JAK1/2 and Pan-Deacetylase Inhibitor Combination Therapy Yields Improved Efficacy in Preclinical Mouse Models of JAK2\textsuperscript{V617F}‐Driven Disease

Emeline Evrot\textsuperscript{1}, Nicolas Ebel\textsuperscript{1}, Vincent Romanet\textsuperscript{1}, Claudia Roelli\textsuperscript{1}, Rita Andraos\textsuperscript{1}, Zhiyan Qian\textsuperscript{1}, Arno Dölemeyer\textsuperscript{2}, Ernesta Dammassa\textsuperscript{1}, Dario Sterker\textsuperscript{1}, Robert Cozens\textsuperscript{1}, Francesco Hofmann\textsuperscript{1}, Masato Murakami\textsuperscript{1}, Fabienne Baffert\textsuperscript{1}, and Thomas Radimerski\textsuperscript{1}

Abstract

Purpose: The myeloproliferative neoplasm myelofibrosis is characterized by frequent deregulation of Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling, and JAK inhibitors were shown to reduce splenomegaly and ameliorate disease‐related symptoms. However, the mutant clone and bone marrow fibrosis persist in the majority of patients. Using preclinical models, we explored whether JAK and pan‐deacetylase inhibitor combination yielded additional benefits.

Experimental Design: The combination of the JAK1/2 inhibitor ruxolitinib and panobinostat was investigated using two different mouse models of JAK2\textsuperscript{V617F}‐driven disease. A Ba/F3 JAK2\textsuperscript{V617F} cell‐driven leukemic disease model was used to identify tolerated and efficacious doses. The drugs were then evaluated alone and in combination in a mouse model of myeloproliferative neoplasm–like disease based on transplantation of bone marrow transduced with a retrovirus expressing JAK2\textsuperscript{V617F}. Exposures were determined in blood and tissues, and phosphorylated STAT5 and acetylated histone H3 pharmacodynamic readouts were assessed in spleen and bone marrow. Histologic analysis was conducted on spleen and bone marrow, including staining of reticulin fibers in the latter organ.

Results: The combination of ruxolitinib and panobinostat was found to have a more profound effect on splenomegaly, as well as on bone marrow and spleen histology, compared with either agent alone, and the analysis of pharmacodynamic readouts showed that ruxolitinib and panobinostat have nonoverlapping and complementary effects.

Conclusion: Combining JAK1/2 and pan‐deacetylase inhibitors was fairly well tolerated and resulted in improved efficacy in mouse models of JAK2\textsuperscript{V617F}‐driven disease compared with the single agents. Thus, the combination of ruxolitinib and panobinostat may represent a promising novel therapeutic modality for myeloproliferative neoplasms.

Introduction

The discovery in 2005 of the somatic activating JAK2\textsuperscript{V617F} mutation in chronic myeloproliferative neoplasms (MPN) polycythemia vera, essential thrombocythemia, and primary myelofibrosis (PMF) provided a rationale for the development of Janus kinase (JAK)2 inhibitors (1), which rapidly entered clinical trials for patients with myelofibrosis (2). Consistent with data obtained in preclinical animal models (3–5) JAK inhibitors have shown a rapid and durable reduction of splenomegaly in patients with myelofibrosis and also a substantial improvement of constitutional symptoms (6–8). However, the impact of JAK inhibitors on the JAK2\textsuperscript{V617F}‐mutant allele burden and bone marrow fibrosis has been modest (9). To yield additional benefits for patients with myelofibrosis, combinations of JAK inhibitors with other agents seem warranted. Several other drugs are being explored clinically, including deacetylase inhibitors, immunomodulatory agents, smoothened antagonists, recombinant IFN‐α, and the rapalog everolimus (10). Encouragingly, some of these drugs have shown early signs of clinical activity, offering opportunities for eventual combinations with JAK inhibitors. However, given the number of different agents being investigated, the choice and prioritization of the combination partner represents a considerable challenge.
Ruxolitinib and Panobinostat in Mouse MPN Models

