Clinical efficacy of ruxolitinib and chemotherapy in a child with Philadelphia chromosome-like acute lymphoblastic leukemia with *GOLGA5-JAK2* fusion and induction failure

Patients with Philadelphia chromosome-like B-cell lymphoblastic leukemia (Ph-like or *BCR-ABL1*-like B-ALL) experience high relapse rates and are difficult to cure with conventional chemotherapy.[1,2] The Ph-like ALL subtype comprises 15-25% of B-ALL in older children and adolescents/young adults (AYAs) and 20-40% of cases in older adults.[1,2] Ph-like ALL is driven by alterations in cytokine receptor signaling pathway genes that induce oncogenic kinase signaling, suggesting therapeutic potential for kinase inhibitors.[1] Approximately 50% of Ph-like ALL has rearrangements in *CRLF2* (*cytokine receptor-like factor 2*), often with concomitant *JAK2* or *JAK1* point mutations, which leads to constitutive JAK/STAT signaling targetable by JAK inhibitors (JAKi) such as ruxolitinib. *JAK2* and *EPOR* translocations comprise an additional 8-12% of Ph-like ALL in children/AYAs and adults.[1-3] *JAK2*- or *EPOR*-rearranged ALLs are also characterized by hyperactive JAK/STAT signaling and are potentially very sensitive to JAK inhibition.[3,4] While JAKi addition to multi-agent chemotherapy is under current clinical investigation in children and adults with newly-diagnosed or relapsed *CRLF2*-R/JAK-mutant Ph-like ALL via early-phase trials (*clinicaltrials.gov identifier 02723994, 03117751, 02420717*), whether this therapeutic strategy
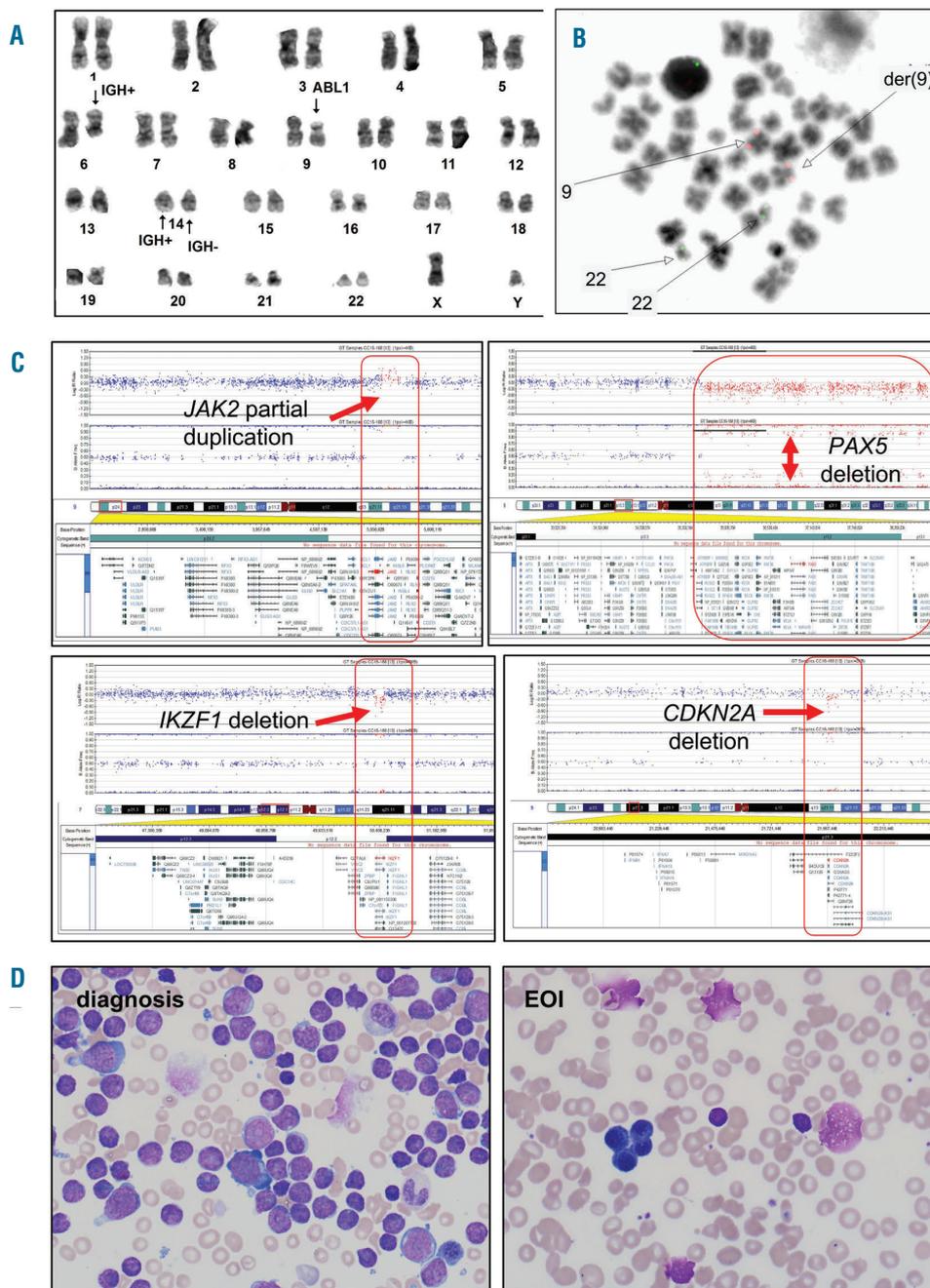

