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A Phase 1 Dosing Study of Ruxolitinib in Children with Relapsed or Refractory Solid tumors, Leukemias, or Myeloproliferative Neoplasms: A Children's Oncology Group Phase 1 Consortium Study (ADVL1011)

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Abstract

Background—Ruxolitinib, an orally bioavailable JAK1/JAK2 inhibitor, may treat cancers with *CRLF2* and/or JAK pathway mutations.

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Conflict of Interest: Dr. Loh served on a Novartis Advisory Board to discuss the development of ruxolitinib in pediatrics in April 2013.

Procedure—A phase 1 trial of ruxolitinib was performed to determine the maximum-tolerated or recommended phase 2 dose, dose-limiting toxicities (DLTs), pharmacokinetics (PK), and pharmacodynamics (PD) in children with recurrent/refractory solid tumors (STs). Ruxolitinib was administered twice daily (BID) in 28-day cycles at five dose levels (15, 21, 29, 39, and 50 mg/m²/dose). PK and PD studies were performed during Cycle 1. Toxicity, preliminary efficacy, and PK/PD were also assessed in children with relapsed/refractory hematologic malignancies (HMs).

Results—Forty-nine patients were enrolled, 28 with STs (dose escalation cohort) and 21 with HMs. Ruxolitinib was well-tolerated with one DLT per cohort of 6 patients at dose levels (DLs) 2-5. One patient with a ST had grade 5 multi-organ failure at DL2. One patient each at DL3 and DL4 had a grade 4 neutropenia, and one patient at DL5 had a grade 4 creatinine phosphokinase elevation. No objective responses were observed in patients with STs. One patient with polycythemia vera achieved a partial response and received 18 cycles of ruxolitinib. The PK of ruxolitinib were similar to that in adults. Partial inhibition of phosphorylated JAK2, STAT5, and S6 was observed in *in vitro* plasma inhibitory activity PD assay.

Conclusion—Ruxolitinib was well-tolerated in children with refractory cancer. The recommended phase 2 dose for continuous BID oral administration is 50 mg/m²/dose. Subsequent evaluation of ruxolitinib in combination with cytotoxic chemotherapy in children, adolescents, and young adults with JAK-mutant leukemias is planned.

Keywords

childhood cancer; JAK inhibitor; ruxolitinib; pediatric; pharmacokinetics

INTRODUCTION

Activation of tyrosine kinases and associated proteins commonly occurs in solid tumors, leukemias, and myeloproliferative neoplasms (MPNs). Phosphorylation of Janus kinase and signal transducer and activator of transcription (JAK/STAT) proteins is critical for cytokine signaling and signal transduction in mammalian cells, and constitutive activation of JAK/STAT and related pathway signaling has been identified in several hematologic malignancies and some solid tumors [1,2].

While contemporary cure rates for childhood acute lymphoblastic leukemia (ALL) approach 85%, relapse remains the most frequent cause of treatment failure and death [3]. Outcomes for adults with newly-diagnosed disease are substantially worse and possibly attributable to the heterogeneous biologic subsets in adult ALL [4,5]. Consequently, identifying genomic alterations that may be targeted with specific therapies in children, adolescents, and young adults at the highest risk of relapse has been an area of intense focus. Over the past decade, we have studied genomic profiles of primary leukemic blasts in association with clinical variables to develop powerful relapse predictors and potential avenues for new therapies. One example is the discovery of a Ph-like gene expression profile in approximately 15% of high-risk pediatric B-ALL patients enrolled on Children's Oncology Group (COG) AALL0232 who lack the classic *BCR-ABL1* fusion gene [6,7]. Only 63% of patients with Ph-like ALL remain in remission at 5 years vs. 86% of those without this signature, highlighting the need for alternative therapeutic approaches [7].

