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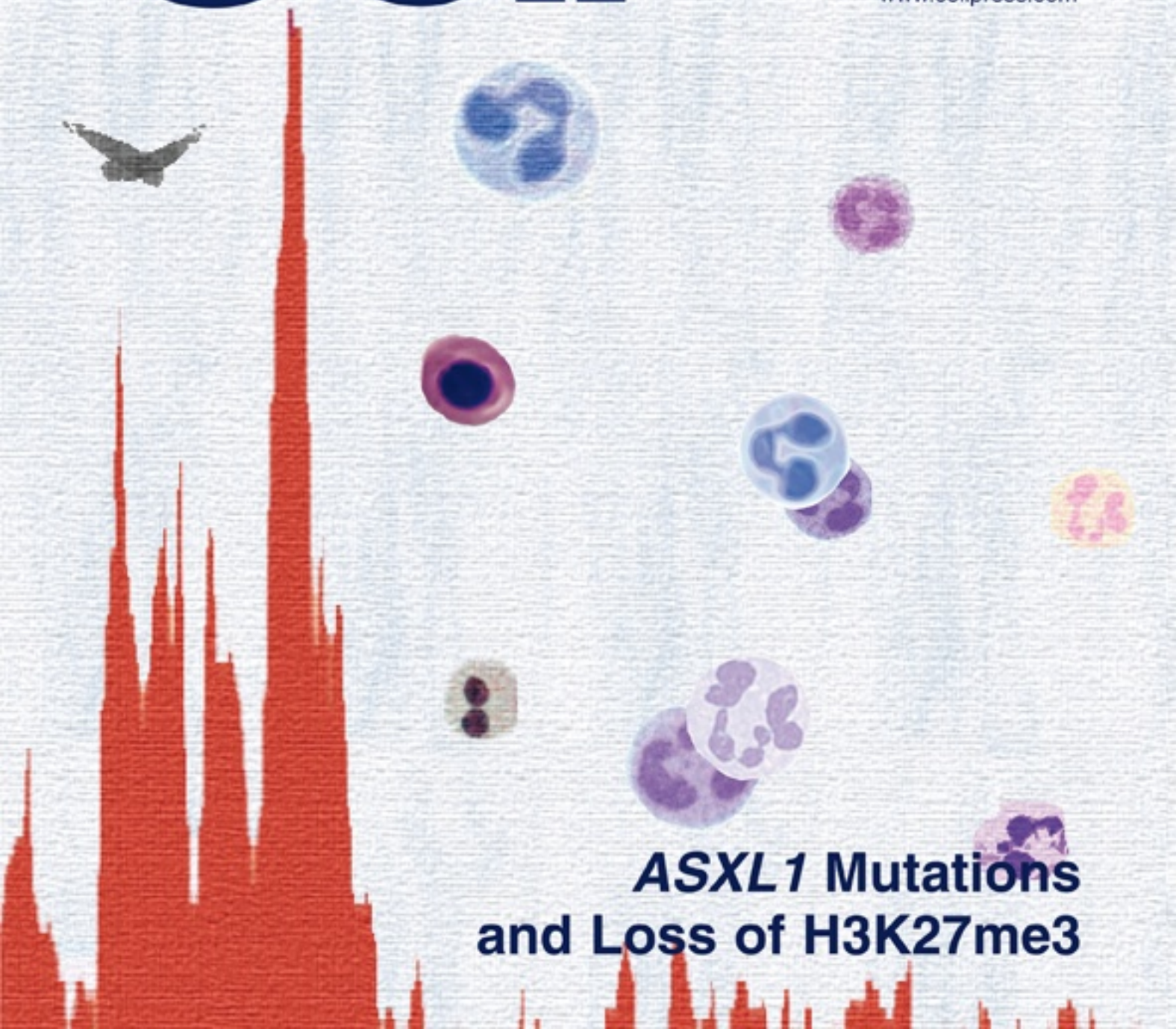

ASXL1 Mutations and Loss of H3K27me3

Genomic Analysis Drives Tailored Therapy in Poor Risk Childhood Leukemia

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Ph-like acute lymphoblastic leukemia (ALL) is a novel subgroup of high-risk childhood ALL. In this issue of *Cancer Cell*, Roberts et al. describe the identification of genetic alterations that lead to activated kinase and cytokine receptor signaling in Ph-like ALL and demonstrate that this aberrant signaling can be inhibited effectively.

