Review

The Current Genomic and Molecular Landscape of Philadelphia-like Acute Lymphoblastic Leukemia

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Abstract: Philadelphia (Ph)-like acute lymphoblastic leukemia (ALL) is a high-risk B-cell Acute Lymphoblastic Leukemia (B-ALL) characterized by a gene expression profile similar to Ph-positive B-ALL but lacking the BCR-ABL1 translocation. The molecular pathogenesis of Ph-like B-ALL is heterogenous and involves aberrant genomics, receptor overexpression, kinase fusions, and mutations leading to kinase signaling activation, leukemogenic cellular proliferation, and differentiation blockade. Testing for the Ph-like signature, once only a research technique, is now available to the clinical oncologist. The plethora of data pointing to poor outcomes for this ALL subset has triggered investigations into the role of targeted therapies, predominantly involving tyrosine kinase inhibitors that are showing promising results.

Keywords: acute lymphoblastic leukemia; CRLF2; PI3K/AKT/mTOR; targeted therapy

1. Introduction

Tremendous progress has been achieved in childhood acute lymphoblastic leukemia (ALL) with long-term survival currently approaching 90% [1]. Outcomes in adult ALL have also improved, albeit, not to the same extent, with long-term survival ranging from 40–60% depending on the age group, ALL subtype, and the regimen used [2,3]. Retrospective and prospective data have demonstrated improved outcomes with pediatric inspired regimens in the adolescent and young adult (AYA) ALL population [2,4–7]. However, B-ALL with the Philadelphia-like signature (Ph-like ALL) stands out as a distinct high-risk entity despite the use of pediatric inspired regimens [2]. Ph-like ALL, also termed BCR-ABL1-like ALL, is a subset of B-ALL defined by the presence of a gene expression profile similar to Ph-positive ALL but without the t(9;22) or BCR-ABL1 translocation. It is characterized by a heterogenous genetic and molecular landscape that includes chromosomal translocations, loss of tumor suppressor genes, kinase gene fusions and mutations, kinase pathway activation, and dysregulated lymphoid transcription factors. Ph-like ALL is recognized as a provisional entity in the 2016 revision to the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia [8]. Ph-like ALL comprises approximately 15% of B-ALL in children [9–13], increases to 20–27% B-ALL in the AYA population [14], and 20% of B-ALL in older adults [15,16], with the highest incidence being in the AYA population. This entity almost always occurs in the subgroup of ALL without BCR-ABL1, ETV6-RUNX1, MLL/KMT2A, and TCF3 rearrangements [12]. In this review, we will describe the genomic landscape, molecular aberrations, and potential therapeutic avenues for treatment of Ph-like ALL.
2. Initial Description and Discovery of Ph-like B-ALL

In the early 2000s, it was recognized that chromosomal abnormalities alone were insufficient to initiate leukemogenesis in ALL and did not fully account for the poor treatment outcomes in certain ALL subsets. This led to a search for other genetic alterations in childhood and adult ALL. The late 1990s and early 2000s also saw rapid progress and automation of DNA microarray technology. This enabled many investigator groups to study the gene expression profiles of different ALL subsets [17–20]. However, it was not until 2009 that the molecular and clinical description of Ph-like B-ALL was reported simultaneously by two independent groups of investigators: St. Jude’s/Children’s Oncology Group (COG) in the United States (US) and the Dutch Childhood Oncology Group (DCOG). These two studies reported a similar gene expression profile as Ph-positive ALL but without the hallmark BCR-ABL1 translocation [12,21]. Although their methodologies differed (Table 1), both groups clearly demonstrated the biological complexity and poor clinical outcomes associated with Ph-like ALL in children.

The COG investigators led by Mullighan et al. [21] identified a group of high-risk B-ALL from the COG P9906 study and used the Affymetrix single-nucleotide-polymorphism (SNP) array analysis with 257 probe sets to perform gene-expression profiling. Deletions in CDKN2A/B, PAX5, IKZF1, ETV6, RB1, and BTG1 were commonly detected. Supervised principal-components analysis was used to associate gene copy number and treatment outcome. Among the genetic aberrations detected, only IKZF1 alteration was associated with poor outcomes. The 10-year incidence of relapse was ~50% in the IKZF1 altered cases compared to ~20% in the IKZF1 intact cases.