Figure 1. Clinical cytogenetic and fluorescence *in situ* hybridization (FISH) analyses of the patient's diagnostic bone marrow aspirate. (A) Karyotype shows multiple cytogenetic abnormalities, including abnormalities of chromosome 9 and t(6;14) involving *IGH* and an unknown fusion partner on chromosome 6. (B) FISH analysis demonstrates absence of *BCR-ABL1* translocation with two normal signals for *ABL1* on chromosome 9 (red probes) and BCR on chromosome 22 (green probes). (C) Single nucleotide polymorphism (SNP) array analysis of the patient's diagnostic bone marrow aspirate demonstrates partial duplication of *JAK2* and deletions of *IKZF1*, *PAX5*, and *CDKN2A*. (D) Photomicrographs of diagnostic (left) and end-of-induction (EOI, right) Wright-Giemsa-stained bone marrow aspirates. Images were captured at 100X via an Olympus BX53 microscope and UPlanSApo camera using CellSens Standard software. End of induction marrow blasts are markedly smaller and have a normal hematogone-like appearance by morphology.
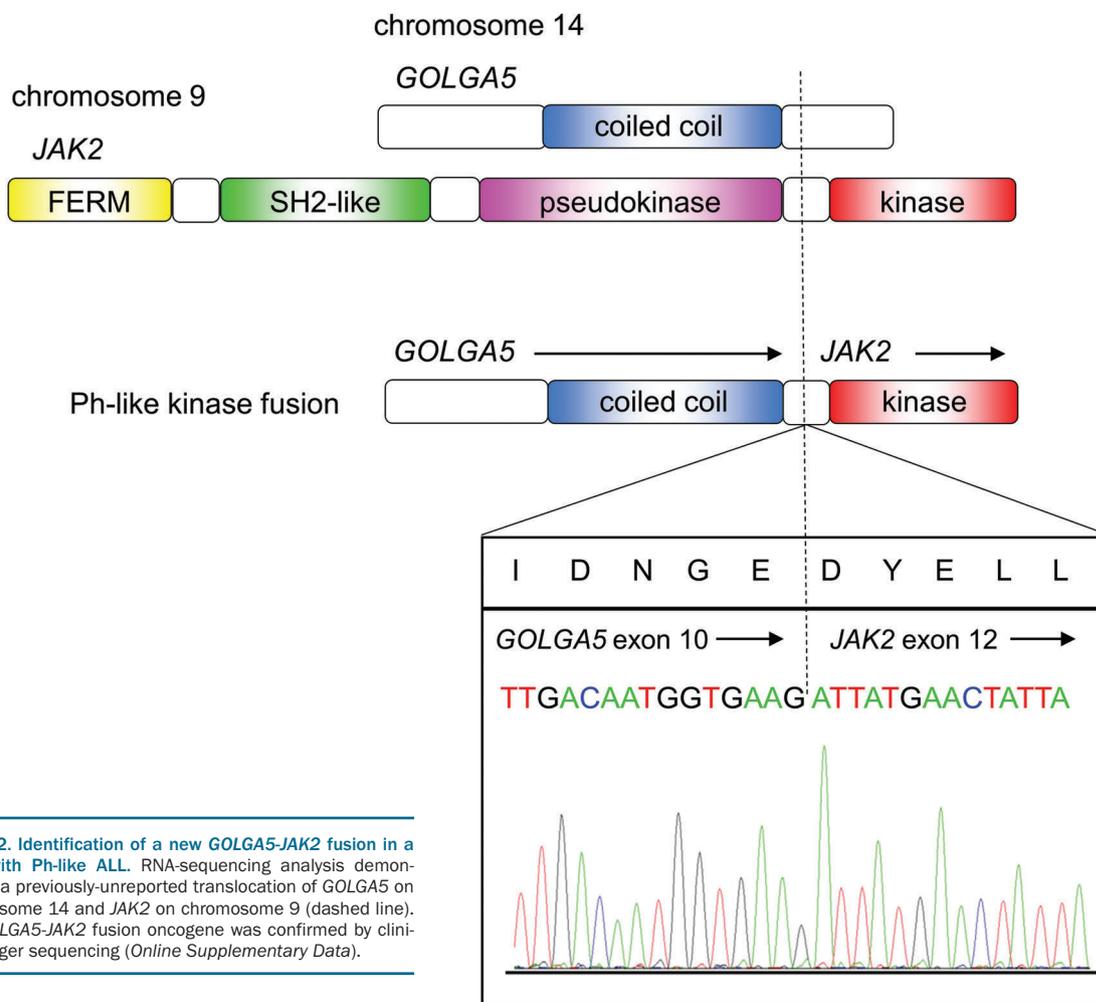
Figure 2. Identification of a new *GOLGA5-JAK2* fusion in a child with Ph-like ALL. RNA-sequencing analysis demonstrated a previously-unreported translocation of *GOLGA5* on chromosome 14 and *JAK2* on chromosome 9 (dashed line). The *GOLGA5-JAK2* fusion oncogene was confirmed by clinical Sanger sequencing (*Online Supplementary Data*).

