LYMPHOID NEOPLASIA

Janus kinase inhibition by ruxolitinib extends dasatinib- and dexamethasone-induced remissions in a mouse model of Ph+ ALL

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Key Points

- In a Ph+ ALL mouse model, dasatinib inhibition of the BCR-ABL kinase resensitizes residual leukemic B cells to Janus kinase inhibition.
- Dasatinib, ruxolitinib, and dexamethasone together limit emergence of dasatinib-resistant BCR-ABL mutants and extend long-term survival.

Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL) is initiated and driven by the oncogenic fusion protein BCR-ABL, a constitutively active tyrosine kinase. Despite major advances in the treatment of this highly aggressive disease with potent inhibitors of the BCR-ABL kinase such as dasatinib, patients in remission frequently relapse due to persistent minimal residual disease possibly supported, at least in part, by salutary cytokine-driven signaling within the hematopoietic microenvironment. Using a mouse model of Ph+ ALL that accurately mimics the genetics, clinical behavior, and therapeutic response of the human disease, we show that a combination of 2 agents approved by the US Food and Drug Administration (dasatinib and ruxolitinib, which inhibit BCR-ABL and Janus kinases, respectively), significantly extends survival by targeting parallel signaling pathways. Although the BCR-ABL kinase cancels the cytokine requirement of immature leukemic B cells, dasatinib therapy restores cytokine dependency and sensitizes leukemic cells to ruxolitinib. As predicted, ruxolitinib alone had no significant antileukemic effect in this model, but it prevented relapse when administered with dasatinib. The combination of dasatinib, ruxolitinib, and the corticosteroid dexamethasone yielded more durable remissions, in some cases after completion of therapy, avoiding the potential toxicity of other cytotoxic chemotherapeutic agents. (Blood. 2015;125(9):1444-1451)

Introduction

The oncogenic fusion protein BCR-ABL, a constitutively active tyrosine kinase driven by a balanced translocation between chromosomes 9 and 22 (the Philadelphia chromosome, Ph), initiates both chronic myeloid leukemia (CML) and Ph-positive acute lymphoblastic leukemia (Ph+ ALL).1 De novo Ph+ ALL closely resembles the aggressive lymphoid blast crisis of CML and is prone to relapse even after combined treatment with potent second-generation inhibitors of the BCR-ABL kinase and intense chemotherapy.2-4 In addition to Ph, deletions of the CDKN2A/B (INK4A/ARF) tumor suppressor locus (encoding p16INK4a, p15INK4b, and p14ARF)5,6 and the IKFZ1 (IKAROS) gene7 (encoding a transcription factor required for lymphoid progeny under B-cell selective-culture conditions, yields polyclonal, cytokine-independent leukemia-initiating cells (LICs). Only 20 such LICs initiate ALL when infused into the tail veins of healthy, nonconditioned syngeneic mice and kill recipient animals within a month of inoculation.11 Therefore, BCR-ABL expression and Arf inactivation are sufficient to guarantee leukemogenesis in healthy recipient animals that initially retain normal hematopoietic and immune function. Leukemic mice respond very poorly to imatinib (Gleevec),10,11 but enter hematologic remission in response to treatment with much more potent second-generation tyrosine kinase inhibitors (TKIs) such as dasatinib (Sprycel).12 However, like human patients with Ph+ ALL,13 continuously treated animals ultimately relapse with the emergence of drug-resistant leukemic clones containing clinically relevant BCR-ABL mutations.12 Premature withdrawal of dasatinib when animals are in remission results in reemergence of leukemia, demonstrating that an occult reservoir of residual LICs has not been eliminated. However, unlike mice that relapse on continuous therapy, leukemic B cells from the latter animals lack BCR-ABL mutations and remain sensitive to TKIs ex vivo,10-12 suggesting that minimal residual disease (MRD) is dependent on salutary signaling within the hematopoietic microenvironment.


I.A. and C.D.R. contributed equally to this study.

