Limited efficacy of BMS-911543 in a murine model of Janus kinase 2 V617F myeloproliferative neoplasm

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Activation of Janus kinase 2 (JAK2), frequently as a result of the JAK2V617F mutation, is a characteristic feature of the classical myeloproliferative neoplasms (MPNs) polycythemia vera, essential thrombocythemia, and myelofibrosis, and it is thought to be responsible for the constitutional symptoms associated with these diseases. BMS-911543 is a JAK2-selective inhibitor that induces apoptosis in JAK2-dependent cell lines and inhibits the growth of CD34+ progenitor cells from patients with JAK2V617F-positive MPN. To explore the clinical potential of this inhibitor, we tested BMS-911543 in a murine retroviral transduction-transplantation model of JAK2V617F MPN. Treatment was initiated at two dose levels (3 mg/kg and 10 mg/kg) when the hematocrit exceeded 70%. Following the first week, white blood cell counts were reduced to normal in the high-dose group and were maintained well below the levels in vehicle-treated mice throughout the study. However, BMS-911543 had no effect on red blood cell parameters. After 42 days of treatment, the proportion of JAK2V617F-positive cells in hematopoietic tissues was identical or slightly increased compared with controls. Plasma concentrations of interleukin 6, interleukin 15, and tumor necrosis factor α were elevated in MPN mice and reduced in the high-dose treatment group, whereas other cytokines were unchanged. Inhibitor activity after dosing was confirmed in a cell culture assay using the plasma of dosed mice and phosphorylated signal transducer and activator of transcription 5 flow cytometry. Collectively, these results show that BMS-911543 has limited activity in this murine model of JAK2V617F-driven MPN and suggest that targeting JAK2 alone may be insufficient to achieve effective disease control. Copyright © 2015 ISEH - International Society for Experimental Hematology. Published by Elsevier Inc.
imperfect models of human MPN, we decided to test BMS-911543 in a retroviral transduction–transplantation model of MPN. This model closely resembles human PV and progresses to secondary MF [13].

Materials and methods

Induction of myeloproliferative neoplasm, study design, and drug administration

A PV-like MPN was induced in female Balb/c mice as previously described [12,13,14]. Once the average hematocrit of the cohort exceeded 70%, four mice were euthanized and examined to confirm MPN through the presence of splenomegaly and green fluorescent protein (GFP)–positive cells in the spleen and bone marrow. Mice were randomly assigned to three treatment groups: vehicle control, 3 mg/kg (low dose [LD]), and 10 mg/kg (high dose [HD]). The 3 and 10 mg/kg doses were chosen based on the near-complete suppression of STAT5 phosphorylation observed following 2, 5, and 10 mg/kg dosing [7]. BMS-911543 (N,N-dicyclopentyl-4-[(1,5-dimethyl-1H-pyrazol-3-yl)amino]-6-ethyl-1-methyl-1,6-dihydropyridazino[4,5-d]pyrrolo[2,3-b]pyridine-7-carboxamide; Bristol-Myers Squibb, New York, NY) was prepared in a solution of 20% citrate/80% PEG400 (polyethylene glycol 400, J.T. Baker, Center Valley, PA) with brief sonication and aliquots were stored at −20°C. Mouse weight was recorded weekly, and drug dilutions were made according to the average weight of each group. Details of the compound, including structure and IC50 values, are provided elsewhere [7]. Pharmacodynamic studies were performed on three mice per group (nine total) 4 hours after administration of the first dose of BMS-911543. Mice were dosed by oral gavage (100 μL/dose) once daily for 42 days, at which point one healthy control, six vehicle-treated mice, and seven mice per treatment group were harvested for examination. Two mice from each drug treatment group were observed for an additional 32 days after discontinuation of drug. Complete blood counts were performed once or twice weekly on a Guava 6HT flow cytometer (Millipore, Billerica, MA) in phosphate-buffered saline (PBS) for 24 hours, then moved to 70% ethanol. Bones were placed in 4% paraformaldehyde in PBS overnight, moved to Formaldehyde-2000 (Decal Chemical Corp., Tallman, NY) for 7–9 days, then placed in 70% ethanol. Tissues were embedded in paraffin, sectioned at 5 micrometers, and stained with hematoxylin and eosin. Separate bone marrow sections were also stained with Chandler’s Reticulin Stain Kit (American MasterTech, Lodi, CA). For reticulin scoring, three images per mouse were captured at 20× and blindly scored according to the 0–3 semiquantitative method described by Thiele et al. [15]. Reticulocyte counts were performed on 10 μL whole blood after 10 min incubation with 8 μL 0.5% new methylene blue solution (Ricca Chemical Company, Arlington, TX). Two hundred red blood cells were scored.

