

## LYMPHOID NEOPLASIA

## Targetable kinase gene fusions in high-risk B-ALL: a study from the Children's Oncology Group

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## Key Points

- Ph-like ALL is characterized by a diverse array of genetic alterations activating cytokine receptor and tyrosine kinase signaling.
- Pediatric patients with Ph-like ALL can be identified in real time for effective treatment stratification.

**Philadelphia chromosome-like (Ph-like) acute lymphoblastic leukemia (ALL) is a high-risk subtype characterized by genomic alterations that activate cytokine receptor and kinase signaling. We examined the frequency and spectrum of targetable genetic lesions in a retrospective cohort of 1389 consecutively diagnosed patients with childhood B-lineage ALL with high-risk clinical features and/or elevated minimal residual disease at the end of remission induction therapy. The Ph-like gene expression profile was identified in 341 of 1389 patients, 57 of whom were excluded from additional analyses because of the presence of *BCR-ABL1* (n = 46) or *ETV6-RUNX1* (n = 11). Among the remaining 284 patients (20.4%), overexpression and rearrangement of *CRLF2* (*IGH-CRLF2* or *P2RY8-CRLF2*) were identified in 124 (43.7%), with concomitant genomic alterations activating the JAK-STAT pathway (*JAK1*, *JAK2*, *IL7R*) identified in 63 patients (50.8% of those with *CRLF2* rearrangement). Among the remaining patients, using reverse transcriptase polymerase chain reaction or transcriptome sequencing, we identified targetable ABL-class fusions (*ABL1*, *ABL2*, *CSF1R*, and *PDGFRB*) in 14.1%, *EPOR* rearrangements or *JAK2* fusions in 8.8%, alterations activating other JAK-STAT signaling genes (*IL7R*, *SH2B3*, *JAK1*) in 6.3% or other kinases (*FLT3*, *NTRK3*, *LYN*) in 4.6%, and mutations involving the Ras pathway (*KRAS*, *NRAS*, *NF1*, *PTPN11*) in 6% of those with Ph-like ALL. We identified 8 new rearrangement partners for 4 kinase genes previously reported to be rearranged in Ph-like ALL. The current findings provide support for the precision-medicine testing and treatment approach for Ph-like ALL implemented in Children's Oncology Group ALL trials. (*Blood*. 2017;129(25):3352-3361)**

## Introduction

B-lineage acute lymphoblastic leukemia (B-ALL) is characterized by recurring sentinel chromosome abnormalities that are important markers for prognosis and treatment stratification and frequently deregulate oncogenes or encode proteins that play a critical role in leukemogenesis, some of which are viable therapeutic targets.<sup>1-3</sup> Although long-term survival in childhood ALL now exceeds 85%,

several genetically defined subgroups continue to have poor survival. One high-risk subtype is Philadelphia chromosome-like (Ph-like) or *BCR-ABL1*-like ALL, defined by the presence of a gene expression profile (GEP) similar to that of Ph<sup>+</sup> ALL but lacking the canonical *BCR-ABL1* fusion.<sup>4-8</sup> Ph-like ALL accounts for approximately 15% of childhood B-ALLs, with the frequency increasing to 20% to 25%

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in adolescents and young adults, and is associated with a higher risk of relapse and death.<sup>6,9-13</sup> A majority of patients with Ph-like ALL harbor somatic genetic alterations that activate kinase and cytokine receptor signaling.<sup>6,9,11,14-16</sup>

Previous work by our group described the genomic landscape of Ph-like ALL in children, adolescents, and young adults.<sup>6,14,15,17</sup> Approximately 50% of patients with Ph-like disease overexpress cytokine receptor–like factor 2 (*CRLF2*), resulting from a rearrangement that fuses the immunoglobulin heavy chain locus (*IGH*) with *CRLF2* or, alternatively, an interstitial deletion within the pseudoautosomal region of chromosome X/Y that fuses *CRLF2* to the *P2RY8* gene promoter.<sup>18-22</sup> Approximately half of patients with *CRLF2* rearrangements harbor activating point mutations of *JAK1* or *JAK2*.<sup>9,18,20,23,24</sup> This subset may be amenable to targeted therapy with *JAK2* inhibitors, such as ruxolitinib, although response has varied in preclinical studies of *CRLF2*-rearranged ALL.<sup>25,26</sup> The remaining patients with Ph-like ALL harbor a variety of kinase alterations, including fusions involving ABL-class genes (*ABL1*, *ABL2*, *CSF1R*, and *PDGFRB*) sensitive to ABL1 tyrosine kinase inhibitors (TKIs) or rearrangements that create *JAK2* fusion proteins or truncating rearrangements of the erythropoietin receptor (*EPOR*) that are sensitive to ruxolitinib in vitro.<sup>6,11,14,16,27,28</sup> *JAK2* inhibitors have demonstrated efficacy in patients with myelofibrosis harboring *JAK2* mutations<sup>29</sup>; however, clinical data regarding their efficacy in patients with ALL harboring *JAK*-activating alterations are limited.

Anecdotal reports describe substantial clinical responses to TKIs in patients with Ph-like ALL and specific genomic rearrangements, providing a compelling rationale for developing strategies to identify such patients in real time and test TKI therapy in genetically defined subsets.<sup>6,16,28,30</sup> However, a wide variety of genomic alterations are present in patients with Ph-like ALL, making diagnostic molecular characterization complex. Our analysis of 1725 patients with B-ALL identified multiple fusion partners for *ABL1* ( $n = 6$ ), *ABL2* ( $n = 3$ ), *PDGFRB* ( $n = 4$ ), and *JAK2* ( $n = 10$ ), some with different genomic breakpoints producing distinct fusion transcripts,<sup>6</sup> as well as 4 different genomic rearrangements that produce an activated, truncated *EPOR*.<sup>14</sup> Other studies have demonstrated a wide variety of ABL-class and *JAK2* fusions.<sup>31-33</sup> Herein we describe the development of an algorithm for genomic testing to identify Ph-like ALL and targetable kinase and cytokine receptor alterations in B-ALL suitable for treatment stratification. This algorithm was applied to an unselected cohort of 1389 consecutive patients with high-risk B-ALL enrolled in Children's Oncology Group (COG) clinical trials.

