



## Genomic analysis of acute lymphoblastic leukemia - insights from next generation sequencing

C. Mullighan

Department of Pathology, St Jude Children's Research Hospital  
Memphis, TN, USA

Hematology Education:  
the education program for the  
annual congress of the European  
Hematology Association

2012;6:1-8

A B S T R A C T

Acute lymphoblastic leukemia (ALL) remains the commonest childhood tumor and the leading cause of cancer death in children and young adults. Current therapies are mostly non-targeted and at maximal intensity, and novel approaches are required to improve cure rates and reduce toxicities. The last decade has witnessed tremendous activity in the use of high-resolution genomic profiling approaches to identify genetic alterations contributing to leukemogenesis and identify novel therapeutic targets. In particular, microarray profiling of structural genetic alterations, coupled with gene expression profiling and candidate gene sequencing has transformed ALL from a disease classified by karyotypic analysis to one defined by constellations of gross and submicroscopic structural alterations and sequence alterations. These novel alterations influence leukemogenesis, responsiveness to therapy and in several instances, have identified entirely new therapeutic approaches. Moreover, these studies have defined several novel disease entities. Second generation sequencing techniques are now being employed to comprehensively identify all genetic alterations in leukemogenesis and examine the interplay between structural genetic alterations, gene expression, and epigenetic alterations. While these studies are still in their infancy, they are already generating important insights into disease biology, and are likely to transform our understanding of ALL. Recent insights from these studies and prospects for the future will be discussed.

### The genetic basis of ALL

Acute lymphoblastic leukemia (ALL) is the commonest childhood tumor,<sup>1</sup> and while the majority of children with ALL enjoy long-term disease free survival, relapse remains a major problem, particularly in specific subgroups including infants and older individuals with ALL. Thus, while ALL is considered one of the “success stories” of modern cancer therapy, there remains an urgent need to understand better the biologic basis of leukemogenesis and the determinants of treatment failure and relapse, and ultimately, to identify new targets and approaches for therapy.

This review addresses the hypothesis that ALL is a genetic disease, and that in order to advance cure rates for ALL, a comprehensive understanding of the genetic basis of ALL is required. It is possible, and even likely, that some new treatments in ALL will derive from complementary or orthogonal approaches – such as high throughput gain- and loss-of-function and/or synthetic lethal screens – that may be performed with no knowledge of underlying tumor genetics.<sup>2</sup> However, the insights obtained from genome-wide profiling approaches in the last few years have transformed our understanding of the biology of ALL,<sup>3,4</sup> and have already resulted in the identification of novel prognostic markers and therapeutic targets,<sup>5-8</sup> as well as providing crucial new insights into other aspects of tumor biology of broad relevance, such as the nature of clonal heterogeneity,<sup>9</sup> tumor evolution,<sup>9-11</sup>

and the role of tumor genetics in the cancer stem cell hypothesis.<sup>12</sup> Next generation sequencing approaches may now be used to profile large number of tumor genomes at a tractable cost,<sup>13,14</sup> and are already providing important insights in acute leukemia not evident from “pre-sequencing” genetic profiling approaches.<sup>15-20</sup> Prior to discussing these findings, this review will describe the insights and limitations obtained from cytogenetic and array-based profiling studies.

### Cytogenetics of ALL

The majority of childhood B-progenitor and T-lineage ALL cases are characterized by gross numerical or structural genetic alterations, including high hyperdiploidy, hypodiploidy, and translocations that commonly activate, disrupt or perturb key tumor suppressors, transcription factors, oncogenes, and tyrosine kinases<sup>1,21,22</sup> (Figure 1). It is also well known that these alterations, with rare exception (*e.g.*, rearrangement of *MLL*) fail to induce leukemia alone in experimental models, and may be present years before the onset of leukemia, indicating that additional genetic or epigenetic alterations must be present. Moreover, the factors driving the acquisition of several of these alterations (*e.g.*, aneuploidy) and the mechanism by which these contribute to leukemogenesis are poorly understood. Thus, while detection of the cytogenetic alterations is a mainstay of ALL diag-

nosis and contemporary risk stratification approaches, they are unable to explain all aspects of disease biology.

## Microarray profiling of ALL

Initial studies profiling genomic aberrations in ALL used microarrays to examine transcriptional profiles of leukemic cells and demonstrated that known cytogenetic subtype has distinct transcriptional signatures; that unsupervised hierarchical clustering could identify new ALL subgroups; and that expression profiling could be used to predict cytogenetic subtypes and outcome; outcome.<sup>23-25</sup> A limitation of these data was that identification of novel submicroscopic genetic alterations and rearrangements in ALL driving these signatures was difficult, or impossible from interrogation of gene expression data alone.

These limitations, in part, were overcome with the advent of microarray approaches to profile structural genetic alterations in ALL, namely array-based comparative genomic hybridization and single nucleotide polymorphism (SNP) microarrays. Since 2007, multiple groups have performed detailed profiling of submicroscopic genetic alterations in large cohorts of both B-progenitor and T-lineage ALL. These studies have been reviewed in detail elsewhere<sup>26,27</sup> but key points are reviewed here.

### ALL genomes harbor multiple submicroscopic genetic alterations

The results of the microarray profiling studies performed by multiple groups are remarkably consistent – both B- and T-ALL genomes harbor focal recurring genetic alterations that often involve a single gene or few genes<sup>3,4,28-32</sup> (Table 1). While experimental data confirming a role for most of these alterations in ALL pathogenesis are limited, several observations suggest these lesions are important in leukemogenesis. Many of the genes involved encode proteins with key roles in lymphoid development (*e.g.*, *PAX5*, *IKZF1*, *EBF1*, *LMO2*), cell cycle regulation and tumor suppression (*CDKN2A/CDKN2B*, *PTEN*, *RB1*), and lymphoid signaling (*BTLA*, *CD200*, *TOX*). In addition, several genes are involved by multiple types of genetic alteration, including copy number alteration (with deletions being more common than amplification in ALL), translocation, and sequence mutation – examples include *PAX5*, *WT1*, and *PTEN*.

