Combined Inhibition of Janus Kinase 1/2 for the Treatment of JAK2V617F-Driven Neoplasms: Selective Effects on Mutant Cells and Improvements in Measures of Disease Severity

Phillip C.C. Liu,1 Eian Caulder,2 Jun Li,3 Paul Waeltz,1 Alex Margulis,1 Richard Wynn,1 Mary Becker-Pasha,1 Yanlong Li,1 Erin Crowgey,2 Gregory Hollis,1 Patrick Haley,2 Richard B. Sparks,4 Andrew P. Combs,4 James D. Rodgers,4 Timothy C. Burn,1 Kris Vaddi,2 and Jordan S. Fridman2

Abstract

Purpose: Deregulation of the Janus kinase-signal transducers and activators of transcription (JAK-STAT) pathway is a hallmark for the Philadelphia chromosome–negative myeloproliferative diseases polycythemia vera, essential thrombocythemia, and primary myelofibrosis. We tested the efficacy of a selective JAK1/2 inhibitor in cellular and in vivo models of JAK2-driven malignancy.

Experimental Design: A novel inhibitor of JAK1/2 was characterized using kinase assays. Cellular effects of this compound were measured in cell lines bearing the JAK2V617F or JAK1V658F mutation, and its antiproliferative activity against primary polycythemia vera patient cells was determined using clonogenic assays. Antineoplastic activity in vivo was determined using a JAK2V617F-driven xenograft model, and effects of the compound on survival, organomegaly, body weight, and disease-associated inflammatory markers were measured.

Results: INCB16562 potently inhibited proliferation of cell lines and primary cells from PV patients carrying the JAK2V617F or JAK1V658F mutation by blocking JAK-STAT signaling and inducing apoptosis. In vivo, INCB16562 reduced malignant cell burden, reversed splenomegaly and normalized splenic architecture, improved body weight gains, and extended survival in a model of JAK2V617F-driven hematologic malignancy. Moreover, these mice suffered from markedly elevated levels of inflammatory cytokines, similar to advanced myeloproliferative disease patients, which was reversed upon treatment.

Conclusions: These data showed that administration of the dual JAK1/2 inhibitor INCB16562 reduces malignant cell burden, normalizes spleen size and architecture, suppresses inflammatory cytokines, improves weight gain, and extends survival in a rodent model of JAK2V617F-driven hematologic malignancy. Thus, selective inhibitors of JAK1 and JAK2 represent a novel therapy for the patients with myeloproliferative diseases and other neoplasms associated with JAK dysregulation. (Clin Cancer Res 2009;15(22):6891–900)

The myeloproliferative diseases (MPD) are clonal proliferative disorders of hematopoietic progenitor cells with unique pathobiologies. Their clinical presentations include an increase in circulating functional, differentiated myeloid cell lineages that largely define the particular MPD. Broadly, the MPDs can be categorized into those that that are Philadelphia chromosome negative or positive, the latter of which defines nearly all cases of chronic myeloid leukemia (CML; refs. 1, 2). The seminal discovery that the Philadelphia chromosome resulted from a 9;22 translocation and produces the BCR-ABL fusion oncoprotein defined the first MPD-associated mutant allele (reviewed in ref. 3).
Translational Relevance

The discovery that activating mutations in the Janus kinase family (JAK) pathway occur in the vast majority of patients with Philadelphia chromosome–negative myeloproliferative neoplasms has resulted in the rapid development of a number of small molecule inhibitors of JAK kinases. Here, we describe the effects of potent JAK1/2 inhibition and the rational for developing a compound with activity on both JAKs. This is of particular interest with the recent description of (a) highly elevated levels of circulating JAK1/2-activating inflammatory cytokines in myelofibrosis patients, which likely contribute to constitutional symptoms and which may alter the effectiveness of selective JAK2 inhibition, and (b) activating JAK1 and JAK2 mutations in pediatric acute lymphoblastic leukemia patients. Compounds with the ability to affect JAK1/2 signaling may therefore have broad therapeutic potential in neoplasias with dysregulated JAK signaling that can occur both within the tumor cells and systemically.

