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Genomic characterization of childhood acute lymphoblastic leukemia

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Abstract

Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy and a leading cause of childhood cancer death. The last decade has witnessed a transformation in our understanding of the genetic basis of ALL due to detailed integrative genomic profiling of large cohorts of childhood ALL. Initially using microarray based approaches, and more recently with next-generation sequencing, these studies have enabled more precise sub-classification of ALL, and have shown that each ALL entity is characterized by constellations of structural and sequence mutations that typically perturb key cellular pathways including lymphoid development, cell cycle regulation, tumor suppression, Ras- and tyrosine kinase driven signaling, and epigenetic regulation. Importantly, several of the newly identified genetic alterations have entered the clinic to improve diagnosis and risk stratification, and are being pursued as new targets for therapeutic intervention. Studies of ALL have also led the way in dissecting the subclonal heterogeneity of cancer, and have shown that individual patients commonly harbor multiple related but genetically distinct subclones, and that this genetically determined clonal heterogeneity is an important determinant of relapse. In addition, genome-wide profiling has identified inherited genetic variants that influence ALL risk. Ongoing studies are deploying detailed integrative genetic transcriptomic and epigenetic sequencing to comprehensively define the genomic landscape of ALL. This review describes the recent advances in our understanding of the genetics of ALL, with an emphasis on those alterations of key pathogenic or therapeutic importance.

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy, accounting for at least 3000 cases of childhood cancer per year in North America (1). Contemporary risk-directed trials of multiagent chemotherapy have resulted in long term event free survival rates exceeding 85%, however relapse occurs in approximately 20% of children, and is associated with a high rate of treatment failure and death, particularly when occurring in the first 18 months of therapy. Several factors have driven an explosion of interest in the use of detailed, genome-wide profiling approaches to comprehensively define all genomic alterations contributing to tumorigenesis in the last decade. These include the knowledge that ALL is characterized by recurring gross structural chromosomal alterations including aneuploidy and translocations whose detection is critical in diagnosis and risk

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stratification, but that up to a quarter of children and a higher proportion of adults lack one of these recurring alterations (2, 3). Also, twin studies have shown that chromosomal translocations may be present years prior to the onset of leukemia (4), and that these chromosomal changes are often insufficient to induce leukemia in mice, suggesting that additional genetic alterations must contribute to tumorigenesis. In addition, it has been known for many years that ALL tumor genomes are not static but evolve over time (5). Low-resolution and candidate gene studies had identified a number of recurring genomic alterations in the “pre-genomics” era but the completion of the human genome project and the development of relatively cheap microarray platforms to profile DNA copy number alterations at high resolution, gene expression and epigenetic changes enabled systematic study of thousands of ALL genomes (6–8). These studies have identified a remarkable diversity of unsuspected submicroscopic structural genetic alterations and deletions in both B-progenitor and T-lineage ALL. Next generation sequencing (NGS) approaches: whole genome (WGS), exome (WES) and transcriptome sequencing are being actively pursued in childhood ALL, particularly in two large collaborative studies: the St Jude – Washington University Pediatric Cancer Genome Project (9–12), and the Children’s Oncology Group – National Cancer Institute TARGET initiative (Therapeutically Applicable Research to Generate Effective Treatments; <http://ocg.cancer.gov/programs/target>) (13), as well as multiple other smaller efforts. While less mature than the microarray based studies, these efforts have already provided critical new data in ALL in the last two years, and it is expected that in the next 2–3 years the landscape of somatic genetic alterations in childhood ALL will be well defined.

A detailed review of the established cytogenetic alterations in ALL, their role in leukemogenesis and their prognostic implications is beyond the scope of this review (14), but key features are summarized below, before recent findings from genomic profiling studies are reviewed.

