New Discoveries in Biology and Molecular Markers

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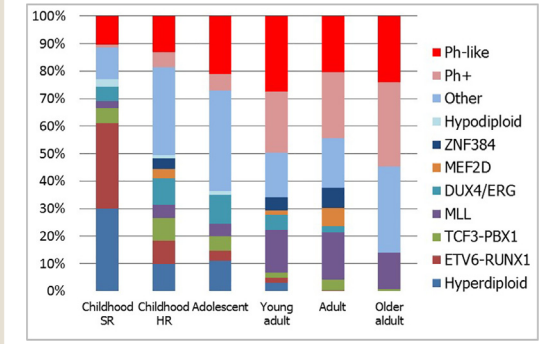
Abstract

Approximately 25% of childhood ALL cases, and a higher proportion of adult ALL cases, lack a unifying chromosomal alteration on cytogenetic analysis. Several new subtypes of B-ALL have been recently described that exhibit distinct leukemic cell gene expression profiles, but diverse, often cytogenetically cryptic founding alterations (Figure 2).

Figure 2 Currently recognized subtypes of B-progenitor ALL from over 2000 cases of ALL subjected to RNA-sequencing



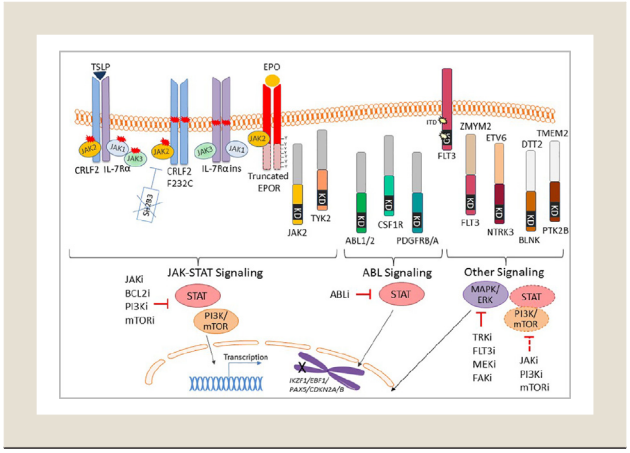
Ph-Like ALL

BCR-ABL1-like or Philadelphia-like (Ph-like) ALL exhibits a gene expression profile similar to Ph+ ALL and adverse prognosis.[1-3] The prevalence of Ph-like ALL increases with age and varies from 10% in standard-risk childhood ALL to over 20% of adults, with a peak prevalence of 27.9% in young adults (21-39 years).[4,5] In both children and adults, Ph-like ALL is associated with high-risk clinical features, a poor response to induction chemotherapy, elevated minimal residual disease (MRD) levels, and/or poor survival.[6]

Genetic alterations deregulating cytokine receptor and tyrosine kinase signaling include: rearrangements and mutation of *CRLF2* (~50%), rearrangements of ABL-class tyrosine kinase genes (12%), rearrangements of *JAK2* (7%) and the erythropoietin receptor gene (*EPOR*) (3-10%), mutations activating JAK-STAT signaling (11%) and Ras (6%), and less common kinase alterations (*FLT3*, *NTRK3*, *BLNK* and *PTK2B*). All kinase fusions retain an intact tyrosine kinase domain and typically exhibit constitutive kinase activation (Figure 3).

Keywords
Acute lymphoblastic leukemia, genomics, next-generation sequencing, tyrosine kinase inhibitors, BCR-ABL1, BCR-ABL1-like, Ph-like, MEF2D, ZNF384, DUX4, ERG

Figure 3 Schematic of targetable signaling pathways in Ph-like ALL. Red stars refer to proteins mutated in JAK-STAT driven Ph-like ALL.