will decrease relapse and improve survival is not yet known. We report an exceptional case of a child with chemoresistant Ph-like ALL and induction failure in whom we identified a novel *GOLGA5-JAK2* fusion. We further demonstrate molecular clearance of the *GOLGA5-JAK2* fusion and leukemia remission induction after addition of ruxolitinib to high-risk B-ALL chemotherapy.

A 10-year-old Caucasian male was diagnosed with National Cancer Institute high-risk B-ALL in June 2015 after presenting with fatigue, weight loss, and dyspnea. Physical examination was pertinent for pallor, orthopnea, and hepatosplenomegaly. Diagnostic laboratory studies showed white blood cell (WBC) count 578,000/μL with 92% blasts, hemoglobin 5.7 g/dL, and platelets 89,000/μL. His dyspnea and hypoxia improved following emergent leukopheresis.

Bone marrow aspirate analysis demonstrated B-ALL (CD45+/CD9+/CD10+/CD19+/CD20(subset)+/CD34+/cCD 79a+/HLA-DR+/TDT+/MPO−) with microscopic leukemia involvement of the cerebrospinal fluid (CNS2a). The patient's parents provided informed consent for research specimen banking on the Children's Oncology Group (COG) AALL08B1 study and induction chemotherapy on the COG AALL1131 trial (*clinicaltrials.gov identifier 02883049; Online Supplementary Methods*). Cytogenetic analysis demonstrated a complex karyotype with 4 6 , X Y , d e r (6) t (6 ; 1 4) (p 2 3 ; q 3 2) d e l (6) (q 1 5 q 2 3) , d e r (9) i n v (9) (p ? q ? 3 4) d e l (9) (p21p13)del(9)(q22q31),der(14)t(6;14)(p23;q32)[14].ish,d

er(6)(IGH+),der(14)(IGH−)/46,XY[4]. Fluorescence *in situ* hybridization studies were negative for *BCR-ABL1*, *ETV6-RUNX1*, and *IGH-MYC* translocations and *CRLF2*, *KMT2A* (*MLL*), *NUP98*, and *PDGFRB* rearrangements (Figure 1A-B). Single nucleotide polymorphism array analysis demonstrated chromosome 9 duplication involving *JAK2* and deletions of *IKZF1*, *PAX5*, and *CDKN2A* concordant with the recently-described *IKZF1*(plus) subtype (Figure 1C, *Online Supplementary Table S1*).[5] The child experienced poor initial response to chemotherapy with 43.3% peripheral blood minimal residual disease (MRD) on induction day 8. While his induction day 29 bone marrow appeared morphologically normal (M1 remission), flow cytometry (FC) analysis revealed 40% MRD, consistent with induction failure (Figure 1D).