Flow cytometry and antibodies

For pSTAT analysis, blood, spleen, and bone marrow cells were harvested 4 hours after treatment with vehicle, 3 mg/kg, or 10 mg/kg BMS-911543 (n = 3 for each group), fixed in 2% paraformaldehyde at 37°C for 10 min, stored on ice for 1 min, permeabilized with 90% cold methanol for 30 min on ice, and stored at −20°C until analyzed. Cells were washed twice in PBS supplemented with 0.5% bovine serum albumin (BSA), then resuspended in 100 μL PBS/BSA at room temperature. Following incubation for 10 min, antibody was added at 1:50 for 1 hour. After two washes in PBS/BSA, the cells were analyzed on a BD FACSCanto (BD Biosciences, San Jose, CA). Alexa Fluor 647–conjugated antibodies directed against pSTAT1Y701, pSTAT3Y705, and pSTAT5Y694 were purchased from Cell Signaling Technology (Danvers, MA). Data were analyzed for each pSTAT, as well as separately, for GFP+ and GFP− cells. Ba/F3 cells were cytokine starved overnight, then simultaneously stimulated with interleukin (IL) 3 (50 ng/mL) and IL-6 (100 ng/mL) for 5 min to serve as species-specific controls. HEL (human erythroleukemia) cells served as an additional control. Lineage antibodies were purchased from BD Biosciences (Ter119–V450, CD19–phycoerythrin (PE)–cytin (Cy) 7, cKit–peridinin chlorophyll–Cy5.5, CD34–Alexa Fluor 647, CD19–PE–Cy7, CD41–PE, CD3–V450, CD71–PE) and eBiosciences (San Diego, CA; granulocyte receptor-1 (Gr1)–peridinin chlorophyll–Cy5.5, B220–allophycocyanin, Fc block). Aqua Live/Dead (Life Technologies, Carlsbad, CA) was included at 1:100 per sample and only live (negative) cells were analyzed. For detection of lineage markers, freshly isolated cells were incubated in a 96-well plate in PBS/BSA and 0.5 μg Fc block for 10 min, antibody for 30 min, washed twice with PBS, fixed with 2% paraformaldehyde, and analyzed on a BD FACS Canto the following day. Compensation was set with OneComp eBeads from eBiosciences.

Cytokine profiling and statistics

Cytokine profiling was performed with the Milliplex Mouse Cytokine/Chemokine kit by Millipore and analyzed with a MAGPIX

Table 1. Summary of JAK-family inhibitors

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<td>73</td>
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instrument (Luminex Corporation, Austin, TX). Cytokine quantifications more than threefold above or below the dataset median were not used for analysis. All datasets in this publication were compared in Microsoft (Redman, WA) Excel with a Student’s t-test using two-tailed distribution and two-sample equal variance. Significance was assigned to p-values ≤0.05.

