Published in final edited form as:

Leukemia. 2017 April ; 31(4): 981–984. doi:10.1038/leu.2016.375.

High Incidence of Philadelphia Chromosome-Like Acute Lymphoblastic Leukemia (Ph-like ALL) in Older Adults with B-ALL

Sarah K Tasian¹, Christian Hurtz², Gerald B Wertheim³, Nathanael G Bailey⁴, Megan S Lim^{4,*}, Richard C Harvey⁵, I-Ming Chen⁵, Cheryl L Willman⁵, Rachel Astles², Alexis Zebrowski², Shalini C Reshmi⁶, Marilyn M Li⁷, Noelle V Frey², Selina M Luger², Martin Carroll², and Alexander E Perl²

¹University of Pennsylvania Perelman School of Medicine, Department of Pediatrics; Children's Hospital of Philadelphia, Division of Oncology and Center for Childhood Cancer Research

²University of Pennsylvania Perelman School of Medicine, Department of Medicine, Division of Hematology and Oncology and Abramson Cancer Center

³University of Pennsylvania Perelman School of Medicine, Department of Pathology and Laboratory Medicine; Children's Hospital of Philadelphia, Division of Hematopathology

⁴University of Michigan, Department of Pathology

⁵University of New Mexico, Department of Pathology and Hematologic Malignancies Research Program

⁶Nationwide Children's Hospital, Department of Laboratory Medicine; the Ohio State University, Department of Pathology

⁷Children's Hospital of Philadelphia, Division of Genomic Diagnostics

Treatment of patients with Philadelphia chromosome-positive B-cell acute lymphoblastic leukemia (Ph+ B-ALL) with tyrosine kinase inhibitors (TKIs) such as imatinib and dasatinib has improved survival in younger adults and allowed de-escalation of conventional chemotherapy, particularly in older adults.¹ Similar approaches may also benefit patients with the Philadelphia chromosome-like (Ph-like) B-ALL subtype recently described in

Users may view, print, copy, and download text and data-mine the content in such documents, for the purposes of academic research, subject always to the full Conditions of use: http://www.nature.com/authors/editorial_policies/license.html#terms

Correspondence to: Sarah K Tasian, MD, Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, 3501 Civic Center Boulevard, CTRB 3010, Philadelphia, Pennsylvania 19104, Phone: 267.425.0118, Fax: 215.590.3770, tasians@email.chop.edu.

*Current affiliation for MSL is University of Pennsylvania Perelman School of Medicine, Department of Pathology and Laboratory Medicine; Children's Hospital of Philadelphia, Division of Hematopathology

Conflict of interest: The authors declare no competing financial interests relevant to this work.

AUTHOR CONTRIBUTIONS

SKT designed and directed the study, performed experiments, analyzed data, and wrote the manuscript. CH and GBW performed experiments, analyzed data, and edited the manuscript. NGB, MSL, RA, AZ, NVF, and SML contributed key reagents and analyzed data. RCH, I-MC, CLW, SCR, and MML performed experiments and analyzed data. MC oversaw conduction of the study and edited the manuscript. AEP directed the study, analyzed data, and wrote the manuscript. All authors approved the final version of the manuscript.

children and adolescents/young adults (AYAs)^{2, 3}. Ph-like ALL has a “kinase-activated” gene expression profile similar to Ph+ ALL, but distinguishes itself by absence of *BCR-ABL1* fusion and presence of activating mutations in kinase- and cytokine receptor-associated signaling pathways.^{3, 4} In children and AYAs, approximately 50% of Ph-like ALL activates JAK/STAT and PI3K/mTOR signaling via rearrangement of *cytokine receptor like factor 2* (*CRLF2*-R) with common co-occurrence of *JAK2* or *JAK1* point mutations. An additional 10–15% of Ph-like ALL harbors *JAK2* or *EPOR* rearrangements, while another 15–20% has translocations or fusions involving *ABL1*, *ABL2*, *CSF1R*, or *PDGFRB* (ABL-class lesions).^{3, 5} As in Ph+ ALL, deletions of *IKZF1*, *CDKN2A/B*, and other lymphoid-associated transcription factors are common.^{4, 6} While Ph-like ALL comprises 20–25% of B-ALL in AYAs and is associated with high relapse rates and poor survival,^{2, 4} the frequency, biology, genetics, and clinical features of Ph-like ALL in older populations remain incompletely defined.⁷