Translational Relevance

Although Janus kinase (JAK) inhibitors have shown rapid and durable reductions in splenomegaly, as well as improvement in symptoms and quality of life in patients with the myeloproliferative neoplasm myelofibrosis, the impact on the mutant allele burden and bone marrow fibrosis has been modest, indicating that combinations with other agents may yield additional benefits. Recently, the pan-deacetylase inhibitor panobinostat has shown encouraging single-agent activity in phase I/II myelofibrosis trials. Using mouse models of JAK2V617F -driven diseases, we show that the combination of the JAK1/2 inhibitor ruxolitinib with the pan-deacetylase inhibitor panobinostat results in markedly improved efficacy compared with the single agents. Our preclinical findings suggest that combining JAK1/2 with pan-deacetylase inhibitors warrants clinical evaluation for myelofibrosis therapy.

On the one hand, combination strategies with JAK inhibitors could be devised to target parallel pathways activated by aberrant JAK signaling, such as the phosphoinositide 3-kinase (PI3K)/mTOR and mitogen-activated protein kinase (MAPK) pathways, and/or downstream STAT effector molecules and their targets (e.g., anti-apoptotic Bcl-2 family members, Pim kinases, c-Myc, etc.; refs. 2, 11). These approaches may result in more profound and/or more sustained inhibition of JAK2V617F signaling. Alternatively, combination strategies with JAK inhibitors could also be aimed at addressing biologic pathways and processes that may cooperate with aberrant JAK signaling. Along these lines, the immunomodulatory drug lenalidomide plus prednisone (12) and the deacetylase inhibitor panobinostat (13–15) have shown promising activity in terms of disease-modifying potential, evidenced by anemia responses, reduction of mutant allele burden, and reversal of bone marrow fibrosis in some patients with myelofibrosis, thus demonstrating potentially appealing combination partners for JAK inhibitors.

To gain confidence in a particular combination modality, preclinical models may provide useful guidance. For instance, in vitro studies using JAK2V617F-mutant cells have shown that pan-deacetylase inhibition resulted in enhanced JAK2 inhibitor–mediated suppression of aberrant JAK/STAT signaling and synergistic apoptosis induction (16). Furthermore, the combination also displayed greater cytotoxicity than either agent alone on primary CD34+ cells from patients with myelofibrosis. Interestingly, combined pan-deacetylase and JAK2 inhibition triggered less apoptosis in CD34+ cells from healthy subjects as compared with CD34+ cells from patients with myelofibrosis (16). These in vitro results suggest that combined JAK and deacetylase inhibition could be promising for the treatment of MPNs; however, the combination has not yet been explored in respective in vivo disease models.

Here, we evaluated the activity and tolerability of the JAK1/2 inhibitor ruxolitinib (INCB018424/INC424; ref. 17) in combination with the pan-deacetylase inhibitor panobinostat (LBH589; ref. 18) in animal models of JAK2V617F-dependent disease. The combination was first explored in a mouse mechanistic model of Ba/F3 JAK2V617F cell–driven leukemic disease, followed by assessment in a bone marrow transplant model of JAK2V617F-mediated MPN-like disease. The combination of ruxolitinib with panobinostat showed superior suppression of splenomegaly, as well as improved bone marrow and spleen histology, as compared with the single agents. Furthermore, ruxolitinib and panobinostat showed nonoverlapping and complementary effects on the proximal pharmacodynamic readouts phosphorylated STAT5 (p-STAT5) and acetylated histone H3.