Through the TARGET (Therapeutically Applicable Research to Generate Effective Treatments) initiative sponsored by the National Cancer Institute (NCI), other novel genomic alterations in childhood ALL have been discovered using a variety of sequencing technologies [7-10]. For example, *JAK1* and *JAK2* point mutations have been detected in children with Down Syndrome-associated and non-Down Syndrome-associated ALL [9,11,12]. JAK point mutations are frequently accompanied by genomic rearrangements and occasionally point mutations of *CRLF2* (*cytokine receptor like factor-2*), which leads to overexpression of the encoded protein, thymic stromal lymphopoietin receptor (TSLPR) [9,13,14]. JAK mutations and TSLPR overexpression confer growth factor independence *in vitro*, which can be overcome by pharmacologic JAK inhibition [9,11,12]. In addition, primary patient ALL cells have been shown to have high basal levels of phosphorylated JAK2 and STAT5 that can be inhibited *in vitro* by the JAK inhibitor ruxolitinib [15]. Constitutive activation of JAK-STAT signaling has also been reported in solid tumors and lymphoma, and decreased tumor growth with JAK inhibition has been demonstrated *in vitro* and *in vivo* in preclinical models [16,17], as well as in an early phase clinical trial for patients with refractory lymphoma [18]. Ruxolitinib (INCB018424) is a potent, orally bioavailable inhibitor of the JAK family of kinases with particular efficacy against JAK1 and JAK2. In clinical trials for adults with MPNs, single-agent ruxolitinib has been well-tolerated with reversible thrombocytopenia as its major dose-limiting toxicity (DLT) [19].

We report the results of a Children's Oncology Group (COG) phase 1 trial (ADVL1011) of ruxolitinib with BID continuous oral dosing in children with refractory or recurrent solid tumors (Part A) or relapsed leukemia or MPNs (Part B). Secondary aims included evaluation of the pharmacokinetics (PK) of ruxolitinib, as well as a preliminary assessment of the pharmacodynamic (PD) effects of ruxolitinib upon intracellular kinase signaling.

METHODS

Patient eligibility

Patients >12 months and <22 years of age with recurrent or refractory solid tumors (STs; Part A), including lymphomas, were eligible. In addition, patients with hematologic malignancies (HMs), including relapsed or refractory ALL or acute myeloid leukemia (AML), and patients with chronic MPNs (polycythemia vera [PV], essential thrombocythemia, juvenile myelomonocytic leukemia [JMML]) without known curative therapies (Part B) were eligible to enroll at one dose level below that at which patients with STs were enrolling. Other eligibility criteria included a Lansky or Karnofsky score $\geq$ 50, recovery from acute toxic effects of prior therapy, and $\geq$ 7 days from prior growth factor therapy (if short-acting or 14 days if long-acting). Patients with STs had to be $\geq$ 3 weeks from receipt of myelosuppressive chemotherapy. Patients with HMs had to be $\geq$ 2 weeks from prior cytotoxic therapy with the exception of maintenance-type ALL therapy for which there was no washout period. Patients had to be >3 months from prior stem cell transplant and without evidence of graft-versus-host disease. At least two weeks from local radiation had to have elapsed. Patients who were on corticosteroids must have been taking a stable or decreasing dose for 7 days prior to enrollment. Hydroxyurea use was permitted until 24 hours prior to the first dose of ruxolitinib.

Other requirements for patients with STs without known bone marrow involvement included adequate bone marrow function (peripheral absolute neutrophil count $>1,000/\mu\text{L}$, platelet count $>100,000/\mu\text{L}$, hemoglobin $>8.0\text{ g/dL}$, and transfusion independence). Patients with STs with marrow involvement and transfusion-dependent hematologic malignancies patients could enroll on study, but did not contribute data to the assessment of hematologic DLTs. Other standard organ function requirements included an age-adjusted normal serum creatinine or glomerular filtration rate (GFR) $\geq 70\text{ mL/min/1.73 m}^2$ and normal liver function (total bilirubin $\leq 1.5\times$ the institutional upper limit of normal for age, ALT $\leq 110\text{ U/L}$, and albumin $\geq 2\text{ g/dL}$).

The institutional review boards of participating institutions approved the protocol. Written informed consent from patients ≥ 18 years or from parents/legal guardians of children age <18 years was obtained (and assent as appropriate) according to individual institutional policies.

Drug administration and study design

The primary objectives of the study were to 1) estimate the maximum tolerated dose (MTD) or recommended phase 2 dose (RP2D) of ruxolitinib administered orally continuously twice daily to children with relapsed or refractory solid tumors, 2) define and describe the toxicities of ruxolitinib, and 3) characterize the PK of ruxolitinib in children with relapsed/refractory malignancies or MPNs. To achieve these objectives, a rolling six dose-escalation scheme in which the ruxolitinib dose was increased in increments of approximately 20% was used [20]. Monitoring for ruxolitinib-related toxicity included physical examinations (weekly during Cycle 1, then prior to subsequent cycles), serum chemistries, triglycerides and cholesterol, albumin before each cycle, and complete blood counts (twice weekly during Cycle 1, then weekly).

