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Acute lymphoblastic leukaemia

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Summary

Acute lymphoblastic leukaemia (ALL) is seen in both children and adults, but its incidence peaks between ages 2 and 5 years. The causation of ALL is considered to be multi-factorial, including exogenous or endogenous exposures, genetic susceptibility, and chance. The survival rate of paediatric ALL has improved to approximately 90% in recent trials with risk stratification by biologic features of leukaemic cells and response to therapy, therapy modification based on patient pharmacodynamics and pharmacogenomics, and improved supportive care. However, innovative approaches are needed to further improve survival while reducing adverse effects. While most children can be cured, the prognosis of infants and adults with ALL remains poor. Recent genome-wide profiling of germline and leukaemic cell DNA has identified novel submicroscopic structural genetic alterations and sequence mutations that contribute to leukaemogenesis, define new ALL subtypes, influence responsiveness to treatment, and may provide novel prognostic markers and therapeutic targets for personalized medicine.

Introduction

An estimated 6000 new cases (3400 male and 2600 female) of acute lymphoblastic leukaemia (ALL) are diagnosed annually in the US.[1] Patients are predominantly children; approximately 60% of cases occur at age <20 years.[2–5] The survival rate of childhood ALL is approaching 90% (appendix, figure 1),[4,6] although the treatment of infants and adults needs improvement.[5,7] Here we review recent advances in the epidemiology, pathobiology, and clinical management of ALL.

Epidemiology

ALL, like cancer in general, is likely to arise from interactions between exogenous or endogenous exposures, genetic (inherited) susceptibility, and chance (figure 1). These factors account for the approximately 1 in 2000 risk of childhood (0–15 years) ALL. The challenge is to identify the relevant exposures and inherited genetic variants and to decipher

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how and when they contribute to the multi-step natural history of ALL from its initiation (usually *in utero*) through its largely covert evolution to overt disease.[8] The task is complicated by the relative rarity of ALL and the existence of biologically distinct subtypes that may not share common aetiological mechanisms.[9] For example, ALL in infants (<12 months) is usually associated with *MLL* gene rearrangement, and the remarkably high concordance rate in monozygotic twins (approaching 100% in those with a single or monochorionic placenta) suggests that leukaemogenesis is largely complete at birth.[10] In contrast, non-*MLL*-rearranged B-ALL has a peak incidence between 2 and 5 years and a concordance rate of 10–15%, suggesting that, although initiation *in utero* is common, other "promotional" exposures are probably required for the later emergence of disease.[10]

Exposures contributing to ALL

The topic of exposures and their role remains contentious. Epidemiological and case-control studies have found more than twenty candidate exposures that contribute to childhood ALL,[11] but very few are based on reproducibly significant data or are biologically plausible. Some are of public concern, especially ionising and non-ionising (eg, electromagnetic field [EMF]) radiation. Ionising radiation is an established causal exposure for childhood ALL, as evidenced by the impact of the 1945 atomic bombs in Japan[12] and by the modestly but significantly elevated risk caused by X-ray pelvimetry during pregnancy.[13] These exposures are not currently relevant, although some argue that background or natural radiation could be significant.[14] EMF exposures (eg, power lines) have been particularly controversial, and concern or confusion may be exacerbated by uncritical news reporting. Meta-analysis suggests a modestly elevated risk at high levels (>0.2μT),[15] but the reliability of this finding is uncertain. It is impossible to prove that EMF never causes ALL, but at most, it might be involved in only a small minority of cases. Further, its credibility as a causal (promotional, late stage?) exposure is weakened by the lack of any known biological mechanism or credible modelling *in vitro* or *in vivo*.

Infection was the first suggested causal exposure for childhood ALL[16] and remains the strongest candidate. Two specific hypotheses have been proposed, often referred to by their eponymous titles, and both are supported by epidemiological data (table 1).[11,17,18] Both postulate that ALL results from an abnormal response to a common infection. The hypotheses differ in detail but are not mutually exclusive as explanations of rare time/space clusters of leukaemia[19,20] or of ALL in the general community.[11] There is no evidence to date of a unique or single transforming virus in ALL,[11] as is the case of leukaemia in some animal species.[21] Instead, it is likely that ALL is promoted indirectly by an abnormal or dysregulated immune response to one or more common infections (viral or bacterial) in a susceptible individual. Influenza viruses are plausible candidates.[22] Susceptible children would be defined as having minimal prior exposure to infection during infancy and have a persistent *in utero*-generated pre-leukaemic clone[11] plus a variable degree of genetic susceptibility, as described below. It is important to continue exploring the possible biological mechanisms of infectious promotion of ALL,[23] as they could eventually lead to prophylactic interventions.

Inherited susceptibility

There is very little evidence of inherited predisposition to ALL via highly penetrant mutations in children or adults.[24] The high concordance in identical twin children has a non-genetic explanation (blood cell chimaerism).[10] Infants born with constitutive trisomy 21 or Down's syndrome are, however, at substantially elevated risk of ALL (~40-fold at age 0–4 years) and acute myeloid leukaemia (AML).[25] The apparent absence of familial clustering of ALL or of greatly elevated sibling risk (maximum 2-fold) does not, however, argue against inherited susceptibility. Previous attempts to identify inherited genetic susceptibility

to ALL have used a candidate gene approach. These studies found interesting potential candidates in, for example, folate metabolism and the immune response, but most were statistically underpowered or not consistently reproducible.[26] More recent genome-wide association studies (GWAS) of childhood ALL compare the whole genome (usually remission blood-derived DNA) in a large series of patients to that in an ethnically matched control group, focusing on single-nucleotide polymorphisms in DNA sequences (with ~80% genome coverage).[27–29] These studies demand hundreds or thousands of patients and controls, and, given the thousands of pan-genome markers being compared, a robust result is generally regarded as requiring a P value $<10^{-7}$. They also require validation in a second, independent series of patients and independent confirmation by another group of researchers. To date, common allelic variants in four genes (*IKZF1*, *ARID5B*, *CEBPE*, and *CDKN2A*) have been significantly and consistently associated with childhood ALL (appendix, table 1).[27–29] It is likely that variants in other genes have odds ratios (or impact) of <1.2, but these can be identified only in very large cooperative studies (3000–5000 cases). The gene variants have additive effects, so that an individual inheriting one copy of a variant will have risk elevated at ~50% above the norm while one who inherits all four variants in homozygous form (or double dose) would have an approximately10-fold increase in the risk of ALL. The overall conclusion from these studies is that the risk of childhood ALL is influenced by co-inheritance of multiple low-risk variants. The lower incidence of B-ALL with high hyperdiploidy in African-American children in the US may at least partially reflect lower prevalence of the *ARID5B* risk allele (appendix, table 1).[30] Inherited allelic variation may also affect response to treatment.[31]