**Indirect plasma drug activity assay**

Given that BMS-911543 was minimally effective at altering the natural course of murine MPN, we sought to determine whether the drug was bioavailable. We dosed four mice at 30 mg/kg and harvested plasma 90 min later. Plasma was centrifuged twice at 21,000 g for 10 min to remove all cellular debris, incubated with 1.8 mg/mL of Proteinase K (QIAGEN, Venlo, Netherlands) at 37°C for 1 hour to digest proteins (including cytokines), and centrifuged through an Amicon Ultra-0.5 centrifugal filter unit with Ultracel-3 membrane (3 kDa nominal molecular weight cut-off, Millipore) to ensure removal of all cytokines. Ba/F3 cells (1 × 107/well, 96-well plate, 100 µL) were IL-3 starved overnight, incubated for 30 min with 50 µL of either vehicle plasma (n = 3) or with plasma from mice dosed with BMS-911543 at 30 mg/kg (n = 4). This dose was chosen to accommodate the need to dilute the plasma 1:3 in cell culture medium (100 µL plasma plus 50 µL plasma) while maintaining a sufficiently high concentration to allow for a clear effect to be observed. Following the 30-min incubation period, murine IL-3 (50 ng/mL; Peprotech, Rocky Hill, NJ) was added to each well and incubated for 5 min at 37°C; then, cells were prepared for flow cytometry following standard fixation/permeabilization steps and anti-pSTAT5 (Y694)-Alexa Fluor 647 (Cell Signaling Technology). Data was collected on a BD FACSCanto and analyzed with FlowJo X (Treestar, Ashland, OR).

**Results and discussion**

**Induction of myeloproliferative neoplasm**

Janus kinase 2 V617F-positive MPN was induced in lethally irradiated Balb/c mice by transplantation of bone marrow.
cells retrovirally transduced with MIG-JAK2V617F (murine stem cell virus, internal ribosome entry site, green fluorescent protein), as described (Fig. 1) [13]. An average hematocrit over 70% was predefined as the trigger for the initiation of treatment. Twenty-one days after transplant, the hematocrit was 74 ± 6%, red blood cells 10.7 ± 0.8 × 10^12/L, hemoglobin 195 ± 11.6 g/L, and white blood cells 31 ± 27.9 × 10^9/L, with 27.7 ± 3.0% granulocytes and 68.4 ± 5.8% lymphocytes. Fluorescence-activated cell sorting revealed 34.2 ± 12.2% GFP+ cells in the peripheral blood. The following day, four mice were sacrificed and subjected to autopsy and histopathology. Spleen weights were increased to 304.5 ± 46.9 mg (healthy control = 76.8 mg), bone marrow was hypercellular, and splenic architecture was effaced. The proportions of GFP+ cells in bone marrow and spleen were 26.1 ± 7.6% and 16.7 ± 2.9%, respectively. Together, these parameters established a diagnosis of MPN. On the following day, BMS-911543 treatment was initiated at two dose levels (LD = 3 mg/kg and HD = 10 mg/kg, once daily by oral gavage) based on the pharmacokinetic data and maintained for 42 days (except in mice harvested for pharmacodynamic studies). Vehicle control mice received carrier only.