## Methods

### Patients and samples

We retrospectively studied 1389 consecutively enrolled, newly diagnosed patients with B-ALL between 2010 and 2014. Samples were selected based on availability of cryopreserved leukemia blasts from patients meeting eligibility criteria for COG clinical trial AALL1131 (NCT01406756). The criteria for enrollment in AALL1131 included National Cancer Institute (NCI) high-risk status at diagnosis (age  $\geq 10$  years and/or initial white blood cell [WBC] count  $\geq 50\,000/\mu\text{L}$ ; 884 [64%] of 1339 patients). The remaining 36% of patients (505 of 1389) analyzed had NCI standard-risk ALL (age  $< 10$  years and WBC  $< 50\,000/\mu\text{L}$ ) and were originally enrolled in COG AALL1131 at the time of diagnosis ( $n = 37$ ) because of central nervous system or testicular leukemia or prior corticosteroid treatment. Alternatively, patients were enrolled in the COG standard-risk ALL trial AALL0932 (NCT01190930) at the time of diagnosis ( $n = 468$ ) and became eligible for COG AALL1131 at the end of induction

because of minimal residual disease (MRD) levels  $\geq 0.01\%$  (Figure 1).<sup>34,35</sup> Because we were interested in identifying patients with new targetable kinase fusion genes amenable to precision-medicine therapies, NCI standard-risk patients with known *BCR-ABL1* (targetable fusion already known) or *ETV6-RUNX1* fusions (known not to have targetable kinase fusion genes) were not included in the samples selected for analysis. Patients with Down syndrome were also excluded from these analyses. Patients and/or their parent(s)/guardian(s) provided informed consent for clinical trial participation, banking, and future research. All analyses were approved by the institutional review boards of Nationwide Children's Hospital, the University of New Mexico, and St. Jude Children's Research Hospital.

### Identification of Ph-like ALL and genomic characterization

All patient cases were analyzed using a TaqMan LDA polymerase chain reaction (PCR) assay on a 384-well microfluidic card (supplemental Figure 1, available on the *Blood* Web site). The GEP used to define Ph-like ALL by prediction analysis for microarray<sup>36</sup> was used to develop an 8-gene quantitative assay optimized to identify patients with Ph-like disease with targetable kinase gene alterations.<sup>11,37</sup> An integrated score between 0 and 1 was generated from the 8-gene assay, with a predictive score  $\geq 0.5$  considered Ph-like. Assays for expression of the 8 genes involved in the LDA predictor (*JCHAIN*, *SPATS2L*, *CA6*, *NRXN3*, *MUC4*, *CRLF2*, *ADGRF1*, *BMPRI1B*) were performed in duplicate along with individual reverse transcriptase PCR (RT-PCR) assays for *P2RY8-CRLF2* (2 splice forms), *BCR-ABL1* (b2a2/b3a2 and e1/a2 isoforms), and *ETV6-RUNX1* (4 isoforms). Both *BCR-ABL1* and *ETV6-RUNX1* fusions were included on the LDA card as validation for use in future prospective screening algorithms. Expression of predictor and fusion genes was internally normalized to an endogenous control gene (*EEF2*), and levels were expressed in units of  $\Delta C_t$  (predictor/fusion  $C_t - EEF2 C_t$ ). Patient cases of Ph-like disease that were *BCR-ABL1*<sup>+</sup> or *ETV6-RUNX1*<sup>+</sup> were excluded from further analysis because either a targetable kinase lesion had already been identified (*BCR-ABL1*) or a targetable kinase fusion had not been identified in comprehensive genome/RNA-sequencing analyses of *ETV6-RUNX1* ALL.<sup>38-40</sup>

Any patient case determined to have elevated *CRLF2* expression (*CRLF2*<sub>high</sub>; defined as  $\Delta C_t \leq 6$  by LDA) was assessed for *P2RY8-CRLF2* by Taqman PCR on the LDA card or for *IGH-CRLF2* by fluorescence in situ hybridization (FISH).<sup>9</sup> Those with *CRLF2*<sub>high</sub> were also assessed for the presence of a concomitant *JAK1* or *JAK2* mutation by Sanger sequencing.<sup>9</sup> All identified coding variants were confirmed to be somatic by comparison with matched remission DNA.

### RT-PCR for kinase fusions

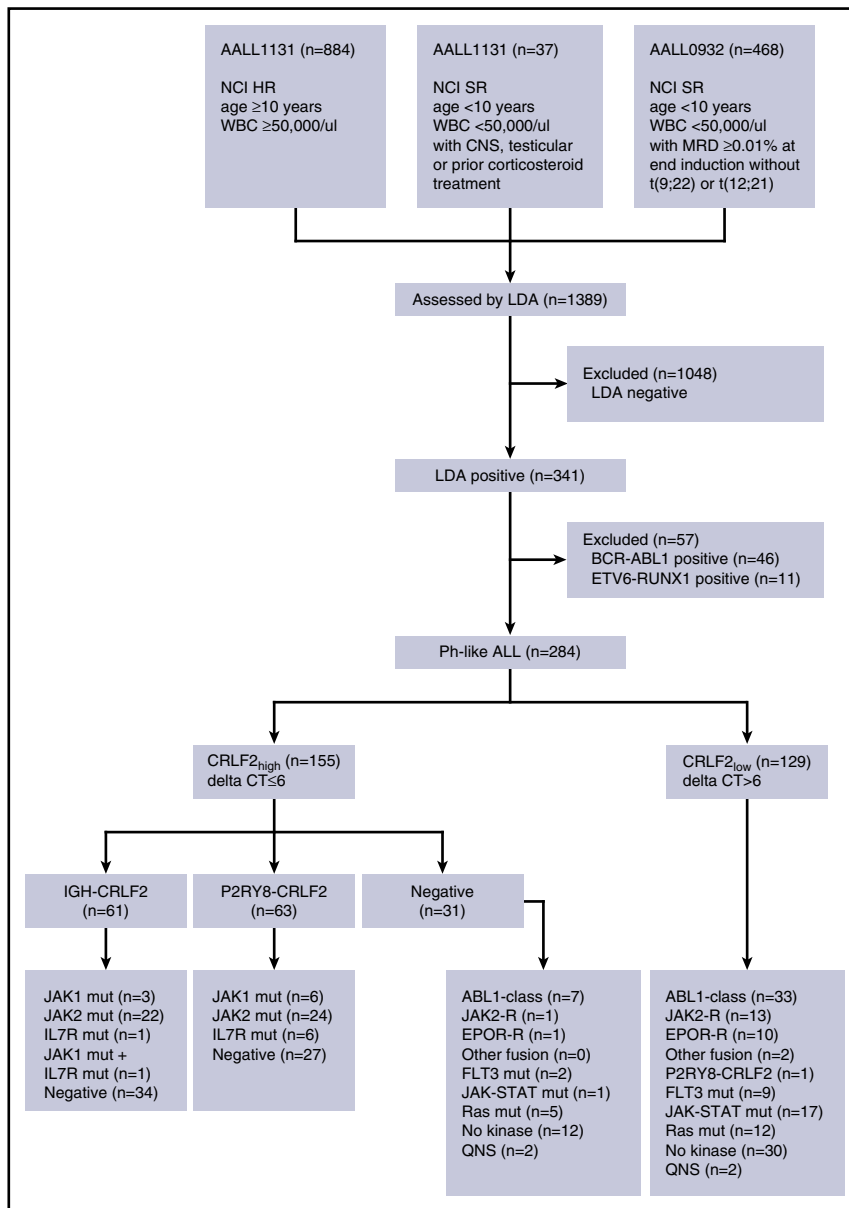
The remaining Ph-like *CRLF2*<sub>low</sub> patient cases were subject to multiplex and singleplex RT-PCR assays for previously identified kinase fusions; 2  $\mu\text{g}$  of total RNA isolated using TRIzol (Life Technologies, Carlsbad, CA) was used to prepare complementary DNA (cDNA) with a High-Capacity cDNA Reverse Transcription Kit (Life Technologies), and 2.5  $\mu\text{g}$  of cDNA (equivalent to 50 ng of starting RNA) was used to test samples for previously identified kinase fusions involving *ABL1*, *ABL2*, *CSF1R*, *JAK2*, *NTRK3*, and *PDGFRB* by RT-PCR (supplemental Table 1).<sup>6,15</sup>