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Cytokine signaling triggers Janus kinase (JAK)-mediated phosphorylation of signal transducers and activators of transcription (STATs) to induce expression of genes that support B-cell proliferation and survival.14 By directly stimulating STAT phosphorylation,15-17 BCR-ABL bypasses cytokine dependence18 and maintains JAK-independent expression of cyclin D2 and McI1, both of which are essential for LIC maintenance.10,19,20 Conversely, dasatinib-mediated inhibition of the BCR-ABL kinase might restore the requirement for cytokine-dependent JAK signaling and sensitize residual LICs to the JAK inhibitor ruxolitinib (Jakafi).21,22 We now report that the survival of dasatinib-treated mice with BCR-ABL-induced ALL is significantly extended in response to coadministration of ruxolitinib, even though ruxolitinib alone has no antileukemic activity. Addition of dexamethasone further reduced the leukemic burden, prevented central nervous system (CNS) relapse, and led to more prolonged survival, concordant asone further reduced the leukemic burden, prevented central nervous system (CNS) relapse, and led to more prolonged survival, concordant with its established efficacy as a mainstay of multidrug regimens for ALL treatment.2-4,23 Prevention of relapse achieved by a nongenotoxic combination of targeted treatments provides a preclinical rationale for employing dasatinib, ruxolitinib, and dexamethasone in older Ph+ ALL patients who are ineligible for BM transplant or cannot tolerate cytotoxic chemotherapy.

Materials and methods

BM cell transduction, adoptive cell transfer, and leukemia development

Mice were housed at St. Jude Children’s Research Hospital and Memorial Sloan Kettering Cancer Center in facilities accredited by the American Association of Laboratory Animal Care. Procedures were performed in accordance with Institutional Animal Care and Use Committee and National Institutes of Health guidelines. BM cells from ArfΔ/− mice14 backcrossed onto a C57BL/6 background and expressing or lacking the interleukin (IL) receptor common γ chain15 (γc) were transduced with a replication-defective mouse stem cell virus coexpressing human BCR-ABL (p185) and luciferase (Luc2) and plated on autologous stroma for 7 days in IL-7 to select for lymphoid progenitor cells.10,25 On IL-7 withdrawal, polyclonal cytokine-independent BCR-ABL-positive LICs11 were expanded and cryopreserved in fetal bovine serum (FBS) and 10% dimethylsulfoxide. LICs were thawed and recovered in liquid culture for 3 days before intravenous injection into the tail veins of healthy, nonconditioned 8- to 10-week-old C57BL/6 mice (Jackson Laboratory). After intraperitoneal injection of d-luciferin (100 mg/kg; Caliper Life Sciences, Hopkinton, MA), leukemia infiltration was documented by bioluminescence as described previously12 using a Xenogen IVIS imager (selected on isopropyl l-thio-β-D-galactopyranoside) and an individually designed probe set to detect vector-encoded Luciferase-2 DNA (forward primer: TGAACGCGCTACGTGAACAC; reverse primer: CACCATGAAAGTGTGCTGT, and TaqMan probe: 6-FAM-CAGCCAGCCGCTCTTTGCGA-TAMRA; Applied Biosystems), as well as an internal control primer set (VIC-TAMRA) detecting the mouse Tfr gene (4458367; Applied Biosystems). Standard curves were derived by mixing BCR-ABL (p185) positive LICs with mixtures of nucleated BM cells or splenocytes at defined ratios; the reproducible detection limit is 1 LIC in 10 000 total cells (see supplemental Figure 1, available on the Blood Web site).

Preclinical therapeutics

Dasatinib (LC Laboratories, Woburn, MA) and ruxolitinib (Incyte, Wilmington, DE) were administered by oral gavage to recipient animals. Dasatinib dissolved in 80 mM citric acid (pH 3.1) was given at 10 mg/kg per dose.12,26 Ruxolitinib prepared in phosphate-buffered saline containing 0.1% Tween-20 was administered at 60 mg/kg per dose.21,22 Dexamethasone sodium phosphate solution (APP Pharmaceuticals, Schaumberg, IL) was added to drinking water at an initial concentration of 6 mg/L for the first week of treatment and at 3 mg/L thereafter.12 All drugs were administered starting 10 days after intravenous cell infusion when animals had developed a significant leukemic burden as documented by bioluminescence. Dosing schedules are indicated in the text and figure legends. Mice were observed daily; those that became moribund during trials (intense leukemic burden, dehydration, respiratory distress, hind limb paralysis, generalized seizures) had extensive leukemic involvement (elevated peripheral white blood cell counts, splenic and lymph node enlargement, leukemic BM and CNS infiltration seen histologically and confirmed by quantitative polymerase chain reaction (qPCR)) as documented previously.10-12 Kaplan-Meier curves were generated using GraphPad Prism (v.6.0c for Mac), and the log-rank Mantel-Cox test was applied for pairwise comparisons of survival data.