Chromosomal alterations in ALL

Approximately three-quarters of childhood ALL cases harbor one or more gross chromosomal alterations, detectable by conventional cytogenetic approaches. In B-progenitor ALL, these include: high hyperdiploidy with gain of at least 5 chromosomes, and less commonly, hypodiploidy with less than 44 chromosomes; a spectrum of translocations including t(12;21)(p13;q22) encoding *ETV6-RUNX1* (*TEL-AML1*), t(1;19)(q23;p13.3) encoding *TCF3-PBX1* (*E2A-PBX1*), t(9;22)(q34;q11.2) encoding *BCR-ABL1*; rearrangement of *MLL* at 11q23 with a diverse range of partner genes (15); rearrangement of *CRLF2* at Xp22.3/Yp11.3 to *P2RY8* or the immunoglobulin heavy chain locus (*IGH*) in B-progenitor ALL (16, 17); and, rearrangements of *IGH* with a range of partner genes including *BCL2*, *EPOR*, *ID4*, *IL3* and *CEBPE* (18, 19) (Figure 1). In T-lineage ALL, common alterations include rearrangement of the T cell receptor gene loci to transcription factor genes including *TLX1* (*HOX11*), *TLX3* (*HOX11L2*), *LYL1*, *TAL1* and *MLL* (2, 20). High hyperdiploidy and *ETV6-RUNX1* are associated with favorable outcome (indeed, relapse of *ETV6-RUNX1* ALL is now rare) (21). In contrast, *MLL* rearrangement, *BCR-ABL1* and hypodiploidy are associated with poor outcome (22–25).

Microarray based profiling of DNA copy number alterations (deletions and gains) using single nucleotide polymorphism (SNP) microarrays (6) or array-based comparative genomic hybridization (array-CGH) (26) have provided two key advances in our understanding of ALL: 1.) the identification of new subtypes of ALL that harbor previously cryptic or submicroscopic structural genetic alterations; and, 2.) the identification of submicroscopic genomic alterations that target key cellular pathways, which are often associated with specific ALL subtypes. These approaches typically examine up to 2–3 million probes across

the genome, and identify DNA copy number abnormalities and loss-of-heterozygosity at a resolution of 1–5 kilobases in size(27). Recently identified subtypes include B-progenitor ALL with intrachromosomal amplification of chromosome 21 (iAMP21), *CRLF2*-rearranged ALL, BCR-ABL1-like ALL, and ALL with deregulation of the ETS family transcription factor *ERG*, each of which is discussed in more detail below.

Submicroscopic genetic alterations in ALL

Since 2007, several groups have reported SNP array and array-CGH profiling results in childhood ALL (6, 26, 28, 29). These studies have shown that while ALL genomes typically harbor fewer structural alterations than many solid tumors, over 50 recurring deletions or amplifications have been identified, many of which involve a single gene or few genes (Figure 2). Importantly, mutation load is not simply a function of patient age, and mutation frequency is highly dependent on tumor type (6, 9).

Many of the involved genes encode proteins with key roles in lymphoid development (e.g., *PAX5*, *IKZF1*, *EBF1*, *LMO2*), cell cycle regulation and tumor suppression (*CDKN2A/CDKN2B*, *PTEN*, *RB1*, *TP53*), putative regulators of apoptosis (*BTG1*), lymphoid signaling (*BTLA*, *CD200*, *TOX*), the glucocorticoid receptor *NR3C1*, transcriptional regulators and co-activators including *TBLIXR1*, *ETV6* and *ERG*, and regulators of chromatin structure and epigenetic regulators (*CTCF*, *CREBBP*) (6, 26). Sanger sequencing studies have identified recurring sequence mutations, which in B-lineage ALL, most commonly affect lymphoid development (*PAX5*, and less commonly, *IKZF1*), Ras signaling (*NRAS*, *KRAS* and *NFI*), cytokine receptor signaling (*IL7R*, *JAK2*) and tumor suppression (*TP53*) (30). Similarly, a number of targets of structural genetic alteration and/or sequence mutation have been identified in T-lineage ALL, including activating mutations of *NOTCH1* (31), deletion/mutation of *PTEN* (32), *WT1* (33), *FBXW7* (34), and amplification of *MYB*. Importantly, several genes are involved in different types of genetic alteration, including copy number alterations, translocation, and sequence mutation (e.g. *PAX5*, *WT1*, and *PTEN*), indicating that microarray profiling is alone incapable of detecting all genetic alterations in ALL.

The nature and frequency of genetic lesions is subtype dependent. *MLL*-rearranged leukemias harbor very few additional structural or sequence alterations (6, 12, 35). In contrast, the majority of non-*MLL* ALL cases harbor recurring submicroscopic deletions, for example at least 6–8 per case in *ETV6-RUNX1* and *BCR-ABL1* ALL (6, 7, 36). In B-lineage ALL, this is in part driven by the activity of the recombinase activating genes (RAG) that cause focal deletions that presumably result in a selective advantage in lymphoid progenitors. Emerging experimental data has shown that several alterations can cooperate in leukemogenesis. For example, deletion of *Pax5* and *Ikzf1* accelerates the onset of leukemia in retroviral bone marrow transplant and transgenic models of *BCR-ABL1* ALL, and in chemical and retroviral models of leukemia (37, 38).