### ALL subtypes harbor distinct submicroscopic genetic alterations

MLL-rearranged leukemias harbor very few additional structural or sequence alterations.<sup>3,33</sup> In contrast, subtypes, such as *ETV6-RUNX1* ALL, and *BCR-ABL1* ALL, harbor more alterations, but the alterations are distinct between subtypes, notably with *IKZF1* alteration being a hallmark of *BCR-ABL1* lymphoid leukemia.

### DNA copy number alterations define novel subtypes of ALL

One quarter of childhood ALL cases and a higher proportion of adult cases lack a known recurring cytogenetic alteration. Detailed cytogenetic/fluorescence *in situ* hybridization and CGH/SNP array analyses have identified several novel subtypes of ALL. These include B-

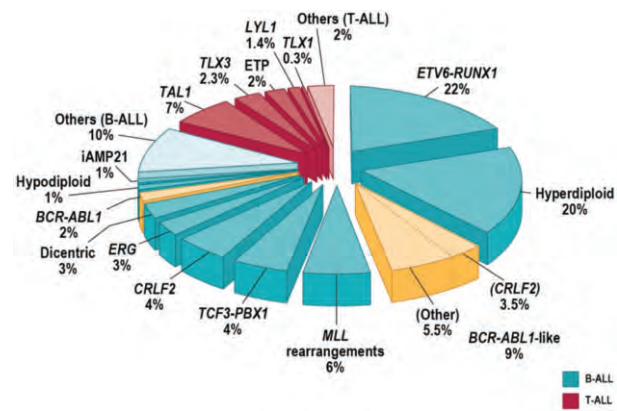


Figure 1. Frequency of cytogenetic subtypes of pediatric ALL.

progenitor ALL with intrachromosomal amplification of chromosome 21 (iAMP21), which is associated with poor prognosis;<sup>35-37</sup> B-ALL with deletion of the ETS family gene *ERG*;<sup>38</sup> and B-ALL with rearrangement of *CRLF2* (cytokine receptor-like factor 2, or *TSLPR*, thymic stromal lymphopoietin receptor).<sup>6,7,39</sup> The latter subtype of ALL is notable for being particularly common in Down syndrome associated ALL (DS-ALL), and in a subtype of ALL with a gene expression profile similar to that of *BCR-ABL1* ALL (“*BCR-ABL1*-like”, or “Ph-like” ALL).<sup>40</sup> Moreover, *CLRF2* alterations are commonly associated with activating<sup>41</sup> mutations in the Janus kinase genes *JAK1*, *JAK2*, and *JAK3* (most commonly at or near *JAK2* R683).<sup>6,7</sup>

### Genetic alterations influence prognosis in ALL

Despite the diversity of novel submicroscopic genetic alterations identified in ALL, remarkably few have been found to influence treatment responsiveness and outcome.<sup>40</sup> One potential explanation is that many of these studies have been underpowered and unable to detect important associations. An alternative explanation is that many alterations are important in establishing the leukemic clone, but have no subsequent role in influence treatment outcome. In contrast, other alterations may be important in leukemogenesis, but also have key roles in determining treatment outcome. Important examples of this are the alterations identified in the transcriptional regulation of B-lymphoid development, which are present in at least two-thirds of B-ALL cases.<sup>3,40</sup> The most commonly involved gene, *PAX5*, is targeted by deletions, focal amplifications, translocations, and sequence mutations in over one-third of ALL cases, and emerging data indicate that *PAX5* haploinsufficiency contributes to the pathogenesis of ALL.<sup>42,43</sup> However, *PAX5* alterations are not associated with poor outcome in B-ALL.<sup>40</sup> In contrast, deletions, or sequence mutations of *IKZF1*, a gene that encodes IKAROS, a zinc finger containing transcription factor essential for normal lymphoid development are less common than alterations of *PAX5*, but are associated with a dramatic increase in the risk of treatment failure and relapse in ALL.<sup>40,44</sup> Indeed, *IKZF1* alterations are associated with at least two subtypes of very high risk ALL: *BCR-ABL1* lymphoid leukemia (either *de novo* or at lymphoid

blast crisis of chronic myeloid leukemia (CML),<sup>4,34</sup> and BCR-ABL1-like ALL,<sup>40,45</sup> discussed below.

#### **ALL is commonly clonally heterogeneous**

It has been recognized for many years that genetic alterations in individual ALL patients may change over time, most notably from initial diagnosis to relapse.<sup>46</sup> However, these original studies performed using cytogenetic analysis were unable to perform a detailed analysis of focal genetic alterations, and recent SNP array profiling of matched diagnosis and relapsed ALL samples has clearly shown that the majority of ALL cases exhibit substantial changes in the nature of genetic alterations from diagnosis to relapse.<sup>9-11</sup> These serial profiling studies have also shown that relapse in ALL often arises from the emergence of a minor subclone at diagnosis that harbors distinct genetic alterations from the predominant clone at diagnosis. In some cases, the predominant clone at relapse is identical to that at diagnosis, or appears completely different. More frequently however, the relapse clone exhibits genetic evolution from the diagnostic clone, or shares only some of the genetic alterations present at diagnosis, while acquiring new changes. Coupled with lesion-specific PCR backtracking confirming the presence of these “relapse-acquired” lesions at diagnosis, these findings provide compelling evidence in support of

the hypothesis that at diagnosis, patients harbor multiple genetically distinct clones that share a common clonal origin, that then respond differently to selective pressure – here, anti-leukemic therapy. This has important clinical implications, as among the lesions that may not be detected at diagnosis, yet emerge at relapse are alterations of *IKZF1*. Thus, while many patients with *IKZF1* alteration at high risk of relapse may be identified by genomic profiling of bulk leukemic samples by standard approaches, others will require highly sensitive methods to identify these alterations (which may be deletions or mutations) at very low levels at the time of diagnosis. Moreover, the full range of genetic alterations contributing to relapse remains to be defined.