A striking finding in these GWAS is the nature of the four genes implicated (appendix, table 1). Their products do not impinge directly on potential exposure pathways, such as immune or liver detoxification pathways, but rather are key regulators of blood cell development, proliferation, and differentiation. Further, acquired or somatic mutants of each of these genes have been detected in cases of ALL. This fact suggests that the inherited gene variants contribute to the intrinsic vulnerability of stem or precursor blood cells to transforming events either *in utero* at initiation and/or with subsequent post-natal promotion and clonal evolution (figure 1). There is suggestive evidence that the risk-conferring gene variants have lowered expression of their product,[27] but their functional aspects remain to be explored. These data provide valuable new insights into the aetiology of childhood ALL, but, at the moment, they do not carry strong enough predictive value to merit screening of the whole population of children.

Pathobiology

Genetic basis of ALL

High-resolution profiling of genetic alterations has transformed our understanding of the genetic basis of ALL. It has been known for several decades that the majority of childhood ALL cases harbour gross chromosomal alterations (figure 2).[32] In B-ALL, these include high hyperdiploidy with non-random gain of at least five chromosomes (including X, 4, 6, 10, 14, 17, 18, and 21), hypodiploidy with fewer than 44 chromosomes, and recurring translocations, including t(12;21)(p13;q22), encoding *ETV6-RUNX1* (*TEL-AML1*); t(1;19)(q23;p13), encoding *TCF3-PBX1* (*E2A-PBX1*); t(9;22)(q34;q11), encoding *BCR-ABL1; MLL* rearrangement involving 11q23 with a wide range of partner genes; and rearrangement of *MYC* into antigen receptor gene loci. Dysregulation of *TAL1*, *TLX1*, *TLX3*, and *LYL1*, particularly by rearrangement into T cell antigen receptor loci, is common in T-ALL. These alterations are of key importance in both the pathogenesis and clinical management of ALL (figure 3). Many chromosomal rearrangements disrupt genes that regulate normal hematopoiesis and lymphoid development (eg, *RUNX1* and *ETV6*), activate oncogenes (eg, *MYC*), or constitutively activate tyrosine kinases (eg, *ABL1*). Several of these alterations

are significantly associated with outcome, particularly in B-ALL, and are used in risk stratification. Notably, high hyperdiploidy and *ETV6-RUNX1* are associated with favorable outcome, whereas low hypodiploidy, and *MLL* rearrangement (especially in infants and adults) are associated with a dismal prognosis, in both children and adults.

However, many of these alterations alone do not induce leukaemia in experimental models, and many cases of ALL lack a gross chromosomal alteration, indicating that additional submicroscopic genetic alterations contribute to leukaemogenesis. High-resolution microarray profiling of DNA copy number alterations (deletions and gains) and sequencing have identified several novel structural genetic alterations and sequence mutations that define new subtypes of ALL, contribute to leukaemogenesis, and influence treatment responsiveness; in several cases, these are being explored as novel prognostic markers and therapeutic targets.[33–38] Importantly, many ALL subtypes are characterized by distinct constellations of structural genetic alterations that together drive establishment of the leukaemic clone.

Submicroscopic genetic alterations in ALL

More than 50 regions of recurring DNA copy number alteration have been identified in ALL.[33,34,39–43] Deletions are more common than amplification and typically are focal, often involving only a single gene. The nature and frequency of these alterations are associated with cell lineage (B or T) and cytogenetic ALL subtype. Notably, *MLL*-rearranged ALL, which is typically aggressive and arises early in life, harbors <1 additional genetic alteration per case, whereas *ETV6-RUNX1* and *BCR-ABL1* leukaemias manifest later in childhood and typically have at least 6–8 additional genetic alterations. Many of the involved genes encode regulators of lymphoid development, cell cycle, tumor suppressors, and lymphoid signaling molecules (figure 3, table 2). Most commonly altered are transcriptional regulators of B lymphoid development (eg, *PAX5, IZKF1,* and *EBF1*) in more than two-thirds of B-ALL cases and the *CDKN2A/CDKN2B* loci encoding the INK4/ARF tumor suppressors in more than 80% of T-ALL cases. While our understanding of sequence mutations in ALL is incomplete, existing data show that several genes are altered by multiple mechanisms, including deletion or amplification, sequence mutation, and translocation. The nature of genetic alteration is highly gene-dependent; for example, structural alterations are more common than sequence mutations in *PAX5* and *IKZF1* in B-ALL, whereas several genes (eg, *WT1*, *PHF6*, and *NOTCH1* in T-ALL) are more commonly targeted by sequence alteration.

Several genetic alterations have well-established roles in leukaemogenesis, such as activating mutations in *NOTCH1*; however, the roles of many other genes remain unknown, in part because of the paucity of mouse models that faithfully recapitulate human ALL. However, several alterations are known to disrupt the activity of the encoded proteins *in vitro* or to result in dominant negative activity, and recent studies have shown that loss-of-function mutations in *Pax5*[44] and *Ikzf1*[45] accelerate the onset of B-ALL in mouse models.

Prognostic genetic alterations in ALL

As cytogenetic alterations alone do not accurately predict the risk of relapse, there is great interest in the relation between novel genetic alterations and ALL outcome. The most consistent association is that of *IKZF1* alterations and poor outcome (table 2). *IKZF1* encodes IKAROS, the founding member of a family of pleiotropic zinc finger-containing transcription factors, and is required for lymphoid lineage development (figure 3). *IKZF1* is altered in 15% of B-ALL cases and is a hallmark of two high-risk ALL subtypes: *BCR-ABL1* lymphoid leukaemia (either *de novo* or chronic myeloid leukaemia in lymphoid blast crisis)[34,46] and a new subtype termed “BCR-ABL1-like ALL”, described below.[47,48]

IKZF1 alterations include loss-of-function deletions of the entire *IKZF1* locus, focal deletions resulting in expression of dominant negative IKZF1 alleles, and less commonly, sequence mutations. Alteration of *IKZF1* is associated with an increased risk of treatment failure and relapse in both *BCR-ABL1*-positive and -negative ALL; hence, there is considerable interest in testing for *IKZF1* alterations at diagnosis, although current treatment protocols do not consider *IKZF1* status in risk stratification.