Analysis of BCR-ABL mutations

Mice injected with 2 × 105 LICs were allowed to develop disease for 10 days and then were left untreated (5 mice) or treated continuously with dasatinib, or dasatinib plus ruxolitinib, with or without dexamethasone for up to 180 days. Mice were euthanized when they showed overt signs of leukemic relapse. BM cells were harvested and genomic DNA was prepared from cells derived from 5 to 7 individual relapsed mice per treatment group. A 2-step nested PCR strategy was used to amplify the human ABL kinase domain (KD) from 200 ng of each genomic DNA by first amplifying the BCR-ABL junction (forward primer: GTGGGCGGCTCCGCAAGACCCG; reverse primer: GCCAGGCTCTCGGGCGTGGG; TaqMan probe: 6-FAM-TGAGCGGCTACGTTAACAAC, reverse primer: CACGATGAAGAAGTGCTCGT, and TaqMan probe: 6-FAM-CAGCCAGCCGCTCTTTGCGA-TAMRA; Applied Biosystems), as well as an individually designed probe set to detect vector-encoded Luciferase-2 DNA (forward primer: TGAACGCGCTACGTGAACAC; reverse primer: CACCATGAAAGTGTGCTGT, and TaqMan probe: 6-FAM-CAGCCAGCCGCTCTTTGCGA-TAMRA; Applied Biosystems), as well as an internal control primer set (VIC-TAMRA) detecting the mouse Tfr gene (4458367; Applied Biosystems). Standard curves were derived by mixing BCR-ABL (p185) positive LICs with mixtures of nucleated BM cells or splenocytes at defined ratios; the reproducible detection limit is 1 LIC in 10 000 total cells (see supplemental Figure 1, available on the Blood Web site).

Results

Abrogated cytokine signaling improves dasatinib response of BCR-ABL-positive LICs

The BCR-ABL kinase circumvents cytokine receptor–mediated JAK signaling by phosphorylating specific STAT family members,15-17 reducing the dependency of cultured LICs on interleukins for their proliferation and survival.18 Nonetheless, BCR-ABL-positive LICs still respond to IL-7,10 which at saturating doses, lowered their sensitivity to dasatinib more than 20-fold (Figure 1A). Inhibition of

Assessment of leukemic burden by quantitative polymerase chain reaction

Leukemic cell infiltration at necropsy in BM and spleen was measured in genomic DNA using a TaqMan Fast Advanced Master Mix (Applied Biosystems) and an individually designed probe set to detect vector-encoded Luciferase-2 DNA (forward primer: TGAACGCGCTACGTGAACAC; reverse primer: CACCATGAAAGTGTGCTGT, and TaqMan probe: 6-FAM-CAGCCAGCCGCTCTTTGCGA-TAMRA; Applied Biosystems), as well as an internal control primer set (VIC-TAMRA) detecting the mouse Tfr gene (4458367; Applied Biosystems). Standard curves were derived by mixing BCR-ABL (p185) positive LICs with mixtures of nucleated BM cells or splenocytes at defined ratios; the reproducible detection limit is 1 LIC in 10 000 total cells (see supplemental Figure 1, available on the Blood Web site).
STAT5 and STAT3 phosphorylation by dasatinib (Figure 1B, lane 2) was antagonized by IL-7 (lane 6). Although ruxolitinib treatment had no effect on STAT5 phosphorylation, it partially inhibited phosphorylation of STAT3 (lanes 3 and 7); however, concomitant inhibition of the BCR-ABL kinase with dasatinib resensitized IL-7-treated LICs to ruxolitinib (Figure 1A) and inhibited STAT phosphorylation (Figure 1B, lanes 4 and 8).

Consistent with the effects of IL-7 on cultured LICs, injected donor LICs lacking γc (required for IL-2, -4, -7, -9, -15, and -21 signaling) were less leukemogenic than their γc+/+ counterparts, and their serial dilution established that infusion of fourfold more γc−/− than γc+/+ LICs would generate an equivalent leukemia burden 10 days posttransplant (Figure 2Ai-ii). Additional groups of mice were infused with either 2 × 10^6 γc+/+ LICs or with a 10-fold greater number of γc−/− LICs (Figure 2Aiii), and dasatinib therapy was initiated in leukemic mice 10 days later. Although all untreated mice died within 3 weeks of receiving LICs, dasatinib treatment with a low-intensity regimen (10 mg/kg of drug once per day, 5 days per week) that is ineffective in maintaining remission was led to survival benefits in both cohorts. Notably, even when challenged with 10-fold more γc−/− LICs, γc-null leukemias exhibited an improved response to dasatinib compared to γc+/+ leukemias (P = .014) (Figure 2B). Hence, interleukin signaling can facilitate leukemogenesis and insulate the response of LICs to targeted TKI therapy in vivo.