CRLF2 encodes cytokine receptor like factor 2, also known as the thymic stromal-derived lymphopoietin receptor (TSLPR) that forms a heterodimeric receptor with the interleukin-7 receptor alpha chain (IL7R) for thymic stromal lymphopoietin (TSLP). *CRLF2* is deregulated by translocation into the immunoglobulin heavy chain locus (*IGH-CRLF2*); focal deletion upstream of *CRLF2,* resulting in formation of a *P2RY8-CRLF2* fusion; and less commonly *CRLF2* point mutations (F232C).[7] *CRLF2* rearrangements are most common in Ph-like and Down-syndrome associated ALL and are age-dependent, with *P2RY8-CRLF2* associated with young age, and *IGH-CRLF2* with older age and Hispanic ancestry.[8,9] CRLF2 is overexpressed on the cell surface of leukemic lymphoblasts and detectable by flow cytometric immunophenotyping. The majority of *CRLF2*-rearranged cases have additional alterations driving JAK-STAT or Ras signaling, particularly activating *JAK1* or *JAK2* mutations. Other mutations observed in *CRLF2*-rearranged cases include *FLT3* and *IL7R* sequence mutations, *SH2B3* deletions, *TSLP* rearrangements and Ras mutations.[4,5,10] In most studies, *CRLF2* rearrangements are associated with poor prognosis, particularly in cases with concomitant *IKZF1* alteration.[11,12] *CRLF2*-rearranged cells exhibit activated JAK-STAT, PI3K/mTOR and BCL-2 signaling, and therapies targeting these pathways alone or in combination have shown efficacy in preclinical models.[13,14]

Another major Ph-like ALL genetic subgroup involves ABL-class rearrangements which encode fusion genes involving *ABL1*, *ABL2* (*ARG*), *CSF1R* (encoding the macrophage colony stimulating factor receptor), *PDGFRA* and *PDGFRB* that are all targetable by inhibitors of ABL1 such as imatinib and dasatinib.[4,5,15,16]

Genomic rearrangements that produce *JAK2* fusion genes or rearrangements targeting *EPOR* are highly sensitive to JAK2 inhibitors, including ruxolitinib in preclinical models. *JAK2* is rearranged to at least 14 different partner genes in Ph-like ALL. *EPOR* rearrangements include reciprocal or cryptic translocations with immunoglobulin and other loci (e.g. *IGH, IGK, LAIR1, THADA*) that deregulate receptor expression and also truncate the cytoplasmic tail of the receptor, resulting in augmented JAK-STAT signaling.[4,5,15,17] The extensive preclinical data showing activation of signaling pathways, inhibition with JAK-STAT or ABL inhibitors, synergy with conventional chemotherapy, and anecdotal responsiveness to TKI therapy in patients with Ph-like ALL has led to the multiple prospective studies examining the efficacy of TKIs (particularly the JAK inhibitor ruxolitinib and the ABL1 inhibitor dasatinib) in Ph-like ALL.

A minority of Ph-like cases have mutations activating Ras signaling (*NRAS, KRAS, PTPN11* and *NF1*), although these are not exclusively observed in Ph-like ALL. Several kinases are infrequently rearranged in Ph-like ALL, including *NTRK3* and *TYK2.*[4,5]

DUX4 and ERG-Deregulated ALL

Approximately 7% of childhood BCP-ALL cases have a distinct immunophenotype and gene expression profile characterized by deregulation of the homeobox transcription factor gene Double Homeobox 4, *DUX4*, and the ETS transcription factor gene *ERG.*[18-21] *DUX4* encodes a double homeobox transcription factor located in a macrosatellite *D4Z4* repeat in the subtelomeric region of 4q.

Translocation of *DUX4* to *IGH* results in of expression of a truncated *DUX4* isoform in the B-cell lineage. [18-21] Less commonly *ERG-DUX4* fusions have also been described.[21] Prior studies had reported intragenic deletions of the *ERG* gene in about 5% of childhood ALL which are now known to be restricted to *DUX4*-rearranged cases. *DUX4*-rearranged cases exhibit gross transcriptional deregulation of *ERG* and commonly express truncated C-terminal ERG proteins irrespective of the presence of *ERG* deletions. *DUX4* rearrangement is an early initiating event in leukemogenesis, and aberrantly expressed DUX4 binds to an intragenic region of *ERG* resulting in expression of a non-canonical first exon and transcript, ERGalt. This encodes a truncated C-terminal ERG protein that retains the DNA-binding and transactivating domains of ERG, inhibits wild-type ERG transcriptional activity and is transforming.[20] Notably, *DUX4/ERG* deregulated ALL is associated

Figure 4 Schematic for the mechanism of deregulation of DUX4 and ERG in ALL.