### *IL7R* mutation analysis

*IL7R* mutations were analyzed in 173 Ph-like samples as previously described.<sup>41</sup> These included patient cases that were LDA<sup>+</sup> but were either negative by other assays (eg, *JAK* mutational analysis, multiplex PCR fusion testing) or not analyzed using RNA sequencing methods.

### Detection of fusion transcripts by RNA kinome capture and transcriptome sequencing

*CRLF2*<sub>low</sub> patient cases with no kinase gene fusion identified by multiplex or singleplex PCR and *CRLF2*<sub>high</sub> patient cases without *CRLF2* rearrangement underwent RNA kinome capture and total-stranded whole-transcriptome sequencing (RNAseq), performed in parallel. All identified fusions were confirmed via bidirectional Sanger sequencing. RNA kinome capture and sequencing were performed using an Agilent SureSelect RNA Kinome Capture Kit (Agilent Technologies, Inc., Santa Clara, CA) including 612 genes (571



**Figure 1. Algorithm used for analysis of patient cases.** Testing pipeline developed for downstream characterization of low-density array<sup>+</sup> (LDA<sup>+</sup>) patient cases for identification of specific genetic events to allocate subgroups for specific targeted therapies with ABL-class TKIs and JAK inhibitors. CNS, central nervous system; HR, high risk; mut, mutation; QNS, quantity not sufficient; R, rearrangement; SR, standard risk.

kinases). Library preparation was performed using capture baits and RNA-sequencing library preparation kits (Agilent Technologies or Illumina; New England Biolabs, Inc., Ipswich, MA) and sequenced using the Illumina HiSeq 2000. Fusion screening was accomplished by Geospiza GeneSifter (Geospiza, Inc. [PerkinElmer], Seattle, WA) with ChimeraScan.<sup>42,43</sup> RNAseq was performed using the TruSeq library preparation on the Illumina HiSeq 2000 platform. To detect kinase fusions, samples were analyzed using CICERO<sup>6</sup> and Fusion-Catcher.<sup>44</sup> Sequence mutations were analyzed using the GATK pipeline.<sup>45</sup> Genomic data have been deposited at the European Genome Phenome archive accession EGAS00001001952. Sequencing metrics (supplemental Table 2) and processing details are provided in the supplemental data.

## Results

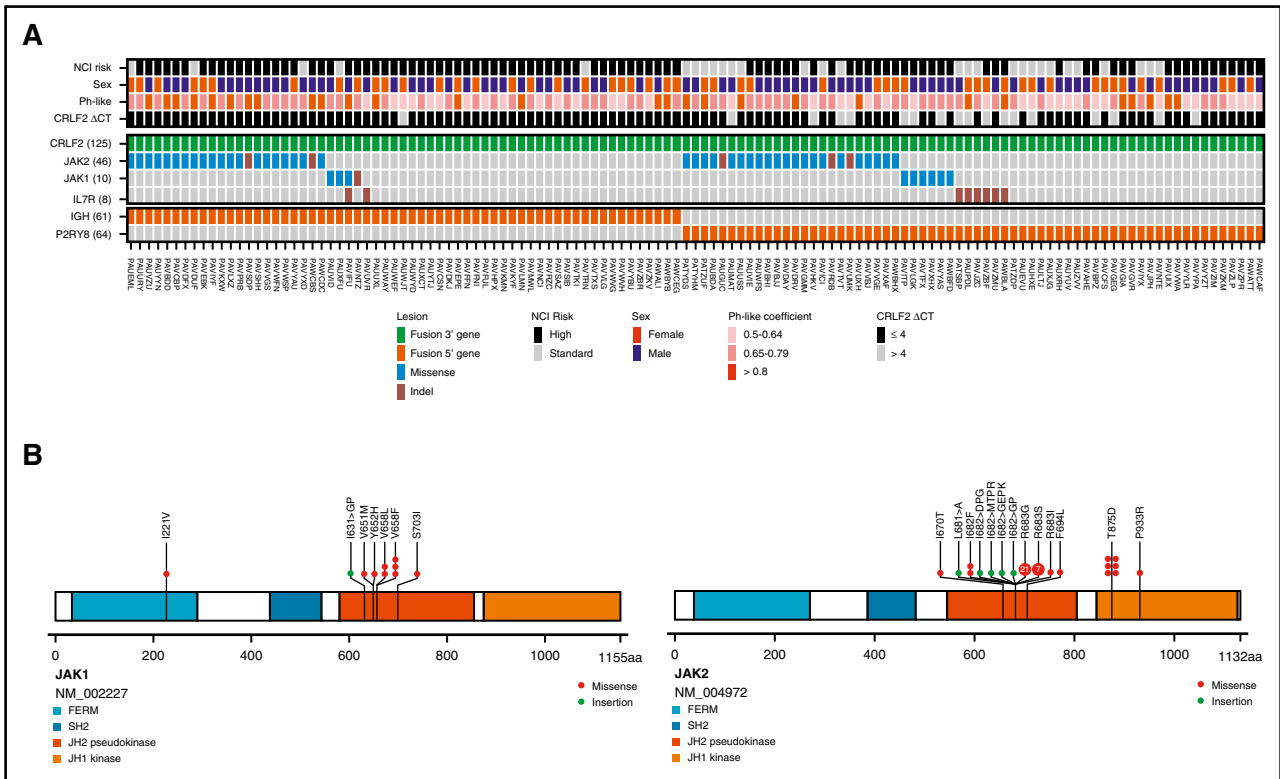
### Identification of Ph-like ALL and clinical characteristics