Effects of dasatinib, ruxolitinib, and dexamethasone during induction therapy

Ten days after infusion of 2 × 10^5 LICs, there is widely disseminated disease, including high peripheral white blood cell counts and significant leukemic infiltration into the BM, spleen, lymph nodes, and meninges. Bioluminescent imaging of living mice allows detection of leukemic cells over a 3-log range, whereas qPCR using TaqMan primers directed to the vector-containing Luc2 gene used for in vivo imaging can detect 1 LIC per 10^9 normal cells (supplemental Figure 1), enabling an independent and more sensitive measure of leukemic burden. Treatment of leukemic mice with twice-daily dasatinib (10 mg/kg) led to a drastic reduction in disease burden in both BM and spleen that fell below the limit of detection after 13 days (Figure 3A). Nonetheless, significant numbers of leukemia cells re-emerged by 9 days after discontinuation of treatment, as indicated by the reappearance of intense whole-body bioluminescence signals in moribund mice that were roughly equivalent to those seen at start of therapy and by TaqMan qPCR analysis documenting 26% to 45% leukemic replacement in BM (data not shown). The posttherapeutic rebound reflects the rapid doubling time of residual leukemic cells, which leads to an ~10-fold increase in leukemic burden every 3 days.

Because 7-day dasatinib treatment is less effective in reducing the number of leukemic cells (Figure 3A), we reasoned that any additive therapeutic benefit of additional drugs could be determined at this time. Accordingly, we treated leukemic mice for 7 days with dasatinib (10 mg/kg twice daily), ruxolitinib (60 mg/kg once daily), and the corticosteroid dexamethasone, a mainstay of ALL treatment, either alone or in combination. Mice were euthanized after treatment, and TaqMan qPCR was performed to quantify MRD (Figure 3B). Dexamethasone alone, administered continuously in the drinking water, reduced the leukemic burden in BM and improved the response to dasatinib in lowering leukemic cell numbers in both BM and spleen. In marked contrast, ruxolitinib treatment had no immediate effect in reducing the leukemic burden during 7 days of induction therapy, either when administered alone or when combined with dasatinib. Moreover, further addition of ruxolitinib did not improve the response to dasatinib plus dexamethasone during the induction phase (Figure 3B).

Ruxolitinib and dexamethasone each extend survival of dasatinib-treated mice

Because efficient inhibition of the BCR-ABL kinase by dasatinib allows reengagement of cytokine-mediated JAK-STAT signaling (Figure 1), we reasoned that any salutary effects of cytokines in maintaining MRD within the hematopoietic microenvironment after dasatinib treatment might well be overcome by ruxolitinib. To test this, leukemic mice that had received LICs were randomized after 10 days into different cohorts for treatment. Animals received either 10 mg/kg dasatinib, 60 mg/kg ruxolitinib, or both drugs combined once daily 5 days per week (weekdays only, as in Figure 2B). Animals were observed daily for signs of disease and imaged weekly, and relapses were documented at necropsy (see “Materials and methods”). As predicted, we did not observe a significant antileukemic effect with ruxolitinib alone (Figure 4A). However, overall survival was significantly improved in leukemic mice treated with the combination of both targeted therapies compared with dasatinib as a single agent (Figure 4A; P < .0001). Thus, although ruxolitinib had no effect in inducing leukemic remission, it had marked activity in improving progression-free survival.

All mice treated as in Figure 4A succumbed to leukemia, in many cases accompanied by hind limb paralysis, doming of the head, and intermittent generalized seizures, implicating meningeal or CNS involvement. Although dasatinib penetrates the blood-brain barrier, an active efflux of the drug from brain capillary endothelial
cells into the blood via 2 different exporters limits drug delivery to the CNS and impedes systemic therapy.\textsuperscript{30,31} Histopathologic examination of tissues from moribund mice confirmed significant leukemic infiltration of the meninges in all animals, whether they failed due to CNS-centered or more disseminated disease (supplemental Figure 2). Therefore, the CNS is a “safe haven” for Ph+ ALL cells that efficiently escape from combined treatment with dasatinib and ruxolitinib.