Subsequent development of selective chemical inhibitors against ABL kinase (imatinib or Gleevec) led to the initial proof-of-concept studies that the targeted inhibition of the dysregulated BCR-ABL protein in CML was highly efficacious (4, 5). Genetic mutations in tyrosine kinases have also been identified in a number of other MPDs and leukemias (reviewed in ref. 6).

Increased activity from the Janus kinase family (JAK) has been suspected in the three most common Philadelphia chromosome–negative MPDs: polycythemia vera (PV), essential thrombocytopenia, and myelofibrosis (MF). The observation that peripheral blood from PV patients had the ability to form colonies in semisolid media in the absence of exogenous JAK-dependent cytokines (7) and demonstration of hypersensitivity to cytokine stimulation (reviewed in ref. 8) suggested dysregulated JAK signaling in MPD. In 2005, a number of independent research laboratories simultaneously identified a single nucleotide mutation (1849G>T) encoding a valine to phenylalanine allele constitutively activates signaling (STAT) and is capable of transforming cells to cytokine independence (8–10). In mice, germline or somatic expression of JAK2V617F induces phenotypes similar to human PV, essential thrombocytopenia, and even MF (14–17). Additional activating (18, 19) mutations have been identified in JAK2 (e.g., exon 12 mutations), as well as in MPL (e.g., MPLW515 mutations), further supporting that constitutive activation of the JAK2 pathway is important for MPD pathogenesis (20, 21). Based on the paradigm shifting impact of imatinib on the treatment of CML (22), it is intriguing to think of the therapeutic potential of JAK inhibitors for the treatment of these diseases. Furthermore, mutations in JAK1, JAK2, and JAK3 have been identified in acute myeloid, lymphoblastic leukemias and acute megakaryoblastic leukemia, indicating that inhibition of dysregulated JAK kinases may have broader therapeutic benefits in other hematologic neoplasms (23–26).

More recently, this concept has been broadened to include solid tumors with the identification of JAK-STAT activating mutations in the gp130 cytokine receptor of hepatocellular tumors (27).

Here, we evaluate the therapeutic potential of JAK inhibition for the treatment of Philadelphia chromosome–negative MPDs using a potent and selective inhibitor of JAK kinases, INCB16562. We find that a selective JAK1/2 inhibitor can reduce malignant cell burden, reverse splenomegaly and normalize splenic architecture, prevent weight loss, and extend survival in a rodent model of JAK2V617F-driven hematologic malignancy. The effects on JAK2V617F-expressing cells were not limited to the murine setting as we show that JAK inhibition reduces endogenous erythroid colony formation from PV patient samples at concentrations below that required to inhibit colony formation from healthy controls. Strikingly, mice bearing cells with the activating JAK2 mutation suffer from markedly elevated levels of numerous inflammatory cytokines, similar to MPD patients (28, 29), and selective JAK inhibition suppresses these cytokine levels. This state of chronic cytokine elevation is thought to contribute to symptoms associated with MPDs including constitutional symptoms (e.g., cachexia and pruritis) and fibrosis (30). Therefore, selective inhibitors of JAK1/2 may have therapeutic potential in MPDs by selectively reducing cells expressing the mutated JAK2 and reversal of a profound disease-associated inflammatory state.

Materials and Methods

The kinase domains of human JAK1, JAK2, JAK3, and TYK2 were cloned by PCR, were epitope tagged, and were expressed recombinantly using baculovirus in Sf21 cells. A full-length human JAK2 clone was cloned with a carboxyl-terminal hemagglutinin epitope into the pMSPC-vpu Simmons for cellular assays. The JAK2V617F mutation was generated by site-directed mutagenesis and sequence confirmed. The Ba/F3 (DSMZ) cell models were generated by nucleoporation of plasmids encoding human Epo receptor clone (pMSCV-neo-EPOR) and JAK2 (pMSPC-vpu-JAK2 or JAK2V617F) followed by selection and isolation of individual clones.