Of 1389 patient cases analyzed, 341 (24.6%) had a Ph-like GEP by LDA. Those with *BCR-ABL1* (n = 46; all NCI high risk) or *ETV6-*

*RUNX1* (n = 11; all NCI high risk) were excluded from further analysis. The remaining 284 patient cases (20.4%) of Ph-like ALL, including 198 (22.4%) of 884 with NCI high-risk ALL and 86 (17.0%) of 505 with NCI standard-risk ALL, that met eligibility criteria for AALL1131 underwent testing according to the algorithm (Figure 1; supplemental Table 3). The sex distribution of patients with Ph-like ALL (57.4% male) was similar to that of patients with non-Ph-like disease (55.0% male; *P* = .473). Patients with Ph-like disease comprised a higher proportion of those ≥10 years of age (25.8%) as compared with those age <10 years (16.7%; *P* < .0001) and had a significantly higher diagnostic WBC count using a cutoff of  $50 \times 10^9/L$  (40.5% vs 30.4%; *P* < .001),  $100 \times 10^9/L$  (24.3% vs 13.8%; *P* < .001), or  $200 \times 10^9/L$  (8.5% vs 5.4%; *P* = .049).

### CRLF2 alterations

Among these 284 patient cases of Ph-like ALL, 155 (54.6%) were *CRLF2*<sub>high</sub>, with *P2RY8-CRLF2* in 63 (22.2% of those with Ph-like disease) and *IGH-CRLF2* rearrangement in 61 (21.5%). Among the 61 patient cases with confirmed *IGH-CRLF2* fusions, 60 (98.4%) had a



**Figure 2. Genetic alterations in patients with Ph-like ALL with *CRLF2* rearrangement.** (A) Genomic landscape of *CRLF2* rearrangements. (B) Protein plot of sequence mutations in *JAK1* and *JAK2*.

*CRLF2* ΔC<sub>t</sub> ≤ 4, whereas 47 (74.6%) of 63 patient cases with *P2RY8-CRLF2* had *CRLF2* ΔC<sub>t</sub> ≤ 4 (*P* = .0001). Overall, rearrangement of *CRLF2* was identified in 124 (80%) of 155 LDA<sup>+</sup> *CRLF2*<sub>high</sub> patient cases, with an overall frequency of 43.7% (124 of 284) in patient cases of Ph-like ALL. Of the 124 patient cases of *CRLF2*-rearranged disease, 63 (50.8%) harbored concomitant activating mutations in either *JAK1* (*n* = 10), *JAK2* (*n* = 46), or *IL7R* (*n* = 8). Notably, 1 patient harbored activating alterations in both *JAK1* and *IL7R* (Figure 2).

**Additional kinase alterations in Ph-like ALL**

The 160 patients with Ph-like disease without *CRLF2* rearrangement, including 129 with *CRLF2*<sub>low</sub> disease and 31 with *CRLF2*<sub>high</sub> disease lacking *CRLF2* rearrangement, were assessed for additional kinase alterations, initially by RT-PCR for known fusions and, if negative, by kinome capture and RNA sequencing (supplemental Table 1).<sup>6</sup> Of these, 35 had kinase fusions involving ABL-class genes, *JAK2*, or *EPOR* identified by RT-PCR. Identification of other kinase fusions in RT-PCR-negative patients was achieved primarily with RNA sequencing. Of the 30 patients with a fusion involving ABL-class genes, *JAK2*, or *EPOR* identified by RNA sequencing, kinome capture was performed in parallel in 27, with the kinase fusion identified in 16 of the 27, whereas 11 were negative by kinome capture. Those not identified by kinome capture included 2 new fusions (known kinases with new 5' partners) and 9 patients with *IGH-EPOR* rearrangement. Two patients with *IGH-EPOR* identified by RNA sequencing were not tested using kinome capture. Similarly, JAK-STAT-activating mutations were only identified by RNA sequencing. Two patient cases with a kinase fusion identified by kinome capture did not undergo further RNA sequencing (*NUP153-ABL1* and *TNIP1-PDGFRB*). Together, 113 of 156 patient cases with Ph-like ALL without *CRLF2* rearrangement (72.4%) with evaluable

material harbored a genomic alteration predicted to activate kinase or cytokine receptor signaling (Figure 3), including 40 with ABL-class fusions (*ABL1*, *n* = 17; *ABL2*, *n* = 4; *CSF1R*, *n* = 4; and *PDGFRB*, *n* = 15), 14 with *JAK2* fusions, 11 with *EPOR* rearrangement, 1 each with an *NTRK3* and *LYN* fusion, and 11 with an activating *FLT3* mutation (10 internal tandem duplications similar to those identified in acute myeloid leukemia and 1 missense V579G mutation). We also observed sequence mutations and copy-number alterations in genes activating JAK-STAT signaling in 20 patients, including *IL7R* (*n* = 14), *SH2B3* (*n* = 7), and *JAK1* (*n* = 2). Notably, 3 patients harbored a concomitant *IL7R* mutation and *SH2B3* deletion, and 2 patients harbored an *FLT3* internal tandem duplication mutation with *SH2B3* deletion. Nineteen patients harbored missense mutations in Ras pathway genes, including *KRAS* (*n* = 11), *NRAS* (*n* = 6), *NF1* (*n* = 1), and *PTPN11* (*n* = 1). One of the *NRAS* mutations was identified in a patient with *ZMIZ1-ABL1* and another in a patient with an *SH2B3* missense mutation. We also identified 1 *P2RY8-CRLF2* fusion by LDA in a patient with *CRLF2*<sub>low</sub> gene expression (ΔC<sub>t</sub>, 7.4). Forty-two patients cases with Ph-like disease (14.8%; including 12 [7.7%] of 155 *CRLF2*<sub>high</sub> and 30 [23.3%] of 129 *CRLF2*<sub>low</sub>; *P* < .001) lacked a kinase alteration by RNAseq analysis, 39 of which had an LDA value ≤ 0.65. Four patient cases lacked material for complete evaluation (supplemental Figure 2).