#### **Sequence mutations contribute to the risk of relapse in ALL**

Most recent studies of genetic alterations in ALL have used microarrays that have several important limitations: they do not provide nucleotide level resolution (current SNP arrays have 2-3 million features that provide 500-1000 base pair resolution); they do not directly identify chromosomal rearrangements (although they may detect focal DNA copy number alterations present at the break-points of chromosomal rearrangements); and they do not detect sequence mutations. Thus, how important are sequence mutations in ALL? Candidate gene sequencing

**Table 1. Key genetic alterations in B-progenitor ALL.**

Gene	Alteration	Frequency	Pathway and consequences of alteration	Clinical relevance	References
<i>PAX5</i>	Focal deletions, translocations, sequence mutations	31.7% of B-ALL	Transcription factor required for B-lymphoid development. Mutations impair DNA binding and transcriptional activation		3,4,28
<i>IKZF1</i>	Focal deletions or sequence mutations	15% of all pediatric B-ALL cases	Transcription factor required for development of HSC to lymphoid precursor. Deletions and mutations result in loss of function or dominant negative isoforms		3
		Over 80% <i>BCR-ABL1</i> ALL and 66% CML in lymphoid blast crisis		Associated with poor outcome	4,34,66
		One-third of high-risk <i>BCR-ABL1</i> negative ALL		Tripling in CIR	40,44,45
	Inherited variants			Increased risk of ALL	67,68
<i>JAK1/2</i>	Pseudokinase and kinase domain mutations	18-35% DS-ALL and	Constitutive JAK-STAT activation. Transforms mouse Ba/F3-EpoR lymphoid hematopoietic cell line		5,69-71
		10.7% High-risk <i>BCR-ABL1</i> -ALL			5
<i>CRLF2</i>	Rearrangement as <i>IGH@-CRLF2</i> or <i>P2RY8-CRLF2</i> resulting in overexpression	5-16% pediatric and adult B-ALL, and >50% DS-ALL	Associated with mutant <i>JAK</i> in up to 50% of cases. <i>CRLF2</i> mutations and <i>JAK</i> mutations cotransforming in Ba/F3 cells and results in constitutive STAT activation		6,7,39,72
		14% pediatric high-risk ALL	Associated with <i>IKZF1</i> alteration and <i>JAK</i> mutations	Associated with poor outcome	41,73
<i>CREBBP</i>	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	8,74

CIR, cumulative incidence of relapse; CML, chronic myeloid leukemia; DS, Down syndrome.

studies, in which genes have been selected for sequencing based on *a priori* knowledge of the role of a gene product in lymphoid development, tumorigenesis, or the knowledge that a gene is involved by structural genetic alterations have identified multiple targets of mutation in ALL (e.g., *PAX5*,<sup>3</sup> *IKZF1*,<sup>4</sup> *LEF1*, *WT1*,<sup>47</sup> *PTEN1*, *NFI*) but in general, have shown that DNA copy number alterations are more common than sequence mutation in ALL. Until recently, it has not been technically feasible to examine very large numbers of genes, or all coding genes, in large cohorts of ALL. However, several recent studies have performed extensive sequencing of candidate genes or chromosomal regions in ALL and have provided compelling evidence that sequence mutations are important in ALL, and that genome-wide sequencing is warranted.

To extend our studies of the genetic basis of relapse in ALL, we selected 300 genes for Sanger (PCR and capillary) sequencing in 24 matched B- and T-lineage ALL cases.<sup>8</sup> Genes were selected based on the presence of structural genetic alterations in individual genes or the pathways in which the genes function, and prior evidence from the literature and databases such as the Catalog of Somatic Mutations in Cancer (COSMIC). This identified multiple novel targets of somatic (tumor-acquired) sequence mutations, but several findings were notable. The frequency of sequence mutation was low (0-5 per case). In addition, as observed for DNA-copy number alterations, many presumed causal lesions present at diagnosis were no longer evident at relapse, including mutations in the Ras signaling pathway (*NRAS*, *KRAS*, *PTPN11*, and *NFI*) and B cell development (*PAX5*, although notably not *IKZF1*, deletions/mutations of which were always preserved at relapse, or acquired as new lesions). The singular most striking finding from this study was a high rate of deletion and/or mutation in *CREBBP*, encoding the transcriptional coactivators and acetyltransferase CREB binding protein (also known as CBP) in almost 20% of relapsed ALL cases. The mutations identified are enriched in the histone acetyltransferase (HAT) domain, and were shown to attenuate the normal HAT activity of murine Crebbp. Moreover, *CREBBP* mediates the transcriptional response to glucocorticoid therapy, and the mutations were shown to disrupt the normal transcriptional response to glucocorticoids. Thus, *CREBBP* mutations may represent an important mechanism underlying treatment failure in ALL, and may be targeted with agents that modulate the level of histone acetylation in leukemic cells, such as histone deacetylase inhibitors.

A second powerful example of the role of unbiased sequence analysis of ALL arises from the work of Adolfo Ferrando and colleagues, who sought to investigate the basis of the sex bias of T-lineage ALL, which exhibits a peak in incidence in older male children. This group captured coding DNA of genes located on the X chromosome in T-ALL followed by second generation massively parallel sequencing of captured DNA using the SOLiD platform.<sup>48</sup> This identified a high frequency of deleterious mutations in *PHF6*, which encodes PHD finger protein 6, a zinc finger containing putative transcriptional factor that is also mutated in the developmental disorder Borjeson-Forssman-Lehmann syndrome.<sup>49</sup> The role of *PHF6* alterations in T-cell leukemogenesis is unclear.