The Dutch Group led by Den Boer et al. studied all risk groups of newly diagnosed childhood ALL from the German Cooperative ALL (COALL) 92/97 and DCOG-ALL-8/9 studies [12]. They used the Affymetrix U133 GeneChips for RNA hybridization. Hierarchical clustering with 110 gene-probe sets revealed that 15% of B-ALL cases co-clustered with BCR-ABL1-positive cases but lacked the BCR-ABL1 translocation, yet had similar poor outcomes with relapse rates of approximately 50%. In vitro drug toxicity assays revealed the BCR-ABL-like leukemic cells were 73 times more resistant to l-asparaginase and 1.6 times more resistant to daunorubicin when compared to other precursor B-ALL cases. No differences were seen in the sensitivity to prednisolone and vincristine. Differences between the two cohorts, methodologies, and outcomes are summarized in Table 1. Since the initial description, other groups have identified similar genomic profiles in additional cohorts of Ph-like ALL in both children and adults [10,22,23].

Table 1. Differences between the cohorts and methodologies used in the initial description of Philadelphia (Ph)-like B-ALL [12,21].

| Feature                  | US COG Cohort [21]                  | DCOG Cohort [12]                  |
|--------------------------|------------------------------------|-----------------------------------|
| Number of genes probed  | 257 gene probe sets                | 110 gene probe sets               |
| Methodology              | Prediction analysis of microarrays  | Hierarchical clustering           |
| Patient characteristics  | High-risk patients (CNS or testicular disease, MLL rearrangement, age >10 years, male sex and WBC > 50,000) in the original cohort and all risk groups in the validation cohort | All risk groups                   |
| Racial/ethnic differences| Presence of Hispanic/Latino ethnicity | Lack of Hispanic/Latino patients  |
| CRLF2 aberration/JAK2 mutation | Higher frequency                  | Lower frequency                   |
| Outcomes                 | Poor outcomes in IKZF1-deleted or mutated cases | Six genetic subtypes of ALL identified BCR/ABL1-like had poor outcomes compared to other groups |
3. Clinical Features and Outcomes

Multiple studies have identified poor outcomes for patients with Ph-like B-ALL across all age groups. Ph-like B-ALL is associated with a higher risk of induction failure, measurable residual disease (MRD) positivity \[24\], higher relapse rates (as high as 70% at 3 years), and lower overall survival \[4,9,10,12–14,16,22,25–31\]. In the initial description of Ph-like B-ALL in pediatric patients reported by investigators from St. Jude’s Children’s Research Hospital, deletion or mutation of \(IKZF1\) was associated with poor outcomes. The 10-year incidence of relapse was 48% and 25% in the \(IKZF1\)-altered and unaltered groups, respectively \((p = 0.004)\). High level MRD at day 29 was significantly more frequent in the \(IKZF1\)-altered group at 23% compared to 6% in the \(IKZF1\)-intact group \((p = 0.001\%\) \[21\]). In a retrospective analysis of pediatric patients enrolled in the COG AALL0331 between 2006 and 2008, the Ph-like gene signature was identified in 13% of National Cancer Institute (NCI) standard risk B-ALL, and these patients had inferior 7-year event-free survival (EFS) compared to those without the Ph-like gene signature (82% versus 90%, \(p = 0.0022\)). However, there was no difference in overall survival (OS, 93% versus 95%, \(p = 0.14\)) \[9\].

Data compiled from multiple cooperative group studies of children, adolescents, and young adults with ALL reveal that for Ph-like ALL subgroups, children have better outcomes compared to adolescents who in turn fare better than young adults. The median 5-year EFS for children, adolescents, and young adults was 58%, 41%, and 24%, respectively, and the 5-year OS was 72%, 65%, and 25%, respectively \[14\]. Among patients >16 years of age with Ph-like B-ALL treated on the Dutch–Belgian HOVON (Stichting Hemato-Oncologie voor Volwassenen Nederland) study group clinical trials from 1993–2009, more than half (57%) of those who were subsequently identified as Ph-like ALL patients were categorized as standard risk at the time of diagnosis and only 71% attained complete remission (CR) following front-line treatment. The 5-year cumulative incidence of relapse, EFS, and OS were 67%, 19%, and 28%, respectively \[13\]. A single-institution analysis from the MD Anderson Cancer Center of 56 patients >15 years of age with Ph-like B-ALL also revealed poor outcomes \[16\]. Although over 90% of these patients achieved complete remission, only 30% achieved an MRD-negative remission. MRD negativity had no impact on outcomes with the median OS being 26 months in the MRD-negative group compared to 23 months in the MRD-positive group \((p = 0.318)\). The 5-year OS was 23% in the Ph-like B-ALL group compared to 59% in the B-other group \((p = 0.006)\). Finally, among AYAs 18–40 years treated on the US Intergroup C10403 trial, 2-year EFS was 52% versus 81% \((p = 0.04)\) \[4\] and 3-year overall survival (OS) was 63% versus 81% \[2\] for patients with and without the Ph-like signature, respectively. In a Japanese cohort of pediatric B-ALL patients harboring Ph-like kinase fusion genes, the 5-year event-free and overall survival rates were 48% and 73%, respectively, underscoring the inferior outcomes in this subset across the globe \[10\]. Despite the inferior outcomes, when using the traditional ALL criteria for risk stratification (e.g., age, White Blood Cell (WBC) count, high-risk cytogenetics) at least half of Ph-like B-ALL patients would fall under low or standard risk groups, underscoring the need to incorporate this feature as a separate poor risk factor \[32\].