The improving survival rate for children with acute lymphoblastic leukemia (ALL) is one of the greatest success stories of cancer treatment. However, a small proportion of patients will relapse; relapsed disease has a poor response to current therapy, resulting in reduced survival. Thus, to ensure optimum treatment, it is essential that patients at high-risk of relapse are identified at the time of diagnosis.

The prognostic relevance of specific chromosomal abnormalities in B-lineage ALL has been known for some time. To date, this information remains an important factor in risk stratification of these patients for treatment (Moorman et al., 2010). In particular, patients with the translocation, t(9;22)(q34;q11.2), resulting in the *BCR-ABL1* fusion, known as Philadelphia chromosome (Ph)-positive ALL, with rearrangements of the *MLL* gene, or with hypodiploidy (<40 chromosomes) are stratified as high-risk and treated on intensive therapy. More recently, using fluorescence in situ hybridization and genomic approaches, novel genetic aberrations have been identified, including those leading to dysregulated expression of CRLF2 or those targeting genes involved in transcriptional regulation of lymphoid development (Mullighan et al., 2007, 2009a; Russell et al., 2009). It was noted that alterations of one of these genes, *IZKF1*, was strongly associated not only with the poor-risk Ph-positive ALL but also with poor-risk among Ph-negative ALL. Many of these Ph-negative cases show a gene expression profile similar to that of the Ph-positive ALL and share the same high-risk of relapse and poor outcome. These cases of ALL were defined as *BCR-ABL1*-like or Ph-like (Den Boer et al., 2009; Mullighan et al., 2009b) and account for ~10% of childhood B-ALL. A pattern of genetic aberrations was beginning to emerge among these Ph-like ALL to facilitate their identification in the many study groups for which gene expression profiling is not available. In addition to the high incidence of *IKZF1* deletions, another genomic pointer of Ph-like ALL so far identified is dysregulated CRLF2 expression. This abnormality is present in ~50% of Ph-like cases, of which ~50% also harbor Janus kinase (*JAK*) mutations. However, in the remaining cases, the genetic alterations were unknown.

In the paper by Roberts and colleagues in this issue of *Cancer Cell* (Roberts et al., 2012), the authors provide a comprehensive genomic definition of Ph-like ALL with genetic alterations of a range of kinase and cytokine receptors. Initially, transcriptome and whole genome sequencing approaches were used to interrogate the genomes of 15 Ph-like cases. The altered genes discovered from this analysis included previously reported, albeit rarely, and novel fusions and mutations: for example *ABL1*, including the *NUP214-ABL1* fusion that had been previously shown to be amplified in T-ALL, *EPOR*, *JAK2*, *PDGFRβ, EBF1, FLT3, IL7R*, and *SH2B3.* Largely, these genes facilitate leukemic transformation by inducing constitutive kinase activation and signaling through the Ras and JAK/STAT5 pathways. All 15 cases showed at least one of these abnormalities (Figures 1A and 1B), indicating that more extensive screening would be required to determine a more accurate incidence as well as the distribution of these alterations. A range of techniques was then applied to screen an extended high-risk cohort from the Children's Oncology Group. The same types of aberrations were identified, and their estimated incidence is shown in Figure 1C.

Although a range of aberrations were identified in Ph-like ALL, the activation of ABL1 and/or JAK/STAT signaling pathways was the common mechanism for transformation. An exciting observation was that the transformation induced by these alterations was attenuated by tyrosine kinase inhibitors (TKI). The basal level of substrate phosphorylation as determined by phosphoflow cytometry was reduced by several TKI in two primary patient samples harboring the *NUP214-ABL1* fusion and by a JAK2 inhibitor in two *JAK2* rearranged samples. In addition, the therapeutic efficacy of the JAK2 inhibitor, ruxolitinib, was demonstrated in a xenograft model of a *JAK2* rearranged primary ALL sample. Another xenograft model of a human B-ALL harboring both an *IL7R* activating mutation and deletion of *SH2B3* responded to ruxolitinib. Also of interest was that a *NUP214-ABL1* ALL xenograft model responded to the ABL1 inhibitor, dasatinib, confirming that cells expressing *NUP214-ABL1* are sensitive to TKI within both T- and B-lineage ALL.