All fusion-positive patient cases were confirmed to have in-frame fusion of the 5' partner to the 3' kinase gene and retention of the tyrosine kinase domain of the 3' fusion partner. The 17 *ABL1* fusions identified included 5 known 5' partners (*ETV6*, *NUP214*, *RANBP2*, *RCS1*, and *ZMIZ1*), with alternate breakpoints identified in *ETV6* and *NUP214* and 3 novel 5' partners: *CENPC1*, *NUP153*, and *LSM14A*; the 4 *ABL2* fusions involved previously known 5' partners (*RCS1* and *ZC3HAV1*). The 4 *CSF1R* fusions involved 1 known 5' partner (*SSBP2*) and 1 novel partner (*TBLIXR1*); the 15 *PDGFRB* fusions

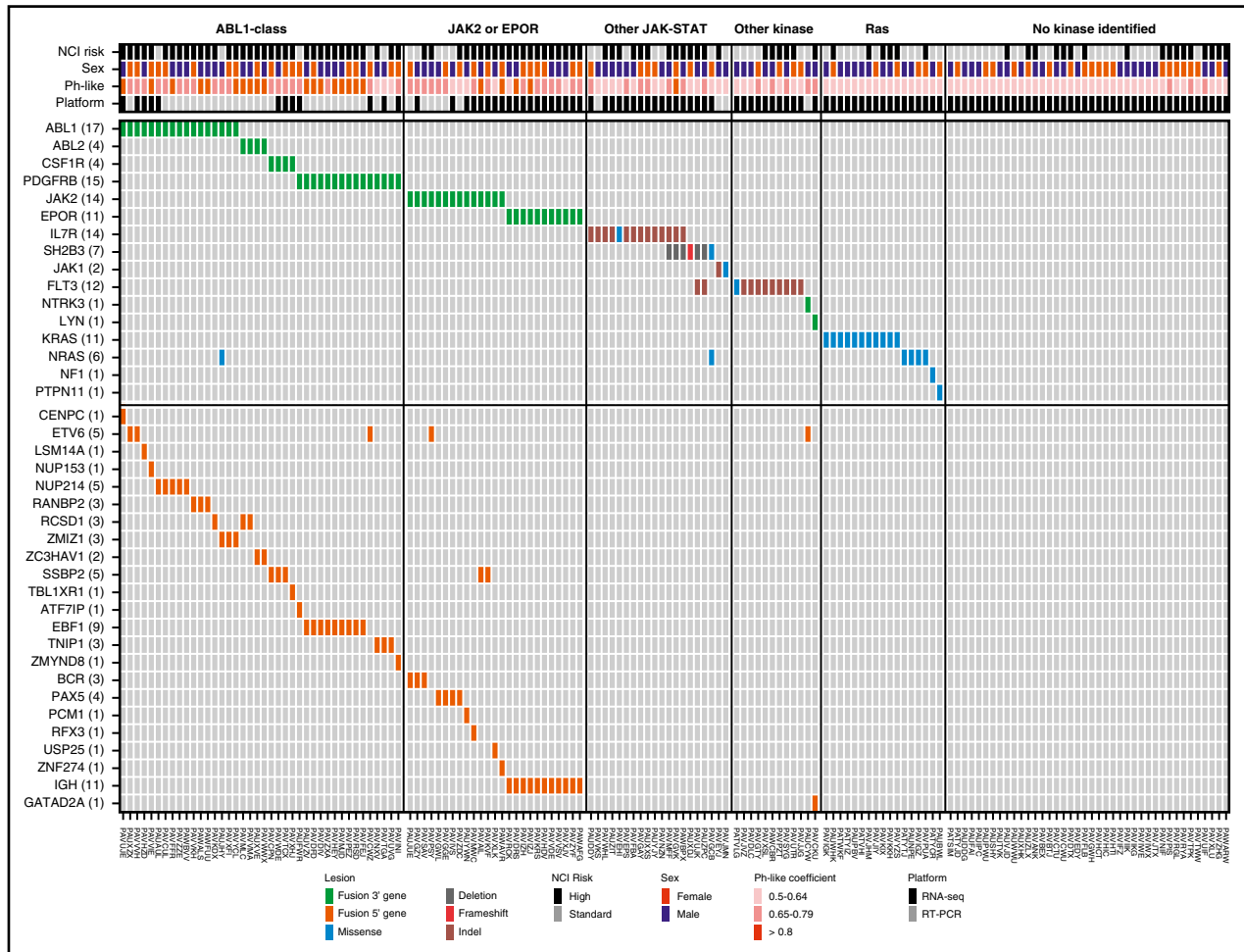


Figure 3. Genetic alterations in patients with Ph-like disease without *CRLF2* rearrangement.

included 1 previously known 5' partner (*EBF1*) and 3 novel partners (*ETV6*, *TNIP1*, and *ZMYND8*). The 14 *JAK2* fusions involved 8 different 5' partners, 5 of which were known (*ETV6*, *SSBP2*, *BCR*, *PAX5*, and *PCM1*) and 3 of which were novel (*RFX3*, *USP25*, and *ZNF274*), with alternate breakpoints identified in *SSBP2*, *BCR*, and *PAX5*. We detected the previously reported *ETV6-NTRK3* fusion in 1 patient and a *GATAD2A-LYN* fusion in another (Table 1; Figures 3 and 4). Although a majority of the fusions involved interchromosomal translocations, several fusions could have arisen from interstitial deletions within 1q24.2-q25.2 (*RCSD1-ABL2*), 5q14.1-q23.2 (*SSBP2-CSF1R*), 5q32 (*TNIP1-PDGFRB*), 5q32-q33.3 (*EBF1-PDGFRB*),

9p24.1-p24.2 (*RFX3-JAK2*), 9p13.2-p24.1 (*PAX5-JAK2*), or 9q34.12-q34.13 (*NUP214-ABL1*).

In total, 238 (83.8%) of 284 patients with Ph-like ALL harbored a genetic alteration activating kinase or cytokine receptor signaling. Those with higher LDA values were more likely to have targetable kinase alterations and particularly *ABL*-class fusions; 98% of patients (50 of 51) with LDA values  $\geq 0.8$  harbored a *CRLF2* rearrangement (n = 28) or other kinase fusion (n = 22), whereas 30.2% (26 of 86) with LDA values between 0.5 and 0.59 harbored a targetable fusion involving *CRLF2* (n = 21) or another kinase (n = 5;  $P < .0001$ ; supplemental Figure 3). We observed differences in the spectrum and

Table 1. Kinase fusions identified in Ph-like ALL

Kinase	TKI	Novel partners, n	Partners to date, n	Patient cases in current study, n	5' Fusion partners in current study
<i>ABL1</i>	Dasatinib	3	12	17	<i>CENPC</i> ,* <i>ETV6</i> , <i>LSM14A</i> ,* <i>NUP153</i> ,* <i>NUP214</i> , <i>RANBP2</i> , <i>RCSD1</i> , <i>ZMIZ1</i>
<i>ABL2</i>	Dasatinib	0	3	4	<i>RCSD1</i> , <i>ZC3HAV1</i>
<i>CSF1R</i>	Dasatinib	1	2	4	<i>SSBP2</i> , <i>TBL1XR1</i> *
<i>PDGFRB</i>	Dasatinib	1	6	15	<i>ATF7IP</i> , <i>EBF1</i> , <i>TNIP1</i> , <i>ZMYND8</i> *
<i>LYN</i>	Dasatinib	1	2	1	<i>GATAD2A</i> *
<i>JAK2</i>	JAK2 inhibitor	3	14	14	<i>BCR</i> , <i>PAX5</i> , <i>PCM1</i> , <i>RFX3</i> ,* <i>USP25</i> ,* <i>ZNF274</i> *
<i>EPOR</i>	JAK2 inhibitor	0	2	11	<i>IGH</i>
<i>NTRK3</i>	TRK inhibitor	0	1	1	<i>ETV6</i>

\*Indicates new 5' fusion partner identified.