**BMS-911543 suppresses leukocytosis but not erythrocytosis in JAK2V617F-induced myeloproliferative neoplasm**

Four hours after the first treatment, three mice per group were euthanized for pSTAT analysis. Spleen, bone marrow, and blood were harvested, and cells were analyzed by flow cytometry to evaluate pSTAT1Y701, pSTAT3Y705, and pSTAT5Y694. Phosphorylated STAT5Y694 was reduced in the peripheral blood of treated mice compared with controls, both in GFP+ and GFP− cells, although the difference did not quite reach statistical significance (p < 0.10 for each; Supplementary Figure E1, online only, available at www.exphem.org). In contrast, pSTAT5Y694 was identical in the marrow and spleen. pSTAT1Y701 and pSTAT3Y705 in treated mice were generally comparable to controls (Supplementary Figures E1–E3, online only, available at www.exphem.org). The mice were followed by daily inspection and one or two weekly complete blood counts. After an initial decrease in all groups (Fig. 2), the white blood cells (WBCs) in the vehicle-treated group steadily increased, reaching 40 × 10^9/L on day 25 of treatment. In contrast, the WBCs in the LD, and more so in the HD, treatment group were reduced, although differences became smaller toward the end of the study. Relative proportions of granulocytes decreased over the treatment period. Erythrocytosis, hemoglobin, and hematocrit gradually decreased into the normal range, but were not different between treatment groups and controls (Fig. 2). Reticulocyte counts were also identical between treated mice and controls (Supplementary Figure E4, online only, available at www.exphem.org), and platelet counts remained in the normal range, as previously described with this model [13]. Thus, BMS-911543 suppressed the leukocytosis associated with JAK2V617F MPN without affecting red cell parameters. Following the 42-day treatment period, two randomly selected HD and LD mice were observed for an additional 32 days after discontinuation of BMS-911543. All mice showed a further increase in WBCs that was maintained for the 32-day observation period (Supplementary Figure E5, online only, available at www.exphem.org). Red cell parameters remained stable, probably reflecting the fact that the disease had entered the spent phase and transformed to myelofibrosis. At autopsy, spleens were enlarged, but their weight was reduced (~184 mg; data not shown) compared with the spleens harvested at day 1 (~300 mg) or day 42 (~230 mg; Supplementary Figure E4, online only, available at www.exphem.org). Five healthy mice were dosed with 10 mg/kg BMS-911543 and five with vehicle in a similar, separate experiment, and no changes in spleen mass, spleen or bone marrow histology (Supplementary Figure E6, online only, available at www.exphem.org), or blood parameters (Supplementary Figure E7, online only, available at www.exphem.org) were observed. Granulocyte receptor 1–positive cells were reduced from 11.6% to 8.6% (p = 0.026) in the BMS-911543 group, whereas B- and T-cell populations were not altered (data not shown), suggestive of mild splenic and/or myeloid toxicity.

**BMS-911543 treatment does not significantly alter myeloproliferative neoplasm histology**

On treatment day 42, spleens, livers, and femurs were harvested from seven mice in each of the two treatment groups and from six vehicle controls. Spleen weight was similar in all groups (Supplementary Figure E4, online only, available at www.exphem.org). Histology showed trilineage hematopoiesis with prominent megakaryocytes (Fig. 3). Spleens from the vehicle and LD (not shown) groups exhibited complete effacement of splenic architecture, whereas the HD group showed some restoration of germinal centers (Fig. 3). Liver histology revealed megakaryocytes and extramedullary hematopoiesis, especially near portal veins, without significant differences between groups. Bone marrows were uniformly hypocellular, and reticulin staining showed severe fibrosis in each group, with average scores near 2.5 on the 0–3 scale (Supplementary Figure E4, online only, available at www.exphem.org) [15]. We observed a higher percentage of total GFP+ cells (JAK2V617F-positive) in the spleen (85.1 ± 0.3% vs. 75.6 ± 8.3%; p = 0.014) and bone marrow (67.1 ± 6.7% vs. 57.2 ± 7.3%; p = 0.026) of mice treated with HD BMS-911543 compared with the vehicle control group (Supplementary Figure E4, online only, available at www.exphem.org).
Figure 2. BMS-911543 lowered and maintained WBC counts in murine MPN. (A) The high dose (HD) group maintained WBC counts that were nearly normal and well below the vehicle-treated group for the treatment period. (B) Granulocytes, as a percentage of WBCs, were reduced, and (C) relative lymphocyte counts were elevated by HD BMS-911543 early in the treatment period, but all groups became indistinguishable after 15 days of treatment. (D) The percentage of peripheral WBCs positive for GFP increased steadily in all groups throughout the treatment period and were slightly higher in the HD group at day 31. (E–G) Red blood cells, hematocrit, and hemoglobin decreased steadily throughout the treatment period. (H) Platelet counts did not vary between groups. Data from 10 vehicle, 10 low dose (LD), and 9 HD mice are shown. *p ≤ 0.05 HD vs. vehicle; #p ≤ 0.05 LD vs. vehicle; ¥p ≤ 0.05 LD vs. HD. Error bars represent SEM. PB = peripheral blood; RBC = red blood cell.
BMS-911543 partially restores lineage composition of spleen but not marrow or blood