Alteration of transcription factor genes in B-ALL

Deletion, sequence mutation or rearrangement of genes encoding transcriptional regulators of lymphoid development is a hallmark of B-ALL. Alteration of *PAX5* (~35%), *IKZF1* (~15%) and *EBF1* (~5%) are the commonest alterations, with at least two thirds of B-ALL cases harboring one or more such lesions (6, 39). These alterations result in either loss of function or dominant negative lesions that lead to arrested lymphoid maturation, which is characteristic of leukemic cells. Notably, while *PAX5* alterations are the most common genetic alteration in B-ALL, they are not associated with outcome (39, 40). In contrast, alteration of *IKZF1* (IKAROS) is a hallmark of two types of high risk ALL: BCR-ABL1 positive ALL (7, 41, 42), and BCR-ABL1-like (Ph-like) ALL (8, 13, 43). *IKZF1* encodes IKAROS, the founding member of a family of zinc finger transcription factors that is

required for the development of all lymphoid lineages (44). The *IKZF1* alterations include focal or broad deletions that result in loss of expression of *IKZF1*, and deletions of coding exons 4–7 that remove the N-terminal DNA-binding zinc fingers, leading to expression of a dominant negative isoform, IK6. *IKZF1* alterations are present in over 70% of *BCR-ABL1* lymphoid leukemia, including *de novo* ALL and in chronic myeloid leukemia (CML) at progression to lymphoid blast crisis (7). Also, *IKZF1* alterations are associated with poor outcome in *BCR-ABL1* positive ALL (42).

***CRLF2* rearrangements and Janus kinase mutations in ALL**

The cytokine receptor gene *CRLF2* is rearranged or mutated in approximately 7% of childhood B-ALL (Figure 1), and in 50% of cases associated with Down syndrome (DS-ALL) (16, 17, 45). *CRLF2* is located in the pseudoautosomal region (PAR1) at Xp22.3/Yp11.3 and encodes cytokine receptor-like factor 2 (thymic stromal lymphopoietin receptor, TSLPR). With interleukin-7 receptor alpha, *CRLF2* forms a heterodimeric receptor for TSLP (thymic stromal lymphopoietin). *CRLF2* is rearranged by translocation into the immunoglobulin heavy chain locus (*IGH-CRLF2*), or by a focal deletion upstream of *CRLF2* that result in expression of *P2RY8-CRLF2* that encodes full-length *CRLF2*. Both rearrangements result in aberrant overexpression of *CRLF2* on the cell surface of leukemic lymphoblasts that may be detected by immunophenotyping (17). Less commonly a *CRLF2* p.Phe232Cys mutation results in receptor dimerization and overexpression (45).

Approximately half of *CRLF2*-rearranged ALL cases harbor activating mutations of the Janus kinase genes *JAK1* and *JAK2* (16, 17, 46), which with the exception of T-lineage ALL are otherwise uncommon in ALL (10, 47). The JAK mutations are most commonly missense mutations at or near R683 in the pseudokinase domain of *JAK2*, and are distinct from the *JAK2* V617F mutations that are a hallmark of myeloproliferative diseases. Less common are activating mutations in the kinase domain of *JAK1* and *JAK2*. The *JAK1/2* mutant alleles observed in ALL are transforming *in vitro*, and co-expression of *CRLF2* and *JAK1/2* mutations is transforming *in vitro* suggesting that these two lesions are central in lymphoid transformation (48–50). *CRLF2*-rearranged leukemic cells harboring *CRLF2* deregulation exhibit activation of JAK-STAT and PI3K/mTOR pathways, and are sensitive to JAK and mTOR inhibitors *in vitro* and *in vivo* (51, 52). An early phase trial of the JAK inhibitor ruxolitinib in relapsed and refractory childhood tumors, including cases with *CRLF2* rearrangement and/or JAK mutations, ADVL1011, has been initiated (clinicaltrials.gov identifier NCT01164163).

In non-DS ALL, *CRLF2* alterations and JAK mutations are associated with *IKZF1* deletion/mutation and poor outcome, particularly in cohorts of high risk B-ALL (53–56). Recent studies performed by the Children's Oncology Group (COG) have shown that *CRLF2* and *IKZF1* alterations are associated with inferior outcome in multiple cohorts, and notably, that elevated *CRLF2* expression in the absence of rearrangement is also an adverse prognostic feature (57).