---

## Genome sequencing of acute lymphoblastic leukemia

These findings strongly suggest that sequencing the genome of childhood ALL is likely to identify additional important targets of inherited and/or somatic mutation. This is now feasible with the falling costs of second generation sequencing, in which hundreds of thousands of template molecules are sequenced simultaneously (massively parallel sequencing), resulting in the generation of gigabases of sequence per sequencing run. Next generation sequencing may be used to sequence a variety of nucleic acid templates to examine structural and coding variation, gene expression, and epigenetic alterations. Widely used approaches to examine coding variation in cancer include whole genome sequencing, transcriptomic sequencing, and exome sequencing. Exome sequencing uses solid or liquid phase baits to capture coding exons to profile coding variation selectively. As the exome represents only a small fraction of the genome, coding variation may be investigated in large numbers of samples for a fraction of the cost of whole genome sequencing. However, this approach is incapable of detecting all structural variations and translocations, which are a hallmark of ALL. Sequencing of messenger RNA (transcriptome sequencing or mRNA-seq) represents a powerful approach to identify chromosomal rearrangements in cancer, as well as providing sensitive quantitative estimates of gene expression and sequence mutation. Whole genome sequencing (WGS), in which the entire genome of a tumor (or non-tumor sample) is sequenced represents the most comprehensive platform for analyzing cancer genomes, but is the most demanding in terms of the amount of sequencing and downstream analysis. Moreover, while WGS provides a mechanism to identify all genetic alterations in cancer, coverage of the genome is not uniform and is influenced by factors, such as regional GC content and the methods used to construct sequencing libraries, perform sequencing, align data, and detect variations.

The utility of second generation sequencing approaches in leukemia have been clearly demonstrated in acute myeloid leukemia (AML), where several studies from the Washington University Genome Institute have identified novel mutations, notably those targeting epigenetic regulators *IDH1/2* and *DNMT3A* in AML, and more recently characterized the mutation spectrum during disease progression and evolution in myelodysplasia and relapsed acute myeloid leukemia.<sup>15-17,19</sup> Several groups are now pursuing WGS, exome sequencing, and mRNA-seq in a variety of subtypes of ALL, and the first results of these studies have recently been described.

---

## Whole genome sequencing of early T-cell precursor ALL

In 2009, Dario Campana and colleagues at St Jude described a subtype of T-ALL with an immature immunophenotype, with expression of cytoplasmic CD3, but lack of expression of CD1a and CD8, weak or absent expression of CD5, and the aberrant expression of stem cell and myeloid markers.<sup>50</sup> The gene expression profile



of these cases was distinctive and bore some similarity to the published gene expression profile of the most immature murine thymic T cell progenitor,<sup>51</sup> the early T-cell precursor, a stage of maturation that retains the ability to differentiate into non T-cell lineages. Hence this entity was named early T-cell precursor ALL (ETP ALL) and was notable for very poor outcome. Detailed microarray profiling and candidate gene sequencing analysis documented genomic instability, with frequent large deletions in a subset of ETP ALL cases, but otherwise no recurring chromosomal translocation or sequence mutation was identified. Indeed, ETP cases have a lower frequency of lesions that are hallmarks of non-ETP T-ALL, such as deletion of *CDKN2A/CDKN2B* (INK4/ARF) and activating mutations of *NOTCH1*.

To identify the genetic basis of ETP ALL, we performed WGS of tumor and matched non-tumor DNA from 12 children with ETP ALL, and coupled this with comprehensive validation of all putative somatic and structural variants identified by two parallel analysis pipelines.<sup>18</sup> Novel, recurrent, and known genetic alterations were also examined in a recurrence cohort of 54 ETP and 42 non-ETP ALL cases by SNP arrays and Sanger sequencing. The findings of this study, the first whole genome sequencing study of ALL, were striking. Despite the homogeneity in many of the clinicopathologic features of ETP ALL, we did not identify a single unifying genetic alteration, such as a recurring translocation. In contrast, there was a marked diversity in the nature and frequency of somatic sequence and structural alterations in ALL. Some cases had few or no somatic structural variants, whereas other cases had a high burden of SVs, several of which had the complex pattern of rearrangements suggestive of a single cellular catastrophe termed “chromothripsis”.<sup>52</sup> Notable, two cases with this pattern had concomitant sequence mutations of genes involved in DNA repair, suggesting a causal link between these alterations and chromothripsis.

Despite this genetic diversity, several themes emerged. ETP ALL is enriched for mutations in three pathways: loss of function alterations in genes regulating hematopoietic and lymphoid development, activating mutations driving Ras and/or cytokine receptor/JAK-STAT signaling, and inactivating mutations in histone modifying genes. Several groups have also identified mutations in several of these genes/and pathways using non-WGS approaches.<sup>53,54</sup> Fifty-eight percent of ETP cases (compared with 17% of non-ETP T-ALL cases) harbored loss of function or dominant negative mutations in developmental genes, including *RUNX1*, *IKZF1*, *ETV6*, *GATA3*, and *EP300*. Several of these genes are well-credentialled targets of mutation in hematopoietic malignancies (e.g., *RUNX1* in myeloid disorders and *ETV6* and *IKZF1* in B-progenitor ALL) but have not previously been implicated in T-ALL. The mutations in *GATA3* were novel and are logical, as *GATA3* regulates egress from the ETP stage of T lymphoid development to later stages of T cell maturation.<sup>55</sup> The mutations identified were clustered in one of the DNA binding zinc fingers, have previously identified in a multiorgan inherited developmental disorder, and shown to impair binding of *GATA3* to its DNA targets.<sup>56</sup> Mutations known or predicted to activate signaling were identified in 67% of cases (compared with 19% of non-ETP T-ALL cases), including known activating

mutations in *NRAS*, *KRAS*, *JAK1*, *NF1*, and *PTPN11*, as well as novel mutations in *JAK3*, *SH2B3* (encoding LNK, a negative regulator of JAK2 signaling)<sup>57,61</sup> and *IL7R*, encoding the interleukin 7 receptor. Several groups have now identified mutations in *IL7R* in both T-lineage and B-progenitor ALL.<sup>62,63</sup> The mutations clustered in highly conserved residues in the transmembrane domain are frequently complex but always inframe, and usually introduce a cysteine that results in constitutive dimerization. The mutations result in constitutive JAK-STAT activation and transformation of model cell lines to IL-7 independent proliferation, and importantly, this transformation is abrogated by pharmacologic JAK inhibitors, such as ruxolitinib, currently in use for other JAK-mutated disorders, suggesting that patients harboring *IL7R* mutations may be candidates for JAK inhibitor therapy.