Spleen, marrow, and peripheral blood were harvested after 42 days of treatment and analyzed by flow cytometry for lineage markers. A dose-dependent reduction of granulocytes (Gr1+), and partial restoration of B-cell (B220+, CD19+) and T-cell (CD3+) distributions was observed in the spleen (Fig. 4), but not in the blood or bone marrow.
No significant differences were observed in other lineage markers, except a small reduction of peripheral blood B cells in the HD group (1.3 ± 0.4% vs. 0.8 ± 0.3%; p < 0.05). To evaluate the impact of BMS-911543 treatment on MPN burden, we measured GFP expression as well as immunophenotypically defined subsets of blood, bone marrow, and spleen cells. BMS-911543 had little effect on the percentage of GFP+ cells in each cellular subset or even led to a mild increase (e.g., GFP+ bone marrow granulocytes increased to 20.3 ± 4.8% in the HD group compared with 15.0 ± 3.2% in the vehicle group; p = 0.0377; Supplementary Figure E9, online only, available at www.exphem.org). These results suggest that BMS-911543 fails to provide JAK2 wild-type cells with a competitive advantage over MPN cells throughout all cell compartments analyzed.

**BMS-911543 partially normalizes plasma cytokine concentrations**

Janus-kinase-family inhibitors like TG101209 or momelotinib (formerly Cytopia387) have been shown to partially normalize cytokine concentrations in mice with JAK2V617F-induced MPN [13,16]. We therefore measured plasma cytokine profiles after 42 days of treatment with BMS-911543. Tumor necrosis factor α (TNFα), IL-6, and IL-15 concentrations were significantly reduced in the HD group compared with the vehicle control, with TNFα and IL-15 reaching the normal range (Fig. 5). Granulocyte colony-stimulating factor and KC (keratinocyte-chemoattractant) concentrations were reduced in MPN mice compared with normal mice, with further reduction upon treatment with BMS-911543 (Supplementary Figure E10, online only, available at www.exphem.org). Interleukin 10, IL-17, and vascular endothelial growth factor concentrations were also elevated in MPN mice but were not reduced by treatment (Supplementary Figure E10, online only, available at www.exphem.org).

**BMS-911543 is present and active in the plasma of treated mice**

The limited efficacy of this inhibitor mandated verification of the presence of BMS-911543 in the plasma of mice following oral dosing. Although this has been reported elsewhere [7], we sought to confirm bioavailability in our system. To this end, we developed a functional, indirect plasma drug activity assay, using plasma from mice treated with BMS-911543 in a cell culture assay with phosphorylation of STAT5 (Y694) as the endpoint. At a dose of BMS-911543 equivalent to 10 mg/kg, we observed a 48% reduction of pSTAT5 median fluorescence intensity compared with controls (p = 0.002; Supplementary Figure E11, online only, available at www.exphem.org), confirming the presence of active drug.

Our data show that BMS-911543 has limited efficacy in our murine model of JAK2V617F-induced MPN. Although we did not perform a direct comparison, the results are clearly inferior to those of momelotinib tested in the same disease model [12]. Since momelotinib is equipotent...
against JAK1 and JAK2, but BMS-911543 is highly selective for JAK2, it is conceivable that JAK1-inhibitory activity is required for more profound effects in this mouse model of MPN. Direct in vivo validation of this notion would require treating mice with drug concentrations that are equipotent toward JAK2, which would be challenging, owing to differences in their pharmacokinetic properties. Similar to CYT387 [12], treatment was initiated when the hematocrit of the cohort exceeded 70%. Given that this is a late model of MPN, more profound effects may have been observed with earlier treatment initiation. The limited activity of BMS-911543 against the MPN phenotype is reflected by pharmacodynamic studies. BMS-911543 reduced pSTAT5Y694 only in the peripheral white blood cells, but not the bone marrow or spleen cells, even at high doses (10 mg/kg). This is in contrast to its strong effects in cell line xenografts, where a 90% suppression of pSTAT5Y694 was reported after a single dose of 10 mg/kg [7], suggesting that pSTAT5Y694 in these key disease sites is not, or is not exclusively, under the control of JAK2, and that the physiologic microenvironmental context is critical for pharmacodynamic assessment. Similarly, BMS-911543 did not reduce pSTAT3Y705, suggesting that JAK1 inhibition may be required to suppress this key regulator of inflammatory cytokine responses. A phase 1/2 study of BMS-911543 is ongoing, and it will be interesting to see whether limited activity of the compound in murine JAK2V617F-induced MPN model is reflected clinically [17].