Quantitative genotyping of JAK2V617F cell burden in vivo. Genomic DNA was used in a quantitative real-time PCR assay to determine the abundance of Ba/F3-JAK2V617F cells in blood and tissue samples. qPCR primer and probe sets were designed against the pMSPC-puro-JAK2V617F expression cassette. The abundance of pMSPC-puro-JAK2V617F served as a measure of mutant cell burden, with all values being normalized to the mass of input genomic DNA using an assay for the mouse Ccr2 genomic locus that is present in both the Ba/F3/JAK2V617F cells and endogenous mouse cells.

Biochemical assay. Enzyme assays were done using a homogeneous time-resolved fluorescence assay with affinity-purified recombinant kinases and peptide substrate. Each enzyme reaction was carried out with test compound or control, JAK enzyme, 500 mmol/L peptide, ATP (at the Km specific for each kinase or 1 mmol/L), and 2.0% DMSO in assay buffer. The calculated IC50 value is the compound concentration required for inhibition of 50% of the fluorescent signal. Glutathione S-transferase fusion proteins composed of the kinase domain with either wild-type JAK2 or JAK2V617F JH2 domain were purchased from Invitrogen and assayed essentially as described above.

Cell proliferation assay. Ba/F3 cells were seeded at 2,000 cells per well of white bottom 96-well plates, treated with compounds from DMSO stocks (0.2% final DMSO concentration), and incubated for 48 h at 37°C with 5% CO2. Viability was measured by cellular ATP

Readability: 3.2

Clin Cancer Res 2009;15(22) November 15, 2009 6892 www.aacrjournals.org

Downloaded from clincancerres.aacrjournals.org on May 28, 2017. © 2009 American Association for Cancer Research.
Results

Biochemical potency and selectivity of INCB16562. A dipyrindiazepine compound INCB16562 (Fig. 1A) was identified as a potent inhibitor of JAK2 (0.3 nmol/L), although it possessed activity against all members of the JAK kinase family (Table 1). When kinase assays were done at 1 nmol/L ATP to approximate the intracellular concentration and to account for differences among enzyme KmATP values, the potency against JAK2 was 2.9 nmol/L, and INCB16562 showed modest (4-fold), intermediate (13-fold), and marked (385-fold) selectivity over JAK1, Tyk2, and JAK3, respectively. The selectivity of INCB16562 was characterized at 100 nmol/L against a broad representation (50 kinases) of the kinome (Supplementary Table S1). Inhibition of TrkA and TrkB and Lck was observed at the high concentration tested. Because the compound is an ATP-competitive molecule (data not shown), we sought to determine whether the MPD-associated mutant JAK2V617F protein would have any different biochemical characteristics from wild-type JAK2. The Km for ATP was indistinguishable (90 nmol/L) between JAK2 and JAK2V617F, and the IC50 values were also similar for the two proteins (2.3 and 1.0 nmol/L for JAK2 and JAK2V617F, respectively). These data indicate that the V617F mutation does not alter the affinity of the mutant protein for ATP or ATP-competitive inhibitors.

Cellular potency and selectivity of INCB16562. As inhibition of JAK2V617F was a key goal, we established a Ba/F3 cell line dependent on JAK2V617F for proliferation and survival (10–13). Strict dependence upon JAK2V617F for viability was confirmed by use of siRNAs specific for human JAK2. This was reversed by addition of IL-3 to activate endogenous murine JAKs (Supplementary Fig. S1). Addition of INCB16562 potently reduced the basal level of phosphorylation on JAK2 and STAT5 proteins with an IC50 of approximately 50 to 128 nmol/L (Fig. 1B). To determine the effect of INCB16562 on erythropoietin (Epo) signaling, cells were pretreated with compound before Epo stimulation. Addition of Epo stimulates not only the JAK-STAT pathway but also the Ras-MAPK and AKT pathways (33). As shown in Fig. 1C, JAK inhibition by INCB16562 dose dependently abrogated signaling through the Ras/MEK/ERK pathway. Likewise the Epo-induced phosphorylation of AKT was reduced by the JAK inhibitor. Attempts to detect phosphorylated JAK1 in these cells were not successful and may reflect either technical limitations or a limited role for JAK1 in this cell system (Supplementary Fig. S1). The use of EpoR as the requisite type I cytokine receptor likely precluded the ability of JAK2 and JAK1 to interact; however, this interaction has been shown in other relevant model systems (34).