Novel subtypes of ALL

Rearrangement of *CRLF2*—As many as 8% of childhood ALL cases have *CRLF2* rearrangement at the pseudoautosomal region 1 (PAR1) of Xp/Yp (figure 2 and table 2). *CRLF2* encodes cytokine receptor-like factor 2, the receptor for thymic stromal lymphopoietin (TSLP).[36,37] The arrangement occurs either as a rearrangement of *CRLF2* into the immunoglobulin heavy chain locus at 14q32, or as a focal deletion immediately upstream of *CRLF2* that results in expression of a novel fusion, *P2RY8-CRLF2*. Both events dysregulate CRLF2 expression, resulting in increased expression by lymphoblasts that may be detected by diagnostic immunophenotyping. Less common is a p.Phe232Cys mutation that results in receptor dimerization.[49] *CRLF2* rearrangement is particularly common (>50% of cases) in ALL associated with Down syndrome (DS-ALL). In both DS and non-DS ALL, approximately 50% of cases with *CRLF2*-rearrangement harbor concomitant activating mutations in the Janus kinase genes *JAK1* or *JAK2* (table 2).[36,37,50] Particularly in non-DS-ALL, *CRLF2* and JAK alterations are also associated with deleterious *IKZF1* alterations and poor outcome.[51] The JAK mutations are located either at or near the active site of the JAK kinase domain or in the pseudokinase domains of JAK1/2, most commonly at p.Arg683 of JAK2. Importantly, JAK2 mutations common in ALL are distinct from those observed in myeloproliferative neoplasms.[52] Co-expression of CRLF2 and JAK mutant alleles transforms model cell lines and activates downstream Jak-Stat signaling, suggesting that these alterations are co-transforming in B-ALL.[37] In DS-ALL, alterations of *IKZF1*, but not of *CRLF2* or *JAK2*, were associated with a worse prognosis.[53]

BCR-ABL1-like ALL—Ten to 12 percent of B-ALL cases exhibit a gene expression profile similar to that of BCR-ABL1 ALL but are BCR-ABL1-negative, commonly show *IKZF1* alteration, and have a poor outcome (figure 2).[47,48] As many as 50% of BCR-ABL1-like cases have *CRLF2* rearrangements and *JAK* mutations. Next-generation sequencing, including transcriptome and whole-genome sequencing, has shown that the remaining cases harbour a diverse range of rearrangements, deletions, and sequence mutations that activate cytokine receptor and kinase signaling (eg, those involving *ABL1, EPOR, IL7R, JAK2,* and *PDGFRB*). Several of these were shown to be transforming in vitro and to activate kinase signaling in primary leukaemic cells.[54]

Intrachromosomal amplification of chromosome 21 (iAMP21) occurs in approximately 2% of BALL cases (figure 2).[55] This entity was originally defined by fluorescence in-situ hybridization (FISH) as gain of at least three copies of *RUNX1*.[56] Subsequent FISH and microarray studies showed that the region of amplification is typically large and complex and is often accompanied by deletion of the subtelomeric regions of chromosome 21.[57] The functional consequences of iAMP21 are largely unknown, but identification is important as it is associated with poor outcome on standard-risk regimens.[55,58]

Genetics of relapse and clonal heterogeneity in ALL

Cytogenetic[59] and genomic profiling of samples collected at diagnosis and relapse has shown that the majority of ALL cases exhibit substantial changes in the nature of genetic alterations during the disease course and that relapse often arises from the emergence of a minor subclone with genetic alterations distinct from those of the predominant clone at

diagnosis (figure 3).[60–62] In most cases, the relapse clone shares lesions with the predominant clone at diagnosis, indicating a common "ancestral" or pre-leukaemic origin, but other lesions are discordant. Sensitive assays specific for individual alterations demonstrate that the relapse clone is often present at a low level at diagnosis, suggesting that the alterations that emerge at relapse confer resistance to therapy. Moreover, several of the alterations that most commonly emerge at relapse are those also associated with poor treatment outcome when present at diagnosis, such as deletions of *IKZF1* and *CDKN2A/CDKN2B.* Less commonly, the relapse clone appears identical to or completely dissimilar to that at diagnosis. Similar findings were observed in candidate gene sequencing studies of relapsed ALL samples, which identified enrichment of loss-of-function mutations of the transcriptional coactivator and acetyltransferase *CREBBP* (encoding CREB-binding protein) and of *TP53* (table 2, figure 3).[38,63,64]

Genome sequencing of ALL

Recent studies have shown that next-generation sequencing approaches are required to comprehensively identify the genetic alterations in leukaemia. Simultaneous sequencing of hundreds of thousands of nucleic acids ("massively parallel" sequencing) may be used to identify sequence mutations and structural variants in the encoding portion of the genome ("exome" sequencing), the transcriptome (mRNA sequencing), or the entire genome. A recent study sequenced 120 candidate genes and pathways targeted by DNA copy number alterations in 187 high-risk B-ALL cases and identified a high frequency of alterations targeting B lymphoid development (68%), the *TP53/RB1* tumour suppressor pathway (54%), Ras signalling (50%), and Janus kinases (11%), as well as recurring mutations in genes including *ETV6, TBL1XR1, CREBBP, MUC4, ASMTL*, and *ADARB2*.[65]

Early T-cell precursor ALL (ETP-ALL) is an aggressive leukaemia characterized by an immature immunophenotype reminiscent of the murine thymic early T cell precursor,[66] aberrant expression of myeloid and stem cell markers, a distinct gene expression profile, and dismal outcome.[67] Whole genome sequencing of 12 ETP-ALL cases and mutational recurrence testing in an additional 94 ETP and non-ETP T-ALL cases showed that three pathways commonly mutated in AML were mutated at high frequency in ETP-ALL.[68] These were inactivating mutations targeting haematopoietic and lymphoid development (including *GATA3*, *ETV6*, *RUNX1* and *IKZF1*), mutations driving aberrant cytokine receptor and Ras signaling (*NRAS, KRAS, FLT3, JAK1, JAK3* and *IL7R*), and deleterious mutations in chromatin-modifying genes, most notably components of the polycomb repressor complex 2 (PRC2) (*EZH2*, *EED*, and *SUZ12*). PRC2 normally mediates trimethylation of lysine 27 of histone 3 (H3K27), resulting in transcriptional repression; thus, these mutations are predicted to de-repress transcription. These results extend recent findings of *PHF6* mutations in T-ALL which were obtained by sequencing X chromosome genes and explained increased incidence of T-ALL in males.[69]

These findings provide compelling evidence that whole-genome sequencing of the entire spectrum of ALL subtypes is required to identify all genetic alterations contributing to leukaemogenesis. In addition, studies investigating the nature of non-coding genetic mutations and the interaction of genetic, epigenetic, and transcriptomic factors in ALL will be of great interest.