Ruxolitinib, supplied by the Incyte Corporation (Wilmington, DE) in 5 mg or 25 mg tablets, was administered twice daily (BID) continuously in cycles of 28 days in duration. Tablets were swallowed whole or crushed and mixed with applesauce or OraPlus (Paddock Laboratories, Minneapolis, MN). Patients $<0.6\text{ m}^2$ were excluded from enrollment at the first dose level. The starting dose was $15\text{ mg/m}^2/\text{dose BID}$ (Dose Level 1; DL1), 100% of the equivalent maximum tolerated dose in adults [19]. Dose levels for subsequent groups of patients were $21\text{ mg/m}^2/\text{dose BID}$ (DL2), $29\text{ mg/m}^2/\text{dose BID}$ (DL3), $39\text{ mg/m}^2/\text{dose BID}$ (DL4), and $50\text{ mg/m}^2/\text{dose BID}$ (DL5). No intra-patient dose escalation was allowed. In the absence of disease progression and if laboratory parameters as defined in the eligibility section were met, each 28-day cycle could be repeated without interruption.

Toxicities were graded according to the Common Terminology Criteria for Adverse Events version 4.0 (<http://ctep.cancer.gov>). Non-hematologic DLT was defined as any grade 3 or 4 non-hematological toxicity attributable to ruxolitinib with the specific exclusion of grade 3 nausea or vomiting of <3 days' duration, transaminase elevation that returned to grade 1 or baseline prior to the next treatment cycle, grade 3 fever or infection, grade 3 electrolyte abnormalities responsive to oral supplementation, or grade 3 fatigue ≥ 3 days' duration. In addition, any grade 2 non-hematological toxicity lasting ≥ 7 days that was considered medically significant or sufficiently intolerable by patients and required treatment

interruption was considered a DLT. Hematologic DLT for patients with STslohm was defined as grade 4 neutropenia or thrombocytopenia for >14 days. DLT for patients with leukemia or MPNs was defined as failure to recover an ANC >500/mm³ and platelets >20,000/mm³ by 42 days after first treatment day that was not due to malignant infiltration. The observation period for the purposes of dose-escalation was the first cycle of therapy.

Disease responses for patients with STs were evaluated using the response evaluation criteria in solid tumors (RECIST) [21]. Complete remissions for patients with leukemia included a bone marrow with <5% blasts, adequate marrow cellularity, and no evidence of extramedullary disease. Patients with MPNs were evaluated by disease-specific response criteria [22]. Disease evaluations were obtained at baseline, at the end of Cycle 1, and after every other cycle.

Pharmacokinetic Studies

Serial blood samples (2–4 mL) for ruxolitinib concentrations were obtained Cycle 1, Day 1 prior to the dose and at 1, 2, 4, 8, 12, and 24 hours following the dose. Since ruxolitinib dosing was BID, the 2nd dose was held on the day of PK sampling. A pre-dose blood sample was obtained in Cycle 1, Day 15 and on Day 1 of each subsequent cycle. Blood was collected and centrifuged at 2,000 x g for 15 minutes at 4 °C, then plasma was stored at –20 °C. Ruxolitinib concentrations were measured as previously described [23]. Concentration-time data were analyzed via standard non-compartmental analysis methods using Phoenix WinNonLin 6.0 (Pharsight Corporation; Mountain View, CA). Associations between dose, PK variables, and toxicity were performed using SAS version 9.1 (SAS Institute, Inc.; Cary, NC). The plots were generated using Prism 3.0 (GraphPad Software; La Jolla, CA).

Pharmacodynamic Studies

Measurements of *ex vivo* target inhibition—Phosphoflow cytometry analyses were performed to assess ruxolitinib-induced target inhibition. For the *ex vivo* plasma inhibitory activity (PIA) assays, plasma from consenting Part A and Part B patients was prepared from peripheral blood (3–5 mL) at Cycle 1, Day 1 prior to dosing (C1D1H0) and 4 hours post-dosing (C1D1H4) and at Cycle 1, Day 15 prior to dosing (C1D15H0) and stored at –80°C for subsequent batched analyses as described [24].