Materials and Methods

Compounds, formulations, and treatment schedules

Ruxolitinib monophosphate was formulated in 0.5% HPMC (Pharmacoat 603, Dow Chemical) at a concentration of 7.9 mg/mL and was administered orally (per os) twice a day (q12h/twice a day) in an application volume of 10 mL/kg at a free-base equivalent dose of 60 mg/kg. The lactate salt of panobinostat was formulated in isotonic D5W (5% dextrose; B. Braun Medical Inc.) at a concentration of 1.5, 1, and 0.5 mg/mL and administered 3 times a week [Monday/Wednesday/Friday (M/W/F)] in a volume of 10 mL/kg by intraperitoneal (i.p.) injection. The final free-base equivalent doses were 11.90, 7.94, and 3.97 mg/kg, respectively (rounded up values reported in text, figures, and tables). For combination treatments, ruxolitinib and panobinostat were administered simultaneously and with the same dosing regimen used for the single agents.

Mouse models of JAK2V617F-driven disease

The mouse model of Ba/F3 JAK2V617F-luc cell–driven leukemic disease (19) and the mouse bone marrow transplant model of JAK2V617F-driven MPN-like disease were used as previously described (20–22). All animal experiments were carried out in strict adherence to Swiss laws for animal welfare and approved by the Swiss Cantonal Veterinary Office of Basel-Stadt.

Detection of GFP-positive cells in blood samples by flow cytometry

Ten microliters of whole blood was used to detect circulating GFP-positive cells. Blood was distributed into a 96-well round bottom plate (#3795; Corning Life Sciences) and RBCs were lysed with 200 μL of RBC lysis buffer (R-7757; Sigma). After 7-minute incubation in the dark on a plate agitator, cells were centrifuged (5 minutes, 300 g) and the supernatant discarded by inversion. After 3 washes in fluorescence-activated cell-sorting (FACS) buffer (D-PBS, 3% FBS, and 0.02% sodium azide), nucleated cells were resuspended in 200 μL of cold FACS buffer, and processed for GFP detection using a LSRII flow cytometer (BD Biosciences).
Western blotting

Western blotting was carried out as previously described (19). Typically, 40 to 100 μg of protein lysates were resolved by NuPAGE Novex 4% to 12% Bis–Tris Midi Gels (Life Technologies) and transferred to polyvinylidene difluoride (PVDF) membranes by semidyed blotting. The following antibodies were used: JAK2 (#3230), Phosphorylated-STAT5 (Tyr694) (#9359), acetylated lysine (#9441), PARP (#9542), and GAPDH (#2118) were from Cell Signaling Technology. STAT5 (#sc-835) and β-tubulin (#T4026) were from Santa Cruz Biotechnology and Sigma, respectively.

Statistical analysis

Results in figures and tables represent mean ± SEM. Absolute values or transformed values (log_{10} as specified) for all parameters were analyzed by one-way ANOVA analysis followed by Dunnett test to compare all treatment groups to vehicle group. Multiple comparisons were conducted using the Tukey test to compare each single agent to combination groups (all treatment groups were included in the test). The significance level was set to $P < 0.05$. Calculations were conducted using GraphPad Prism 5.00.

Results

Activity and tolerability of ruxolitinib and panobinostat, alone and in combination, in a mouse model of Ba/F3 JAK2V617F cell–driven leukemic disease

A mechanistic mouse model of JAK2V617F-driven leukemic disease (19) was used to assess the activity and tolerability of ruxolitinib and panobinostat alone or in combination. Ruxolitinib was given at 60 mg/kg orally twice daily, whereas panobinostat was given at i.p. doses of 4, 8, and 12 mg/kg. For ruxolitinib, a somewhat lower dose than that previously reported in a similar model (17) was chosen to be able to observe modulation of efficacy readouts upon titration of panobinostat in the combination setting. Animals were treated for 1 week, and bioluminescence signals were measured during the course of the treatment and at study end. Ruxolitinib and panobinostat single agents produced significant antitumor effects, reflected by the reduction in light emission (Fig. 1A). Enhanced efficacy was observed upon combination of panobinostat with ruxolitinib, particularly with the 2 higher panobinostat dose levels. The combination of ruxolitinib with the 12 mg/kg panobinostat regimen was most efficacious with a treatment over control ratio (T/C) of 3% (near stasis), which was significantly different from the efficacy seen with the single agents (Fig. 1A and Supplementary Table S1).