To investigate the impact of JAK1/2 inhibition on cellular proliferation and survival, we measured proliferation of cells expressing either the wild-type or mutant JAK2. INCB16562 inhibited the proliferation of Ba/F3-JAK2V617F cells with an IC50 of 133 ± 35 nmol/L (Fig. 2A), whereas cells expressing wild-type human JAK2 that require exogenous cytokine supplementation were inhibited with IC50 values of 638 and 1213 nmol/L in the presence Epo and IL-3, respectively. The compound also inhibited proliferation of human cell lines that endogenously express the mutant JAK2V617F allele Hel92.1.7 erythroblenemia (IC50 value, 590 nmol/L) and SET2 (IC50, 63 nmol/L). Suppression of cell proliferation correlated with decreased phosphorylation of STAT3 and STAT5 proteins in both cell lines.
As described above, technical limitations precluded us from studying the signaling effects of INCB16562 on JAK1 in JAK2V617F-expressing cells. Therefore, to directly determine the cellular effect of INCB16562 on JAK1, we generated a Ba/F3 cell line that was driven by an activating JAK1 mutation found in pediatric acute lymphoblastic leukemia (ALL), JAK1V658F; in these cells, INCB16562 suppressed proliferation with an IC₅₀ of 208 nmol/L (±70 nmol/L). Consistent with the selectivity of INCB16562 for JAK family enzymes, INCB16562 did not significantly reduce proliferation of Ba/F3-BCR-ABL cells at concentrations up to 5 μmol/L or reduce survival of human embryonic kidney cells, resting T cells, or bone marrow stromal cells at similar concentrations.

Impact of JAK inhibition on primary PV patient cells. To assess the impact of JAK inhibition on the proliferation of hematopoietic progenitor cells from PV patients, we performed clonogenic colony forming assays. Cells isolated from three PV donors were JAK2V617F positive and exhibited cytokine-independent colony formation, whereas no cytokine-independent colonies emerged from three normal donors (data not shown). INCB16562 treatment suppressed the number of erythroid colonies (burst-forming units-erythroid and colony-forming units-erythroid) from PV patient samples with an IC₅₀ value of 110 nmol/L (Fig. 2B). Progenitor cells from normal donors require supplementation with cytokines that activate JAK pathways. Under these conditions, there was no significant difference in the IC₅₀ for suppression of erythroid colony formation between control donors and PV patients, consistent with what was observed in Ba/F3 cells driven by JAK2V617F stimulated with cytokines that signal through wild-type JAKs.

INCB16562 stimulates apoptosis of JAK2V617F-expressing cells. To study the mechanism for reduced cell number by JAK inhibition, we analyzed cellular apoptosis in Ba/F3-JAK2V617F cells treated with INCB16562. After 24 hours of treatment with INCB16562, there was a dose-dependent increase in the number of Annexin V–positive cells, a marker for early apoptosis (Fig. 3A). Concomitant with increased Annexin V staining, there was dose- and time-dependent activation of effector caspases 3 and 7 as shown by increased FAM fluorescence from cells irreversibly labeled with a modified peptide containing a canonical caspase 3 and 7 recognition motif. Thus inhibition of JAK kinases by INCB16562 leads to programmed cell death in Ba/F3-JAK2V617F cells (Fig. 3B).