While some studies indicate that MRD-directed therapy can improve outcomes in these patients \[10, 24\], other studies demonstrate otherwise \[2,24,25,33\]. Data from a limited number of patients derived from the HOVON study group clinical trials indicate that allogeneic stem cell transplant (ASCT) in the first complete remission may improve outcomes in Ph-like B-ALL. Only one relapse was seen among five patients who underwent ASCT, whereas nine of the 15 patients treated with chemotherapy alone relapsed \[13\]. Nevertheless, additional therapies to target the unique biological aberrations of Ph-like ALL are necessary, in addition to MRD-directed therapy, to improve outcomes in this high-risk ALL subgroup.

4. Role of Lymphoid Transcription Factors IKAROS and PAX5

IKAROS is a zinc finger protein that binds to and regulates DNA transcription \[34,35\]. It has recently been implicated as an epigenetic regulator \[36\] with the ability to induce active enhancers and
super-enhancers [37]. IKAROS plays key roles in hematopoiesis and lymphopoiesis [38,39] and is of importance for B-ALL as it is essential for the differentiation of B cell precursors [40].

IKAROS is encoded by the IKZF1 gene which is deleted in 60–80% of Ph-positive ALL [17,41] and is associated with failure of tyrosine kinase inhibitor (TKI) therapy [41]. IKZF1 deletion has been reported in 80% [17] of Ph-like B-ALL and contributes to the poor prognosis of this disease entity [12,21,31]. Several types of IKZF1 aberrations have been observed in Ph-like B-ALL [21] including deletion of the entire locus, of subgroups of exons, or of genes upstream. Deletion of exons 3 through 6 results in the expression of a dominant negative isoform, which lacks the N-terminal DNA binding domain but retains the C-terminal dimerization domain. This short isoform binds to other IKAROS proteins, sequesters them from DNA, and thereby inhibits their function [42]. Although the extent of IKAROS tumor suppressor mechanisms are unclear, it has been shown to bind DNA and regulate the expression of genes involved in cellular proliferation [43]. Further, the loss of IKAROS regulatory activity in B cell precursors is believed to contribute to the block in B cell differentiation that is characteristic of B-ALL cells [40]. IKZF1 deletion also contributes to chemoresistance in the leukemic cells [12].

A genetic aberration in IKZF1 alone may be insufficient for poor outcomes, which likely requires a synergistic effect with other molecular factors. The oncogenic kinase Casein Kinase 2 (CK2) is over-expressed in B-ALL [43]. CK2 impairs the ability of Ikaros to bind DNA and exert its tumor suppressor function [44–46]. Song et al. [43] demonstrated the antileukemic effects of CK2 inhibitors in patient derived xenograft models of Ph-like ALL where CK2 inhibition increased the activity of IKAROS protein produced in cells with monoallelic IKZF1 deletion.

The PAX5 gene encodes for the PAX5 (paired-box 5) protein which is also a DNA binding transcription factor [47] essential for B cell precursor differentiation [48]. PAX5 is an important tumor suppression gene in ALL and is altered in a third of Ph-like B-ALL cases [12,21]. Mutations in PAX5 haplo-insufficient mice increase the penetrance and reduce the latency of development of B-ALL [49]. IKZF1 and PAX5 alterations frequently occur together [50,51]. While only IKZF1, and not PAX5 alterations, was independently associated with poor outcomes in one large study, defects in multiple B cell differentiation regulators (such as PAX5 in combination with others) were also associated with worse outcomes [21].