Diagnosis

Morphological identification of lymphoblasts by microscopy and immunophenotypic determination of lineage commitment and developmental stage by flow cytometry are essential for correct diagnosis of ALL.[2] Chromosomal analysis still plays an important role in the initial cytogenetic work-up. RT-PCR, FISH/multiplex ligation-dependent probe

amplification, and flow cytometry are used to identify leukaemia-specific translocations, submicroscopic chromosomal abnormalities, and cellular DNA content, respectively. After genome-wide analysis becomes time- and cost-effective, it may replace many current diagnostic techniques. Appendix table 2 lists the tests that have prognostic and therapeutic implications.

Risk assignment

Clinical and biological factors

Age (infant or ≥10 years old), presenting leukocyte count (≥50×10^9/L), race (Hispanic or black), male sex, and T-cell immunophenotype have been considered adverse clinical prognostic factors in children, although their effect is diminished by contemporary risk-adapted therapy and improved supportive care.[2–6] Infants with *MLL* rearrangement, especially those < 6 months old with a leukocyte count >300×10^9/L at diagnosis, still have a dismal prognosis.[7] Cytogenetic and molecular risk factors have been discussed above.

Racial/ethnic differences in prognosis have been linked not only to socioeconomic factors but also to differences in genomic alterations.[51,70,71] For example, germline single-nucleotide polymorphisms of *PDE4B*[70] and *ARID5B*[71] were shown to be associated with Native American genetic ancestry, and somatic *CRLF2* rearrangements in ALL blasts[51] were overrepresented in children from a Hispanic ethnic background; these alterations were found to contribute to inferior outcomes in Hispanics. Adverse prognosis conferred by genetic ancestry was mitigated by adding a course of delayed intensification therapy.[70]

Adolescents and adults have a greater prevalence of biologically high-risk leukaemia (eg, *BCR-ABL1* and *MLL* rearrangement), a low incidence of favorable subtypes (eg, *ETV6-RUNX1* and hyperdiploidy), and poorer adherence and tolerance to therapy.[72] Older age (especially ≥60 years) and high presenting leukocyte count are also poor prognostic factors in this population. Recent studies showed that they had better outcomes when treated on paediatric rather than adult regimens.[72–76] Typically, paediatric regimens provide higher doses of non-myelosuppressive drugs, early and frequent intrathecal therapy, reinduction and long maintenance phases, and strict oversight of adherence. Appendix figure 2 shows ALL survival according to age group.

Response to therapy

Early treatment response is predictive of the risk of relapse and is used to assign patients to subsequent risk-adapted therapy.[77] Methods that track residual leukaemic cells by flow cytometry (detecting aberrant immunophenotypes) and by PCR amplification (detecting leukaemia-specific immunoglobulin and T-cell receptor genes or fusion transcripts) allow the recognition of ALL cells present at levels well below those detectable by microscopic morphologic assessment, ie, minimal residual disease (MRD). MRD is currently the most powerful prognostic indicator in childhood and adult ALL, even in patients with low-risk features at presentation.[6,78–82] The kinetics of MRD clearance in response to identical remission-induction chemotherapy differed between B- and T-ALL; negative MRD on day 33 (after administration of 4 drugs) was the strongest prognostic factor in B-ALL,[79] while negative MRD on day 78 (after 7 drugs) was also predictive in T-ALL, regardless of positive MRD on day 33.[80]

PCR is typically more sensitive than flow cytometry for measurement of MRD (~0.001% *vs* ~0.01%), and PCR-measurable low levels of MRD (0.001 to < 0.01%) after remission-induction therapy showed prognostic significance in childhood ALL.[83] However, flow cytometry is faster, generally less expensive, and applicable to a larger proportion of patients,[77] allowing early tailoring of therapy.[81] The sensitivity of flow cytometry can be

improved by using multi-color combinations of additional leukaemia-associated markers identified from differently expressed genes in ALL cells, yielding a detection threshold of ~0.001%.[84]

Treatment

Treatment of ALL typically spans 2–2.5 years, comprising 3 phases: remission-induction, intensification (or consolidation), and continuation (or maintenance).[2] Most of the drugs used were developed before 1970. However, their dosage and schedule of administration in combination chemotherapy have been optimized on the basis of leukaemic-cell biological features, response to therapy (MRD), and patient pharmacodynamic and pharmacogenomic findings, resulting in the current high survival rate. Central nervous system (CNS)-directed therapy is administered to prevent relapse caused by leukaemia cells sequestered in this sanctuary site. Allogeneic haematopoietic stem-cell transplantation is considered for patients at very high risk. This section will focus on the most important advances in ALL treatment over the past 5 years.

Remission-induction therapy

Four to 6 weeks of remission-induction treatment eradicates the initial leukaemic cell burden and restores normal haematopoiesis in 96–99% of children and 78–92% of adults.[2–5] The chemotherapy agents typically include a glucocorticoid (prednisone or dexamethasone), vincristine, and asparaginase, with or without anthracycline. This regimen appears to be sufficient for standard-risk ALL if intensified post-remission treatment is given. Patients at high or very high risk receive four or more drugs.