An unexpected finding was a high frequency of mutations in epigenetic regulators in ETP ALL, a finding confirmed by other studies. The most frequent targets of mutation were genes encoding regulators of the polycomb repressor complex 2 (PRC2) a H3K27 trimethylase that normally induces transcriptional repression and antagonizes the transcriptional activating effects of MLL, a target of rearrangement in ALL and AML, and an H3K4 trimethylase.<sup>64</sup> The most commonly mutated gene was *EZH2*, which encodes the catalytic component of the complex. *EZH2* is also mutated in follicular lymphoma, but in contrast to the highly recurrent Y641 mutations observed in FL that are gain of function,<sup>65</sup> the mutations in T-ALL occur in other sites in *EZH2* and are predicted to disrupt the catalytic SET domain and result in loss of function.

Although several of the targets of mutation are novel, the pathways – hematopoietic development, signaling and epigenetic regulation – are also frequently mutated in AML. In addition, detailed comparison of the transcriptional profile of ETP ALL to normal and leukemic hematopoietic progenitors demonstrated highly significant similarity to normal hematopoietic stem cells and granulocyte macrophage progenitor cells, as well as high risk AML and ALL signatures, but *not* the normal human ETP. Together, these genetic and transcriptomic findings suggest that ETP arises from transformation of an immature hematopoietic stem or progenitor cell, and exhibits greater similarity with AML cells than previously suspected. These findings have important implications for how therapy for ETP ALL might be improved, for example with the addition of AML-directed therapy, the use of targeted therapies against Janus kinases, or epigenetic modulators. In addition, ongoing studies examining the genetics and biology of other leukemias of ambiguous or immature lineage (e.g., biphenotypic and bilineal leukemia) will be of great interest.

### Next generation sequencing of BCR-ABL1-like ALL

Up to 15% of childhood B-progenitor ALL cases lack a previously identified chromosomal rearrangement, but exhibit a gene expression profile highly similar to that of BCR-ABL1-positive ALL, and often have deletion/mutation of *IKZF1*, which is also common in BCR-ABL1-ALL.<sup>40,45</sup> These cases commonly have a dismal prognosis with current combination chemotherapy, and novel thera-

peutic approaches are urgently required. Pre-NGS approaches using microarrays and Sanger sequencing identified *CRLF2* rearrangement/mutation and JAK mutations in up to half of “BCR-ABL1-like” ALL cases, but the kinase-activating alterations in the remaining cases were unknown.

In a collaborative study between the US National Cancer Institute TARGET (Therapeutically Applicable Research to Generate Effective Treatments) initiative and multiple North American Institutions, we performed second generation transcriptome sequencing (mRNA-seq) and selected WGS in 12 BCR-ABL1-like cases, and identified a range of novel rearrangements, copy number alterations, and sequence mutations activating kinase signaling in all cases.<sup>20</sup> These included rearrangements of *PDGFRB*, *ABL1*, *JAK2*, and *EPOR*, as well as deletion/mutation of *SH2B3* and the *IL7R*. Several of these alterations were studied in *in vitro* model systems and shown to activate JAK-STAT signaling and result in transformation that was attenuated with JAK or ABL1/PDGFRB inhibitors. These results are preliminary and much work remains to be done, including defining the full range of kinase activating lesions in BCR-ABL1-like ALL and examining the role of kinase inhibitors in patients bearing these lesions, but these results provide compelling initial data to support that these lesions drive leukemogenesis, and that pathway-based screening should be pursued at the time of diagnosis to identify patients with Ph-like ALL and identify those patients that may benefit from kinase inhibitor therapy.

## Conclusions

The application of molecular profiling approaches continues to transform our understanding of the genetic basis of childhood acute lymphoblastic leukemia. The studies described above clearly emphasize the utility – but also limitations of – microarray and candidate gene sequencing approaches, and provide proof of principle that next generation sequencing is required to identify the full repertoire of genetic alterations driving leukemogenesis and treatment responsiveness in ALL. Thus far, very few NGS analysis in ALL has been reported, but multiple large efforts are in progress. At present, most studies are focused on characterizing non-silent genetic variation in leukemic genomes, but future studies characterizing both coding and non-coding variation, and examining the relationship between genetic alterations, gene expression, and epigenetic variations will be of great interest.