For each timepoint, 500 µL of thawed plasma was incubated with 4 × 10⁶ MUTZ5 cells (human *IGH@-CRLF2* and *JAK2* R683G ALL cell line) for 60 minutes at 37°C. Cells were subsequently washed and fixed with paraformaldehyde and permeabilized with methanol for phosphoflow cytometry analysis [15] or lysed for immunoblotting [24]. MUTZ5 cells incubated with normal human plasma were used as basal signaling control, while cells incubated *in vitro* with 1 µM ruxolitinib for 30 minutes at 37°C were used to delineate maximal phosphorylation inhibition.

For phosphoflow cytometry analyses, MUTZ5 cells were washed, rehydrated, and stained with antibodies against phosphorylated (p) JAK2, pSTAT5, and pS6 prior to multi-parametric analysis as described [15]. Digital flow cytometry data were recorded and

analyzed in Cytobank (Palo Alto, CA) as described [15,25]. Percent inhibition of the C1D1H0 baseline was calculated for the C1D1H4 and C1D15H0 timepoints via Prism 6.0. The Kruskal-Wallis test with Dunn's post-test for multiple comparisons was used to assess differences among ruxolitinib dose levels for each phosphoprotein and timepoint. Comparison of percent phosphoprotein inhibition between C1D1H4 and C1D15H0 timepoints was performed with the Wilcoxon matched-pairs signed rank test.

Immunoblotting of MUTZ5 lysates with densitometry quantification of total and pSTAT5 was performed as described [24]. Percent inhibition of the C1D1H0 pSTAT5 band was calculated for the C1D1H4 and C1D15H0 timepoints as above [24]. The Spearman rank correlation was performed to assess the linear relationship between phosphoflow cytometry and immunoblotting data.

Measurements of *in vivo* target inhibition—As an exploratory PD analysis, whole blood phosphoflow cytometry assays were performed on some specimens (n=6) from consenting Part B patients to assess leukemia-specific *in vivo* target inhibition. Blood was obtained at the C1D1H0, C1D1H4, and C1D1H15 timepoints and prepared as previously described.[26] Briefly, 100 μ L aliquots of blood were fixed with formaldehyde (basal measurements) or exposed *ex vivo* to 1 μ M ruxolitinib for 30 minutes at 37 °C prior to fixation. Specimens were lysed with Triton X-100 and permeabilized in methanol prior to flow cytometry data acquisition as above [15]. Leukemia cells were gated by cell surface markers and light scatter properties for the intracellular signaling analyses.

Targeted Mutation Analyses—Samples (3-5 mL) were obtained at study entry from consenting patients with HMs for JAK mutation analyses. DNA was isolated and subjected to polymerase chain reaction amplification with primers for *JAK1* (exons 13, 14, 15, 18, 19) or *JAK2* (exons 16, 20, 21), then subjected to Sanger sequencing as described [6,9].

RESULTS

Patient Characteristics

Forty-nine patients enrolled between September 2010 and August 2012. Twenty-eight patients with STs were enrolled in the dose escalation component of the trial (Part A), and 27 were fully evaluable for toxicity. The inevaluable patient with a ST withdrew from study prior to starting ruxolitinib. Seventeen patients with leukemia and 4 patients with MPNs were enrolled in Part B; ten of these were evaluable for toxicity. The one Part A and eleven Part B subjects were inevaluable due to refusal of further therapy or early disease progression, respectively. None of these patients experienced DLTs. Patients received a median of one cycle of therapy (range 1 – 18 cycles). Patient characteristics are summarized in Table I and Supplemental Table I.

Toxicity

Table II summarizes the observed Cycle 1 DLTs for Part A and Part B patients. DLTs were reported as AEs with accompanying lab results where relevant. Tables III and IV summarize toxicities across all cycles of therapy. No more than 1 of 6 patients per cohort experienced a

DLT in either part of the trial. One patient with a ST treated at DL2 (21 mg/m²) developed nausea/vomiting and severe dehydration within 4 days of starting ruxolitinib. Ruxolitinib was discontinued, but the patient, who had a large left lung sarcoma burden with resultant extrinsic cardiac compression, refused hospitalization after initial supportive care measures. She subsequently experienced acute renal failure followed by hepatic failure and death. Post-mortem examination revealed changes consistent with hypotensive shock. Other Cycle 1 DLTs across the five dose levels evaluated in Part A included neutropenia (n=2) and increased creatine phosphokinase (n=1). The MTD was not exceeded at any dose level evaluated. One Part B patient treated at DL4 (39 mg/m²) experienced DLTs of grade 3 creatinine increase and grade 3 acute kidney injury and subsequently died of leukemia progression. A second patient at DL4 experienced a grade 5 bronchopulmonary hemorrhage after discontinuing protocol therapy, which was attributed to progressive disease. Ruxolitinib was not held in other patients, including Part A patients with grade 3 cytopenias who were allowed blood product transfusions.