Efficacy and tolerability of ruxolitinib in a mouse model of JAK2V617F-driven MPN-like disease

On the basis of the encouraging results obtained in the mouse mechanistic model, we next set out to establish the efficacious and tolerated regimens of ruxolitinib and panobinostat, first given as monotherapies, in a mouse bone marrow transplant model of JAK2V617F-driven MPN-like disease (20, 21). These studies also served to establish the doses of the agents to be assessed in combination. Transplanted mice were randomized on day 27 based on hematocrit (67%–68% on average per treatment group) and treated with ruxolitinib at 30, 60, or 90 mg/kg twice daily for 21 consecutive days. The spleen weight was significantly reduced after ruxolitinib therapy, with a trend for dose dependency (Fig. 3A). Body weight changes were modest (Supplementary Fig. S3A), indicating that
Figure 1. Activity of ruxolitinib (RUX) and panobinostat (PAN), alone and in combination, in a mouse model of Ba/F3 JAK2V617F cell–driven leukemic disease. SCID beige mice were intravenously inoculated with Ba/F3 JAK2V617F-luc cells. On day 4 after cell injection, detectable luciferase signals could be measured and mice were randomized and treated with vehicle, panobinostat at 4, 8, and 12 mg/kg i.p. on a M/W/F dosing schedule, alone or in combination, with ruxolitinib at a dose of 60 mg/kg per os twice a day for 7 days. A, representative bioluminescence images on day 11 after cell injection (7 days of treatment). The difference from control was calculated by the ratio of bioluminescence fold-increase (treated/control) × 100. *, P < 0.05 versus vehicle-treated animals (one-way ANOVA followed by post hoc Dunnett test on log10-transformed values); †, P < 0.05 versus ruxolitinib-treated animals; and ‡, P < 0.05 versus panobinostat-treated animals at the same dose (one-way ANOVA followed by post hoc Tukey test on log10-transformed values). B, spleen weights (mean ± SEM, n = 7/group) were recorded at sacrifice, which was 2 hours after receiving the final dose after 7 days of treatment. Average spleen weight of age-matched SCID beige mice is 75 mg. *, P < 0.05 versus vehicle-treated animals (one-way ANOVA followed by post hoc Dunnett test on log10-transformed values); †, P < 0.05 versus ruxolitinib-treated animals; ‡, P < 0.05 versus panobinostat-treated animals at the same dose (one-way ANOVA followed by post hoc Tukey test on log10-transformed values). Similar results were obtained in an independent experiment.
ruxolitinib was well tolerated. Elevated hematocrit (Supplementary Fig. S3B), as well as increased reticulocyte and WBC counts (Fig. 3B and C), were reduced after ruxolitinib therapy, and the latter 2 parameters were within the normal ranges at study end. Platelet count was not affected by ruxolitinib treatment and slightly below normal range in all groups (Supplementary Fig. S3C). The percentage of circulating GFP-positive cells was determined by FACS analysis after 2 weeks of treatment as a surrogate readout for JAK2V617F mutant allele burden changes. Treatment with ruxolitinib was found to reduce the fraction of GFP-positive cells (Fig. 3D), although dose dependency was not observed.