Acknowledgments

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Figure 5. Effect of BMS-911543 on cytokine concentrations in MPN. Plasma was evaluated after the 42-day treatment period and (A) IL-6, (B) TNFα, and (C) IL-15 were elevated compared with normal mice and reduced in the high dose group compared with the vehicle-treated group. Data from six vehicle, seven low dose, and seven high dose mice are shown. Error bars represent SEM. *p ≤ 0.05.
Conflict of interest disclosure
This work was supported by funding provided to MW Deininger by Bristol-Myers Squibb. His laboratory is also funded by Novartis, Celgene, Genzyme, and Gilead. He is a consultative/advisory board member of Bristol-Myers Squibb, Novartis, Pfizer, ARIAD Pharmaceuticals, and Incyte. No potential conflicts of interest were disclosed by the other authors.

References
Pharmacokinetic analysis revealed that BMS-911543 is orally bioavailable and very stable in Balb/c mice.

### Supplementary Table E1

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*AUC* = area under curve.

### Supplementary Figure E1

**Effect of BMS-911543 on STAT phosphorylation in GFP<sup>+</sup> and GFP<sup>-</sup> cells.**

(A) Blood, (B) spleen, and (C) bone marrow cells were isolated from the MPN mice that received a single dose of BMS-911543 and analyzed for STAT phosphorylation within the GFP<sup>+</sup> and GFP<sup>-</sup> compartments. Mice were treated and harvested 1 day after the hematocrit for the cohort exceeded 70%. Data are for three vehicle, three LD, and three HD mice. For each analysis, the MFI of the vehicle was set to 100%, and the low and high doses are expressed relative to this. Bars represent SEM. *MFI* = Median fluorescence intensity.
Supplementary Figure E2. Validation of the pSTAT antibodies. To validate pSTAT flow cytometry assays, cells were serum starved overnight and (A, B) Jurkat cells were stimulated with interferon α or (C) Ba/F3 cells stimulated with IL-3 and IL-6. In A and B, control is isotype and no antibody for C. (A) pSTAT1-Y701, Jurkat, (B) pSTAT3-Y705, Jurkat, and (C) pSTAT5-Y694, Ba/F3.
**Supplementary Figure E3.** Examples of histograms from pSTAT flow cytometry. The GFP percentage of fixed, permeabilized cells was set as closely as possible to that observed with fresh tissue on the day of harvest, and the MFI of the pSTAT-Alexa Fluor 647 signal was determined for GFP^+^ and GFP^-^ populations. The example shown is peripheral blood, with GFP^-^ cells on the left and GFP^+^ cells on the right. (A, B) pSTAT1, (C, D) pSTAT3, and (E, F) pSTAT5. MFI = median fluorescence intensity.
Supplementary Figure E4. Pathology after 42 days of treatment with BMS-911543. (A) Spleen weight, (B) reticulin scores, (C) reticulocytes, (D) %GFP^+ cells in the spleen, (E) %GFP^+ cells in the bone marrow. Data are for six vehicle, seven LD, and seven HD mice. Bar represents SEM. *p ≤ 0.05.
Supplementary Figure E5. Blood counts after discontinuation of treatment. Two mice per treatment group were observed for 32 days after the 42-day treatment period, and complete blood count results are shown. (A) WBCs, (B) percent granulocytes, (C) percent lymphocytes, (D) red blood cells, (E) hematocrit, (F) hemoglobin, (G) platelets, and (H) percent of peripheral WBCs positive for GFP. Shaded area indicates the time without treatment. Data represent two mice per group. One LC and one HD mouse died between days 60 and 67. RBC = red blood cells.