BCR-ABL1-like ALL

Recently, a new subgroup of B-ALL was described characterized by a leukemic cell expression profile similar to *BCR-ABL1* positive ALL, deletion of *IKZF1*, and poor outcome (8, 43). *BCR-ABL1*-like ALL is common, comprising up to 10–15% of childhood B-ALL, and up to one third of B-ALL in adolescents and young adults (unpublished data), and is associated with poor outcome (58). Approximately half of *BCR-ABL1*-like ALL cases harbor *CRLF2* rearrangements and concomitant *JAK1/2* mutations. Recent transcriptome and whole genome sequencing has shown that non-*CRLF2*-rearranged *BCR-ABL1*-like ALL cases harbor a diverse range of genomic alterations that activate cytokine

receptors and tyrosine kinases including *ABL1*, *ABL2*, *EPOR*, *JAK2* and *PDGFRB* (ref (13) and unpublished data). These alterations are most commonly chromosomal rearrangements resulting in chimeric fusion genes deregulating tyrosine kinases (*NUP214-ABL1*, *ETV6-ABL1*, *RANBP2-ABL1*, *RCSD1-ABL1*, *BCR-JAK2*, *PAX5-JAK2*, *STRN3-JAK2* and *EBF1-PDGFRB*) and cytokine receptors (*IGH-EPOR*). Up to 20% of BCR-ABL1-like cases lack a chimeric fusion, and additional alterations activating kinase signaling, including activating mutations of *FLT3* and *IL7R*, and focal deletions of *SH2B3*, or LNK, which constrains JAK signaling (59), have been identified in fusion-negative cases. These diverse genetic alterations activate a limited number of signaling pathways, notably *ABL1* and *PDGFRB* and JAK-STAT signaling, and it is predicted that the majority of BCR-ABL-like ALL cases will be amenable to therapy with a limited number of tyrosine kinase inhibitors (TKIs): imatinib-class TKIs for *ABL1*, *ABL2* and *PDGFRB* rearrangements, and JAK inhibitors such as ruxolitinib for alterations activating JAK-STAT signaling (*EPOR*, *IL7R*, *JAK2* and *SH2B3*). These rearrangements have been shown to activate signaling pathways in model cell lines and in primary leukemic cells (13, 52), and xenografts of BCR-ABL1-like ALL are highly sensitive to TKIs *in vivo*. There are also emerging anecdotal reports of responsiveness of refractory BCR-ABL1-like ALL to appropriate TKI therapy, for example *EBF1-PDGFRB* ALL to imatinib (60). Ongoing studies are performing NGS of childhood and adult ALL to comprehensively identify all kinase-activating alterations in BCR-ABL1-like ALL, and to implement TKI therapy in clinical trials.

Hypodiploid ALL

Hypodiploidy with less than 44 chromosomes is observed in up to 3% of ALL cases and is associated with poor prognosis (24, 25, 61, 62). Two subtypes of hypodiploid ALL have been described according to the severity of aneuploidy: near-haploid cases with 24–31 chromosomes, and low hypodiploid cases with 32–39 cases (24, 25, 61). A recent study of over 120 hypodiploid ALL cases incorporating SNP and gene expression microarray analysis, candidate gene sequencing, and NGS (WGS, WES and mRNA-seq) of approximately 50 cases clearly demonstrated that near haploid and low hypodiploid ALL have distinct transcriptomic signatures and submicroscopic DNA copy number alterations and sequence mutations, which differ from other B-ALL subtypes (11). The majority of near haploid cases harbor mutations activating Ras signaling (*NF1* in 40% of cases, but also *NRAS*, *KRAS* and *PTPN11*), and inactivating deletions and mutations of the IKAROS family gene *IKZF3* (AIOLOS). Low hypodiploid cases have near universal mutation of the tumor suppressor *TP53*, and inactivating mutations of a third IKAROS family member *IKZF2* (HELIOS). Hypodiploid cells from both near-haploid and low hypodiploid cases exhibit activation of Ras-Raf-MEK-ERK and phosphatidylinositol-3-OH kinase (PI3K) signaling that is sensitive to PI3K and PI3K/mTOR inhibitors, suggesting that PI3K inhibition represents a novel therapeutic approach. An unexpected finding was that the *TP53* sequence mutations identified in low hypodiploid ALL are commonly present in matched non-tumor cells, suggesting germline inheritance. This has been confirmed in a limited number of kindreds, indicating that low hypodiploid ALL is a manifestation of Li-Fraumeni syndrome (11, 63). Additional deleterious germline mutations were identified in other hypodiploid ALL cases, including activating mutations of *NRAS* and *PTPN11*. Thus, detailed analysis of the role of inherited mutations in the pathogenesis will be of interest. This is supported by a recent study identifying a germline *PAX5* mutation in two kindreds with autosomal dominant pre-B ALL (64).