Prednisone (or prednisolone) has traditionally been used in ALL treatment, but dexamethasone is increasingly considered.[85] However, the optimal doses and bioequivalence of these drugs are unclear. In prospective randomized trials, dexamethasone provided better control of CNS leukaemia and, at a prednisone-to-dexamethasone dose ratio <7, yielded better event-free survival, especially in children with T-ALL who responded well to prednisone pre-phase treatment and children age <10 years with B-ALL.[86–89] However, when a higher dose of prednisolone (dose ratio >7) was used, the two drugs showed no difference in efficacy.[90,91] Glucocorticoid treatment is associated with adverse effects, including infection, osteonecrosis, fracture, psychosis, and myopathy, whose incidence is generally higher with dexamethasone than with prednisone. Thus, high-dose dexamethasone (eg, 10 mg/m^2/day) is not recommended for adolescent B-ALL.[92]

Three preparations of asparaginase are currently available: *Escherichia coli*-derived, *Erwinia caratovora*-derived, and a monoethoxypolyethylene glycol succinimidyl conjugate of *E. coli* L-asparaginase (PEG-asparaginase).[93] As these formulations have different half-lives (PEG-asparaginase > *E. coli* > *Erwinia*), it is crucial to maintain asparagine depletion by optimizing the dose intensity and schedule of the asparaginase used. PEG-asparaginase has largely replaced the native product, as it provides at least 2 weeks of therapeutic activity after a single dose and less frequently induces antibodies.[94,95] Native *E. coli* and PEG-asparaginase activity were reported to be inversely related to anti-*E. coli* asparaginase antibody titers, although PEG-asparaginase was inhibited only at high antibody titers.[96] Therefore, PEG-asparaginase may be considered when antibody titers are low to intermediate, and *Erwinia* asparaginase should be considered when titers are high. A significant pharmacokinetic interaction has been observed between glucocorticoid and asparaginase.[97,98] Higher systemic exposure to asparaginase was found to be associated with higher exposure to dexamethasone, presumably because of impaired hepatic synthesis of proteins involved in dexamethasone clearance. Thus, anti-asparaginase antibodies can reduce exposure to both drugs and may increase the risk of relapse.[98]

Patients with *BCR-ABL1*-positive ALL have been considered to have a poor prognosis but benefit from early administration of a tyrosine kinase inhibitor (eg, imatinib, dasatinib). When this agent is added to multiagent chemotherapy, complete remission rates are >90% and event-free survival is superior to that of historical controls.[99,100] Unlike imatinib, dasatinib targets both ABL1 and Src kinases; it also has more potent activity against BCR-ABL1, is active against imatinib-resistant BCR-ABL1 (except for T315I mutation), and has better CNS penetration.[100–102]

Intensification (consolidation) therapy

Intensification (consolidation) therapy is administered after remission-induction to eradicate residual leukaemic cells.[2,3] This phase commonly uses high-dose (ie, 1–8g/m^2) methotrexate (MTX) with mercaptopurine, frequent pulses of vincristine and glucocorticoid, uninterrupted asparaginase for 20–30 weeks, and reinduction therapy with agents similar to those used during remission-induction.

The accumulation of the active MTX metabolites, MTX polyglutamates ($MTXPG_{1–7}$), in leukaemic cells is associated with anti-leukaemic activity, the results of which can be affected by somatic and germline genetic factors, dose and duration of MTX administration, and leucovorin rescue. Functional enzyme and somatic genetic studies show that $MTXPG_{1–7}$ accumulation varies widely among ALL subtypes, being low in *TCF3-PBX1* ALL, T-ALL, and *ETV6-RUNX1* ALL and high in hyperdiploid B-ALL, especially with gain of chromosome 18 or 10; therefore, the former group may benefit from higher MTX doses.[103–105] Germline single-nucleotide polymorphisms of the organic anion transporter polypeptide *SLCO1B1* were found to be associated with high MTX clearance.[106,107] In patients with high-risk ALL, high-dose MTX (5 g/m^2, 4 doses, every 14 days) plus mercaptopurine was more effective than escalating-dose MTX (initial dose 100 mg/m^2, increasing by 50 mg/m^2, 5 doses, every 10 days) plus PEG-asparaginase, without increased acute toxicity.[108] The duration of an effective serum MTX level is also important; accumulation of $MTXPG_{1–7}$ was less with 4-hour infusions of high-dose MTX than with 24-hour infusions.[109] Leucovorin rescue is required after high-dose MTX administration; however, its excessive use can counteract the anti-leukaemic effects of MTX and increase the risk of relapse.[110]

Reinduction therapy has proven to be a crucial element of ALL protocols. Intensified reinduction therapy with vincristine and asparaginase improved the outcome of patients with high-risk ALL.[111] However, an identical second reinduction cycle did not improve the outcome of patients with high-risk ALL and a rapid marrow response to 7 days of induction therapy or of those with standard-risk ALL, suggesting that residual leukaemia clones after a course of reinduction therapy may represent intrinsic drug resistance.[111,112] It is not clear whether this second reinduction cycle offers a benefit to patients with high-risk ALL and a slow early response, in the context of contemporary therapy. Osteonecrosis frequently occurs after reinduction therapy, especially in children 10 years and older. Alternative-week (10 mg/m^2/day on days 0–6 and 14–20, 2 courses) rather than continuous (on days 0–20, 1 course) administration of dexamethasone significantly reduced osteonecrosis despite a higher cumulative dose.[113]

Hematopoietic stem cell transplantation and cellular therapy

Allogeneic haematopoietic stem cell transplantation (HSCT) is considered for children with very high-risk ALL and/or persistent disease.[114] Contemporary HSCT protocols with high-resolution HLA typing, case-based conditioning, and improved supportive care have reduced relapse-related mortality, regimen-related toxicity, and infection.[115,116] Further,

survival is comparable regardless of stem cell source (matched related, matched unrelated, cord blood, or haploidentical donor).[116–118]

In view of the ongoing development of disease detection and frontline therapies, the indications for allogeneic HSCT should be reassessed continuously. A level of MRD $\geq 10^{-4}$ before HSCT is strongly associated with relapse, and new strategies are needed to reduce the disease burden before and/or after HSCT.[119,120] Patients with *BCR-ABL1*-positive ALL who obtain remission after multi-agent chemotherapy with ABL1 kinase inhibitors and young children (age <6 years) with B-ALL in delayed remission after induction failure can be treated without HSCT.[99,100,121] The benefit of HSCT for infants with ALL is controversial; the role of HSCT, if any, is limited to a small high-risk group.[122,123] Although many adult centers have considered HSCT during first complete remission a key element of therapy, treatment with paediatric-based regimens will decrease its use.[5,72]

Continuation therapy

Continuation therapy typically lasts 2 years or longer and comprises mainly daily mercaptopurine and weekly methotrexate with or without pulses of vincristine and dexamethasone.