5. Role of CRLF2

CRLF2 (Cytokine receptor-like factor 2) [52] partners with IL7Rα to form a heterodimeric receptor complex that binds the ligand thymic stromal lymphopoietin receptor (TSLP) [53–55]. This ligand–receptor interaction leads to downstream activation of the JAK2/STAT5 and the PI3K/AKT/mTOR pathways [56–58]. Figure 1 demonstrates the CRLF2 receptor complex and downstream pathways. Rearrangement of the CRLF2 gene located on Xp22.3 and Yp11.3, with the resulting overexpression of the CRLF2 receptor complex is seen in 10–15% [28,30] of all high-risk B-ALL. Among Ph-like B-ALL, the prevalence of CRLF2 rearrangement can be as low as 24% in children with NCI-standard risk disease to as high as 60% in adolescents [9,14]. It was first described by Russell et al. in 2009 [59] in a UK cohort of B-ALL and subsequently by Harvey et al. in a US cohort [30].

Two types of CRLF2 rearrangements have been reported. IgH-CRLF2 involves translocation of the immunoglobulin heavy chain gene IG@ on 14q32 to CRLF2 in the pseudo-autosomal region 1 (PAR1) of Xp22.3/Yp11.3. P2RY8-CRLF2 involves fusion due to an interstitial deletion of the pseudo-autosomal region 1 (PAR1) centromeric of CRLF2 in chromosomes X and Y. The latter is the predominant abnormality in Down syndrome associated B-ALL (DS-ALL) being present in ~50% of such cases [11]. Patients with IgH-CRLF2 tend to be older (median age of 16 years), whereas those with P2RY8-CRLF2 tend to be younger with a median age of 4 years [59]. In a single institution retrospective analysis of patients >15 years with Ph-like B-ALL, 66% were CRLF2 overexpressing. Among them, 76% had IgH-CRLF2, whereas only 17% had P2RY8-CRLF2 [16]. JAK mutations are present in more than 50% of CRLF2 rearranged Ph-like ALL [9,14,16,30,32,58,60].
CRLF2 rearrangement and overexpression predict poor outcomes with 4-year relapse-free survival of 35% with CRLF2 versus 71% without CRLF2 alterations [28,30,59]. CRLF2 overexpression, JAK mutations, and IKZF1 deletions orchestrate leukemogenesis and contribute to dismal prognosis with survival rates of 25–30%. CRLF2-overexpressing B-ALL harboring IKZF1 deletion is associated with increased risk of relapse even with low MRD levels [33]. Therefore, risk adapted therapy using MRD alone may not be sufficient in reducing relapse in these patients, underscoring the need for novel targeted therapies [2].

IKAROS protein has been shown to directly bind to the CRLF2 promoter and regulate CRLF2 expression in leukemic cells. CK2 (Casein kinase 2) inhibitor has been shown to increase IKAROS activity and binding to the CRLF2 promoter and to suppress CRLF2 expression [29]. Preclinical in vivo models of CRLF2 B-ALL are challenging due to the low homology between mouse and human CRLF2 and its ligand, TSLP. Patient-derived xenograft (PDX) models are even more challenging since mouse TSLP does not activate the human TSLP receptor complex. A novel PDX model engineered by Francis et al. [65] provides human TSLP and thus provides an important preclinical model for understanding disease mechanisms and identifying therapies that can be effective against Ph-like B-ALL with CRLF2 rearrangements.

Figure 1. Schematic representation of the CRLF2-receptor complex.

6. Kinase Activating Alterations in Ph-like B-ALL

Roberts et al. [14] reported on the kinase activating genetic alterations in Ph-like ALL that were noted in 10% of children and 27% of young adults with B-ALL and mirrored the poor outcomes seen by other groups. Kinase activating gene alterations were noted in 91% of patients with Ph-like B-ALL and included ABL fusions (ABL1, ABL2, CSF1R, PDGFRB) in 12%, EPOR rearrangement in 4%, JAK2 rearrangement in 7%, CRLF2 rearrangement in 50%, other JAK/STAT alterations (IL7R, FLT3, SH2B3, JAK1, JAK3, TYK2, IL2RB) in 12%, RAS mutations in 4%, and uncommon fusions (NTRK3 or DGKH) in 1%. These aberrations, depicted in Figure 2, lead to phosphorylation and activation of STAT5 and cytokine independent proliferation. ABL rearrangements were noted to be more common in children while JAK2 rearrangements were more common in young adults. Druggable targets were identified in vitro with ABL1, ABL2, CSF1R, and PDGFRB fusions sensitive to Dasatinib, EPOR and JAK2 rearrangements sensitive to JAK inhibitor Ruxolitinib, and ETV6-NTRK3 fusion responding to
Crizotinib. Other studies have shown efficacy of Ruxolitinib and Rapamycin (mTOR inhibitors) in CRLF2 and JAK aberrant B-ALL xenografts [58,66].

Figure 2. Genomic alterations in adults with Ph-like acute lymphoblastic leukemia (ALL). Pie chart constructed with data from Roberts et al. [67].