B-progenitor ALL with intrachromosomal amplification of chromosome 21 (iAMP21)

iAMP21 is characterized by gain of at least 3 copies of a (usually large) region of 21 that always includes *RUNX1* (65–67). This gain is often complex with flanking regions of chromosomal loss, and is usually observed in patients lacking other key cytogenetic

alterations, although is also observed in *ETV6-RUNX1* ALL and BCR-ABL1-like ALL. The presence of iAMP21 is generally associated with an unfavorable outcome, although this can be mitigated with intensive chemotherapy (68). The nature of cooperating lesions and the role of iAMP21 in driving an aggressive leukemia are currently poorly understood.

ERG-altered ALL

While many of these alterations are enriched in specific cytogenetic ALL subtypes, a notable exception is alteration of the ETS-family transcription factor *ERG* (ETS-related gene), which exclusively occur in cases lacking known chromosomal rearrangements and are a hallmark of a novel subtype of B-ALL with a distinct gene expression profile (69). The *ERG* deletions involve an internal subset of exons resulting in loss of the central inhibitory and pointed domains, and expression of an aberrant C-terminal ERG fragment that retains the ETS and transactivation domains, which functions as a competitive inhibitor of wild-type ERG. Notably, despite the presence of *IKZF1* alterations in a proportion of *ERG*-deregulated cases, the outcome of this subtype of ALL is favorable (54).

T-lineage ALL

T-ALL is characterized by an older age of onset than B-ALL, male sex preponderance, and inferior outcome in comparison to B-ALL (20). To gain further insight into the genetic basis T-ALL, Ferrando and colleagues performing targeted capture and sequencing of X chromosome genes, and identified sequence mutations and deletions of *PHF6* in 16% and 38% of childhood and adult T-ALL, respectively (70). The *PHF6* alterations result in loss of *PHF6* expression and are associated with *TLX1/3* and *TAL1* rearranged ALL (70, 71). The role of *PHF6* in leukemogenesis is poorly understood, but has been shown to be a RNA-interacting protein and component of the nucleosome remodeling and deacetylation (NuRD) complex (72, 73). Thus, *PHF6* may have complex and multifactorial roles as a tumor suppressor.

Early T-cell precursor (ETP) ALL is an aggressive subtype of immature leukemia that is associated with very poor outcome (74–76). Various laboratory criteria have been proposed to identify these immature cases, but the original definition utilized immunophenotypic criteria: the expression of T-lineage markers (e.g. cytoplasmic CD3) but lack of expression of markers otherwise characteristic of T-ALL, such as CD1a and CD8, weak or negative CD5 expression, and aberrant expression of myeloid and/or stem cell markers (74). This pattern is reminiscent of the murine early T-cell precursor (77), the earliest stage of thymic T-cell maturation that retains lineage plasticity.

The first WGS study of a lymphoid malignancy performed WGS of tumor and matched non-tumor DNA of 12 ETP ALL cases and mutation recurrence testing of selected genes in 94 additional ETP and non-ETP T-ALL cases(10). Unexpectedly, there was marked diversity in the frequency and nature of genetic alterations, with several cases exhibiting complex multi-chromosomal structural alterations with the hallmarks of chromothripsis (78), but no common genomic alteration identified in all cases. This diversity notwithstanding, three pathways were frequently mutated, which have also been detected in parallel genomic profiling studies of T-ALL: hematopoietic development, cytokine receptor and Ras signaling, and chromatin modification (47, 79–85). Loss-of-function alterations in genes encoding regulators of hematopoietic development are present in two-thirds of ETP T-ALL cases, and most commonly involve *ETV6*, *GATA3*, *IKZF1* and *RUNX1*. It is notable that many of these genes are known targets of mutation and rearrangement in other subtypes of ALL and AML. Similarly deletions, mutations and translocations of these genes were observed in ETP ALL, emphasizing the need for detailed analysis of both structural and sequence alterations to fully define the nature of genetic alterations in hematologic