Response

No objective responses were observed in patients with STs. Twelve of the 37 evaluable patients had stable disease at the end of Cycle 1. The diagnoses for the 10 evaluable Part B patients included ALL (n=4), AML (n=2), JMML (n=3), and PV (n=1). Of these patients, 4 had stable disease at the end of first month, including 2 JMML patients. The patient with PV had a partial response (PR) and received 18 cycles of therapy before meeting criteria for progressive disease. One patient with relapsed JMML received 5 cycles of chemotherapy before meeting disease progression criteria. Of note, 11 of the 21 patients with hematologic malignancies had rapid disease progression and did not complete the first cycle of therapy.

Pharmacokinetics

Forty-two children participated in PK analyses; the results are summarized in Supplemental Table II. Mean $\pm$ standard error (SE) concentration versus time data for each dose level is shown in Figure 1A. Peak plasma concentrations were achieved 1 hour (range 1 – 4 hours) after the first oral dose and decreased in a monoexponential fashion with a mean $\pm$ standard deviation (SD) half-life of 2.3 ± 0.9 hours (h). The mean values for ruxolitinib oral plasma clearance (CL/F) and volume of distribution (V/F) among all patients were 14.8 ± 5.9 L/h and 59.4 ± 29.1 L, respectively, and were independent of dose level (Supplemental Table II and Figure 1B). Ruxolitinib PK disposition appeared linear over the dose range studied based on dose-proportional increases in $C_{\max}$ and $AUC_{0-\infty}$ values and observations of trends, and similar CL/F values were measured across dose levels (Supplemental Table II). Children ≤ 12 years had higher body surface area (BSA)-corrected CL/F values compared with children >12 years (14.4 ± 8.5 L/h/m² versus 10.2 ± 3.1 L/h/m²). The variability in BSA-corrected CL/F values was also greater for children ≤ 12 than in children >12 years (Figure 1B). Interestingly, 7 of 8 CL/F values >15 L/h/m² were found in children ≤ 12 years treated at the intermediate dose levels. No differences in CL/F were observed between males (n=26) and females (n=16) (12.1 ± 6.3 L/h/m² versus 12.5 ± 7.2 L/h/m²).

Pharmacodynamics

PIA assay results showed median peak inhibition of 44.8% for pJAK2 (range 9.4 – 72.6%), 58.9% for pSTAT5 (range 10.0 – 74.3%), and 62.3% for pS6 (range 0.9 – 85.8%) by phosphoflow cytometric analysis at C1D1H4. Median inhibition at C1D15 steady state was 21.5% for pJAK2 (range –50.6 – 80.3%), 46.3% for pSTAT5 (range –50.6 – 80.4%), and 59.2% for pS6 (range –55.5 – 85.7%), demonstrating some loss of JAK/STAT pathway inhibition at trough ($p < 0.0001$ for both pJAK2 and pSTAT5), but sustained PI3K/mTOR pathway inhibition (Figure 2A). A subset of patients demonstrated increased phosphoprotein levels at steady state and loss of signaling inhibition (Figure 2A), suggesting signaling upregulation during treatment (24). With the exception of increased peak pSTAT5 inhibition at DL2 and DL5, further increases in signaling inhibition were not observed with increasing doses of ruxolitinib (Figure 2A). Immunoblotting studies showed median pSTAT5 inhibition of 80% (range 11 – 98%) and 51% (range –154 – 93%) at C1D1H4 ($n=37$ patients) and C1D15H0 ($n=32$ patients), respectively (Figure 2B). Comparison of pSTAT5 phosphoflow cytometry and immunoblotting PIA data demonstrated strong methodological correlation (Figure 2C).