## References

- Pui CH, Robison LL, Look AT. Acute lymphoblastic leukaemia. *Lancet* 2008;371:1030-43.
- Mullenders J, Bernards R. Loss-of-function genetic screens as a tool to improve the diagnosis and treatment of cancer. *Oncogene* 2009;28:4409-20.
- Mullighan CG, Goorha S, Radtke I, Miller CB, Coustan-Smith E, Dalton JD, et al. Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. *Nature* 2007;446:758-64.
- Mullighan CG, Miller CB, Radtke I, Phillips LA, Dalton J, Ma J, et al. BCR-ABL1 lymphoblastic leukaemia is characterized by the deletion of *Ikaros*. *Nature* 2008;453:110-4.
- Mullighan CG, Zhang J, Harvey RC, Collins-Underwood JR, Schulman BA, Phillips LA, et al. JAK mutations in high-risk childhood acute lymphoblastic leukemia. *Proc Natl Acad Sci U S A* 2009;106:9414-8.
- Russell LJ, Capasso M, Vater I, Akasaka T, Bernard OA, Calasanz MJ, et al. Deregulated expression of cytokine receptor gene, *CRLF2*, is involved in lymphoid transformation in B-cell precursor acute lymphoblastic leukemia. *Blood* 2009;114:2688-98.
- Mullighan CG, Collins-Underwood JR, Phillips LA, Loudin MG, Liu W, Zhang J, et al. Rearrangement of *CRLF2* in B-progenitor- and Down syndrome-associated acute lymphoblastic leukemia. *Nat Genet* 2009;41:1243-6.
- Mullighan CG, Zhang J, Kasper LH, Lerach S, Payne-Turner D, Phillips LA, et al. CREBBP mutations in relapsed acute lymphoblastic leukaemia. *Nature* 2011;471:235-9.
- Mullighan CG, Phillips LA, Su X, Ma J, Miller CB, Shurtleff SA, et al. Genomic analysis of the clonal origins of relapsed acute lymphoblastic leukemia. *Science* 2008;322:1377-80.
- Yang JJ, Bhojwani D, Yang W, Cai X, Stocco G, Crews K, et al. Genome-wide copy number profiling reveals molecular evolution from diagnosis to relapse in childhood acute lymphoblastic leukemia. *Blood* 2008;112:4178-83.
- Kawamata N, Ogawa S, Seeger K, Kirschner-Schwabe R, Huynh T, Chen J, et al. Molecular allelotyping of relapsed pediatric acute lymphoblastic leukemia. *Int J Oncol* 2009;34:1603-12.
- Notta F, Mullighan CG, Wang JC, Poepl A, Doulatov S, Phillips LA, et al. Evolution of human BCR-ABL1 lymphoblastic leukaemia-initiating cells. *Nature* 2011;469:362-7.
- Meyerson M, Gabriel S, Getz G. Advances in understanding cancer genomes through second-generation sequencing. *Nat Rev Genet* 2010;11:685-96.
- Mardis ER, Wilson RK. Cancer genome sequencing: a review. *Hum Mol Genet* 2009;18:R163-8.
- Ley TJ, Mardis ER, Ding L, Fulton B, McLellan MD, Chen K, et al. DNA sequencing of a cytogenetically normal acute myeloid leukaemia genome. *Nature* 2008;456:66-72.
- Mardis ER, Ding L, Dooling DJ, Larson DE, McLellan MD, Chen K, et al. Recurring mutations found by sequencing an acute myeloid leukemia genome. *N Engl J Med* 2009;361:1058-66.
- Ley TJ, Ding L, Walter MJ, McLellan MD, Lamprecht T, Larson DE, et al. DNMT3A mutations in acute myeloid leukemia. *N Engl J Med* 2010;363:2424-33.
- Zhang J, Ding L, Holmfeldt L, Wu G, Heatley SL, Payne-Turner D, et al. The genetic basis of early T-cell precursor acute lymphoblastic leukaemia. *Nature* 2012;481:157-63.
- Ding L, Ley TJ, Larson DE, Miller CA, Koboldt DC, Welch JS, et al. Clonal evolution in relapsed acute myeloid leukaemia revealed by whole-genome sequencing. *Nature* 2012;481:506-10.
- Roberts KG, Morin RD, Zhang J, Hirst M, Harvey RC, Kasap C, et al. Novel Chromosomal Rearrangements and Sequence Mutations in High-Risk Ph-Like Acute Lymphoblastic Leukemia. *ASH Annual Meeting Abstracts* 2011;118:67.
- Pui CH, Carroll WL, Meshinchi S, Arceci RJ. Biology, risk stratification, and therapy of pediatric acute leukemias: an update. *J Clin Oncol* 2011;29:551-65.
- Harrison CJ. Acute lymphoblastic leukemia. *Clin Lab Med* 2011;31:631-47.
- Golub TR, Slonim DK, Tamayo P, Huard C, Gaasenbeek M, Mesirov JP, et al. Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. *Science* 1999;286:531-7.
- Ross ME, Zhou X, Song G, Shurtleff SA, Girtman K, Williams WK, et al. Classification of pediatric acute lymphoblastic leukemia by gene expression profiling. *Blood* 2003;102:2951-9.
- Yeoh EJ, Ross ME, Shurtleff SA, Williams WK, Patel D, Mahfouz R, et al. Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene expression profiling. *Cancer Cell* 2002;1:133-43.
- Mullighan CG, Downing JR. Genome-wide profiling of genetic alterations in acute lymphoblastic leukemia: recent insights and future directions. *Leukemia* 2009;23:1209-18.
- Collins-Underwood JR, Mullighan CG. Genomic profiling of high-risk acute lymphoblastic leukemia. *Leukemia* 2010;24:1676-85.
- Kuiper RP, Schoenmakers EF, van Reijmersdal SV, Hehir-Kwa JY, van Kessel AG, van Leeuwen FN, et al. High-resolution genomic profiling of childhood ALL reveals novel recurrent genetic lesions affecting pathways involved in lymphocyte differentiation and cell cycle progression. *Leukemia* 2007;21:1258-66.
- Kawamata N, Ogawa S, Zimmermann M, Kato M, Sanada M,