INCB16562 reduces JAK2V617F cell burden, and splenomega-
ly, in vivo, prevents weight loss, and prolongs survival. To explore the potential of selective JAK inhibition in JAK2V617F-driven malignancies in vivo, we developed a model similar to that previously described by Pardanani et al. (35) We injected Ba/F3 cells expressing the EpoR alone, or in combination with JAK2V617F i.v. into cohorts of mice, and monitored them for morbidity and mortality. Mice injected with 1 × 10⁷ cells expressing just the EpoR showed no signs of morbidity during the duration of the observation period (45 days). In contrast, when cells expressing both EpoR and JAK2V617F were inoculated, mortality was observed as early as 17 days postinoculation and was dependent on the number of malignant cells inoculated (Fig. 4A). Early sacrifice and necropsy of a subset of mice indicated inoculation of these JAK2V617F-expressing cells...
cells results in gross splenomegaly, similar to that which is often observed clinically in advanced MPD. We investigated the efficacy of INCB16562 in this model, initiating treatment within 24 hours of cell inoculation (see Materials and Methods for details). As shown in Fig. 4B, INCB16562 significantly prolonged survival in a dose-dependent manner. Whereas the twice-daily dose of 5 mg/kg had minimal effects, 25 and 75 mg/kg extended the mean survival time by >100% and >300%, respectively. A similar significant and dose-dependent reduction in splenomegaly was observed in a separate cohort of mice sacrificed 9 days following inoculation of the JAK2V617F-expressing cells (Fig. 4C) with the mid and high dose levels restoring spleens to their normal size (based on historical controls). In addition, although vehicle-treated mice failed to gain body weight during the study—indicative of cachexia or a hypermetabolic state—treatment with active doses of INCB16562 restored expected increases in animal weight similar to those in naïve control animals (Fig. 4D).

Histopathologic analysis revealed that the massive increase in spleen weights was echoed by a stunning increase in the diameter of cross-sections taken mid-spleen in vehicle-treated mice injected with JAK2V617F-expressing cells compared with naïve age-matched animals (Fig. 5A). In contrast, INCB16562 resulted in a marked reduction in cross-sectional area as well as a normalization of splenic architecture. Vehicle-treated mice suffered from a total effacement of the red pulp, which had been replaced with neoplastic cells. Treatment with INCB16562 reduced the gross number of neoplastic cells in a dose-dependent manner. This effect, along with a lack of gross lymphodepletion by INCB16562, supported the reversion of drug-treated spleens to a more normal appearance. The livers from vehicle-treated mice also showed significant dissemination of neoplastic cells into the sinusoids—a finding mitigated by drug treatment (Fig. 5B). Finally, though no fibrosis was noted in this rapidly progressing model, the bone marrows of vehicle mice were completely obliterated and replaced with malignant cells (Fig. 5C). As was seen in other tissues, selective inhibition of JAK1/2 reduced the visible tumor burden in the bone without broad cytoreduction.

The burden of BaF3/JAK2V617F cells was determined by quantitative real-time PCR using genomic DNA and an assay for the JAK2V617F expression cassette. Results from this assay show that the low and intermediate dose levels of INCB16562 had a demonstrable but modest reduction in malignant cell burden relative to normal cells (Fig. 5D). In contrast, the highly efficacious dose of INCB16562 dramatically reduced the proportion of malignant cell DNA, consistent with the histopathologic findings. These data imply that INCB16562 selectively affects cells expressing the mutant JAK2, consistent with the “oncogene addiction” hypothesis (36).

Elevated inflammatory cytokines and reversal by INCB16562. It has been suggested that inflammatory cytokines play a fundamental role in the progressive bone marrow pathology of MPD patients as well as in the debilitating symptoms from which MPD patients suffer (30). Clinical data demonstrating a remarkable elevation in multiple cytokines (e.g., IL-6 and MCP-1) support this hypothesis in MF patients (37).

