingly, the incidence of asparaginase-related toxicities (e.g., pancreatitis, central neurotoxicity, and thrombosis) was not associated with asparaginase activity levels except in the case of liver toxicities. In a randomized study to assign non-high-risk patients to either ten continuous doses (2-week intervals) or three intermittent doses (6-week intervals) of intramuscular pegaspargase (1000 IU/m^2), after receiving five doses every two weeks, asparaginase-related toxicities (hypersensitivity, osteonecrosis, pancreatitis, and thromboembolism) were significantly reduced in the latter group without compromising disease-free survival (DFS), suggesting that prolonged continuous administration of pegaspargase might not be necessary in the context of multiagent chemotherapy.[104] Most allergic reactions to pegaspargase occur after two or three doses, and are mediated by the PEG moiety and not by the L-asparaginase, possibly because of the patients' exposure to other PEG-containing products, such as laxatives and tablet coatings, before the diagnosis of ALL.[105] Although adding rituximab to ALL therapy decreased the allergic reaction to native *E. coli* L-asparaginase and improved the outcome in adults with CD20-positive Ph-negative ALL,[106] the efficacy of rituximab on PEG-related allergy is unknown. Discontinuation of planned asparaginase doses is associated with worse prognosis in high-risk patients.[107] In cases of allergy to pegaspargase or native *E. coli* asparaginase, *Erwinia* asparaginase (Erwinase) is considered an acceptable alternative. However, Erwinase is more expensive, is intermittently unavailable, and requires frequent administration because of its short half-life.[108] Universal premedication with diphenhydramine and an H2-blocker can decrease the incidence of allergic reactions,[109] and drug desensitization in patients with previous and persistent anti-PEG antibodies is feasible.[110]

Consolidation (intensification) therapy

Induction with the 3- or 4-drug regimen (the IA phase) is followed by consolidation (the IB phase) with cyclophosphamide, cytarabine, and mercaptopurine. In patients with B-ALL, 5-year EFS was 92.3% for those with negative MRD at the end of both the IA phase (day 33) and the IB phase (day 78), which was better than the 5-year EFS for those with positive MRD at one or both time points but at a level of <10^{-3} on day 78 (77.6%) and for those with positive MRD at a level of ≥10^{-3} on day 78 (50.1%).[111] Interestingly, the MRD level on day 33 in patients with T-ALL was not relevant if MRD was negative on day 78, suggesting the importance of the IB phase in T-ALL.[112] Similarly, in ETP ALL, which is often associated with poor early response to 4-drug induction, IB-phase consolidation is effective at reducing MRD,[113] and outcomes were comparable among patients with ETP, near-ETP, and non-ETP ALL in the Children's Oncology Group AALL0434 study.[114]

Methotrexate is crucial for controlling systemic leukemia and also CNS and testicular disease. Methotrexate is administered as a high dose (2-5 g/m^2) plus leucovorin rescue, together with 6-mercaptopurine, or as escalating intermediate doses of methotrexate (100-300 mg/m^2) without leucovorin rescue followed by asparaginase (the Capizzi regimen). In randomized studies, high-dose methotrexate was superior to the Capizzi methotrexate regimen in patients with high-risk B-ALL (Table 1).[2] Interestingly, in patients with T-ALL, Capizzi methotrexate was associated with better outcomes than high-dose methotrexate (Table 1).[4] However, approximately 90% of the patients received cranial irradiation, and those in the high-dose methotrexate arm underwent irradiation five months later than those in the Capizzi regimen arm. As cranial irradiation can control both CNS and systemic relapses, this difference in timing of irradiation might have contributed to the different outcomes.

In a randomized trial of nelarabine in patients with intermediate- or high-risk T-ALL, the 5-year DFS and CNS relapse (isolated and combined) rates were significantly better in patients who received nelarabine than in those who did not (88.2% *vs*. 82.1% and 1.3% *vs*. 6.9%, respectively).[115]

Consolidation therapy is followed by reinduction (delayed intensification) therapy, which consists of medication similar to that used during the IA and IB phases and is a critical component of ALL therapy in both standard-risk and high-risk patients. Reducing the duration and chemotherapy doses of delayed intensification led to an increased incidence of relapse in standard-risk patients, especially those who did not have *ETV6-RUNX1*-positive ALL or were aged ≥7 years at diagnosis.[116]

Maintenance therapy

Maintenance therapy typically lasts ≥1 year and consists of daily mercaptopurine and weekly methotrexate with or without vincristine and steroid pulses. One study found that completing maintenance therapy at one year after diagnosis resulted in a high relapse rate (38.8±2.8% at 12 years after diagnosis), although this approach cured more than half of the children with ALL, and some genetic subgroups, such as *TCF3-PBX1* and *ETV6-RUNX1*, were associated with excellent DFS.[117]