- Hemminki K, et al. Molecular allelokaryotyping of pediatric acute lymphoblastic leukemias by high-resolution single nucleotide polymorphism oligonucleotide genomic microarray. *Blood* 2008;111:776-84.
30. Strefford JC, Worley H, Barber K, Wright S, Stewart AR, Robinson HM, et al. Genome complexity in acute lymphoblastic leukemia is revealed by array-based comparative genomic hybridization. *Oncogene* 2007;26:4306-18.
  31. Van Vlierberghe P, Homminga I, Zuurbier L, Gladdines-Buijs J, van Wering ER, Horstmann M, et al. Cooperative genetic defects in TLX3 rearranged pediatric T-ALL. *Leukemia* 2008;22:762-70.
  32. van Vlierberghe P, Meijerink JP, Lee C, Ferrando AA, Look AT, van Wering ER, et al. A new recurrent 9q34 duplication in pediatric T-cell acute lymphoblastic leukemia. *Leukemia* 2006;20:1245-53.
  33. Bardini M, Spinelli R, Bungaro S, Mangano E, Corral L, Cifola I, et al. DNA copy-number abnormalities do not occur in infant ALL with t(4;11)/MLL-AF4. *Leukemia* 2010;24:169-76.
  34. Iacobucci I, Storlazzi CT, Cilloni D, Lonetti A, Ottaviani E, Soverini S, et al. Identification and molecular characterization of recurrent genomic deletions on 7p12 in the IKZF1 gene in a large cohort of BCR-ABL1-positive acute lymphoblastic leukemia patients: on behalf of Gruppo Italiano Malattie Ematologiche dell'Adulto Acute Leukemia Working Party (GIMEMA AL WP). *Blood* 2009;114:2159-67.
  35. Strefford JC, van Delft FW, Robinson HM, Worley H, Yiannikouris O, Selzer R, et al. Complex genomic alterations and gene expression in acute lymphoblastic leukemia with intrachromosomal amplification of chromosome 21. *Proc Natl Acad Sci U S A* 2006;103:8167-72.
  36. Moorman AV, Richards SM, Robinson HM, Strefford JC, Gibson BE, Kinsey SE, et al. Prognosis of children with acute lymphoblastic leukemia (ALL) and intrachromosomal amplification of chromosome 21 (iAMP21). *Blood*. 2007;109:2327-30.
  37. Rand V, Parker H, Russell LJ, Schwab C, Ensor H, Irving J, et al. Genomic characterization implicates iAMP21 as a likely primary genetic event in childhood B-cell precursor acute lymphoblastic leukemia. *Blood* 2011;117:6848-55.
  38. Mullighan CG, Miller CB, Su X, Radtke I, Dalton J, Song G, et al. ERG Deletions Define a Novel Subtype of B-Progenitor Acute Lymphoblastic Leukemia. *ASH Annual Meeting Abstracts* 2007;110:691.
  39. Yoda A, Yoda Y, Chiaretti S, Bar-Natan M, Mani K, Rodig SJ, et al. Functional screening identifies CRLF2 in precursor B-cell acute lymphoblastic leukemia. *Proc Natl Acad Sci U S A* 2010;107:252-7.
  40. Mullighan CG, Su X, Zhang J, Radtke I, Phillips LA, Miller CB, et al. Deletion of IKZF1 and Prognosis in Acute Lymphoblastic Leukemia. *N Engl J Med* 2009;360:470-80.
  41. Harvey RC, Mullighan CG, Chen IM, Wharton W, Mikhail FM, Carroll AJ, et al. Rearrangement of CRLF2 is associated with mutation of JAK kinases, alteration of IKZF1, Hispanic/Latino ethnicity, and a poor outcome in pediatric B-progenitor acute lymphoblastic leukemia. *Blood* 2010;115:5312-21.
  42. Miller CB, Mullighan CG, Su X, Ma J, Wang M, Zhang J, et al. Pax5 Haploinsufficiency Cooperates with BCR-ABL1 to Induce Acute Lymphoblastic Leukemia. *ASH Annual Meeting Abstracts* 2008;112:293.
  43. Dang J, Mullighan CG, Phillips LA, Mehta P, Downing JR. Retroviral and Chemical Mutagenesis Identifies Pax5 as a Tumor Suppressor in B-Progenitor Acute Lymphoblastic Leukemia. *ASH Annual Meeting Abstracts* 2008;112:1789.
  44. Kuiper RP, Waanders E, van der Velden VH, van Reijmersdal SV, Venkatachalam R, Scheijen B, et al. IKZF1 deletions predict relapse in uniformly treated pediatric precursor B-ALL. *Leukemia* 2010;24:1258-64.
  45. Den Boer ML, van Slegtenhorst M, De Menezes RX, Cheok MH, Buijs-Gladdines JG, Peters ST, et al. A subtype of childhood acute lymphoblastic leukaemia with poor treatment outcome: a genome-wide classification study. *Lancet Oncol* 2009;10:125-34.
  46. Raimondi SC, Pui CH, Head DR, Rivera GK, Behm FG. Cytogenetically different leukemic clones at relapse of childhood acute lymphoblastic leukemia. *Blood* 1993;82:576-80.
  47. Tosello V, Mansour MR, Barnes K, Paganin M, Sulis ML, Jenkinson S, et al. WT1 mutations in T-ALL. *Blood* 2009;114:1038-45.
  48. Van Vlierberghe P, Palomero T, Khiabani H, Van der Meulen J, Castillo M, Van Roy N, et al. PHF6 mutations in T-cell acute lymphoblastic leukemia. *Nat Genet* 2010;42:338-42.
  49. Chao MM, Todd MA, Kontny U, Neas K, Sullivan MJ, Hunter AG, et al. T-cell acute lymphoblastic leukemia in association with Borjeson-Forssman-Lehmann syndrome due to a mutation in PHF6. *Pediatr Blood Cancer* 2010;55:722-4.
  50. Coustan-Smith E, Mullighan CG, Onciu M, Behm FG, Raimondi SC, Pei D, et al. Early T-cell precursor leukaemia: a subtype of very high-risk acute lymphoblastic leukaemia. *Lancet Oncol* 2009;10:147-56.
  51. Rothenberg EV, Moore JE, Yui MA. Launching the T-cell-lineage developmental programme. *Nat Rev Immunol* 2008;8:9-21.
  52. Stephens PJ, Greenman CD, Fu B, Yang F, Bignell GR, Mudie LJ, et al. Massive genomic rearrangement acquired in a single catastrophic event during cancer development. *Cell* 2011;144:27-40.
  53. Ntziachristos P, Tzirigos A, Vlierberghe PV, Nedjic J, Trimarchi T, Flaherty MS, et al. Genetic inactivation of the polycomb repressive complex 2 in T cell acute lymphoblastic leukemia. *Nat Med* 2012;18:298-303.
  54. Van Vlierberghe P, Ambesi-Impiomato A, Perez-Garcia A, Haydu JE, Rigo I, Hadler M, et al. ETV6 mutations in early immature human T cell leukemias. *J Exp Med* 2011;208:2571-9.
  55. Hosoya T, Maillard I, Engel JD. From the cradle to the grave: activities of GATA-3 throughout T-cell development and differentiation. *Immunol Rev* 2010;238:110-25.
  56. Zahirieh A, Nesbit MA, Ali A, Wang K, He N, Stangou M, et al. Functional analysis of a novel GATA3 mutation in a family with the hypoparathyroidism, deafness, and renal dysplasia syndrome. *J Clin Endocrinol Metab* 2005;90:2445-50.
  57. Baran-Marszak F, Magdoud H, Desterke C, Alvarado A, Roger C, Harel S, et al. Expression level and differential JAK2-V617F-binding of the adaptor protein Lnk regulates JAK2-mediated signals in myeloproliferative neoplasms. *Blood* 2010;116:5961-71.
  58. Bersenev A, Wu C, Balcerak J, Jing J, Kundu M, Blobel GA, et al. Lnk constrains myeloproliferative diseases in mice. *J Clin Invest* 2010;120:2058-69.
  59. Lasho TL, Pardanani A, Tefferi A. LNK mutations in JAK2 mutation-negative erythrocytosis. *N Engl J Med* 2010;363:1189-90.
  60. Oh ST, Simonds EF, Jones C, Hale MB, Goltsev Y, Gibbs KD, Jr., et al. Novel mutations in the inhibitory adaptor protein LNK drive JAK-STAT signaling in patients with myeloproliferative neoplasms. *Blood* 2010;116:988-92.
  61. Pardanani A, Lasho T, Finke C, Oh ST, Gotlib J, Tefferi A. LNK mutation studies in blast-phase myeloproliferative neoplasms, and in chronic-phase disease with TET2, IDH, JAK2 or MPL mutations. *Leukemia* 2010;24:1713-8.
  62. Shochat C, Tal N, Bandapalli OR, Palmi C, Ganmore I, te Kronnie G, et al. Gain-of-function mutations in interleukin-7 receptor- $\alpha$  (IL7R) in childhood acute lymphoblastic leukemias. *J Exp Med* 2011;208:901-8.
  63. Zenatti PP, Ribeiro D, Li W, Zuurbier L, Silva MC, Paganin M, et al. Oncogenic IL7R gain-of-function mutations in childhood T-cell acute lymphoblastic leukemia. *Nat Genet* 2011;43:932-9.
  64. Margueron R, Reinberg D. The Polycomb complex PRC2 and its mark in life. *Nature* 2011;469:343-9.
  65. Morin RD, Johnson NA, Severson TM, Mungall AJ, An J, Goya R, et al. Somatic mutations altering EZH2 (Tyr641) in follicular and diffuse large B-cell lymphomas of germinal-center origin. *Nat Genet* 2010;42:181-5.
  66. Martinelli G, Iacobucci I, Storlazzi CT, Vignetti M, Paoloni F, Cilloni D, et al. IKZF1 (Ikaros) deletions in BCR-ABL1-positive acute lymphoblastic leukemia are associated with short disease-free survival and high rate of cumulative incidence of relapse: a GIMEMA AL WP report. *J Clin Oncol* 2009;27:5202-7.
  67. Trevino LR, Yang W, French D, Hunger SP, Carroll WL, Devidas M, et al. Germline genomic variants associated with childhood acute lymphoblastic leukemia. *Nat Genet* 2009;41:1001-5.
  68. Papaemmanuil E, Hosking FJ, Vijayakrishnan J, Price A, Olver B, Sheridan E, et al. Loci on 7p12.2, 10q21.2 and 14q11.2 are associated with risk of childhood acute lymphoblastic leukemia. *Nat Genet* 2009;41:1006-10.
  69. Bercovich D, Ganmore I, Scott LM, Wainreb G, Birger Y, Elimelech A, et al. Mutations of JAK2 in acute lymphoblastic leukaemias associated with Down's syndrome. *Lancet* 2008;372:1484-92.
  70. Gaikwad A, Rye CL, Devidas M, Heerema NA, Carroll AJ, Izraeli S, et al. Prevalence and clinical correlates of JAK2