Spleen and bone marrow samples were collected posttherapy and sections were assessed for p-STAT5 by immunohistochemistry (IHC). Ruxolitinib was found to suppress levels of p-STAT5 after 2 hours, with the higher doses eliciting stronger p-STAT5 suppression, and levels recovered by 12 hours (Supplementary Fig. S4). Histologic analysis revealed marked proliferation of erythroid and myeloid cells in spleen, leading to a disruption of splenarchic structure (Supplementary Fig. S5A). A decrease in severity of extramedullary hemopoiesis was observed in the spleen of ruxolitinib-treated animals when compared with the vehicle-treated group (Supplementary Fig. S5A, top). In parallel, a decrease in incidence and severity of bone marrow hypercellularity was observed in the sterna of ruxolitinib-treated animals in a dose-dependent manner as compared with the vehicle-treated group (Supplementary Fig. S5A, bottom). Fine reticulin fibers in the bone marrow were reduced to some extent in the ruxolitinib-treated arms, with the best effect seen at the 60 mg/kg dose (Supplementary Fig. S5B).

Taken together, in this model of JAK2V617F-driven MPN-like disease, ruxolitinib therapy was found to suppress splenomegaly, elevated hematocrit, reticulocyte and WBC counts, the mutant allele burden surrogate, hypercellularity, and reticulin fibers in bone marrow, as well as extramedullary erythropoiesis in the spleen. Differences between the ruxolitinib dose levels on these efficacy parameters were either relatively modest or not evident, and all doses were well tolerated.

**Efficacy and tolerability of panobinostat in a mouse model of JAK2V617F-driven MPN-like disease**

Next, panobinostat was evaluated in the mouse MPN model. Mice were randomized on day 27 posttransplantation based on hematocrit (70% on average per treatment group), and panobinostat was given at doses of 4, 8, or 12 mg/kg i.p. on a thrice weekly schedule for 19 consecutive days. A somewhat stronger MPN disease phenotype was observed compared with the ruxolitinib monotherapy experiment, with average spleen weights exceeding 1 g, hematocrits of 76%, and a WBC count of 300 × 10^9/L in the control group at the time of sacrifice. Spleen weight was significantly reduced, but not normalized, in a dose-dependent manner by panobinostat treatment as compared with the vehicle group (Fig. 4A). Panobinostat treatment led to some decrease of body weight (8% on average) in the 12 mg/kg dose group (Supplementary Fig. S6A). Panobinostat had modest, if any, effects on hematocrit (Supplementary Fig. 6B).
Fig. S6B) and reticulocyte count (Fig. 4B) but significantly reduced WBC count (Fig. 4C). Platelet count was at the lower end of the normal range in the vehicle group and significantly reduced below normal range with all panobinostat doses (Supplementary Fig. S6C). Dose dependency of panobinostat-mediated effects on blood parameters was either minimal or not discernible. Finally, the 8 and 12 mg/kg panobinostat dose levels also reduced the percentage of circulating GFP-positive (i.e., JAK2V617F-mutant) cells (Fig. 4D).

Levels of acetylated lysine were markedly increased in spleen samples of panobinostat-treated animals (Supplementary Fig. S7A). Acetylation of histone H3 was assessed in the bone marrow by IHC, revealing a dose-dependent increase of the pharmacodynamic marker in panobinostat-treated animals (Supplementary Fig. S7B). Histologic analysis showed that panobinostat treatment with the 12 mg/kg dose level led to a slight decrease of bone marrow hypercellularity as compared with vehicle-treated animals (Supplementary Fig. S8A). A trend for a decrease of fine reticulin fibers in the bone marrow was noted with all panobinostat doses (Supplementary Fig. S8B).

In summary, panobinostat reduced splenomegaly and aberrant WBC count in the mouse MPN disease model. These changes in efficacy parameters were consistent with deacetylase inhibition, as judged by assessment of pharmacodynamic readouts in target organs. However, treatment of mice with panobinostat also led to some degree of body weight loss and reduced platelet count.