To gain a better understanding of the relationship between JAK2V617F-associated MPDs and cytokines, we analyzed plasma samples from vehicle- or drug-treated mice inoculated with JAK2V617F-expressing cells 9 days after inoculation—a time point associated with marked splenomegaly and disease dissemination (Fig. 5). Compared with control animals, vehicle-treated mice had striking elevations in circulating IL-6, tumor necrosis factor-α, MCP-1, and RANTES (Table 2). This was not a generalized response to malignant cells in tumor-bearing mice as we have examined these cytokines in other models (e.g., PC-3 tumor-bearing mice) and seen no significant elevations.5 Notably, treatment with INCB16562 reduced the levels

---

**Table 1. Inhibition of JAK family kinase activity in vitro**

<table>
<thead>
<tr>
<th>JAK2 IC₅₀ (nmol/L)</th>
<th>JAK1 IC₅₀ (nmol/L)</th>
<th>JAK3 IC₅₀ (nmol/L)</th>
<th>TYK2 IC₅₀ (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP-Km 0.3</td>
<td>ATP-1 mmol/L 2.1</td>
<td>ATP-1 mmol/L 1895.0</td>
<td>ATP-1 mmol/L 28.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

Fig. 2. INCB16562 inhibits proliferation of cells expressing JAK2V617F. A, inhibition of Ba/F3 cell proliferation. Cells expressing JAK2V617F, JAK2, or JAK1V658F were incubated for 48 h with INCB16562, and proliferation was determined by measuring intracellular ATP. A representative IC₅₀ curve is shown with the mean (±SD) of triplicate points. B, INCB16562 inhibits proliferation of erythroid progenitor cells from PV patients. Colony-forming assays were done using mononuclear cells isolated from the peripheral blood of three normal donors and three JAK2V617F-positive PV patients. Cells were plated onto H4531 (no cytokine) or H88434 (+cytokine) methocell with INCB16562 or DMSO (0.1%) and scored 14 d later for colony-forming units of erythroid and burst-forming unit-erythroid colonies.
of these inflammatory cytokines by 48%, 59%, ≥89%, and ≥96%, respectively. Because IL-6 signals through JAK1 and JAK2, treatment with a JAK1/2 inhibitor would also be expected to reduce signaling mediated by this cytokine and improve associated constitutional symptoms. Consistent with this, we have recently reported that infusion of IL-6 hyperactivates JAK/STAT signaling in skeletal muscle and recapitulates several of the biological and physical corollaries of cancer-associated cachexia. Significantly, JAK1/2 inhibition with INCB16562 reduced pSTAT5 in muscle and completely prevented muscle atrophy (38).

These data suggest that cells expressing JAK2V617F elevate systemic inflammatory proteins and that selective JAK inhibition can mitigate this response. Because some of these factors (e.g., IL-6) use JAK1/2 to signal, their inhibition may also reduce abnormal cytokine signaling, thereby improving symptoms associated with their dysregulation. Moreover, inhibition of JAK1/2 may have cell autonomous effects beyond inhibition of JAK2V617F. Although the cell lines used here have not permitted us to query this directly, recent data advocate an important role for JAK1/2-activating cytokines in MPDs, suggesting JAK2-specific inhibition may be rendered less effective by
Fig. 4. INCB16562 treatment prolongs survival and reduces splenomegaly in a mouse model of JAK2V617F-driven hematologic malignancy. A, survival of mice inoculated with JAK2V617F bearing cells. Increasing numbers of Ba/F3 cells expressing the EpoR and JAK2V617F were inoculated i.v. into BALB/c recipient mice, on day 0 and survival was monitored over time. B, INCB16562 treatment protects mice from effects of JAK2V617F cell–induced mortality. Kaplan-Meier survival plot showing dose-dependent improvement in survival of mice inoculated with 1 × 10⁵ Ba/F3-JAK2V617F cells treated orally, twice daily, with vehicle or INCB16562 at the indicated doses. C, reversal of splenomegaly by INCB16562. Spleens from mice treated as above were harvested on day 9, photographed, and weighed. All dose levels significantly reduced spleen weights (5 mg/kg, \( P < 0.01 \); 25 mg/kg, \( P = 0.001 \); 75 mg/kg, \( P < 0.001 \)). Columns, mean spleen weight (\( n = 4 \)); bars, SD. D, restoration of body weight gain by INCB16562. Growth of JAK2V617F-expressing cells prevented normal body weight gain in vehicle-treated mice. Treatment with either 25 or 75 mg/kg INCB16562 permitted normal weight gain to occur. Columns, mean body weights (\( n = 8 \)) taken before initiation of treatment (day 0) and day 14, the first day mortality was noted in vehicle animals; bars, SEM.
increased JAK1/2 signaling mediated by cytokines (39–41). Nonetheless, the paucity of JAK1 signaling data makes the cell autonomous benefits of a JAK1/2 inhibitor theoretical at this time, at least for cells expressing JAK2V617F. In the case of JAK1-mutated pediatric ALL, the benefits of a dual JAK1/2 inhibitor are more immediately apparent.