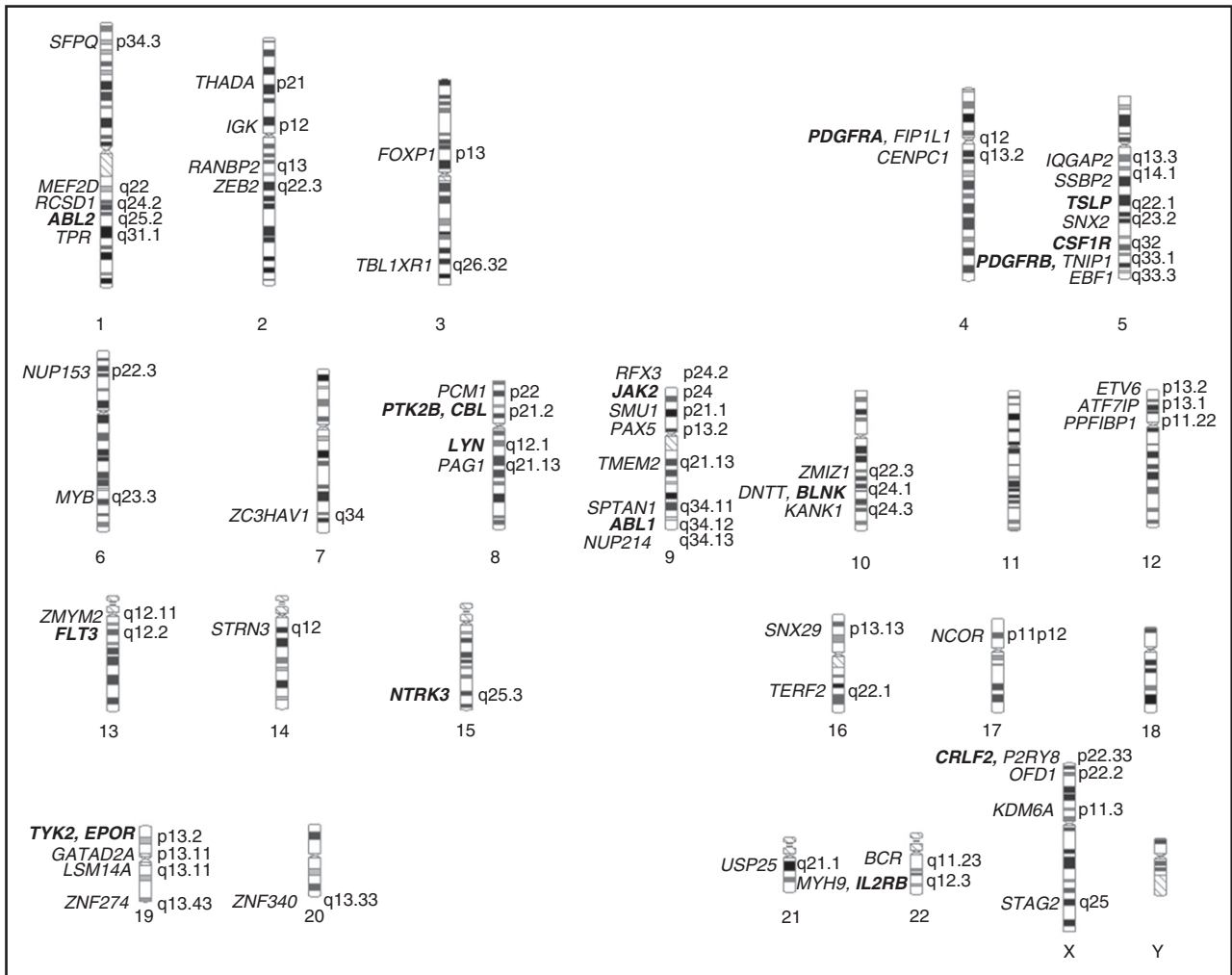


Figure 4. Karyotype ideogram summary of actionable gene fusions identified to date in adult and pediatric high-risk B-ALL. 3' Kinase gene partners are in bold.

distribution of kinase alterations in the standard- vs high-risk cohorts (supplemental Figure 4). Compared with the standard-risk cohort eligible for AALL1131, patients with Ph-like ALL meeting NCI high-risk criteria had a higher prevalence of *CRLF2* rearrangement (48.5% vs 33.7%;  $P < .02$ ) and *ABL*-class fusions (17.7% vs 5.8%;  $P = .0087$ ), with fewer Ras pathway alterations (3.0% vs 12.8%;  $P = .0044$ ) and instances of no kinase alteration being identified (9.1% vs 27.9%;  $P < .0001$ ; Table 2).

Among the remaining 41 patient cases with available material for RNAseq, 12 harbored fusions in additional genes not involved in kinase or cytokine receptor signaling, including *MEF2D* ( $n = 4$ ) and *ETV6* ( $n = 2$ ; supplemental Table 3).

### Analysis of LDA-negative patient cases

To determine whether the screening algorithm in this study was sensitive for detection of potentially targetable kinase fusions, we analyzed patient cases with LDA values below the threshold of 0.5. Of the 1048 LDA-negative patient cases, 6 had *P2RY8-CRLF2* fusion detected by LDA and 3 harbored *IGH-CRLF2* detected by FISH. The LDA values for these patient cases with *CRLF2* rearrangement ranged from 0.419 to 0.488, and all were *CRLF2*<sub>high</sub> with *CRLF2*  $\Delta C_t$  values ranging from 2.2 to 5.8. A total of 194 additional patient cases underwent the multiplex/singleplex PCR panel (3 with LDA values

between 0.25 and 0.3 [all standard risk]; 132 with LDA values between 0.40 and 0.449 [standard risk,  $n = 68$ ; high risk,  $n = 64$ ]; and 59 with LDA values between 0.45 and 0.499 [standard risk,  $n = 31$ ; high risk,  $n = 28$ ], and 63 patient cases underwent RNA sequencing (31 with LDA values between 0.40 and 0.449 and 32 with LDA values between 0.45 and 0.499). The 194 patients analyzed had characteristics similar to the 854 patients not tested, with respect to sex, Hispanic ethnicity, age, and initial WBC count ( $P = .299-1.0$ ) but were more likely to be NCI standard risk ( $P < .001$ ). No potentially targetable kinase fusions involving *ABL1*, *ABL2*, *CSF1R*, *PDGFRB*, *JAK2*, *NTRK3*, or *EPOR* rearrangement were identified. We did identify 1 potentially targetable fusion involving the fibroblast growth factor receptor 1 gene, *HOOK3-FGFR1*, with an LDA value of 0.47. Other fusions identified in this cohort included *TCF3-PBX1* ( $n = 4$ ) and *MEF2D* ( $n = 3$ ) and 2 rearrangements each involving *ETV6*, *PAX5*, *IGH*, and *NUTM1* (supplemental Table 4).