**Efficacy and tolerability of ruxolitinib and panobinostat, alone and in combination, in a mouse model of JAK2V617F-driven MPN-like disease**

Having established the single-agent efficacious doses, we next evaluated the combination of ruxolitinib and panobinostat in the MPN disease model. On the basis of the monotherapy efficacy studies in this model, we tested ruxolitinib at a dose of 60 mg/kg twice a day, as this dose yielded efficacy, but reasoning that it would still enable detecting modulation of efficacy in the combination setting with another drug. Panobinostat was tested at the 8 mg/kg dose level for the same reasons, and to have some margin in terms of tolerability. Mice were randomized on day 27 post-transplantation based on hematocrit (67% on average.
per treatment group) and treated either with vehicle, panobinostat, ruxolitinib, or the combination of ruxolitinib with panobinostat for 21 consecutive days. Panobinostat administered alone reduced spleen weight but not to normal, whereas ruxolitinib showed a trend to reduce spleen weight, but with high variability (range, 67–1153 mg; Fig. 5A). The combination significantly improved efficacy in terms of spleen weight, which normalized or was even somewhat below normal range (in 6 of 9 animals; Fig. 5A). Ruxolitinib was well tolerated as judged by monitoring body weight, whereas panobinostat showed moderate body weight loss (average 5% on average), and body weight decreased further in the combination arm (Supplementary Fig. S9A) but still less than the maximal tolerated body weight loss (15%).

The combination showed a trend for a stronger impact on elevated RBC parameters, particularly on reticulocyte count, although this effect was not significantly different from single-agent arms (Fig. 5B and Supplementary Fig. S9B). WBC count was reduced by panobinostat alone and in combination with ruxolitinib, despite absence of leukocytosis in this experiment, except for single animals in the vehicle and ruxolitinib groups, respectively (Fig. 5C). Platelet count was below normal range in the vehicle arm and reduced further by panobinostat treatment. However, platelet count reduction by panobinostat was not significantly exacerbated upon combination with ruxolitinib (Supplementary Fig. S9C). In this experiment, ruxolitinib had little impact on the mutant allele burden surrogate readout, whereas a trend for a reduction was observed in the panobinostat and combination groups, respectively (Fig. 5D).

Pharmacodynamic marker assessments by IHC showed a clear reduction in p-STAT5 upon treatment with ruxolitinib alone and in combination with panobinostat (Fig. 6A and B), whereas panobinostat markedly increased levels of acetylated histone H3, alone and in combination with ruxolitinib (Fig. 6A and C). However, the 8 mg/kg panobinostat dose level did not appreciably reduce levels of p-STAT5 in the spleen (Fig. 6A), suggesting that the combination of ruxolitinib and panobinostat, at tolerated doses, has nonoverlapping and complementary effects on these proximal pharmacodynamic marker readouts in MPN target organs.
Treatment with either ruxolitinib or panobinostat improved splenic architecture, with the strongest effect being observed with the combination regimen (Supplementary Fig. S11A). A decrease in bone marrow hypercellularity was observed for all drug treatment groups, with combination treatment resulting in the greatest effect, but with mild bone marrow depletion. Both mononuclear megakaryocytes and megakaryocytes with multilobulated nuclei were observed in all treatment groups, without specific differences in their proportion. Despite high variability, the ruxolitinib and the combination groups showed a tendency for reduced fibrosis score, as assessed by staining reticulin fibers on sternum sections (Supplementary Fig. S11B). Cleaved caspase-3 levels in bone marrow sections were highest in the panobinostat-treated group, whereas a trend for increased levels was also observed in the ruxolitinib and the combination groups, although the differences did not reach statistical significance. Importantly, the combination did not impact tissue exposure to each compound (Supplementary Tables S4 and S5).

In summary, the combination of ruxolitinib with panobinostat showed a significant improvement on splenomegaly reduction, as well as on bone marrow and spleen histology, compared with the drugs given alone. Pharmacodynamic marker analyses showed that ruxolitinib and panobinostat were given at active doses and have nonoverlapping and complementary effects on the proximal readouts p-STAT5 and acetylated histone H3, respectively. Panobinostat therapy led to some degree of body weight loss in the animals, which decreased further upon combination with ruxolitinib. However, overall, the combination of ruxolitinib with panobinostat was fairly well tolerated in this model of MPN-like disease.