malignancies. Activating mutations in cytokine receptor and Ras signaling were also present in the majority of cases, including *NRAS*, *KRAS*, *FLT3*, *JAK1*, *JAK3* and *IL7R*. The mutations in Ras, FLT3 and JAK1/3 were similar to those previously reported in leukemia. Several groups concomitantly described activating mutations of IL7R, encoding the alpha chain of the interleukin 7 receptor in T-ALL, and less commonly in B-ALL (81, 82). The mutations are usually complex inframe insertion mutations that introduce a cysteine in the transmembrane domain of IL7R, resulting in dimerization of the receptor and constitutive activation of JAK-STAT signaling in the absence of ligand. In cell lines and primary mouse bone marrow, the IL7R mutations induce cytokine independent proliferation and activation of JAK-STAT signaling that is abrogated by JAK inhibitors such as ruxolitinib (10). Although IL7R mutations are only present in a proportion of ETP ALL cases, evidence of JAK-STAT activation on phosphoflow cytometry or gene expression profiling is present in the majority of cases, suggesting that JAK inhibitors are a rational therapeutic strategy in this high risk leukemia.

An unexpected finding was a high frequency of mutations of epigenetic regulators in ETP ALL. Most common were mutations or deletions of genes encoding components of the polycomb repressor complex 2 (PRC2; *EZH2*, *SUZ12*, *EED*) that normally mediates histone 3 lysine 27 (H3K27) trimethylation. The most common target of alteration was *EZH2* which encodes the catalytic component of PRC2, and contains an MLL-like SET domain that mediates H3K27 methylation. Recurring *EZH2* SET domain p.Tyr641 mutations are characteristic of lymphoma (86). This mutation causes a subtle conformational change in the SET domain that enhances di- and tri-methylation, and is a gain-of-function alteration (87, 88). In contrast, this mutation is not observed in ETP ALL, rather a range of deleterious mutations in the SET domain and elsewhere in *EZH2* are observed that are predicted to be loss-of-function. In support of this observation, loss of *Ezh2* promotes the development of T-ALL in experimental models (89). *EZH2* and PRC2 also interact with the histone methyltransferase DNMT3A, which is mutated in adult AML (90) and adult ETP ALL (91), but not childhood ETP ALL (unpublished data). Also, MLL-rearranged leukemia is dependent on *EZH2* activity for tumor maintenance (92, 93). Thus, perturbation of PRC2-mediated chromatin modification and transcriptional regulation is a hallmark of multiple hematologic malignancies, but targeting of this pathway may require enhancement (e.g. ETP ALL) or inhibition (lymphoma, MLL-rearranged leukemia) depending on the context and nature of mutation (94). Additional recurring epigenetic targets alteration included *SETD2*, encoding a histone 3 lysine 36 trimethylase, and the histone acetyltransferase and CREBBP homolog EP300 (p300). Additional new targets of mutation were identified including *DNM2*, *ECT2L* and *RELN*, and several genes – and indeed specific somatic mutations – were identified that had previously been reported as germline mutations in inherited developmental disorders, notably those in the zinc finger domain of the hematopoietic transcription factor gene *GATA3*. The mutational spectrum of ETP ALL is similar to that observed in myeloid leukemias, and the transcriptional profile of ETP ALL is similar to that of normal and malignant human hematopoietic stem cells and myeloid progenitors, but *not* the normal human early T-cell precursor (10). Thus, “early T-cell precursor” ALL is likely a misnomer, and ETP ALL may be more appropriately considered part of a spectrum of immature leukemias of variable and often ambiguous lineage.

Although similarly comprehensive studies of “typical” T-ALL are awaited, recent studies have performed exome sequencing of T-ALL, that have identified additional targets of mutation including *CNOT3*, a member of transcriptional regulatory complex, and ribosomal proteins (95).