Mercaptopurine and thioguanine are structural analogs of hypoxanthine and guanine, respectively, and inhibit de novo purine synthesis. Although thioguanine requires fewer steps to form the active metabolite thioguanine nucleotides and has greater in vitro cytotoxicity to lymphoblasts, randomized studies have not consistently shown a benefit of thioguanine in event-free survival[124,125] or overall survival,[126] and protracted doses •40 mg/m^2/day were associated with death during remission, veno-occlusive disease, portal hypertension, and thrombocytopenia.[124–126] Thus, mercaptopurine is preferred for continuation therapy. Thiopurine methyltransferase (TPMT) catalyzes S-methylation of thiopurines to inactive methylated metabolites. Patients with homozygous or heterozygous *TPMT* deficiency experience moderate to profound myelosuppression when treated with thiopurines.[127] They may also develop secondary malignancy, especially at higher doses (eg, mercaptopurine 75 mg/m^2/day).[128] Further, an adherence rate <95% to planned mercaptopurine doses is associated with relapse.[129] Therefore, uninterrupted, pharmacogenetics-based mercaptopurine dosing is important.[2] After thioguanine nucleotides are incorporated into DNA, DNA mismatch repair enzymes exert cytotoxicity. Deficiency of such enzymes (eg, MSH2) renders leukaemic cells thiopurine-resistant.[130] MSH2 expression was low or undetectable in approximately 11% of children with newly diagnosed ALL due to partial or complete somatic deletion of genes that regulate MSH2 degradation (*FRAP1, HERC1, PRKCZ*, and *PIK3C2B*). These children experienced a high incidence of relapse.[130]

CNS-directed therapy

Control of CNS disease is a key component of ALL therapy. Prophylactic cranial irradiation (12–18 Gy) effectively controls disease, but its use has recently been reduced or eliminated to prevent acute neurotoxicity, neurocognitive deficits, endocrinopathies, secondary malignancies, and excess late mortality.[131] The St. Jude Total XV[6] and Dutch Childhood Oncology Group ALL-9[132] protocols replaced cranial irradiation with triple intrathecal chemotherapy (methotrexate, hydrocortisone, and cytarabine) for all newly diagnosed patients; the 5-year cumulative risk of isolated CNS relapse was 2.7% and 2.6%, respectively, within the range achieved by prophylactic cranial irradiation (1.5–4.5%). Patients at high-risk of CNS relapse, comprising those with any CNS involvement (including leukemic-cell contamination by traumatic spinal tap) and/or T-ALL,[6] should be treated with intensified intrathecal therapy during early remission-induction. Cranial

irradiation can be reserved only for salvage treatment, as the retrieval rate is high for patients with an isolated CNS relapse who did not receive irradiation with initial treatment.[6]

In a randomized study in standard-risk ALL, triple intrathecal treatment reduced the frequency of CNS relapse compared with single-agent intrathecal methotrexate, but was associated with increased risk of bone marrow and testicular relapse, possibly due to less intensive systemic therapy.[133] In St. Jude Total XV, not only excellent CNS outcomes but also excellent overall outcomes (5-year event-free survival, 85.6%; overall survival, 93.5%) were achieved.[6] As CNS and hematologic relapses are competing events, systemic chemotherapy with high-dose methotrexate, intensive asparaginase, and dexamethasone, plus risk-based early intensive intrathecal chemotherapy, play a substantial role in preventing CNS relapse.[131]

Remaining questions and future directions

There remain subsets of ALL that carry an adverse prognosis. Further intensification of current regimens is unlikely to dramatically improve survival but is certain to increase short- and long-term adverse effects. Reduction of treatment intensity should be sought for patients at low risk. Studies of chronic health complications in the growing number of long-term adult survivors will help to refine therapy to reduce the toxicity of treatment, while functional genomics and proteomics will provide a deeper understanding of the epidemiology and pathogenesis of individual cases, allowing targeted “personalized medicine” (table 3). Although the majority of epidemiology and pathobiology studies have been developed for paediatric ALL, these should be expanded to adult patients.

Pharmacologic inhibitors of Janus kinases, such as ruxolitinib, are being explored in cases of childhood ALL harboring *CRLF2* and *JAK* alterations.[35] Detailed preclinical studies in *BCR-ABL1*-like ALL showed that the ABL1/PDGFRB inhibitors imatinib and dasatinib are effective against ALL cells with *NUP214-ABL1* and that JAK inhibitors are effective against those with *BCR-JAK2* or mutated *IL7R*; therefore, cases harboring these alterations may be candidates for targeted therapy.[54] DNA and histone methyltransferase inhibitors and histone deacetylase inhibitors may reactivate silenced tumor-suppressor genes or augment sensitivity to concomitant chemotherapy. These epigenetic agents may be considered for infants with *MLL*-rearranged ALL, in whom aberrant DNA and histone methylation is frequently observed,[134,135] and for patients with *CREBBP* mutations that encode histone acetyltransferase CREB-binding protein.[38] Mutations in ETP-ALL are also observed in AML, suggesting that AML-directed therapies or agents that target JAK signaling may be beneficial.[68] Monoclonal antibodies to surface antigens such as CD19, CD20, CD22, and CD52 have been used in unconjugated form (eg, rituximab and epratuzumab), conjugated to immunotoxins or chemotherapeutic agents (moxetumomab and inotuzumab ozogamicin), or in the form of a bispecific antibody (blinatumomab).[136] The incorporation of rituximab into the hyper-CVAD regimen (fractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone) appears to improve outcome for younger adults (<60 years) with CD20-positive, *BCR-ABL1*-negative B-ALL.[137] Blinatumomab, a bispecific, single-chain antibody to CD19 and CD3ϵ, recruits and activates CD3 effector cytotoxic T cells and is cytotoxic to CD19-expressing target cells bound to the other arm of the antibody; it has shown activity against relapsed/refractory ALL in an adult phase II study.[138]

Emerging evidence shows that newly diagnosed ALL comprises multiple subclones and that chemoresistance is frequently driven by subpopulations harboring genetic alterations that confer resistance.[60–62] Thus, efforts should be made to identify patients at high risk of relapse by using highly sensitive methods to detect these subpopulations at diagnosis, and

therapy should target these subpopulations to augment the efficacy of the current therapeutic regimen while reducing the intensity.