In vivo inhibition of signal transduction was also observed in Part B patients' blood specimens analyzed by whole blood phosphoflow cytometry (Supplemental Figure 1A). Three B-ALL patient specimens studied in these assays demonstrated modest inhibition of pJAK2, pSTAT5, and pS6 at steady state dosing (representative patient data shown in Supplemental Figure 1B). However, *ex vivo* exposure of blood specimens to 1 μ M ruxolitinib demonstrated additional signaling inhibition, suggesting lack of complete *in vivo* PD inhibition at the doses of ruxolitinib studied (Supplemental 1C). In addition, analysis of samples from a relapsed AML patient treated at DL3 demonstrated no basal activation or inhibition of JAK/STAT signaling proteins, but marked upregulation of PI3K and MAPK pathway proteins (Supplemental Figure 1D).

Targeted Mutation Analysis

All Part B patients who consented for the JAK mutation analyses were assessed. No *JAK1* or *JAK2* point mutations or increased TSLPR staining by flow cytometry were identified in leukemia patients. The patient with PV had a *JAK2* V617F mutation.

DISCUSSION

In summary, ruxolitinib was well-tolerated in children with recurrent or refractory malignancies at all dose levels tested. While no objective responses were documented, 8 patients received >1 cycle of ruxolitinib. Of the 10 evaluable patients with HMs, 4 had stable disease at the end of first month of therapy, including 2 with relapsed JMML. Notably, 11 of the 21 patients with HMs progressed by the defined criteria during the first month of induction, which highlights the challenges associated with enrollment of children with acute leukemias on dose escalation studies. One PR occurred in the patient with PV, but she discontinued ruxolitinib after Cycle 18 with documented disease progression.

Similar PK in children was observed as in healthy adults or adults with myelofibrosis [23]. PK in male and female children were similar with greater variability in younger children possibly due to differences in P450 expression. PD analyses showed moderate JAK/STAT signaling inhibition of pSTAT5 at all dose levels that was relatively dose-independent. Unfortunately, there were no patients with JAK-mutant acute leukemias (either point mutation or fusion) enrolled on the study. Therefore, our correlative data are limited and reflective of what happens in cells that harbor wild-type *JAK2*. The level of phosphoprotein inhibition that may correlate with clinical responses in patients with JAK-mutant cancers thus remains unknown. While 50-60% pJAK2/STAT5/S6 inhibition measured by our indirect PIA assays perhaps seems numerically inadequate, the PD data demonstrate relatively dose-independent phosphoprotein inhibition by ruxolitinib. It is possible that such inhibition may correlate with clinical responses in JAK-mutant leukemias; our data also suggest that it may not be possible to achieve further phosphoprotein inhibition *in vivo* with dose escalation of ruxolitinib. Interestingly, preclinical data in murine xenograft models of JAK-mutant ALL demonstrate inhibition of leukemia proliferation with ruxolitinib monotherapy (24) at doses that produced similar steady-state levels (approximately 500 nM) as in patients in this phase 1 trial.

Despite the lack of a clear response to ruxolitinib in the relapsed/refractory pediatric cancer population, it should be noted that (with the exception of patients with *JAK2*-mutant PV) no patients were enrolled who would have been predicted to respond to ruxolitinib. Extensive data indicate that 10-27% of children, adolescents, and young adults have somatic activating point mutations in JAK family members or *JAK2* fusions [6,7,9,12,15,27], but no patients with these alterations enrolled on this trial. Furthermore, these patients have poor outcomes with currently available therapy, raising therapeutic possibilities of incorporating JAK inhibitors into upfront therapy, similar to the paradigm shift that has occurred with *BCR-ABL1*+ ALL and imatinib [28,29]. Anecdotal data support the use of ruxolitinib in JAK-mutated ALL, as evidenced by preliminary clinical responses of several patients with relapsed or chemorefractory *JAK2*-mutant ALL treated with ruxolitinib monotherapy (personal communication, Loh). In light of the safety and tolerability of this agent in the current study, testing ruxolitinib with traditional chemotherapy is a rational and logical next step in the journey to incorporate targeted therapies into our armamentarium to improve cure rates for leukemia patients with JAK lesions or evidence of dependence on JAK/STAT signaling.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Pharmacokinetics of ruxolitinib in children with relapsed cancers

(A) Mean (SE) ruxolitinib plasma concentrations versus time in children treated on ADVL1011 are stratified by dose level. (B) Area under the concentration from time zero to infinity as a method to calculate mean concentration levels of a therapeutic agent (upper panel), oral clearance per dose/m² (middle panel), and oral clearance per age in years (lower panel) are displayed.