## Discussion

Patients with Ph-like ALL have significantly inferior event-free and overall survival, emphasizing the clinical importance of identifying those who could potentially benefit from targeted therapy.<sup>4-6,10-12,15</sup>

**Table 2. Frequency of kinase alterations**

Subgroup	NCI high risk (n = 884), n (%)	NCI standard risk, (n = 505), n (%)	Total (n = 1389), n (%)	P, high vs standard risk*
Ph-like ALL	198 (22.4)	86 (17.0)	284 (20.4)	.019
<b>CRLF2 rearrangement</b>	96 (10.9)	29 (5.7)	125 (9.0)	.0012
IGH-CRLF2	56 (6.3)	5 (1.0)	61 (4.4)	<.001
P2RY8-CRLF2	40 (4.5)	24 (4.8)	64 (4.6)	.89
CRLF2 rearrangement with JAK-STAT mutation	46 (5.2)	17 (3.4)	63 (4.5)	.14
CRLF2 rearrangement without JAK-STAT mutation	50 (5.7)	12 (2.4)	62 (4.5)	.0043
<b>Non-CRLF2</b>	102 (11.5)	57 (11.3)	159 (11.4)	.93
ABL1-class fusions†	35 (4.0)	5 (1.0)	40 (2.9)	.0012
JAK2 rearrangement	9 (1.0)	5 (1.0)	14 (1.0)	.99
EPOR rearrangement	11 (1.2)	0 (0)	11 (0.8)	.0095
Other kinase‡	7 (0.8)	6 (1.2)	13 (0.9)	.56
Other JAK-STAT§	12 (1.4)	6 (1.2)	18 (1.3)	.99
Ras	6 (0.7)	11 (2.2)	17 (1.2)	.021
No kinase identified	18 (2.0)	24 (4.8)	42 (3.0)	.0055

\*Fisher's exact test.

†ABL1-class: *ABL1*, *ABL2*, *CSF1R*, *PDGFRB*.‡Other kinase: *NTRK3*, *LYN*, *FLT3*.§Other JAK-STAT: *IL7R*, *SH2B3*, *JAK1*.

Here we determined the frequency of kinase-activating lesions in 1389 patients with B-ALL enrolled in or eligible for COG protocol AALL1131 for high-risk ALL identified as Ph-like by LDA (n = 284; 20.4%). Of note, we were not able to assess outcome in these patients, because these data were not yet mature and were not available for analysis. It will be interesting to determine whether MRD-based risk-directed therapy can salvage the poor outcome for these patients, as has previously been reported for patients in St. Jude clinical trials, although a number of patients with Ph-like ALL in those trials underwent stem-cell transplantation because of elevated MRD.<sup>46</sup>

We observed rearrangement of *CRLF2* in 43.7% of patient cases of Ph-like ALL, with similar prevalence of *P2RY8-CRLF2* and *IGH-CRLF2*. This differs from *CRLF2* fusion partner ratios of 2.1:1 and 5:1<sup>18,19</sup> for *P2RY8:IGH* reported in other studies<sup>18,19,47</sup> and was likely a result of the different ethnic composition and NCI risk stratification of our study cohort and the comprehensive FISH analyses performed. Of the 61 *IGH-CRLF2* fusions identified, 56 (91.8%) occurred in NCI high-risk patients and 5 in standard-risk patients. The frequency of self-declared Hispanic/Latino ethnicity was 27.6% among the 1389 patients analyzed, 37.0% (106 of 284) among patients with Ph-like ALL ( $P = .0002$  for Ph-like vs overall), 30.2% (19 of 63) among patients with *P2RY8-CRLF2*, and 59% (36 of 61) among patients with *IGH-CRLF2* ( $P = .0012$  for *P2RY8-* vs *IGH-CRLF2*). Similar to prior reports, point mutations of *JAK2* or *JAK1* were present in approximately half (45.2%) of those with *CRLF2*-rearranged disease.<sup>6,9,15,20,23</sup> Coexpression of genomic lesions causing *CRLF2* overexpression and *JAK1/2* mutations is transforming in vitro and promotes lymphoid transformation.<sup>18,48</sup> Regardless of JAK status, cells with *CRLF2* deregulation are frequently sensitive to JAK and mTOR inhibitors, although variations in response have been observed.<sup>25,49</sup>

An additional 22.8% of patients with Ph-like ALL harbored targetable kinase fusions involving ABL-class genes, *JAK2*, or *EPOR* (45.0% of LDA<sup>+</sup> *CRLF2*<sub>low</sub> patient cases and 5.8% of LDA<sup>+</sup> *CRLF2*<sub>high</sub> patient cases). Functionally, overexpression of fusion proteins encoded by *ABLI*, *ABL2*, *CSF1R*, or *PDGFRB* rearrangement phenocopies *BCR-ABL1* in vitro, with similar transforming capabilities and analogous sensitivity to the ABL inhibitors imatinib and dasatinib.<sup>6,15</sup> These data suggest that patients harboring ABL-class fusions are candidates for testing ABL

inhibitors in combination with chemotherapy, an approach that has revolutionized treatment of pediatric Ph<sup>+</sup> ALL.<sup>50-52</sup> In addition, the novel *GATAD2A-LYN* fusion is predicted to activate SRC signaling and also respond to dasatinib<sup>31,53</sup>; functional studies are ongoing.