Search strategy and selection criteria

We searched Medline and PubMed for articles published between January 2007 and November 2012, using the keywords “acute lymphoblastic leukaemia”, “acute lymphocytic leukaemia”, and “acute lymphoid leukaemia”. Additional information was obtained from abstracts presented to the American Society of Hematology and the American Society of Clinical Oncology. We focused on publications from the past 5 years, but did not exclude commonly referenced and highly regarded older publications. We also searched the reference lists of articles identified by this search strategy and selected those we judged relevant. Review articles and book chapters are cited to provide readers with more details and more references than this Seminar can provide. Our reference list was modified on the basis of comments from peer reviewers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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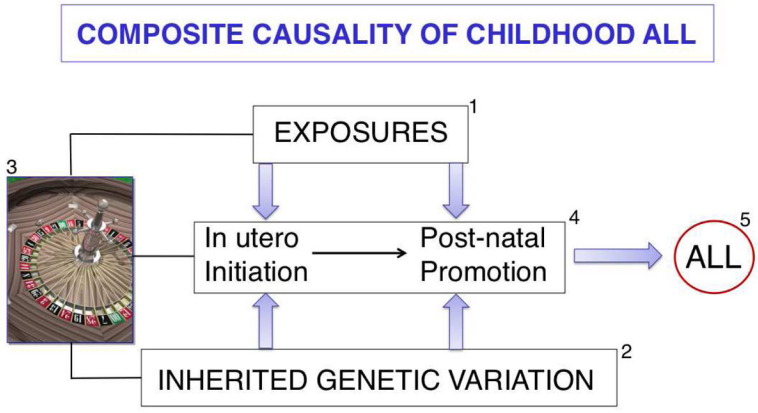

Figure 1.

Composite causality of childhood ALL. 1) Exogenous (eg, infection) and endogenous (eg, inflammation, oxidative stress) exposures, 2) normal allelic variation in inherited genes, and 3) chance all play roles in 4) the covert natural history of childhood ALL,[8,10,11] leading ultimately to 5) overt disease and clinical diagnosis. Cancer causation is riddled with chance,[157] for example, incidental "external" exposure, incidental damage to a relevant oncogene in a relevant cell (stem or progenitor cell), and chance events at conception involving parental gene shuffling and recombination.

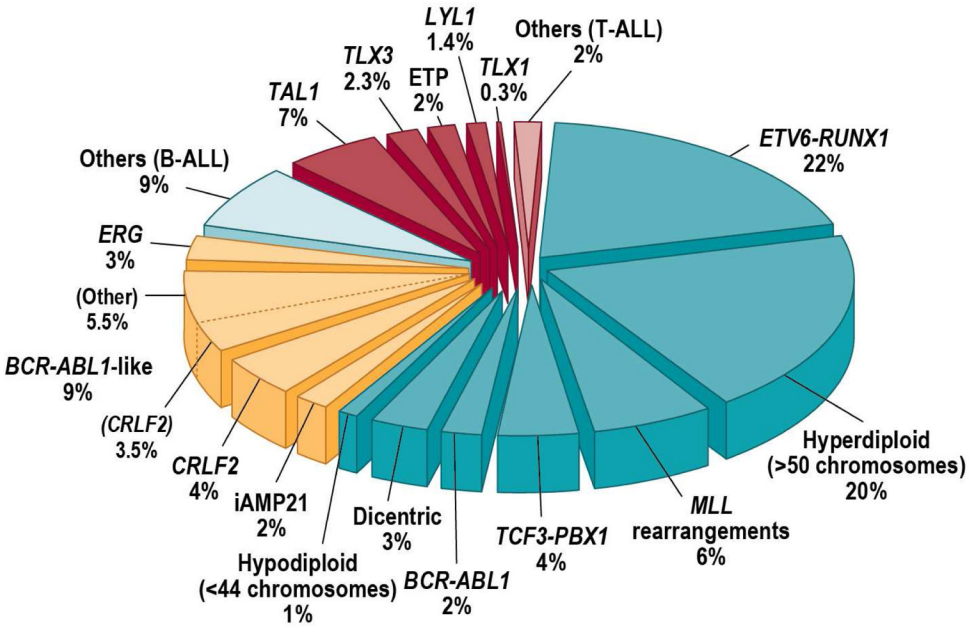

Figure 2.

Cytogenetic and molecular genetic abnormalities in childhood ALL. ALL with rearrangement of *CRLF2* but without the *BCR-ABL1*-like transcriptional profile rarely presents with other classifying karyotypic alterations but may be seen with high hyperdiploidy. The dicentric cases may have a range of heterogeneous translocations, including classifying translocations (eg, *ETV6-RUNX1*). iAMP21, intrachromosomal amplification of chromosome 21.

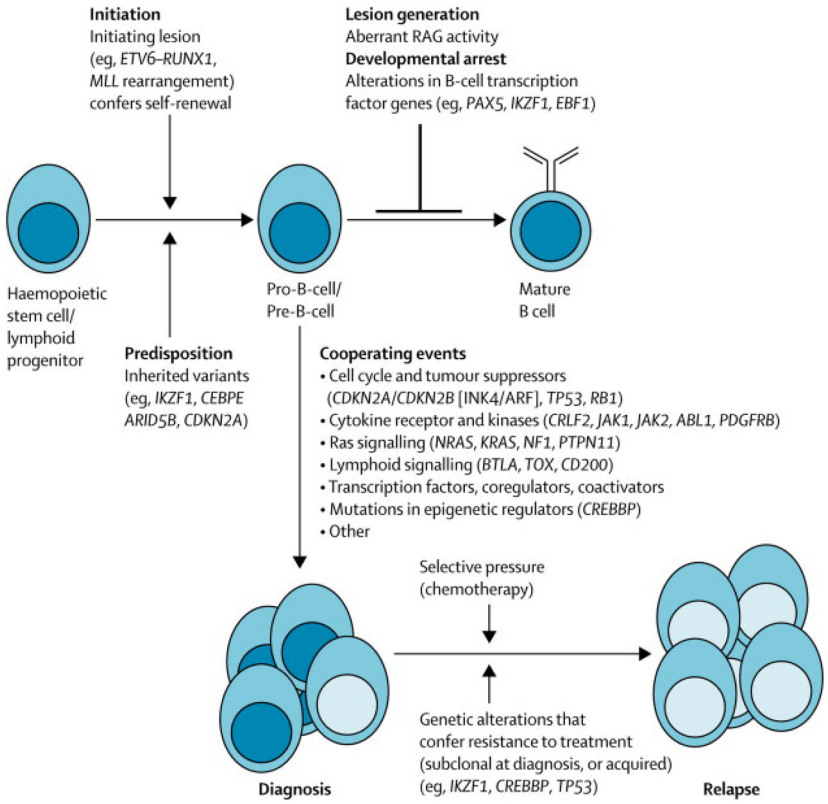

Figure 3.

Schema of genetic pathogenesis of B-ALL at diagnosis and relapse. HSC, hematopoietic stem cell; RAG, recombinase activating gene.