Given the efficacy of dexamethasone during induction therapy (Figure 3) and its ability to cross the blood-brain barrier and reduce the risk of CNS relapse,\textsuperscript{23} it was incorporated into the treatment regimen.

\textbf{Figure 3.} Acute response of leukemic mice to drug treatment. (A) Dasatinib treatment rapidly reduces leukemia burden. Twelve mice injected intravenously with $2 \times 10^5$ LICs were allowed to develop leukemia over a 10-day period. Mice with equal leukemia burdens were randomized into groups of 3 mice, the first of which was left untreated and euthanized when moribund (time 0). Other groups were treated twice a day with dasatinib (10 mg/kg body weight) for 7, 10, or 13 days and euthanized immediately after the indicated times of treatment. Additional treated cohorts were allowed to undergo clinical relapse after dasatinib withdrawal at day 13 and were moribund by day 22 (see the “Effects of dasatinib, ruxolitinib, and dexamethasone during induction therapy” section). Bone marrow cells (top) and splenocytes (bottom) were harvested for TaqMan qPCR analysis performed with primers directed to the vector-containing Luc2 gene. The fraction of leukemic cells was interpolated from a standard curve created by mixing cultured BCR-ABL+ Arf\textsuperscript{-/-} LICs with nucleated cells from BM or spleen at defined ratios (supplemental Figure 1). (B) Dexamethasone (Dex), but not ruxolitinib, reduces leukemic burden during 7-day induction therapy. Mice that received LICs and developed leukemia as in panel A were treated with the indicated drug combinations and euthanized. Because monotherapy with dasatinib for 13 days reduces the frequency of leukemic cells below the limit of detectability (panel A), a 7-day treatment period was chosen to allow any additional effects of ruxolitinib and dexamethasone to be recorded. Leukemia burden in BM (top) and spleen (bottom) was estimated by qPCR (TaqMan) as in panel A. Ruxolitinib treatment alone or in combination with dasatinib had no significant activity in acutely reducing the leukemia burden, whereas dexamethasone potentiated remission induction.
Initially, mice infused with LICs were again randomized to different treatment groups at day 10 and treated with various drug combinations for only 13 days. Bioluminescence imaging was used to verify disease remission after therapy, and then "time to treatment failure" (ie, time to terminal leukemia relapse after drug withdrawal) was evaluated (Figure 4B). We reasoned that differences in survival after treatment termination should serve as a surrogate parameter for the level of residual disease achieved by different treatment schemes. Although dexamethasone alone or with ruxolitinib provided no significant survival advantage, both agents improved the duration of remission in response to dasatinib (Figure 4B). However, disseminated leukemia documented by increasing bioluminescence, clinical criteria, and qPCR (Figure 4B, legend) invariably ensued. Ruxolitinib and dexamethasone each offered additive benefits, indicating that the 3 drugs act through different mechanisms in extending survival. Despite additional immunosuppressive effects of dexamethasone, we did not observe infectious complications. The combination of dasatinib and ruxolitinib led to a significant reduction of spleen weights in moribund mice, an effect enhanced by dexamethasone (Figure 4C). Continuous treatment with the 3-drug regimen for up to 6 months led to a further beneficial effect in leukemic mice, significantly improving their survival during and after therapy (Figure 4D; see figure legend for P values). Indeed, 2 of 12 mice that received continuous therapy for 6 months were alive and healthy 6 months after discontinuation of treatment.

The combination of 3 targeted agents selects for fewer BCR-ABL mutations at relapse