The patient's high WBC count, *IKZF1* deletion, absence of *BCR-ABL1* fusion, and induction failure raised significant suspicion for Ph-like ALL. Low-density microarray (LDA) analysis of leukemia RNA demonstrated a very high Ph-like ALL expression signature (0.835 8-gene and 0.987 15-gene scores) and normal *CRLF2* expression.[6] However, clinical testing for 39 known Ph-like kinase fusions via reverse-transcriptase polymerase chain reaction (RT-PCR) was negative for *CRLF2*, *JAK2,ABL1*, *ABL2*, *CSF1R*, and *PDGFRB* fusions and *JAK1* and *JAK2* point mutations.[6] Given the patient's lack of *CRLF2* rearrangement and LDA score >0.8 (associated with ABL-class kinase fusions in >80% of cases[6]) and reported *EBF1-PDGFRB* fusions in patients with Ph-like ALL induction failure,[7] dasatinib 60 mg/m² orally once-
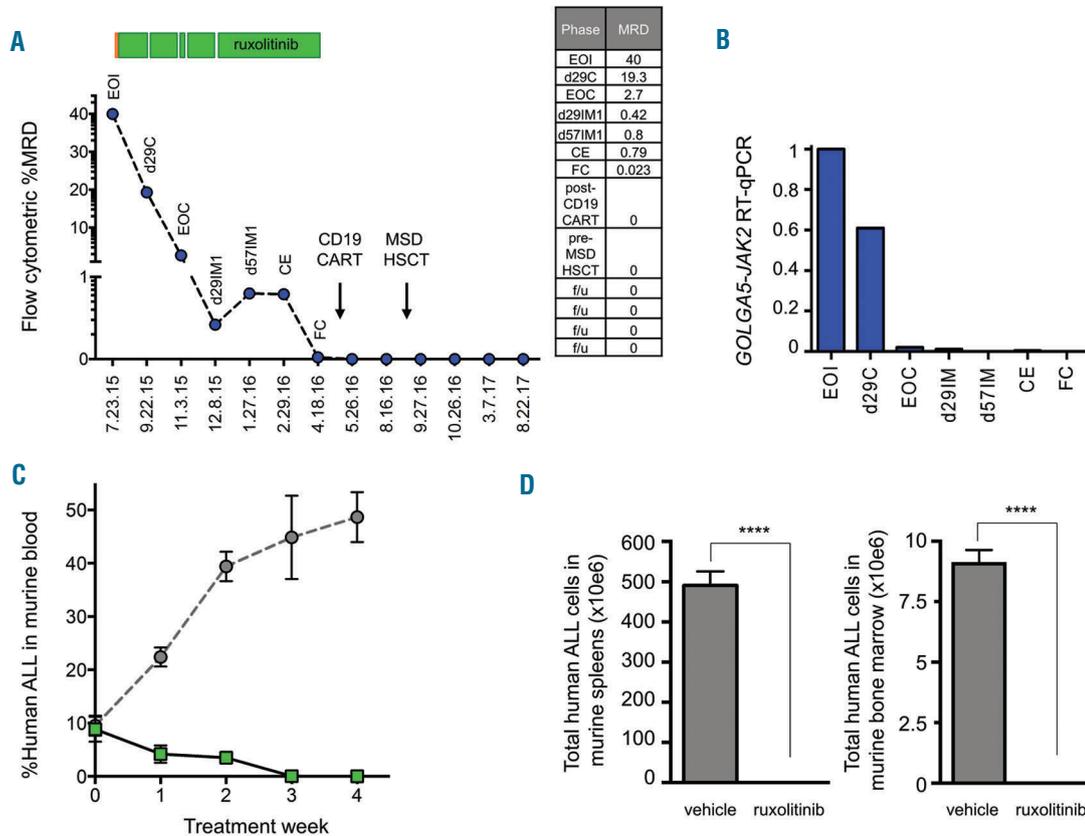
Figure 3. Clinical response to JAK inhibition and chemotherapy and assessment of *in vivo* efficacy of ruxolitinib monotherapy in *GOLGA5-JAK2* Ph-like ALL patient-derived xenograft (PDX) model. (A) Flow cytometric minimal residual disease (MRD) monitoring of patient's bone marrow during combined ruxolitinib and AALL1131-type chemotherapy demonstrates rapid induction of leukemia remission (y-axis and table inset; assay sensitivity 1 x 10⁴). X-axis indicates dates of bone marrow aspirate assessments. Orange bar = dasatinib 60 mg/m² orally once daily. Green bar = ruxolitinib 40 mg/mg/dose orally twice daily. EOI: end of induction; d29C: day 29 of consolidation; EOC: end of consolidation; IM1: interim maintenance 1; CE: cyclophosphamide/etoposide; FC : fludarabine/cyclophosphamide; CD19 CART: CD19-redirected chimeric antigen receptor T-cell immunotherapy; MSD HSCT: matched sibling donor allogeneic hematopoietic stem cell transplantation; f/u: follow-up time points. (B) Quantitative reverse-transcriptase and real-time polymerase chain reaction (RT-qPCR) monitoring of the *GOLGA5-JAK2* fusion transcript in serial bone marrow aspirate specimens demonstrated rapid molecular clearance of *JAK2*-mutant ALL with combination therapy as in (A) (assay sensitivity 1 x 10⁴ - 1 x 10⁵). (C) Eight-week-old female NSG mice were injected via tail vein with 2 x 10⁶ of the child's *GOLGA5-JAK2* ALL cells from diagnostic bone marrow (PAXDBJ) to create primary xenograft models, and murine spleens were harvested for flow cytometry (FC) quantification of CD10⁺ CD19⁺ CD45⁺ human B-ALL cells and subsequent xenotransplantation. Secondary PDX models were created via injection of 1 x 10⁶ viably cryopreserved murine splenocytes from well-engrafted primary PDX mice. After 5% human B-ALL engraftment was detected in murine peripheral blood by FC, secondary PDX mice were randomized to treatment with control or ruxolitinib 2g/kg chow continuously provided for 28 days (n=10 mice/cohort). Rapid clearance of human leukemia cells was observed in murine peripheral blood with ruxolitinib treatment by weekly quantitative FC analysis. (D) Ruxolitinib potently inhibited leukemia proliferation *in vivo* with near-complete elimination of human ALL in murine spleens and bone marrow after 4 weeks of treatment (****P<0.0001 by Student's t-test).