Ph+ and Ph-like ALL are rare in young children, but increase in frequency during adolescence.³ Ph+ ALL incidence rises further in adulthood, peaking at >60 years. Although younger adults can be treated with pediatric protocol-inspired chemotherapy, such intensive regimens are poorly tolerated in middle-aged and older adults.⁸ Robust anti-leukemia activity of JAK inhibition (*e.g.*, ruxolitinib) and SRC/ABL inhibition (*e.g.*, dasatinib) has been demonstrated in preclinical models of *JAK2/EPOR*-mutant and *ABL/PDGFRB*-mutant Ph-like ALL, respectively.^{3, 5, 9} Clinical testing of kinase inhibitors with chemotherapy is now ongoing for children with specific genetic subtypes of Ph-like ALL (NCT02723994, NCT02883049). Improved delineation of the genetic drivers in adult ALL may similarly facilitate development of molecularly-targeted treatment approaches for appropriate patient subsets that can minimize toxicity while retaining potent anti-leukemic efficacy.

Given these therapeutic implications, we sought to define the incidence and clinical characteristics of adult Ph-like ALL via expression profiling of 93 unselected B-ALL specimens. In particular, demonstration of a sizable older adult population with Ph-like ALL would support development of TKI-based therapies alone or with lower-intensity cytotoxic therapies.¹ Bone marrow or peripheral blood specimens were obtained from consenting patients with newly-diagnosed B-ALL via institutional review board-approved research protocols at the University of Pennsylvania, University of Michigan, and Children’s Hospital of Philadelphia (signaling analyses only) in accordance with the Declaration of Helsinki. Patient age, sex, race/ethnicity, diagnostic white blood cell (WBC) count, cytogenetic and molecular testing data, treatment, and clinical outcomes were tabulated when available. Patients with antecedent chronic myeloid leukemia in lymphoid blast crisis were excluded.

Nucleic acids were extracted from B-ALL specimens according to manufacturer’s instructions (Qiagen). Cell pellets for fluorescence *in situ* hybridization (FISH) assays were prepared as described.¹⁰ RNA specimens were first profiled via a 15-gene low density microarray (LDA) classifier that identifies a kinase-activated gene expression signature associated with both Ph+ and Ph-like ALL.¹¹ Ph+ specimens were characterized as *BCR-ABL1* p190 or p210 breakpoints. LDA signature-positive specimens without *BCR-ABL1* fusion (Ph-like ALL) were categorized as *CRLF2*-overexpressing or non-overexpressing via LDA quantification of expression levels.¹¹ *CRLF2*-overexpressing specimens were

subsequently assessed for *IGH-CRLF2* and *P2RY8-CRLF2* rearrangements and *JAK1* and *JAK2* mutations via FISH, polymerase chain reaction (PCR), or reverse transcriptase (RT)-PCR with confirmatory Sanger sequencing as described.¹² Non-*CRLF2*-overexpressing Ph-like specimens were tested for 39 known kinase fusions involving 3' partner genes *ABL1*, *ABL2*, *CSF1R*, *JAK2*, *NTRK3*, *PDGFRB*, and *TYK2* via multiplex RT-PCR with confirmatory sequencing and/or FISH and for *EPOR* and other rearrangements via candidate next-generation sequencing (Archer FusionPlex).^{5, 12}