Reshmi et al. identified the Ph-like gene signature in 20% of children from the COG AALL1131 trial [32]. Among them, CRLF2 rearrangement was noted in 43%, with half of those harboring mutations in the JAK/STAT pathway (JAK1, JAK2, IL7R). ABL class fusions (ABL1, ABL2, CSF1R, PDGFRB) were present in 14%, EPOR rearrangements or JAK2 fusions in ~9%, other JAK/STAT alterations (IL7R, SH2B3, JAK1) in 6%, other kinases (FLT3, NTRK3, LYN) in ~5%, and RAS pathway mutations (KRAS, NRAS, NFI, PTPN11) in 6%. Figure 3 depicts the above alterations.

Figure 3. Genomic alterations in children with Ph-like ALL. Pie chart constructed with data from Roberts et al. and Reshmi et al. [14,32].

Multiple tyrosine kinase fusion genes were also identified by Boer et al. in bcr-abl1-like ALL [68]. These include EBF1-PDGFRB, SSPB2-CSF1R, ZM1Z1-ABL1, FOXP1-ABL1, RCSD1-ABL2, TERF2-JAK2, BCR-JAK2, and PAX5-JAK2. Sequence mutations involving IL7R, KRAS and NRAS are seen in ~5%
of Ph-like ALL [9]. Tyrosine kinase fusions and JAK2 mutations are mutually exclusive, the latter occurring only in CRLF2-overexpressing cases. Tyrosine kinase fusion genes are also commonly associated with IKZF1 deletions and poor prognosis.

EPOR (erythropoietin receptor) rearrangements in Ph-like ALL were further detailed by Iacobucci et al. [69] and involved insertion of the EPOR gene (located on chromosome 19) adjacent to the IGH or IGK loci, a t(14;19) that places IGH in proximity to EPOR, or an intrachromosomal inversion of chromosome 19 that juxtaposes EPOR to LAIR1 (leucocyte-associated Ig-like receptor 1). These changes lead to truncation of the cytoplasmic tail of EPOR, loss of regulatory residues, increased sensitivity to ligand binding by erythropoietin, and JAK/STAT activation. EPOR rearranged B-ALL was sensitive to JAK inhibition (Ruxolitinib) in vitro [69]. ETV6-ABL1 fusion occurs in <1% of childhood and adult ALL cases. Eighty percent of these cases harbor concurrent CDKN2A/B and IKZF1 deletions and have poor outcomes with <40% overall survival, similar to other bcr-abl-like ALL [70].

7. Diagnosis of Ph-like ALL: Screening and Subtype Confirmation

The multiplicity of genetic and molecular aberrations that characterize Ph-like ALL has made it difficult to implement a single set of tests for screening and subtype confirmation that can be used across all settings. Testing can therefore be comprehensive, utilizing whole genome, whole exome, or transcriptome sequencing, or limited to identifying the major subtypes only. The former methods are utilized by a few major research institutions whereas the latter are offered by several commercial and institutional laboratories. An algorithmic approach to the diagnosis of Ph-like ALL is summarized in Figure 4.

![Proposed testing algorithm for Ph-like ALL in the clinical setting](image)

- **Ph negative, MLL negative B-ALL**
  - LDA if feasible
- **FISH or Flow cytometry**
  - For CRLF2 aberration
- **PCR for JAK1/2 mutations**
  - (targeted by Ruxolitinib)
- **Negative**
  - FISH for other aberrations*
  - If ABL rearranged, targeted by Dasatinib

Figure 4. Proposed testing algorithm for Ph-like ALL in the clinical setting. MLL: Mixed Lineage Leukemia; FISH: fluorescent in situ hybridization; PCR: polymerase chain reaction; LDA: low density array. *Other aberrations include—ABL1, ABL2, CRLF2, JAK2, EPOR, PDGFRB, and CSF1R.

7.1. Gene Expression Profiling/Analysis

Gene expression analysis to classify Ph-like ALL was first reported by Den Boer et al. in the Netherlands and Mullighan et al. in the US [12,21]. The former group applied hierarchical clustering using 110 gene probe sets and classified pediatric ALL into six genetic subtypes, one of which was bcr-abl-like. The latter group utilized a 257 gene probe set and a prediction analysis of microarrays to identify Ph-like ALL. A risk score was developed based on supervised principal-components analysis and identified an association between copy number status of 20 genes and treatment outcome.
A 15 gene (IGJ, SPATS2L, MUC4, CRLF2, CA6, NRXN3, BMP1R, GPR110, CHN2, SEMA6A, PON2, SLC2A5, S100Z, TP53INP1, IFITM1) low density array (LDA) classifier developed by Harvey et al. [71] was able to predict Ph-like ALL with high sensitivity (93%) and specificity (89%). The same group also developed an 8-gene assay (JCHAIN, SPATS2L, CA6, NRXN3, MUC4, CRLF2, ADGRF1, BMP1R) to identify Ph-like ALL with targetable kinase gene aberrations [32] and generated an integrated score between 0 and 1, with a score of ≥ 0.5 being predictive of Ph-like ALL. LDA can be used as a screening test to identify Ph-like ALL and further characterization can be done with FISH, RT-PCR, and flow cytometry.