There is interpatient variability in mercaptopurine tolerance. Inherited heterozygous or homozygous deficiency of thiopurine methyltransferase (*TPMT*) leads to higher levels of active thiopurine metabolites and excess hematologic toxicities, which are more common in patients of European descent.[88] For patients with East Asian or Native American ancestry, germline variants of *NUDT15*, which encodes a nucleoside diphosphatase, reduce degradation of active thiopurine nucleotide metabolites and were strongly associated with mercaptopurine intolerance.[88] Therefore, thiopurine dosing adjustments based on *TPMT* and *NUDT15* genotypes are recommended.[118]

Adherence of <95% to planned daily mercaptopurine doses is associated with a 2.7-fold increase in incidence of relapse compared with that seen when adherence is ≥95%.[119] Confirming adherence by directly asking patients or by measuring their erythrocyte thioguanine nucleotide levels is important, especially for patients with persistently high WBC counts or absolute neutrophil counts, although self-reporting can overestimate the true intake in non-compliant patients. Patients were previously instructed to take mercaptopurine in the evening and without food/dairy products, but these restrictions did not affect outcomes or erythrocyte thioguanine

nucleotide levels as long as daily doses were administered at the same time of day.[120]

Central nervous system-directed therapy

Because of the high risk of late neurocognitive sequelae, endocrinopathy, and secondary cancers, cranial irradiation has been largely replaced by intrathecal chemotherapy, in addition to systemic chemotherapy that has CNS effects (e.g., dexamethasone, high-dose methotrexate, and asparaginase). In an international meta-analysis, cranial irradiation decreased the incidence of isolated CNS relapse in patients with overt CNS involvement at diagnosis (CNS 3) but the cumulative incidences of any event and the OS were similar to those in patients who did not receive cranial irradiation.[121]

The St. Jude Total XVI study used intensified intrathecal therapy during induction therapy and obtained a remarkable reduction in 5-year cumulative isolated and combined CNS relapses to 1.3% and 1.5%, respectively, without cranial irradiation (Table 1).[9] In addition to CNS 3 at diagnosis, CNS 2 status (<5 WBC/μL and blasts) at diagnosis is associated with worse outcomes and greater risk of CNS relapse, and augmented intrathecal chemotherapy is required.[122] Traumatic lumbar puncture at diagnosis can introduce circulating ALL blasts into the cerebrospinal fluid (CSF) and is also associated with worse outcomes. Delaying the first intrathecal therapy until circulating blasts had disappeared improved the control of CNS disease.[123]

Acute lymphoblastic leukemia blasts are typically detected in CSF by morphologic evaluation of cytospin samples. Flow cytometric analysis of CSF improved ALL blast detection, and positive results were associated with a higher incidence of any relapse,[124] although another study failed to show such an association.[125]

Molecularly targeted agents

With the increased understanding of genetic alterations in ALL, approaches targeting the driving genetic mutation and/or the associated signaling pathway are emerging (Table 2). Such an approach is attractive as it can augment or replace conventional chemotherapy with fewer off-target effects. In pediatric Ph-positive ALL, adding an ABL1 tyrosine kinase inhibitor, imatinib mesylate (340 mg/m^2/day), to intensive post-induction chemotherapy resulted in outcomes similar to those in patients who received HCT.[126] Newer generations of tyrosine kinase inhibitors are available, and a randomized study showed that pediatric patients who received chemotherapy with 80 mg/m^2/day of dasatinib, a dual ABL/SRC inhibitor with more potent activity against BCR-ABL1 and better CNS penetration than imatinib, had better EFS, OS, and CNS disease control when compared to patients who received imatinib (300 mg/m^2/day).[127] Ponatinib has potent activity in both wild-type and mutant *BCR-ABL1* ALL, including cells harboring the gatekeeper ABL1 T315I mutation. Combination chemotherapy with ponatinib and hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone (hyper-CVAD) alternating with high-dose methotrexate and cytarabine resulted in excellent 2-year EFS in adults with newly diagnosed Ph-positive ALL.[128] Because of the potential adverse effects, such as thrombosis and pancreatitis, the safety of ponatinib in combination with pediatric regimens should be evaluated.