daily was empirically added to the child's AALL1131-type consolidation therapy (*Online Supplementary Methods*). We then performed research-level RNA-sequencing and identified a previously-unreported *GOLGA5-JAK2* fusion from t(9;14) (Figure 2), which was confirmed by clinical Sanger sequencing and Archer anchored multiplex PCR FusionPlex testing.

Given our prior demonstrated sensitivity of Ph-like ALL patient-derived xenograft (PDX) models with *JAK2* fusions to ruxolitinib,[4] dasatinib was discontinued at consolidation day 7 upon detection of the *GOLGA5-JAK2* fusion. Ruxolitinib was started at 40 mg/m²/dose orally twice-daily (PO BID), a safe and pharmacodynamically-active dose identified on the COG ADVL1011 trial[8] and the starting dose on the now-open COG AALL1521/INCB18424-269 trial (*clinicaltrials.gov identifier 02723994*). The patient tolerated combined ruxolitinib and AALL1131-type post-induction chemotherapy well. Ruxolitinib was briefly discontinued after consolidation

for grade 4 (CTCAE v4.0) transaminitis of unclear etiology that resolved in <7 days and empirically between myelosuppressive chemotherapy phases to facilitate blood count recovery. He did not experience prolonged cytopenias or toxicities beyond those typically observed with AALL1131 backbone therapy. Serial bone marrow aspiration with FC MRD testing was performed to assess response. *GOLGA5-JAK2* fusion transcript levels were followed in marrow specimens by quantitative RT-PCR assays (*Online Supplementary Methods*).

Sequential marrow analysis demonstrated significant clinical response to combined ruxolitinib/chemotherapy by the end of consolidation and interim maintenance 1 (IM1), although with persistent MRD ≥0.01% by FC (Figure 3A). Given high relapse risk and poor outcomes of children with MRD positivity prior to allogeneic hematopoietic stem cell transplantation (HCST),[9,10] the child received autologous CD19-redirected chimeric antigen receptor T cells (CD19CART) in April 2016 on a

phase 1 trial (*clinicaltrials.gov identifier 02374333*) and achieved MRD-negative remission. Given his prior chemoresistance and known relapse risk post-CD19CART, he underwent matched sibling donor HSCT in August 2016 following myeloablative conditioning. He remains in continued MRD-negative leukemia remission with full donor chimerism at 20 months post-HSCT.