7.2. Fluorescent in Situ Hybridization (FISH)

The majority of chromosomal rearrangements in Ph-like ALL are cytogenetically cryptic, that is, they cannot be detected by conventional karyotyping. Many labs throughout several countries now offer FISH analysis to detect the commonly known gene translocations. These include ABL1, ABL2, CRLF2, JAK2, EPOR, PDGFRB, and CSF1R.

7.3. Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR)

RT-PCR is a relatively rapid method of confirming the same gene abnormalities detected by gene expression profiling by RNA sequencing. Chiaretti et al. developed a quantitative RT-PCR-based model to predict bcr/abl1-like cases by using a 10 (overexpressed) gene signature (SOCS2, IFITM1, CD99, TP53INP1, IFITm2, JCHAIN, NUDT4, ADGRE5, SEMA6A, CRLF2) [26].

7.4. Flow Cytometry

CRLF2 (TSLPR) is overexpressed in approximately half of Ph-like ALL cases and can be quickly identified by flow cytometry. CRLF2 flow cytometry is part of the standard Ph-like panel of many commercial and institutional laboratories.

7.5. Next-Generation Sequencing (NGS)

Extensive gene sequencing can be done with transcriptome sequencing, whole genome sequencing, or whole exome sequencing to identify Ph-like ALL, including the aberrations that may be missed by the 8- or 15-gene LDA analyses [14]. Transcriptome sequencing involves extensive RNA sequencing. Whole exome sequencing (WES) is limited to the coding regions and therefore misses anomalies that may occur in non-coding regions, for example gene rearrangements involving non-coding regions. Whole genome sequencing (WGS) can capture abnormalities in coding as well as non-coding regions. All three sequencing methods are expensive, time consuming, and used only in a few large research institutions.

8. Therapeutic Avenues

Since multiple studies have demonstrated poor outcomes with pediatric and adult treatment regimens with and without allogeneic stem cell transplant in Ph-like ALL across all age groups [4,9–13,15–17,21,25,27,28,30,32,33,41,62,67,72], there is an unmet need for novel therapies. The plethora of genomic and molecular alterations in Ph-like ALL opens up multiple avenues for targeted therapies. These include, but are not limited to, JAK (Ruxolitinib), ABL (Dasatinib), mTOR and other kinase pathway inhibitors, and immune therapies against surface molecules. Table 2 summarizes preclinical studies and Table 3 summarizes clinical studies using targeted therapies.

8.1. JAK/STAT Pathway Targets

JAK aberrations are present in approximately 40% of all Ph-like ALL, primarily in the CRLF2 subgroup where about half the cases have JAK mutations [14,32,58,72]. This leads to activation of the JAK/STAT/Pi3K/mTOR pathway [65] and opens up opportunities for the use of JAK, mTOR, and PI3K inhibitors alone or in combination as shown in vitro and in vivo preclinical studies [58,66,73].
The combination of JAK inhibition (with Ruxolitinib) and MEK inhibition (with Selumetinib) was also shown to have \textit{in vitro} and \textit{in vivo} efficacy in PDX models of JAK mutated B-ALL [74]. Currently, at least three clinical trials are underway to investigate the addition of Ruxolitinib to combination chemotherapy in CRLF2 rearranged/JAK aberrant Ph-like B-ALL (NCT02723994, NCT03117751, NCT02420717). Total Therapy XVII for Newly Diagnosed Patients with ALL (NCT03117751) is a phase 2/3 clinical trial designed for early identification of patients with targetable lesions and addition of Tyrosine Kinase inhibitors (TKIs) to the chemotherapy backbone. Patients with JAK/STAT signaling pathway activation receive Ruxolitinib and those with ABL class fusions receive Dasatinib. The MD Anderson Cancer Center is evaluating Ruxolitinib or Dasatinib with chemotherapy in treating adult patients with relapsed or refractory Ph-like ALL (NCT02420717). Additionally, investigators at Oregon Health Sciences University are leading a study exploring personalized kinase inhibitors in combination with chemotherapy for patients with newly diagnosed acute myeloid leukemia (AML) or ALL. Based on the results of \textit{in vitro} kinase inhibitor assay, patients in the ALL cohort receive Dasatinib, Ponatinib, or Ruxolitinib (NCT02779283).