For patients with Ph-like ALL and *ABL*-class gene fusions (*ABL1*, *ABL2*, *CSF1R*, *LYN*, *PDGFRA*, or *PDGFRB*), ABL1 inhibitors can be combined with chemotherapy.[129,130] For those patients with alterations that activate the JAK-STAT signaling pathway, such as rearrangements or a mutation of CRLF2 (*IGH-CRLF2*, *P2RY8-CRLF2*, or *CRLF2* F232C), rearrangements of *JAK2*, *EPOR*, or *TYK2*, or mutations/deletions of *IL7R*, *SH2B3*, *JAK1*, *JAK3*, *TYK2*, or *IL2RB*, clinical trials of a JAK inhibitor, ruxolitinib, are ongoing.

Venetoclax inhibits the anti-apoptotic regulator BCL-2. Deregulated cell death pathways contribute to treatment failure in ALL.[131] Preclinical studies have identified activities of venetoclax against high-risk leukemias such as ETP ALL, *KMT2A*-rearranged ALL, *TCF3-HLF*-positive ALL, and hypodiploid ALL (Table 2).[132,133] Proteasome and mTOR inhibitors have shown efficacy in relapsed ALL.[134,135] DOT1L, bromodomain, menin, and histone deacetylate inhibitors have shown promise in preclinical studies as therapies targeting unique molecular characteristics of *KMT2A*-rearranged ALL.[136]

Immunotherapy

Immunotherapy can be given as antibody-based therapy (e.g., blinatumomab or inotuzumab ozogamicin) or T-cell-based therapy (chimeric antigen receptor T [CAR T] cells, e.g., tisagenlecleucel), which have improved the response rate and outcomes in patients with relapsed/refractory B-ALL (Figure 4).[137] Antibodies (e.g., daratumumab directed against CD38) and CAR T cells (e.g., targeting CD1a, CD5, and CD7) against T-ALL are also under investigation.

Blinatumomab has two different single-chain Fv fragments: one binds the CD3 antigen and activates T-cell cytotoxicity, and the other binds the B-cell antigen CD19, which is expressed on most B-ALL cells.[138-140] In a randomized study of adults with refractory/relapsed B-ALL, patients who received blinatumomab had a better complete remission rate and survival than those who received the standard-of-care chemotherapy,[138] and blinatumomab was effective in eradicating MRD.[139] Patients aged 1-30 years with intermediate- or high-risk relapsed B-ALL were randomized to receive either two blocks of intensive chemotherapy or two 4-week blocks of blinatumomab after receiving re-induction chemotherapy.[140] The blinatumomab arm had better 2-year DFS, OS, and MRD clearance and lower incidences of febrile neutropenia, infection, and sepsis when compared with the chemotherapy arm. Cytokine release syndrome and neurotoxicity are adverse effects of blinatumomab, and their incidence and severity can be reduced by decreasing the disease burden before treatment.

Inotuzumab ozogamicin is a humanized anti-CD22 monoclonal antibody conjugated to calicheamicin.[141-143] A randomized study in adults with refractory/relapsed B-ALL showed that patients who received inotuzumab had a better remission rate and survival than did patients who received standard chemotherapy.[141] In a pediatric inotuzumab compassionate-use program, complete remission was seen in 67% of 51 children with relapsed/refractory ALL.[142] Inotuzumab is associated with sinusoidal obstruction syndrome, especially after HCT.[141-143] Fractionated use of low-dose inotuzumab and a longer interval between inotuzumab treatment and HCT can reduce the incidence of this syndrome.