Other targetable genomic lesions in patients with Ph-like ALL lacking *CRLF2* rearrangement include *JAK2* fusions and *EPOR* rearrangement, both of which are sensitive to ruxolitinib in vitro and in vivo, suggesting that small-molecule JAK inhibitors merit testing in this Ph-like ALL subset.<sup>14,25,49</sup> Other subgroups of Ph-like ALL include patients who harbor activating sequence mutations or copy-number alterations activating JAK-STAT signaling, including *IL7R*, *SH2B3*, and *JAK1* (collectively 6.3%). Preclinical studies suggest the efficacy of JAK inhibitors should also be assessed in patients harboring these JAK-STAT pathway alterations.<sup>25</sup> Of note, 3.9% of patients with Ph-like disease in this study harbored *FLT3*-activating alterations, with strong preclinical data suggesting US Food and Drug Administration–approved *FLT3* inhibitors are logical agents to test in this subgroup.<sup>54</sup> The remaining ~15% of patients with Ph-like disease had other genomic lesions that do not seem to target cytokine or receptor signaling, with no obvious therapeutic implications.

The population of patients in this study was based on eligibility criteria for the COG AALL1131 clinical trial, which included those with NCI high-risk ALL and those with NCI standard-risk disease with central nervous system or testicular leukemia, steroid pretreatment, or MRD  $\geq 0.01\%$  at end of induction. This study was not designed to assess the frequency of Ph-like ALL and kinase alterations in an unselected cohort of patients with standard-risk ALL. Despite this, we still observed significant differences in the genetic characteristics of patients with Ph-like ALL based on initial NCI risk stratification, even though 79.5% (66 of 83) of those with Ph-like ALL with NCI standard-risk disease were MRD<sup>+</sup> vs only 56.5% (108 of 191) of patients with high-risk Ph-like ALL. The incidence of Ph-like ALL was modestly lower in the tested NCI standard-risk patients compared with the NCI high-risk patients (17.0% vs 22.4%;  $P = .019$ ). This is likely because patients with Ph-like ALL are older and tend to have higher WBC count at diagnosis and are therefore more likely to present with NCI high-risk features. In total, 10.9% of NCI high-risk patients had Ph-like disease with *CRLF2* rearrangement compared with 5.7% of tested

standard-risk patients ( $P = .0012$ ; Table 2). There were similar overall percentages of patients with *P2RY8-CRLF2* (4.5% of high-risk and 4.8% of tested standard-risk patients); however, poor-prognosis *IGH-CRLF2* status was much more common in high-risk than tested standard-risk patients (6.3% vs 1.0%;  $P < .001$ ). We also observed an increased prevalence of other targetable kinase fusions in the high-risk vs tested standard-risk cohort (6.3% vs 1.0%). The frequency of *JAK2* fusions was similar between the NCI high-risk and tested standard-risk patients, with increased frequency of *ABL*-class fusions (4.0% vs 1.0%;  $P = .0012$ ) and *EPOR* rearrangement (1.2% vs 0%;  $P = .01$ ) observed in NCI high-risk compared with tested NCI standard-risk patients with ALL. Compared with our previous report on unselected patients with NCI standard-risk ALL, where Ph-like status was defined by prediction analysis for microarray analysis of microarray data, the frequency of Ph-like ALL was increased in our current selected standard-risk cohort (10% vs 17%); however, the frequency of targetable kinase fusions was similar in both standard-risk cohorts (0.6% vs 1.0%).<sup>6</sup> These data suggest that targetable kinase fusions are less common in patients classified as having standard-risk ALL based on initial NCI criteria of age and WBC count, even among those with a poor initial response to therapy, and likely help to explain the lower frequencies of these lesions reported by some groups that have screened cohorts of unselected patients with ALL.<sup>46</sup>

Given the ongoing discovery of new fusions and new splice variants of known fusions, screening with RT-PCR assays for known fusions will not identify fusions with novel breakpoints or 5' fusion partners. The most comprehensive approach would be to perform unbiased RNA sequencing on all patient cases of ALL or on the subset of patient cases defined as having a Ph-like GEP signature. This approach is currently challenging, particularly for clinical trials involving large numbers of patients from multiple sites (>1800 patients with B-ALL are enrolled in COG ALL trials by >200 centers per year), but will likely become feasible as sequencing costs continue to decline and the speed of bioinformatic analysis improves. We explored a kinome capture RNA-sequencing approach in the current study but found that this assay lacks significant cost and efficiency advantages over full RNA sequencing. Other technologies that might be used include the assay based on Nanostring (Seattle, WA) and a commercially available anchored multiplex PCR assay (ArcherDx, Inc., Boulder, CO) based on targeted next-generation sequencing with the potential ability to detect any fusion associated with a 3' kinase gene partner.

These data show that 20.4% of children, adolescents, and young adults with B-ALL and high-risk clinical features as defined in this study have Ph-like ALL defined by the LDA  $\geq 0.5$  threshold. Among these patients, 83.8% had cytokine receptor- or kinase-activating alterations, confirming and extending our previous observation that, although Ph-like ALL has a complex range of genomic lesions, the underlying alterations seem to be associated with a limited number of pathways, including *ABL*-class and *JAK*-*STAT* signaling, that are sensitive in vitro to US Food and Drug Administration-approved TKIs and *JAK* small-molecule inhibitors.<sup>6,28</sup> Although somewhat complex, the LDA screening and downstream testing approach we have developed and described in this study is feasible for screening patients in the context of treatment stratification. Our data demonstrate that the LDA threshold of 0.5 is robust, with only 1 of 194 patient cases with an LDA value  $< 0.5$  tested having a kinase fusion (*HOKK3-FGFR1*). Similar to our prior reports,<sup>6,9,18,47</sup> a small subset of patients with *CRLF2* rearrangement (9 [6.8%] of 133) did not have a Ph-like GEP but did exhibit high *CRLF2* expression. On the basis of these

results, we implemented the LDA assay and downstream testing algorithm in COG AALL1131 in 2016. Patients with NCI high-risk Ph-like ALL and *ABL*-class fusions are eligible to have dasatinib added to their backbone chemotherapy regimen. Those patients with Ph-like ALL and *JAK*-*STAT* pathway lesions, including *CRLF2* rearrangement with or without *JAK* mutations, *JAK2* fusions, *EPOR* rearrangement, or *IL7R* rearrangement, are eligible to enroll in COG AALL1521 (NCT02723994), in which ruxolitinib is added to combination chemotherapy.

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## Authorship

Contribution: S.C.R., R.C.H., K.G.R., and S.P.H. prepared the manuscript; E.S., A.S., H.J., I.-M.C., M.V., K.G.R., Y.S., D.P.-T., and Z.G. performed experiments; S.C.R., R.C.H., Y. Liu, Y. Li, J.Z., K.G.R., J.E., M.D., N.A.H., A.J.C., M. J. Borowitz, B.L.W., C.G.M., C.L.W., and S.P.H. analyzed data; E.A.R., A.L.A., M. J. Burke, W.L.S., P.A.Z.-M., K.R.R., W.L.C., M.L.L. and S.P.H. provided patient samples; and all authors edited and approved the manuscript.

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