Dose finding results from the Phase 2 study of Ruxolitinib with chemotherapy in 40 children and AYAs with CRLF2 or other JAK aberrant, newly diagnosed, high-risk Ph-like ALL were reported in 2018 [75]. Ruxolitinib was added to consolidation chemotherapy with an augmented Berlin-Franfurt-Munster (BFM) protocol on the AALL1521 study. Cytopenias (>60%), febrile neutropenia (~70%), and transaminitis (~50%) were commonly observed. Eighty percent of patients experienced grade 3 or 4 events related to Ruxolitinib without identified dose-limiting toxicities. Plasma drug levels at 4 hours were consistent with the known pharmacokinetic profile of Ruxolitinib, and pharmacodynamic studies demonstrated inhibition of phosphorylated STAT5.

### Table 2. Preclinical studies identifying kinase mutations and targeting with kinase inhibitors in Ph-like B-ALL.

| Kinase Target                        | Inhibitor                        | Reference                      |
|--------------------------------------|----------------------------------|--------------------------------|
| JAK, ABL, ETV6-NTRK3 fusion          | Ruxolitinib, Dasatinib, Crizotinib| Roberts et al.; 2014 [14]      |
| JAK + MEK                            | AZD1480 + Selumetinib            | Suryani et al.; 2015 [74]      |
| mTOR + HDAC                          | mTOR inh + Vorinostat            | Beagle et al.; 2015 [76]       |
| mTOR                                 | mTOR inhibitor                   | Hasan et al.; 2015 [77]        |
| RCS1-AVL1 rearr. t(1;9)              | ABL inhibitor Ponatinib          | Collette et al.; 2015 [78]     |
| HSP90/JAK                            | HSP90 inhibitor                  | Kucine et al.; 2015 [79]       |
| ABL1 (NUP214-abl1 fusion)            | Dasatinib                        | Duployez et al; 2016 [80]      |
| SYK (spleen tyrosine kinase)         | PRT318/R406 (Fostamatinib)       | Kohrer et al.; 2016 [81]       |
| ABL1(RCS1-ABL1)                      | Imatinib                         | Perwein et al.; 2016 [82]      |
| AT711/PDGFRB                         | Dasatinib                        | Kobayashi et al.; 2014 [83]    |
| EBF1-PDGFRB fusion                   | Imatinib                         | Weston et al.; 2013 [84]       |
| Lnk negative/ p53 negative blasts    | Ruxolitinib/BEZ235 (dual mTOR and PI3K) | Cheng et al.; 2016 [85] |
| C-myc and JAK2                       | JQ1 (BET bromodomain) and Ruxolitinib | Kim et al.; 2018 [86] |
| SRC/ABL                              | Dasatinib                        | Sarno et al.; 2018 [87]        |

### 8.2. mTOR Inhibition

mTOR inhibition alone was shown to decrease leukemia growth in a preclinical model of high-risk ALL characterized by early relapse [77]. Combining mTOR inhibition with histone deacetylase inhibition was shown to have activity in Ph+ and Ph-negative cell lines [76]. Similarly, combining
Dasatinib with mTOR inhibitor was more effective than Dasatinib alone in a preclinical model of Ph-like ALL harboring ABL1 or ABL2 rearrangements [88]. A phase 1 trial of the mTOR inhibitor, Temsirolimus, with intensive re-induction for relapsed ALL (COG study ADVL1114) induced remission in 50% of the patients. However, it resulted in excessive toxicity and was therefore not tolerable in these heavily pretreated children [89]. A potential strategy to overcome this drawback is to identify mTOR-responsive leukemias at diagnosis and incorporate lower doses of an mTOR inhibitor staggered to the chemotherapy backbone.

8.3. ABL Inhibition

ABL class fusions, involving ABL1, ABL2, CSF1R, and PDGRFB, are present in approximately 10% of Ph-like B-ALL and are targetable with ABL tyrosine kinase inhibitors such as Dasatinib [14,32,83,88]. Clinical trials testing Dasatinib in patients harboring these lesions are also using Ruxolitinib if there is JAK/STAT signaling pathway activation and are detailed in the section above titled JAK/STAT pathway targets.