Chimeric antigen receptor T cells express a synthetic

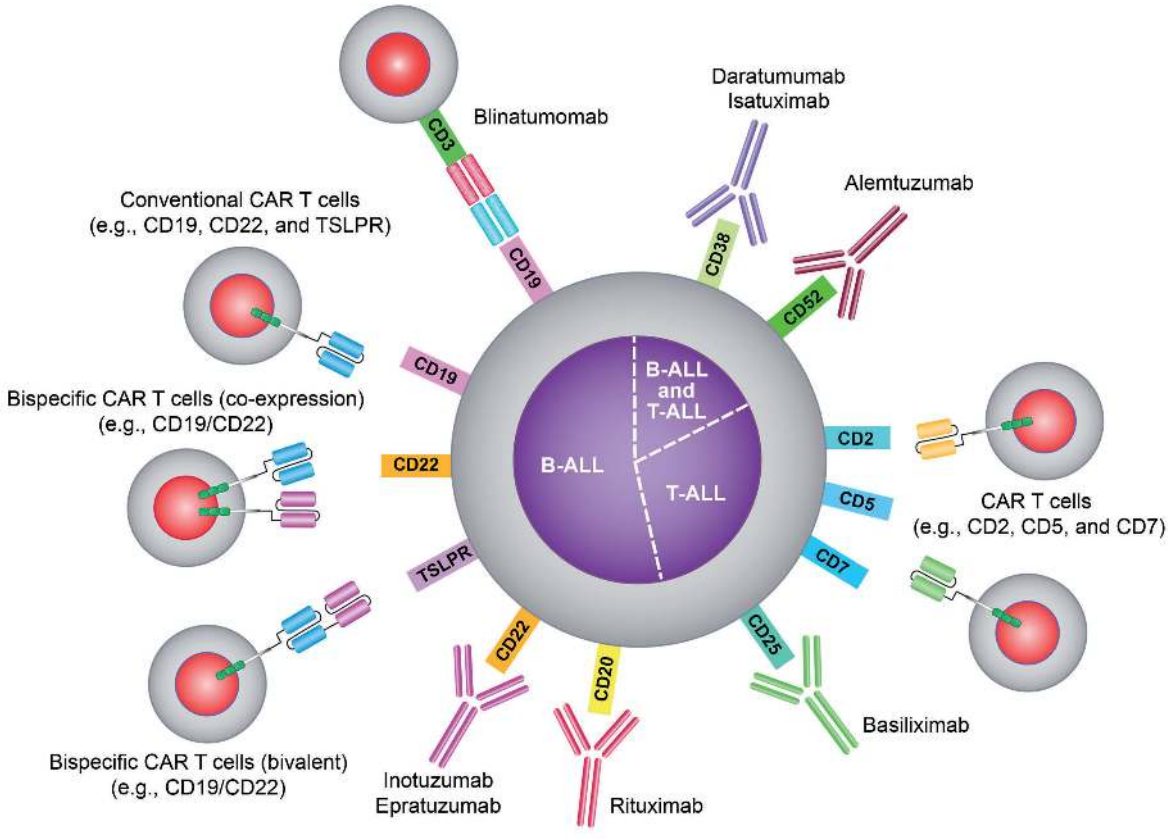

Figure 4. Immunotherapy in acute lymphoblastic leukemia. CAR T cells: chimeric antigen receptor T cells; ALL: acute lymphoblastic leukemia; TSLPR: thymic stromal lymphopoietin receptor.

receptor consisting of a single-chain variant fragment (scFv) domain directed against a B-lineage–associated antigen (e.g., CD19 and CD22) and intracellular signaling domains such as 4-1BB or CD28 with CD3ζ.[144] A study of CD19 CAR T cells in children and young adults with relapsed/refractory B-ALL showed a complete remission rate of 81% with 12-month EFS and OS of 50% and 76%, respectively.[144] CAR T cells can migrate to extramedullary sites such as the CNS and testes; therefore, they can be considered not only for patients with isolated bone marrow relapses but also for those with isolated or combined extramedullary relapses.[145] The persistence of CAR T cells and B-cell aplasia are important factors in long-term remission unless there is loss of the target antigen.[145,146] Therefore, CAR T cells have been developed that can target other antigens (e.g., CD22 or the thymic stromal lymphopoietin receptor) or simultaneously target dual antigens (e.g., CD19/CD22).[146] As with blinatumomab, cytokine release syndrome and neurotoxicity are major adverse effects. Tocilizumab (an anti-IL-6 receptor antibody) and/or steroid have been used to ameliorate these effects, and early intervention in patients who are developing signs of cytokine release syndrome is effective without compromising the anti-leukemia potency of CAR T cells.[147] Although CAR T cells can be curative by themselves, some consider them as a bridging therapy to subsequent HCT.[148]

To monitor MRD and antigen escape, the leukemia population should be characterized by multiparametric flow cytometry without using the targeted antigen prior to immunotherapy.[146] Alternatively, PCR or next-generation sequencing methods can be used.

Future perspectives

Comprehensive sequencing and integrative genome-wide analyses have profoundly refined the taxonomy of ALL, resulting in the identification of new entities with prognostic and therapeutic significance. There are distinct gene expression patterns in ALL caused by a wide range of genetic alterations that converge on specific pathways. Identifying these pathways is crucial for therapeutic targeting and demands the incorporation of gene expression approaches into the clinical diagnostic work-up of ALL. Mutation-agnostic approaches, such as drug sensitivity testing of panels of chemotherapeutic agents *ex vivo* and functional genomic screens, also offer the promise of identifying new therapeutic vulnerabilities and efficacious combinations.[149] Such intervention would lead to new therapeutic strategies incorporating individualized mutation-directed targeted therapy, immunotherapy, and reduced-intensity conventional chemotherapy or a chemotherapy-free regimen, which would ultimately improve patient survival and reduce adverse effects.

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