Research-level RT-PCR monitoring of *GOLGA5-JAK2* transcript levels in the patient's bone marrow demonstrated near-complete clearance of the Ph-like ALL fusion by the end of consolidation and undetectable transcript levels at the end of IM1 (Figure 3B), although he had persistent low-level MRD detected by clinical FC analyses. MRD quantification by currently-available next-generation sequencing or PCR-based immunoglobulin/T-cell receptor rearrangement methodologies may be beneficial in such situations to increase precision of MRD assessments and to inform therapeutic decisions.

Simultaneously, we established a PDX model (PAXD-BJ) using viably cryopreserved diagnostic bone marrow ALL cells from the child engrafted into NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG) mice via informed consent on Institutional Review Board- and Institutional Animal Care and Use Committee-approved research protocols as described.[4,11] Once ≥1% human ALL was detected in murine peripheral blood, secondary PDX mice were randomized to control or ruxolitinib 2g/kg chow (a gift from the Incyte Corporation) treatment continuously provided. Human ALL cells were quantified weekly by FC analysis of retro-orbital murine venous blood samples. Total human ALL burden was enumerated in spleens of sacrificed animals after 28 days of treatment.[11] Data were compared by t-tests using Prism (GraphPad). *GOLGA5-JAK2* ALL PDX models treated with ruxolitinib demonstrated rapid clearance of human leukemia in murine peripheral blood. Residual ALL was effectively undetectable by quantitative FC analysis of murine spleens and bone marrow after 28 days of ruxolitinib versus control chow (P<0.0001) (Figure 3C-D). These data demonstrate a similar magnitude and rate of ruxolitinib-induced leukemia clearance as was observed in the patient, suggesting that preclinical PDX models may predict clinical responses to JAKi.

Prior studies have demonstrated remarkable outcome improvements in children with *BCR-ABL1*-rearranged (Ph+) ALL with imatinib addition to chemotherapy.[12,13] Integration of kinase inhibitor-based approaches for patients with Ph-like ALL may similarly decrease relapse risk and improve survival given analogous hyperactivation of kinase signaling. While recently-opened clinical trials are investigating efficacy of ruxolitinib and dasatinib in patients with JAK pathway-mutant and ABL class-mutant Ph-like ALL, respectively, anecdotal use of kinase inhibitors in Ph-like ALL has primarily been reported in children/AYA with ABL class alterations treated with imatinib or dasatinib.[1] To our knowledge, only one report has described ruxolitinib use in a patient with Ph-like ALL.[14] In that study, a 17-year-old Native American female with CRLF2-overexpressing Ph-like ALL and a *JAK2* F694L point mutation had increasing MRD (10.4%) after consolidation chemotherapy. Ruxolitinib 40 mg/m2/dose PO BID was added to AALL1131-type interim maintenance and delayed intensification therapy. She achieved MRD <0.01% with JAKi/chemotherapy, underwent allogeneic HSCT, and was in continued remission at 100 days post-HSCT.[14] Our concordant clinical data demonstrate safe and clinically effective combination of high-dose ruxolitinib and post-induction multi-agent chemotherapy in a child with Ph-like ALL with a *JAK2* fusion and induction failure, which has been associated with dismal long-term outcomes.[7,15] We observed excellent reduction in the patient's ALL burden and significantly decreased *GOLGA5-JAK2* fusion levels with JAKi/chemotherapy, although persistence of FC-detectable MRD prompted us to pursue subsequent cellular immunotherapy and HSCT. More importantly, our patient's case highlights continued discovery of new Ph-like alterations and need for unbiased genetic testing approaches to diagnose fusions in real-time for potential clinical intervention. Anchored multiplex PCR fusion assays and RNA-sequencing technologies may provide an appreciable detection advantage over current PCR-based clinical assays for known fusions.[6]

In summary, (1) early clinical suspicion for Ph-like ALL and (2) rapid RNA-sequencing analysis capabilities facilitated identification of a previously-unknown and initially clinically-unidentifiable *GOLGA5-JAK2* fusion in a child with significant leukocytosis and induction failure. This timely genetic characterization allowed swift implementation of commercially-available JAKi therapy (prior to current clinical trials), which was well-tolerated with an intensive chemotherapy backbone and induced morphologic remission. Concomitant PDX modeling demonstrating exquisite *in vivo* sensitivity of *GOLGA5-JAK2* ALL cells to ruxolitinib provided additional biologic validation of the child's clinical response. Data from AALL1521 and other trials will determine the potential efficacy of combining ruxolitinib and chemotherapy in larger cohorts of patients with JAK pathway-mutant Ph-like ALL.

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