Discussion

The cMPNs polycythemia vera, essential thrombocythemia, and PMF are characterized by a high incidence of the JAK2V617F mutation (2). JAK inhibitors rapidly entered clinical trials for patients with myelofibrosis following the discovery of aberrant JAK/STAT pathway activation in the disorder and showed encouraging reductions in splenomegaly, improvement in symptoms and quality of life for patients (24). However, the impact of JAK inhibitors on the mutant allele burden and bone marrow fibrosis has been modest so far (25), suggesting that combinations with other drugs may yield additional benefits. More recently,
additional mutations, albeit at lower frequencies and in varying combinations, have been identified in cMPNs, affecting for example, TET2, ASXL1, EZH2, CBL, IDH1, IDH2, LNK, and IKZF1 (26). The loss of function mutations in CBL (27) and LNK (28), which are negative regulators of JAK/STAT signaling, would be expected to raise JAK activity. Interestingly, the remainder of mutations identified to date are thought to affect chromatin states, the epigenome, and/or gene expression, suggesting that alterations at these levels contribute to MPNs and may cooperate with aberrant JAK/STAT activation in MPN pathogenesis (26). Thus, interfering with altered chromatin and gene expression in MPNs may represent yet another potential avenue for MPN therapy. In support of this notion, Akada and colleagues have
recently shown that treatment of JAK2V617F knock-in mice with the pan-deacetylase inhibitor vorinostat led to a reduction in splenomegaly, aberrant blood counts, and mutant allele burden. Encouragingly, anemia responses, as well as reduction of mutant allele burden and bone marrow fibrosis, have been observed in some patients with myelofibrosis treated with the pan-deacetylase inhibitor panobinostat (13–15). In terms of promising combination strategies, preclinical studies have shown that sequential treatment of CD34+ cells from patients with myelofibrosis with the hypomethylating agent 5-aza-2’-deoxycytidine followed by the deacetylase inhibitor trichostatin A was capable of correcting aberrant CXCR4 expression and trafficking behavior of the mutant clone (30). Furthermore, studies with JAK2V617F-mutant cells showed that pan-deacetylase inhibition enhanced suppression of JAK/STAT signaling by a JAK2 inhibitor, and the combination exerted synergistic apoptosis induction (16). Finally, in JAK2- or FLT3-mutant acute myelogenous leukemia (AML) xenograft models, the HDAC inhibitor prinacostatin displayed synergistic tumor growth inhibition in combination with the dual JAK2/FLT3 inhibitor pacritinib (31).