TKI treatment in addition to chemotherapy has led to dramatic improvements in outcome for Ph-positive ALL patients (Schultz et al., 2009). Patients with *PDGFRβ* rearrangements show complete hematological and molecular response to imatinib (Apperley et al., 2002). It had been shown previously that B-ALL cells harboring *CRLF2* rearrangements have enhanced signaling through oncogenic pathways that can be targeted with JAK or P13K inhibitors (Tasian et al., 2012). The Roberts et al. (2012) study now shows

that Ph-like leukemic cells are sensitive to currently available TKI. Thus, taken together, these observations indicate that screening at diagnosis to identify those Ph-like patients who may benefit from the addition of TKI to their current treatment regimen would be a feasible proposition.

At present, gene expression profiling, next generation sequencing, as well as the range of complex molecular techniques required to reliably identify the specific genetic alterations described in the Roberts et al. (2012) paper are not routinely available in most diagnostic laboratories and will no doubt preclude widespread screening for these cases in the near future. However, with the rising implementation of flow cytometry in the diagnostic arena and the increased application of phosphoflow cytometry worldwide, it may be possible to integrate flow cytometric phosphosignaling analysis for activated kinase pathways in conjunction with flow cytometric detection of CRLF2 overexpression as a diagnostic test to identify Ph-like cases.

Alternatively, by making use of the distinctive gene expression profile of Ph-like ALL, it may become possible to design customized targeted low density gene expression arrays suitable for routine diagnostic use. Such arrays would serve as the initial screen for Ph-like ALL, followed by testing for the specific alterations in kinase and cytokine receptors. These patients could then be considered for treatment combining chemotherapy with appropriate TKI, thus improving the outcome for another group of high-risk childhood ALL patients.

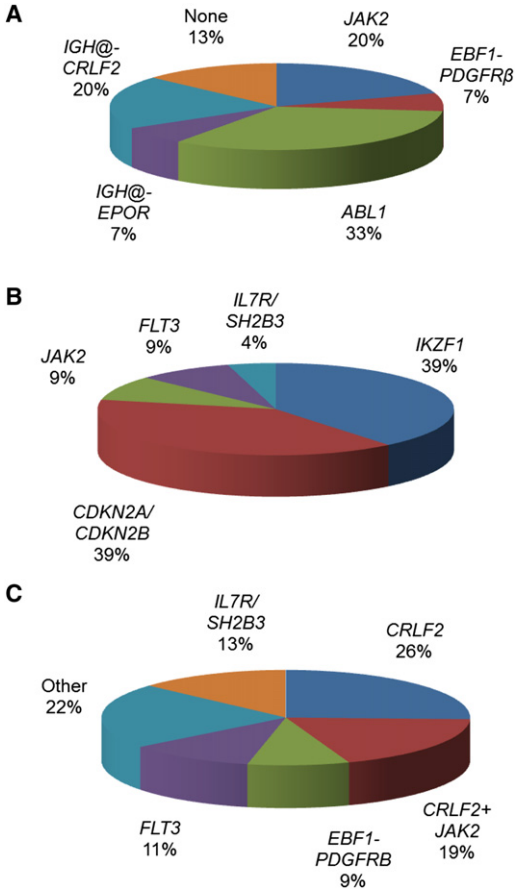

Figure 1. Distribution of Rearrangements, Mutations, and Deletions Affecting Kinase and Cytokine Signaling

(A and B) Distribution of rearrangements (A), mutations, and deletions (B) among the 15 discovery Ph-like ALL patients described in Roberts et al. (2012). The rearrangements of *ABL1* include two cases of *NUP214-ABL1*. The *IKZF1* category includes deletions and mutations; two mutations occurred along with deletions. The *CDKN2A/CDKN2B* category includes deletions only. The remaining aberrations are mutations. The distribution is an estimate as some patients harbored more than one of these aberrations.

(C) Estimated distribution of aberrations among Ph-like ALL calculated from Children's Oncology Group high-risk cohorts. In these high-risk cohorts 15%–20% of patients were classified as Ph-like according to their gene expression signature. In total, ~50% of cases had CRLF2 high expression, of which ~30% have *JAK2* mutations. The "Other" category includes an estimated incidence of *ABL1*, *JAK2*, *PDGFRβ*, and other kinase rearrangements and sequence mutations.

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