Supplementary Figure E6. Histology after 35 days of treating healthy mice with 10 mg/kg BMS-911543. Five healthy mice were treated with 10 mg/kg BMS-911543, and five healthy mice were treated with vehicle for 35 days. (A) Spleen sectioned at 5 microns and stained with hematoxylin and eosin, shown at 10× with 40× insets. (B) Bone marrow sectioned at 5 microns and stained with hematoxylin and eosin, shown at 10× with 40× insets. (C) Peripheral blood smears stained with Wright stain and shown at 40×.
Supplementary Figure E7. Blood counts after 35 days of treating healthy mice with 10 mg/kg BMS-911543. Five healthy mice were treated with 10 mg/kg BMS-911543, five healthy mice were treated with vehicle for 35 days, and blood counts were monitored weekly. (A) White blood cells, (B) red blood cells, (C) hematocrit, (D) hemoglobin. *p < 0.05. HCT = hematocrit; HGB = hemoglobin; RBC = red blood cells.
Supplementary Figure E8. Effect of BMS-911543 on hematopoietic cell lineages in MPN. Lineage markers were evaluated by flow cytometry following 42 days of treatment with BMS-911543. (A) Gr1 (granulocytes), (B) B220/CD19 (B cells), (C) CD3 (T cells), (D) CD41 (megakaryocytes), (E) Ter119 (late erythroid progenitors), (F) CD71 (early erythroid progenitors), (G) cKit⁺/CD34⁺ (stem cells/early progenitors), and (H) cKit⁺/CD34⁻ (late progenitors). Data from six vehicle, seven LD, and seven HD mice are shown. Error bars represent SEM. *p ≤ 0.05.
Supplementary Figure E9. Effect of BMS-911543 on hematopoietic cell lineages in MPN. Lineage markers were evaluated by flow cytometry following 42 days of treatment with BMS-911543, then the percent of cells positive for GFP was determined. (A) Gr1 (granulocytes), (B) B220/CD19 (B cells), (C) CD3 (T cells), (D) CD41 (megakaryocytes), (E) Ter119 (late erythroid progenitors), (F) CD71 (early erythroid progenitors), (G) cKit⁺/CD34⁻ (stem cells/early progenitors), and (H) cKit⁺/CD34⁺ (late progenitors). Flow cytometry data from six vehicle, seven LD, and seven HD mice are shown. Error bars represent SEM. *p ≤ 0.05. ns = not significant.
Supplementary Figure E10. Effect of BMS-911543 on cytokine concentrations in MPN. Cytokine profiling was performed following 42 days of treatment with BMS-911543. Data are for six vehicle, seven LD, and seven HD mice, expressed as pg/mL. Bars represent SEM. *p ≤ 0.05 for vehicle compared with HD. Cytokines shown in (A–C) are grouped according to the pg/mL range for each to allow comparisons along the y axis. pg = picograms.
Supplementary Figure E11. Functional, indirect plasma drug activity assay. Plasma from mice treated with BMS-911543 was analyzed for pSTAT5 phosphorylation inhibitory activity. (A) Schematic of the experimental approach. (B) Representative histograms of the pSTAT5 MFI for vehicle and BMS-911543 mouse plasma. (C) The average pSTAT5 MFI in Ba/F3 cells following incubation with plasma from BMS-911543-treated mice (n = 4) is reduced by 48% compared with controls. Error bars represent SEM. *p < 0.05. FACS = fluorescence-activated cell sorting; MFI = median fluorescence intensity; WEHI = Walter and Eliza Hall Institute, mouse myelomonocytic leukemia cell line, conditioned medium is source of interleukin-3.