To confirm activated kinase signaling previously reported in childhood Ph-like ALL, 31 adult ALL specimens were incubated *in vitro* with human cytokines, ruxolitinib, or dasatinib and fixed and permeabilized as described.^{13, 14} Phosphorylated CrkL, STAT5, and/or S6 were measured by leukemia cell-specific phosphoflow cytometry to identify basal signaling activation and TKI-induced signaling inhibition and analyzed with Cytobank as described.^{13, 14}

Adult Ph-like ALL patient-derived xenograft (PDX) models were established in NOD.Cg-*Prkdcscid Il2rgtm1 Wjl/SzJ* (NSG) mice as described.¹⁴ Engrafted animals (5% human CD19+/CD45+ ALL in peripheral blood) were randomized to vehicle, ruxolitinib 90 mg/kg (LC Labs), or dasatinib 10 mg/kg (LC Labs) treatment twice daily via oral gavage for 28 days to assess *in vivo* inhibition of leukemia proliferation.^{3, 9} Human leukemia in spleens from sacrificed animals at end of treatment was quantified by flow cytometry as described.^{9, 14}

Means, medians, survival, and multivariate analyses for clinical data were calculated using Prism (GraphPad) or STATA. Unpaired two-sided t-tests were used to compare leukemia cell counts in spleens from vehicle- and TKI-treated PDX mice.

In these studies, we profiled 93 diagnostic B-ALL specimens for the Ph-like gene expression signature and associated genetic alterations (Supplemental Table 1). Four samples failed LDA testing due to insufficient material. For the remaining 89 specimens, median patient age at diagnosis was 46 years (range 18–88), 52.4% of patients were male, and median WBC count was 35.1×10^3 cells/uL blood (range 1–436) (Supplemental Table 2). Chemotherapy, TKI, and hematopoietic stem cell transplant (HSCT) status are delineated in Supplemental Table 1.

LDA analysis identified 51/89 patients (57.3%) with a kinase-active expression signature. Of these, 33 (37.1% of the entire cohort) were *BCR-ABL1*-rearranged (Ph+), and 18 (20.2%) were *BCR-ABL1*-negative by FISH or RT-PCR (Ph-like) (Figure 1A). Among the 38 non-Ph+/non-Ph-like specimens, 13 had *KMT2A (MLL)* rearrangements. Patients with Ph+ ALL and Ph-like ALL had similar sex distribution and levels of hyperleukocytosis (Supplemental Table 2).

Fourteen of the 18 (77.8%) Ph-like ALL specimens were *CRLF2*-R, and seven of these had concomitant *JAK2* mutations (Supplemental Table 1). Of the four non-*CRLF2*-R Ph-like ALL specimens, one had a cytogenetically-cryptic *EPOR* rearrangement (UP_240).⁵ Ph-like kinase alterations were not detected in specimen UP_5049 by RT-PCR or FusionPlex assays, although next-generation sequencing identified *FLT3 (fms-related tyrosine kinase 3)* internal

tandem duplication that may have caused its activated kinase signature (Supplemental Table 1). The remaining two specimens (UM_06, UM_07) had insufficient material for further genetic characterization.

Overall survival was assessed for patients for whom outcomes data were available (n=81). Median survival was 1.6, 2.7, 0.7, and 2.7 years for Ph-like, Ph+, *KMT2A*-R, and other B-ALL subsets, respectively (Figure 1B). Log-rank testing demonstrated a trend towards differential survival among the ALL subgroups (p=0.0798), although small patient numbers precluded definitive statistical analysis. Thirty-one patients underwent HSCT, including eight patients with Ph-like ALL (Supplemental Tables 1 and 2).