Table 3. Ongoing clinical trials of Ph-like ALL (Clinicaltrials.gov).

| Sponsor/Collaborator                                      | Trial                                                                 | NCT#               |
|-----------------------------------------------------------|-----------------------------------------------------------------------|--------------------|
| Children’s Oncology Group                                 | Phase 2 study of Ruxolitinib with chemotherapy for CRLF2- or JAK-mutated B-ALL | NCT02723994        |
| University of New Mexico                                  | ALL therapies informed by genomic analysis                            | Single arm, open label NCT02580981 |
| University of Chicago                                     | Phase 1 trial of Ruxolitinib in combination with pediatric-based regimen for AYAs with Ph-like ALL | NCT03571321 (not yet recruiting as of 12/2019) |
| MD Anderson Cancer Center                                 | Ruxolitinib or Dasatinib with chemotherapy in relapsed/refractory Ph-like ALL | NCT02420717        |
| St. Jude’s Children’s Research Hospital                   | Total therapy XVII: Dasatinib for ABL1 class fusion and Ruxolitinib for activated JAK/STAT signaling | NCT03117751        |
| OHSU Knight Cancer Institute                              | Personalized kinase-inhibitor therapy combined with chemotherapy     | NCT02779283        |
| University of Washington                                  | In vitro-sensitivity directed therapy                                 | NCT02551718        |
| St. Jude’s Children’s Research Hospital                   | Treatment with combination chemotherapy for relapsed or refractory ALL | NCT03515200        |
| Nanfang Hospital of Southern Medical University, China    | To evaluate safety and efficacy of adding Chidamide (oral histone deacetylase inhibitor) and Dasatinib (TKI) to pediatric-inspired and MRD-directed pediatric protocol for Ph-like ALL | Open label, two-arm trial |

8.4. Other Therapeutic Avenues

Qin et al. reported on the preclinical efficacy of chimeric antigen receptor expressing T cells against TSLPR (CRLF2) [90]. Birinapant, a SMAC (second mitochondria-derived activator of caspases) mimetic, has shown preclinical activity against Ph-like B-ALL xenografts. SMAC is an endogenous antagonist of IAPs (inhibitors of apoptosis) and is released from mitochondria in response to apoptotic stimuli with subsequent activation of downstream caspases [91].
8.5. Other Molecular Aberrations/Targets

LNK (SH2B3), a lymphocyte adaptor protein, inhibits cytokine signaling during hematopoietic stem cell renewal and B cell development. LNK mutations are seen in ~6% of pediatric and young adult Ph-like ALL. Co-absence of TP53 and LNK genes have been shown to initiate pre-B ALL that resembles Ph-like B-ALL with JAK/STAT/mTOR pathway activation and responds in vitro and in vivo to JAK inhibitor (Ruxolitinib) and the dual mTOR and PI3K inhibitor (BEZ235) [85]. Ge et al. identified a high-risk subset of B-ALL that exhibited high IL7R and low SH2B3 expression that was associated with IKZF1 deletion. Use of a Casein Kinase 2 inhibitor was shown to restore IKAROS function in these cells [92].

9. Summary

Ph-like ALL is a high-risk subgroup of ALL with poor clinical outcomes across all age groups. It is characterized by a heterogenous genetic and molecular landscape populated by IKZF1 deletion, CRLF2 rearrangement and overexpression, JAK mutations, ABL class fusions, EPO rearrangements, tyrosine kinase pathway activation, and several other less common aberrations. The biologic heterogeneity of this disease entity leads to challenges not only in diagnosis but also in the investigation of novel therapies. Several diagnostic laboratory tests are available. However, unlike Ph-positive ALL, there is no uniformity in the diagnostic methodology employed. Several diagnostic panels are employed by different commercial and institutional laboratories. In this review, we have suggested an algorithm for the laboratory diagnosis of Ph-like ALL in the clinical setting (Figure 4).

Although the outcomes are poor, there is an immense potential for targeted therapeutic strategies involving kinase inhibitors as well as immune therapies targeting the CRLF2 molecule on the cell surface. To this effect, there are several ongoing clinical trials testing kinase inhibitors and several more preclinical studies investigating novel therapies. Current clinical trials utilize primarily JAK and ABL kinase inhibitors (Ruxolitinib and Dasatinib). Future trials should incorporate other agents targeting many other genomic/molecular aberrations. Until then, early identification, referral to a clinical trial, MRD-directed therapeutic strategies incorporating CD19 and CD22 targeted agents (Blinatumumab and Inotuzumab, respectively) and chimeric antigen receptor cell therapies, and early referral for hematopoietic stem cell transplant may improve outcomes.

Taken together, what is currently a difficult leukemia to cure will hopefully evolve into a highly curable disease thanks to the efforts of several investigator groups worldwide.

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