- mutations in Down syndrome acute lymphoblastic leukaemia. *Br J Haematol* 2009;144:930-2.
71. Kearney L, Gonzalez De Castro D, Yeung J, Procter J, Horsley SW, Eguchi-Ishimae M, et al. A specific JAK2 mutation (JAK2R683) and multiple gene deletions in Down syndrome acute lymphoblastic leukaemia. *Blood* 2008;113:646-8.
  72. Hertzberg L, Vendramini E, Ganmore I, Cazzaniga G, Schmitz M, Chalker J, et al. Down syndrome acute lymphoblastic leukemia: a highly heterogeneous disease in which aberrant expression of CRLF2 is associated with mutated JAK2: a report from the iBFM Study Group. *Blood* 2010;115: 1006-17.
  73. Cario G, Zimmermann M, Romey R, Gesk S, Vater I, Harbott J, et al. Presence of the P2RY8-CRLF2 rearrangement is associated with a poor prognosis in non-high-risk precursor B-cell acute lymphoblastic leukemia in children treated according to the ALL-BFM 2000 protocol. *Blood* 2010;115:5393-7.
  74. Pasqualucci L, D. D-S, Chiarenza A, Fabbri G, Grunn A, Trifonov V, et al. Inactivating mutations of acetyltransferase genes in B-cell lymphoma. *Nature* 2011;10;471:189-95.