Relapsed ALL

Several chromosomal alterations such as *BCR-ABL1* and *MLL*-rearrangement are associated with a high risk of treatment failure. However relapse occurs across the spectrum of ALL subtypes. It has also long been recognized that ALL genomes are not static but exhibit acquisition of chromosomal abnormalities over time (5). There is thus intense interest in genomic profiling of matched diagnosis and relapse samples to dissect the genetic basis of clonal heterogeneity in ALL, and the relationship of such heterogeneity to risk of relapse. SNP microarray profiling has demonstrated that the majority of ALL cases show changes in the patterns of structural genomic alterations from diagnosis to relapse (5, 96, 97), and that many relapse-acquired lesions, including *IKZF1*, and *CDKN2A/B* are present at low levels at diagnosis (96, 98). Sanger and NGS studies have begun to identify recurring mutations that influence drug sensitivity and risk of relapse.

Sequencing of 300 genes in matched diagnosis-relapse samples identified mutations in the transcriptional coactivator and histone and non-histone acetyl transferase CREBBP (CREB-binding protein, or CBP) as a relapse-acquired lesion in up to 20% of relapsed ALL samples (99, 100). CREBBP mutations are also observed at diagnosis in non-Hodgkin lymphoma, particularly diffuse large B-cell lymphoma (DLBCL) (101) and impair histone acetylation (101). CREBBP has an important role in mediating the transcriptional response to glucocorticoids (102, 103), and histone deacetylase inhibitors were active in steroid resistant ALL cell lines (99). Recently, two groups independently identified relapse-acquired mutations in the 5' nucleotidase gene *NT5C2* that confer increased resistance to purine analogues (104, 105). Thus, mutations that confer resistance to drugs commonly used to treat ALL represent a key mechanism of treatment failure and resistance.

CLINICAL IMPLEMENTATION AND FUTURE DIRECTIONS

The identification of genetic alterations that confer an increased risk of leukemogenesis (*TP53*, *PAX5* germline mutations), relapse (e.g. *IKZF1*, *CREBBP*, *NT5C2*) and intuitively “druggable” lesions (e.g. the kinase activating rearrangements in *BCR-ABL1*-like ALL) have focused attention on the transition of sequencing efforts into clinical diagnostics in ALL. This is being actively pursued in many centers, but a note of caution is warranted. Our understanding of the genetic basis of ALL is far from complete, and ongoing work is sequencing additional cases and subtypes to more fully define the nature of somatic genetic alterations in this disease. Moreover, many of these alterations are not amenable to many currently used genomic sequencing services, such as sequencing of exomes or panels of cancer genes, as they will not identify key structural alterations and rearrangements. At present, efforts are focused on implementing testing of specific genetic alterations using conventional molecular assays, and establishing comprehensive sequencing in reference centers. A major challenge will be the robust detection of variants present in minor subclones at diagnosis and early in therapy – akin to sensitive assays for minimal residual disease (MRD) – that will predict subsequent relapse. NGS approaches for the detection of MRD by sequencing antigen receptor rearrangements have been successfully developed (106), and thus there is no reason why these assays cannot be developed for the other recurring genetic alterations detected in ALL (107).

Thus far current work has largely focused on identifying somatic genetic alterations in the coding genome. An important future area of investigation is the nature of non-coding inherited and somatic genetic alterations in ALL, and their relationship to transcriptional and epigenetic signatures, and leukemogenesis. It is well established that each ALL subtype is characterized by a distinct cytosine methylation signature that is an important determinant of gene expression (108, 109), but thus far sequence level studies have not been reported. Experimental validation of these recurring genetic alterations in models of leukemia is also

an important task – but one that is now tractable as the nature of the ALL genome has now been revealed.

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Figure 1.
Subclassification of childhood ALL. Blue wedges refer to B-progenitor ALL, yellow to recently identified subtypes of B-ALL, and red wedges to T-lineage ALL

Figure 2.

Schema for the nature and timing of acquisitions of genetic alterations in the pathogenesis of B-ALL. It is likely that chromosomal rearrangements are acquired early in leukemogenesis, and drive transcriptional and epigenetic dysregulation and aberrant self-renewal. These lesions and/or secondary genetic alterations disrupt lymphoid development and result in an arrest in maturation. Additional genetic alterations target cellular pathways including cell cycle regulation, tumor suppression, cytokine receptor and kinase signaling, and chromatin modification. Diagnosis ALL samples are commonly clonally heterogeneous, and genetic alterations in minor clones may confer resistance to therapy and promote relapse. A similar schema can be proposed for T-ALL, where lesions targeting lymphoid development, self-renewal, and kinase signaling are also observed; and in which there are multiple targets of mutation of unknown role in leukemogenesis (e.g. *PHF6*, *WT1*).