Concordant with prior observations in childhood Ph-like ALL,¹³ phosphoflow cytometric analysis of adult *CRLF2*-R Ph-like ALL specimens demonstrated basal and/or cytokine-inducible pSTAT5 and pS6, which were inhibited by ruxolitinib (Figure 2A and Supplemental Figure 1). Analogously, *ABL1*-rearranged Ph-like and Ph+ ALL cells had basal pCrkL activation, which was inhibited by dasatinib (Figure 2A). We further observed significant inhibition of leukemia proliferation in adult *CRLF2*-R/*JAK2*-mutant Ph-like ALL PDX models treated with ruxolitinib and in *ABL1*-rearranged models treated with dasatinib (Figure 2B). These results, consistent with earlier pediatric studies,^{3, 9, 14} confirm that JAK- and ABL-targeted TKI therapies also have therapeutic potential in relevant subsets of adult Ph-like ALL.

A potential bias of our study is possible preferential banking of ALL specimens from patients with higher WBC counts, which may have enriched detection of Ph+, Ph-like, and other high-risk subtypes of ALL. Multivariate analyses of pediatric/AYA B-ALL patients suggest that presenting WBC ≥ 100,000 cells/uL is highly suggestive of Ph-like ALL and portends poor prognosis, although leukocytosis alone remains an imperfect clinical predictor.²

Over-representation of *GATA3* ALL susceptibility variants and *IKZF1* deletions has been reported in pediatric/AYA Ph-like ALL with greatest incidence in Hispanic/Latino and Native American patients.¹⁵ While we did not specifically investigate these polymorphisms or other copy number alterations, 8.3% of our cohort was Hispanic/Latino, which may have also influenced the Ph-like frequency identified in our population.

In summary, we identified a 20.2% incidence of Ph-like ALL in adults and, importantly, demonstrate that >50% of adult B-ALL is associated with activated kinase signaling via *BCR-ABL1* or *BCR-ABL1*-like rearrangements. While frequent kinase fusions also occur in pediatric/AYA Ph-like ALL, our study and a recent European study report *CRLF2* rearrangement as the predominant genetic lesion in adult Ph-like ALL.⁷ We thus propose that routine ALL diagnostic testing algorithms include flow cytometric assessment of surface *CRLF2* (TSLPR) overexpression with subsequent genetic confirmation of specific *CRLF2* rearrangements.¹³

Our study used LDA testing as a “gold standard” to define a Ph-like gene expression signature in adult ALL.^{11, 12} This validated test derived from a gene expression array classification defining Ph-like ALL rapidly identifies both Ph+ and Ph-like ALL and enables

prioritization of targeted downstream molecular characterization of Ph-like specimens. While our study analyzed a relatively small patient cohort, we nonetheless demonstrate a substantial population of adults 40 years old with Ph-like ALL and poor outcomes who may benefit from TKI addition to appropriately dose-intensive chemotherapy. Clinical translation of these findings will potentially transform therapeutic approaches and improve clinical outcomes for older adults with Ph-like ALL.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors acknowledge Dr Yong Li, Ms Jenna Harrison, Dr Anne Lehman, and Ms Gisela N Brake-Sillá for assistance with specimen preparation and Dr Fengqi Chang, Ms Eileen Stonerock, and Ms Heather Jenkins for assistance with genetic assays. The authors also thank Ms Joy Cannon and Dr Marlise Luskin for assistance with clinical data acquisition and analysis. This work was supported by the NIH/NCI (K08CA184418; SKT), the Hematologic Malignancies Translational Center of Excellence of the Abramson Cancer Center and the Institute for Translational Medicine and Therapeutics at the University of Pennsylvania (NIH/NCRR UL1RR024134; SKT, GBW, MC), the Rally Foundation for Childhood Cancer Research (SKT), and an American Cancer Society CEOs Against Cancer Pennsylvania Chapter Research Scholar Grant (RSG-16-102-01-LIB to AEP). MC is supported by a Veterans Administration Merit Award 1I01BX000918.

SKT receives research funding from Gilead Sciences. SKT and MC have research collaborations with Incyte Corporation without financial support.