**Discussion**

In the present study, we examined the potential of selective JAK inhibition for the treatment of MPDs with dysregulated JAK activity using both in vitro and in vivo models. The prevalence and penetrance of JAK mutations in MPDs strongly suggests that inappropriate activation of JAK-STAT is a common pathogenic mechanism for these diseases. This hypothesis is supported by murine models in which introduction of the activating JAK2 or Mpl mutations is sufficient to induce pathologies similar to human MPDs (14–17, 20). The apparent fundamental role of JAK signaling in MPDs and the paucity of efficacious therapies for advanced PV and MF compels the discovery and development of new targeted therapeutics. Moreover, the data described here are relevant to additional neoplasms with dysregulated JAK activity; for example, the recent description of JAK1 and JAK2 mutations in a group of high-risk pediatric ALL patients (19).

This study shows that INCB16562 is a potent, selective, orally bioavailable inhibitor of JAK1/2. In cellular assays, INCB16562 inhibits growth of cell lines driven by JAK2V617F, in part, by

**Table 2.** Inflammatory cytokine levels in plasma samples from mice bearing JAK2V617F tumors

<table>
<thead>
<tr>
<th></th>
<th>IL-6 (pg/mL)</th>
<th>TNFα (pg/mL)</th>
<th>RANTES (pg/mL)</th>
<th>MCP-1 (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naive (no tumor cells)</td>
<td>16.9 ± 3.7</td>
<td>46.2 ± 1.0</td>
<td>36.8 ± 20.2</td>
<td>BQL*</td>
</tr>
<tr>
<td>Vehicle</td>
<td>220.1 ± 128.2</td>
<td>111.6 ± 27.7</td>
<td>70.4 ± 15.0</td>
<td>379.9 ± 185.7</td>
</tr>
<tr>
<td>INCB16562</td>
<td>113.5 ± 6.7</td>
<td>45.3 ± 0.8†</td>
<td>70.4 ± 15.0</td>
<td>379.9 ± 185.7</td>
</tr>
</tbody>
</table>

Abbreviation: TNF, tumor necrosis factor.

*Below quantitative limit is 7.5 and 15 pg/mL for RANTES and MCP-1, respectively.
†P < 0.05, nonparametric t test comparing vehicle to INCB16562-treated mice.
inducing apoptosis. Our results show that JAK inhibition is more potent in cell lines and primary cell samples expressing the mutant JAK2V617F than in those driven by wild-type JAKs. INCb16562 also showed potent activity against cells expressing JAK1V656F, a mutation recently identified in ALL (19). In some cases, transactivation between JAK1 and JAK2 may occur, as evidenced by the recent demonstration that JAK2V617F and JAK1 complex with the IL-27 and IL-3 receptors (34, 40). The cell models used here, Ba/F3 (expressing JAK2V617F and requiring EpoR for scaffolding) and SET2, did not show activated JAK1 or a dependence upon JAK1 for growth (data not shown; Supplementary Fig. S1); therefore, a cell-autonomous contribution by JAK1 could not be assessed at this time. Nevertheless, cytokines that activate JAK1/2 have been shown elsewhere to convey resistance to genetic or pharmacologic inhibitors of JAK2 (41). Therefore, we believe that JAK1 inhibition may provide additional cell autonomous benefits beyond selective inhibition of JAK2.