For the long-term treatment study shown in Figure 4D, we used PCR amplification and nucleotide sequencing to evaluate genomic DNA from treated and untreated mice for the presence of BCR-ABL KD mutations. From each treatment cohort, 5 to 7 moribund mice that underwent relapse, as well as 5 untreated leukemic animals (from Figure 4B) were euthanized. The genomic DNA obtained from BM of individual animals was analyzed. No BCR-ABL KD mutations were detected in samples obtained from untreated leukemic mice (Table 1). In contrast, we observed a strong selective effect of dasatinib alone, or dasatinib plus ruxolitinib, for the occurrence of KD mutations. Three mice developed leukemic clones harboring the T315I or the F317L mutations, both of which inhibit dasatinib binding; others developed...
The improved response to TKIs in mice inoculated with LICs that lacked the common \( \gamma_c \) points directly to salutary effects of cytokines in supporting the viability of LICs in the face of dasatinib treatment. Ruxolitinib mimicked effects of abrogated cytokine signaling caused by the \( \gamma_c \) knockout but had no antileukemic effects when administered alone and was unable to augment the immediate effects of dasatinib in rapidly decreasing the leukemia burden during the initial phase of induction therapy. However, when combined with more prolonged dasatinib treatment, which was predicted to reinstate cytokine dependence, ruxolitinib proved effective. In short, effective dasatinib therapy sets a precondition in which ruxolitinib acquires therapeutic efficacy in maintaining disease remission. Recent work of others has suggested that addition of ruxolitinib to TKI therapy might also improve therapeutic efficacy in CML.\(^{35}\) Combining these 2 agents facilitated the apoptotic elimination of cultured human CML CD34\(^+\) cells and prevented their engraftment into immunodeficient mice. Unfortunately, untreated transplanted CML cells were not leukemogenic, limiting any possibility of in vivo treatment trials.\(^{35}\)

The time-to-treatment-failure approach, following a relatively brief 13-day drug exposure, provides a cost-efficient means to evaluate therapeutic options in our model and can be used as a surrogate parameter for MRD status due to the highly predictable population doubling time of untreated leukemic cells in vivo (\(~20\) hours\(^{11}\)). Strikingly, 13-day monotherapy of leukemic mice with twice-daily dasatinib began 10 days after LIC infusion produced at least a 4-log reduction in leukemia burden in BM and spleen, and yet all continuously treated animals underwent relapse. Even though dasatinib penetrates the blood-brain barrier, its efficacy is limited by active efflux from brain capillary endothelia into the blood.\(^{30,31}\) Remissions achieved with the combination of dasatinib and ruxolitinib were more durable, but mice still relapsed, and many exhibited clinical symptoms and signs of CNS involvement. Dexamethasone, a cornerstone of ALL therapy that improves control of leukemia in the CNS,\(^{23}\) further extended progression-free survival. In a cohort that received 6-month treatment with this 3-drug combination, a small subset of leukemic mice lived for an additional 6 months after discontinuation of therapy. The efficacy of this treatment scheme was not diminished by toxicity or more frequent infectious complications, reflecting the benefit of targeted therapy in combination with steroids compared to “conventional” high-dose chemotherapy.

By maintaining a reservoir of LICs that can undergo additional genetic alterations in the face of continued drug treatment, MRD can allow the emergence of clones with BCR-ABL KD mutations that confer resistance to TKI therapy and lead to leukemic relapse. BCR-ABL mutations emerging during therapy depend not only on the duration and intensity of therapy as shown here and in previous studies with this model\(^{12}\) but also on the therapeutic agents used. Although combined treatment with dasatinib and ruxolitinib yielded many mutant clones at relapse, addition of dexamethasone significantly reduced their overall frequency. Therefore, the emergence of BCR-ABL mutations is not requisite for relapse, and treatment with broadly active third-generation TKIs that inhibit even gatekeeper mutations may not be sufficient to cure Ph\(^+\) ALL.\(^{36}\) The mechanisms that confer drug resistance to combinatorial therapy with dasatinib, ruxolitinib, and dexamethasone remain unclear. However, more than 15 different kinase-activating mutations, all of which invoke a BCR-ABL-like program, were identified in 91% of patients with Ph-like ALL,\(^{37}\) implying that a wide variety of resistance mechanisms might restore leukemogenesis in response to targeted therapy. Clearly, the best way to treat Ph\(^+\) ALL is to minimize or eliminate MRD.\(^{4}\)

In summary, our data underscore the relevance of this preclinical model and further elucidate the key role of the hematopoietic...
microenvironment in maintaining MRD. Taken together, our results point to a new therapeutic strategy for the treatment of Ph+ ALL and provide a rationale for a phase 1/2 clinical trial in which patients with Ph+ ALL age ≥40 years will receive dasatinib, nuxolitina, dexamethasone, and intrathecal methotrexate as a first-line remission induction therapy for 12 weeks, followed by continuation of therapy or other treatment options, including conventional chemotherapy or allogeneic stem cell transplant for eligible patients.

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Authorship

Contribution: I.A., C.D.R., and C.J.S. designed the experiments; I.A., C.D.R., E.d.S., C.C., and G.C. performed the work; S.W.L. provided suggestions on the experimental protocols and helped support the work; and I.A., C.D.R., and C.J.S. formulated the data for publication and wrote the manuscript.

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