Here, we expanded on these findings and describe the combination of ruxolitinib with panobinostat in 2 preclinical mouse models of JAK2V617F-driven disease. We first took advantage of a mechanistic model of Ba/F3 JAK2V617F cell–driven leukemic disease, as it readily enables to determine tolerated and efficacious dose ranges of drug combinations. Panobinostat was found to inhibit the growth of JAK2V617F–driven leukemic cells and the combination of panobinostat with ruxolitinib further improved activity, while being well tolerated. Next, we established the efficacious doses for ruxolitinib and panobinostat in a mouse JAK2V617F bone marrow transplant model of MPN-like disease and then tested the drugs in combination. The combination of ruxolitinib with panobinostat markedly reduced splenomegaly in the mouse JAK2V617F-dependent MPN disease model, returning spleen weights to normal. The combination also improved bone marrow hypercellularity and spleen histology. These findings were consistent with modulation of p-STAT5 and acetylated histone H3 by ruxolitinib and panobinostat, respectively, showing that when given at active and tolerated doses, the 2 drugs have nonoverlapping and complementary effects on these proximal pharmacodynamic marker readouts, which may underlie some of the observed beneficial effects of the combination. Depending on the dose of panobinostat combined with ruxolitinib, greater suppression of aberrant JAK2/STAT5 signaling can be achieved compared with ruxolitinib, as exemplified by decreased levels of both phospho- and total STAT5 in the mechanistic model. Recently, it was reported that panobinostat can impact STAT5 levels in patients with myelofibrosis receiving the drug, albeit at a high starting dose that was not well tolerated (32). Thus, patients with MPN may tolerate and benefit from deacetylase inhibitors alone or combined with other agents if they receive the deacetylase inhibitor at lower doses and over prolonged periods of time (15, 32, 33). In the mouse disease model, the combination of panobinostat with ruxolitinib further decreased body weight seen with panobinostat alone, although this was not seen in the mechanistic mouse model, and unlikely to be attributed to enhanced exposure due to drug–drug interaction based on pharmacokinetic and pharmacodynamic analyses. Panobinostat therapy in the MPN disease model decreased the platelet count. Although ruxolitinib alone did not decrease platelet count and did not exacerbate the panobinostat-mediated drop in platelet count when combined with the deacetylase inhibitor, it should be pointed out that both drugs exhibited thrombocytopenia as the dose-limiting toxicity in clinical trials (7, 8, 15). Thus, given this overlapping toxicity, platelet count will have to be carefully monitored in the clinic. Although human exposure is not straightforward to replicate in mouse models, due to species differences in clearance or plasma protein binding for example, the ruxolitinib and panobinostat doses used in our mouse models yielded exposures consistent with those achieved clinically. For instance, in healthy volunteers, the Cmax and exposure (AUC) determined in plasma at the maximum tolerated ruxolitinib doses of 25 mg twice a day or 100 mg every day were 1.16 μmol/L and 4.32 (μmol/L) × h or 4.78 μmol/L and 16.60 (μmol/L) × h, respectively (34). The recommended phase II dose of oral panobinostat in a study in patients with myelofibrosis was determined as 25 mg thrice weekly (15), and at an oral dose of 20 mg, the drug yielded plasma Cmax and exposure (AUC) values of 0.04 μmol/L and 0.44 (μmol/L) × h, respectively (35).

Taken together, our preclinical in vivo studies show that the combination of ruxolitinib with panobinostat is fairly well tolerated, affords a significant reduction in splenomegaly, and ameliorates histology of the bone marrow and spleen, as compared with either drug given alone. The added benefit is seemingly consistent with pharmacodynamic marker analyses, which revealed complementary effects of the ruxolitinib and panobinostat combination. The combination of ruxolitinib and panobinostat is currently being investigated in a phase Ib clinical trial in patients with myelofibrosis (36).

Disclosure of Potential Conflicts of Interest
All authors are full-time employees of Novartis Pharma AG. A patent application on combination of ruxolitinib with panobinostat has been filed.

Authors’ Contributions
Conception and design: R. Cozens, M. Murakami, F. Baffert, T. Radimerski
Development of methodology: E. Evrot, N. Ebel, V. Romanet, Z. Qian, A. Doelmeneyer, F. Baffert
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): E. Evrot, N. Ebel, V. Romanet, C. Roelli, R. Andraos, M. Murakami, F. Baffert
Analysis and interpretation of data (e.g., statistical analysis, bioinformatics, computational analysis): E. Evrot, N. Ebel, V. Romanet, C. Roelli, R. Andraos, Z. Qian, A. Doelmeneyer, D. Sterker, M. Murakami, F. Baffert, T. Radimerski
Writing, review, and/or revision of the manuscript: Z. Qian, A. Doelmeneyer, R. Cozens, M. Murakami, F. Baffert, T. Radimerski
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): V. Romanet, C. Roelli, D. Dammassa, T. Radimerski
Study supervision: R. Cozens, F. Hofmann, F. Baffert, T. Radimerski
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Emeline Evrot, Nicolas Ebel, Vincent Romanet, et al.


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