References

1. Chalandon Y, Thomas X, Hayette S, Cayuela JM, Abbal C, Huguet F, et al. Randomized study of reduced-intensity chemotherapy combined with imatinib in adults with Ph-positive acute lymphoblastic leukemia. *Blood*. 2015 Jun 11; 125(24):3711–3719. [PubMed: 25878120]
2. Loh ML, Zhang J, Harvey RC, Roberts K, Payne-Turner D, Kang H, et al. Tyrosine kinase sequencing of pediatric acute lymphoblastic leukemia: a report from the Children's Oncology Group TARGET Project. *Blood*. 2013 Jan 17; 121(3):485–488. [PubMed: 23212523]
3. Roberts KG, Li Y, Payne-Turner D, Harvey RC, Yang YL, Pei D, et al. Targetable kinase-activating lesions in Ph-like acute lymphoblastic leukemia. *The New England journal of medicine*. 2014 Sep 11; 371(11):1005–1015. [PubMed: 25207766]
4. 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. *The Lancet Oncology*. 2009 Feb; 10(2):125–134. [PubMed: 19138562]
5. Iacobucci I, Li Y, Roberts KG, Dobson SM, Kim JC, Payne-Turner D, et al. Truncating Erythropoietin Receptor Rearrangements in Acute Lymphoblastic Leukemia. *Cancer Cell*. 2016 Feb 8; 29(2):186–200. [PubMed: 26859458]
6. Mullighan CG, Su X, Zhang J, Radtke I, Phillips LA, Miller CB, et al. Deletion of IKZF1 and prognosis in acute lymphoblastic leukemia. *The New England journal of medicine*. 2009 Jan 29; 360(5):470–480. [PubMed: 19129520]
7. Herold T, Schneider S, Metzeler K, Neumann M, Hartmann L, Roberts KG, et al. Philadelphia chromosome-like acute lymphoblastic leukemia in adults have frequent IGH-CRLF2 and JAK2 mutations, persistence of minimal residual disease and poor prognosis. *Haematologica*. 2016 Aug 25.
8. Sive JI, Buck G, Fielding A, Lazarus HM, Litzow MR, Luger S, et al. Outcomes in older adults with acute lymphoblastic leukaemia (ALL): results from the international MRC UKALL XII/ECOG2993 trial. *Br J Haematol*. 2012 May; 157(4):463–471. [PubMed: 22409379]

9. Maude SL, Tasian SK, Vincent T, Hall JW, Sheen C, Roberts KG, et al. Targeting JAK1/2 and mTOR in murine xenograft models of Ph-like acute lymphoblastic leukemia. *Blood*. 2012 Oct 25; 120(17):3510–3518. [PubMed: 22955920]
10. 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 Feb 4.
11. Harvey RC, Kang H, Roberts KG, Chen IM, Atlas SR, Bedrick EJ, et al. Development and Validation of a Highly Sensitive and Specific Gene Expression Classifier to Prospectively Screen and Identify B-Precursor Acute Lymphoblastic Leukemia (ALL) Patients with a Philadelphia Chromosome-Like Signature (“Ph-like” or “BCR-ABL1-Like”) for Therapeutic Targeting and Clinical Intervention. *Blood*. 2013; 123(21) ASH annual meeting abstract #826.
12. Reshmi SC, Harvey RC, Smith A, Chen I-M, Valentine M, Liu Y, et al. Frequency of actionable gene fusions in patients with Philadelphia chromosome-like (Ph-like) B-acute lymphoblastic leukemia (ALL): A retrospective study from the Children’s Oncology Group (COG). *Cancer Research*. 2015 Aug 1.75(15 Supplement) 2015. abstract 4729.
13. Tasian SK, Doral MY, Borowitz MJ, Wood BL, Chen IM, Harvey RC, et al. Aberrant STAT5 and PI3K/mTOR pathway signaling occurs in human CRLF2-rearranged B-precursor acute lymphoblastic leukemia. *Blood*. 2012 Jul 26; 120(4):833–842. [PubMed: 22685175]
14. Tasian SK, Teachey DT, Li Y, Shen F, Harvey RC, Chen IM, et al. Potent efficacy of combined PI3K/mTOR and JAK or ABL inhibition in murine xenograft models of Ph-like acute lymphoblastic leukemia. *Blood*. 2016 Oct 24.
15. Perez-Andreu V, Roberts KG, Harvey RC, Yang W, Cheng C, Pei D, et al. Inherited GATA3 variants are associated with Ph-like childhood acute lymphoblastic leukemia and risk of relapse. *Nat Genet*. 2013 Dec; 45(12):1494–1498. [PubMed: 24141364]