Table 1

Infectious hypotheses for childhood ALL

Hypotheses		Concepts	Timing	Agent	Evidence
The Kinlen "population mixing" hypothesis[17,19]	Unusual demographic mixing of susceptible and infected individuals	**a.** Herd immunity[139] **b.** Animal leukaemia precedents[21]	Perinatal?	Single novel virus?	Increased incidence (transient, 2x) in multiple situations of population mixing or clusters[19]
The Greaves "delayed infection" hypothesis[11,18]	Delayed exposure to common infections in childhood under-exposed as infants	**a.** Mismatch between evolutionary programming of immune system and modern (hygienic) lifestyle[11] **b.** Two-step pre-/post-natal natural history of ALL[11]	Later "promotional" or triggering event[11]	One or (more probably) several common infections (bacterial or viral)	Reduced risk of ALL from day care attendance in infancy[140,141]

Table 2

Key genetic alterations in B-ALL

Gene	Alteration	Frequency	Pathway and consequences of alteration	Clinical relevance	References
PAX5	Focal deletions, translocations, sequence mutations	31.7% of B-ALL	Transcription factor required for B-lymphoid development. Mutations impair DNA binding and transcriptional activation.		33,34,39
IKZF1	Focal deletions or sequence mutations	15% of all paediatric B-ALL	Transcription factor required for development of HSC to lymphoid precursors. Deletions and mutations result in loss of function or dominant negative isoforms.		33
		More than 80% of *BCR-ABL1* ALL and 66% of CML in lymphoid blast crisis		Associated with poor outcome	34,46,142
		One-third of high-risk *BCR-ABL1*-negative ALL		Tripling in cumulative incidence of relapse	47,48,143
	Inherited variants			Increased risk of ALL	27,28
JAK1/2	Pseudokinase and kinase domain mutations	18–35% of DS-ALL and 10.7% of High-risk *BCR-ABL1*-negative ALL	Mutations result in constitutive JAK-STAT activation. Transforms mouse Ba/F3-EpoR hematopoetic cell line.		35,144–146
CRLF2	Rearrangement as *IGH@-CRLF2* or *P2RY8-CRLF2* resulting in overexpression	5–16% of paediatric and adult B-ALL and >50% of DS-ALL	Associated with mutant *JAK* in up to 50% of cases. CRLF2 mutations and JAK mutations cotransforming in Ba/F3 cells and results in constitutive STAT activation.		36,37,49,50
		14% of paediatric high-risk ALL	Associated with *IKZF1* alteration and JAK mutations	Associated with poor outcome	51,147
CREBBP	Focal deletion and sequence mutations	19% of relapsed ALL. Also mutated in non-Hodgkin lymphoma	Mutations result in impaired histone acetylation and transcriptional regulation.	Mutations selected for at relapse, and associated with glucocorticoid resistance.	38,148

CML, chronic myeloid leukaemia; DS, Down syndrome; HSC, hematopoietic stem cells.

Table 3

Targeted antileukaemic drugs in current clinical trials

Class	Agent	Target	Indication
Purine nucleoside analogue			
	Clofarabine[149]	Ribonucleotide reductase; DNA polymerase; mitochondria	All ALL
	Nelarabine[150]	Ribonucleotide reductase; DNA synthesis	T-ALL
	Forodesine	Purine nucleoside phosphorylase	T-ALL
Vinca alkaloid			
	Vincristine sulfate liposome[151]	Tubulin	All ALL
Kinase inhibitor			
ABL1 kinase inhibitor			
	Dasatinib;[100] Nilotinib;[152] Imatinib;[99] Ponatinib	ABL1 kinase; platelet-derived growth factor receptor B	*BCR-ABL1*-positive ALL; *BCR-ABL1*-like ALL (eg, *NUP214-ABL1*)
Aurora kinase inhibitor			
	MLN8237	Aurora A kinase	*BCR-ABL1*-positive ALL
Janus kinase (JAK) inhibitor			
	Ruxolitinib; TG101348; CYT387	JAK	JAK-mutated ALL; *BCR-ABL1*-like ALL (eg, *BCR-JAK2*; mutated *IL7R*)
Tyrosine kinase inhibitor			
	Lestaurtinib; Midostaurin; Sorafenib; Quizartinib; Tandutinib; Sunitinib	FMS-like tyrosine kinase 3	*MLL*-rearranged ALL; hyperdiploid ALL
Other molecular or signaling inhibitor			
Proteasome inhibitor			
	Bortezomib[153]	Ubiquitin-proteasome pathway	All ALL
Mammalian target of rapamycin (mTOR) inhibitor			
	Sirolimus; Temsirolimus; Everolimus	mTOR	All ALL
Farnesyltransferase inhibitor			
	Tipifarnib; Lonafarnib	Ras, lamin A	All ALL
γ-Secreatase inhibitor			
	RO4929097	γ-Secretase	T-ALL
Angiogenesis inhibitor			
	Bevacizumab	Vascular endothelial growth factor	All ALL
Apoptosis inducer			

Class	Agent	Target	Indication
	Obatoclax; Oblimersen	Bcl-2	All ALL
Chemokine receptor (CXCR4) antagonist			
	Plerixafor	CXCL12 (SDF1)/CXCR4 axis	All ALL
Epigenetic therapy			
DNA methyltransferase inhibitor			
	Azacitidine; Decitabine	DNA methyltransferase	All ALL
Histone methyltransferase inhibitor			
	EPZ-5676	DOT1L	*MLL*-rearranged ALL
Histone deacetylase inhibitor			
	Vorinostat; Panobinostat; Depsipeptide; Valproic acid	Histone deacetylase	All ALL
Immune therapy			
Monoclonal antibody			
	Blinatumomab[138]	CD19 (engages CD3 T cells)	CD19-positive ALL
	SAR3419	CD19	CD19-positive ALL
	DT2219ARL	CD19 and 22	CD19/CD22-positive ALL
	Rituximab[137]	CD20	CD20-positive ALL
	Epratuzumab;[154] Moxetumomab; Inotuzumab ozogamicin[155]	CD22	CD22-positive ALL
	Alemtuzumab[156]	CD52	CD52-positive ALL
Cellular therapy			
	Natural killer cells	Killer immunoglobulin-like receptor (KIR)-ligand	Donor KIR-recipient ligand mismatch
	T cells with CD19-specific chimeric antigen receptor	CD19	CD19-positive ALL