Figure 2. Quantification of *in vivo* signaling inhibition

MUTZ5 cells were incubated with plasma obtained at the specified timepoints from patients treated with ruxolitinib, then analyzed by phospho-flow cytometry (**A**) and immunoblotting (**B**) to quantify inhibition of signal transduction proteins at peak (C1D1H4; n=37 patients) and trough (C1D15H0; n=32 patients) timepoints. C1D15H0 plasma samples were not submitted for some patients or were of insufficient volume for analysis. Increased pSTAT5 inhibition was observed with doses of ruxolitinib at 29 mg/m²/dose and 50 mg/m²/dose ($p<0.0001$ via Kruskal Wallis test with Dunn's post-test for multiple comparisons), but

inhibition of phosphoproteins was otherwise dose-independent. Loss of signaling inhibition was observed for pJAK and pSTAT5 between C1D1H4 and C1D15H0 (**** $p < 0.0001$ for both), but not for pS6 ($p = 0.4045$). (C) Linear correlation of phosphoflow cytometry (PF) and immunoblotting (IB) percent pSTAT5 inhibition data was observed at both C1D1H4 ($R = 0.6007$, $p = 0.0002$) and C1D15H0 ($R = 0.6550$, $p = 0.0007$) timepoints. Grey = ruxolitinib 15 mg/m²/dose (n=7), red = 21 mg/m²/dose (n=7), blue = 29 mg/m²/dose (n=8), pink = 39 mg/m²/dose (n=9), black = 50 mg/m²/dose (n=6). Open circles = solid tumor patients, filled circles = leukemia/MPN patients. C = cycle, D = day, H = hour.

Table I

Characteristics for eligible patients (n=49).

Characteristic	Number (%)
Age (years)	
Median	14.4
Range	2.4-21.4
Sex	
Male	31 (63.3)
Female	18 (36.7)
Race	
White	29 (59.2)
Asian	2 (4.1)
American Indian or Alaska Native	1 (2.0)
Black or African American	8 (16.3)
Unknown	9 (18.4)
Ethnicity	
Non-Hispanic	35 (71.4)
Hispanic	12 (24.5)
Unknown	2 (4.1)
Diagnosis *	
Solid Tumor	28
Hematologic Malignancy	21

* See Supplemental Table I for detailed list of diagnoses.

Table II

Dose-limiting toxicity summary for Cycle 1.

Stratum	Dose Level	Number Patients Entered	Number Patients Evaluable	Number Patients with DLT	Toxicity (Grade)
Part A	15 mg/m ²	4	3	0	-
	21 mg/m ²	6	6	1	ALT (4), dehydration (3), hypotension (3), multi-organ failure (5), nausea (3), vomiting (3)
	29 mg/m ²	6	6	1	Neutropenia (4)
	39 mg/m ²	6	6	1	Neutropenia (4)
	50 mg/m ²	6	6	1	CPK increased (4)
Part B	15 mg/m ²	6	4	0	-
	21 mg/m ²	3	1	0	-
	29 mg/m ²	6	3	0	-
	39 mg/m ²	6	2	1	Creatinine (3), acute kidney injury (3)

Non-dose-limiting hematologic toxicities observed in evaluable solid tumor patients (n=27)

Table III

Toxicity Type	Maximum grade of toxicity across Cycle 1 (total = 37 cycles)				Maximum grade of toxicity across Cycles 2 to 18 (total = 36 cycles)			
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4
Anemia	8	6	4		3	4	4	
Lymphopenia	4	5	1	2	3	2	3	1
Lymphocytosis		2						
Leukopenia	9	5	3		3	1	2	
Neutropenia	3	6	4	2	2	5	1	
Thrombocytopenia	10	2	2		4			1

Note: This table consists of non-dose-limiting hematologic toxicities independent of frequency and attribution.

Non-dose-limiting non-hematologic toxicities related to protocol therapy and observed in >10% of evaluable patients during Cycle 1 (n=37).

Table IV

Toxicity Type	Maximum grade of toxicity across Cycle 1 (total = 37 cycles)				Maximum grade of toxicity across Cycles 2 to 18 (total = 36 cycles)			
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4
Abdominal pain	4	1			1			
Alanine aminotransferase	4	3		1	1	1		
Aspartate aminotransferase	8		1		2	1	1	
Bilirubin	3	1				1		
Constipation	4							
Creatinine	3	1	1					
Fatigue	7	3				1		
Headache	3	1			1			
Hypocalcemia	6				1			
Hyponatremia	4				1			
Nausea	8	1	1		2			
Vomiting	3		1					