Figure 1. Ph-like ALL occurs commonly in adults with B-ALL and is associated with poor outcomes

(A) Incidence of Ph-like and other genetic subtypes in adults with B-ALL with subset analyses of patients ≥ 40 and < 40 years of age. Age was not available for two patients. (B) Kaplan-Meier survival analysis with log-rank comparison test of patients with B-ALL for whom outcomes data were available (n=81) with indicated p-value. When appropriate, survival was censored at last known clinical evaluation.

Figure 2. Ph-like ALL in adults demonstrates signaling hyperactivation and sensitivity to tyrosine kinase inhibition

(A) Thirty-one primary adult B-ALL specimens with the designated genetic alterations (n=9 *CRLF2*-R Ph-like, n= 2 *ABL1*-R Ph-like, n=10 Ph+, n=*KMT2A*-R, n=5 other B-ALL) were incubated *in vitro* with 25 ng/mL thymic stromal lymphopoietin (TSLP; ligand for *CRLF2* receptor), 100 nM dasatinib, or 1 uM ruxolitinib for 30 minutes (TSLP) or 60 minutes (inhibitors) at 37 °C, then fixed with paraformaldehyde and permeabilized with methanol.¹³ Stimulated or inhibited intracellular phosphoproteins within human CD19+ CD45+ (and CD10+ and TSLPR+ if applicable) ALL cells were analyzed by phosphoflow cytometry. Data are depicted as %change of basal median fluorescent intensity (MFI) with colorimetric normalization to basal MFI levels (grey) for each phosphoprotein in each specimen and summarized as a heatmap. Red and blue indicate increased and decreased phosphorylation, respectively. Gating strategy and representative histograms for individual samples are

displayed in Supplemental Figure 1. **(B)** Patient-derived xenograft (PDX) models of adult Ph-like ALL established in 6–8 week old male and female NSG mice were randomized to treatment with vehicle, ruxolitinib 90 mg/kg twice daily, or dasatinib 10 mg/kg twice daily (n=5 mice/treatment cohort) via oral gavage for 28 days after initial demonstration of 5% human CD19+CD45+ ALL in murine peripheral blood.^{9, 14} Animals were monitored daily for physical signs of disease and weekly by venous bleeding for flow cytometry (FC) quantification of leukemia in peripheral blood during treatment. Human ALL cells in murine spleens from sacrificed animals were also quantified by FC as above after four weeks of treatment at planned study endpoint. Ruxolitinib or dasatinib markedly reduced total human ALL cell number (y-axis) versus vehicle-treated controls in Ph-like ALL PDX models harboring *CRLF2* or *ABL1* rearrangement, respectively (p<0.0001 via two-tailed t-test). All animal studies were conducted on CHOP Institutional Animal Care and Usage Committee-approved research protocols in compliance with NIH and ARRIVE guidelines. Animals were monitored daily for physical signs of illness and sacrificed at planned study endpoint or sooner if ill-appearing. Note that clinical data associated with specimens from young adults at the Children's Hospital of Philadelphia (CHOP) used for functional studies were not included in the main analyses in Figure 1 or Supplemental Tables. UP = University of Pennsylvania, UM = University of Michigan.