In vivo, our data also suggest that cells expressing JAK2V617F are more susceptible to the effects of JAK inhibition than normal cells. Mice inoculated with Ba/F3 cells expressing the mutant JAK suffer from massive splenomegaly, effacement of the bone marrow, reduced body weight gain, and premature death. When treated with INCb16562, spleen size normalizes, tumor burden decreases, body weights are improved, and survival is markedly prolonged. Importantly, these effects occur without gross lymphopenia or anemia, even at the highest dose tested (data not shown; Fig. 4D). Furthermore, these cells induce a systemic inflammatory state similar to that observed in MF patients (37). Analogue to recent clinical data with INCb184824 (37, 42), INCb16562 reduced inflammatory cytokine levels and dramatically improved constitutional symptoms. Inhibition of JAK1/2 also has the potential of further mitigating the effects of these dysregulated cytokines (e.g., IL-6) by reducing signaling through cytokine receptor–associated JAKs. In aggregate, antagonism of elevated cytokine receptor signaling in MPD patients may relieve constitutional symptoms such as pruritis, night sweats, and cachexia, and may occur independently from direct effects on the malignant clone (43). Indeed, we observed that selective JAK1/2 inhibition enhances retention of muscle and fat and improves functional end points in various cancer cachexia models, including effects brought about solely by the administration of the JAK1/2-activating cytokine IL-6 (38). These data suggest that selective inhibition of JAK1 and JAK2 may impact both cell autonomous and nonautonomous mechanisms in myeloid neoplasms.

The promise of molecularly targeted agents in MPDs has precedent with the unparalleled success of imatinib in CML. The identification of genetic mutations in multiple kinases in clonal MPDs suggest that dysregulation of pathways involved in cell proliferation and survival, such as JAK–STAT, provide a selective advantage. However, as single genetic mutations have been associated with multiple distinct phenotypes—such as JAK2V617F in PV, essential thrombocythemia, and MF—antecedent mutations or disease modifiers likely contribute to the respective diseases and their clinical outcomes (reviewed in ref. 44). Therefore, it is currently unknown if inhibition of the dysregulated JAK activity will be curative. Moreover, the ideal profile of a JAK inhibitor has yet to be determined. Current JAK inhibitors in development vary in their selectivity both within and beyond the JAK family (45–47). Avoidance of JAK3 has been proposed to be important for reducing effects on immunosuppression; likewise, management of JAK2 inhibition through pharmacokinetic properties is thought to be needed to minimize cytopenias (48). Nonetheless, preliminary data from two phase I/II clinical studies seem promising with dramatic effects being reported recently on both splenomegaly and constitutional symptoms (49, 50). These early data support the continued evaluation of selective JAK inhibitors in the MPDs and other disease areas associated with dysregulated JAK activity.

Disclosure of Potential Conflicts of Interest

All authors are or have been employees of the Incyte Corporation.

References

22. Deininger MW, Druker BJ. Specific targeted
Combined Inhibition of Janus Kinase 1/2 for the Treatment of JAK2V617F-Driven Neoplasms: Selective Effects on Mutant Cells and Improvements in Measures of Disease Severity

Phillip C.C. Liu, Eian Caulder, Jun Li, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-09-1298

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2009/11/05/1078-0432.CCR-09-1298.DC1

Cited articles
This article cites 48 articles, 17 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/15/22/6891.full.html#ref-list-1

Citing articles
This article has been cited by 2 HighWire-hosted articles. Access the articles at:
/content/15/22/6891.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.