Selective Inhibition of JAK1 and JAK2 Is Efficacious in Rodent Models of Arthritis: Preclinical Characterization of INCB028050

Jordan S. Fridman, Peggy A. Scherle, Robert Collins, Timothy C. Burn, Yanlong Li, Jun Li, Maryanne B. Covington, Beth Thomas, Paul Collier, Margaret F. Favata, Xiaoming Wen, Jack Shi, Ryan McGee, Patrick J. Haley, Stacey Shepard, James D. Rodgers, Swamy Yeleswaram, Greg Hollis, Robert C. Newton, Brian Metcalf, Steven M. Friedman and Kris Vaddi

J Immunol 2010; 184:5298-5307; Prepublished online 2 April 2010; doi: 10.4049/jimmunol.0902819
http://www.jimmunol.org/content/184/9/5298

Supplementary Material http://www.jimmunol.org/content/suppl/2010/04/02/jimmunol.0902819.DC1

Why The JI?

• Rapid Reviews! 30 days* from submission to initial decision
• No Triage! Every submission reviewed by practicing scientists
• Speedy Publication! 4 weeks from acceptance to publication

*average

References This article cites 32 articles, 9 of which you can access for free at:
http://www.jimmunol.org/content/184/9/5298.full#ref-list-1

Subscription Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852
Copyright © 2010 by The American Association of Immunologists, Inc. All rights reserved.
Print ISSN: 0022-1767 Online ISSN: 1550-6606.
Selective Inhibition of JAK1 and JAK2 Is Efficacious in Rodent Models of Arthritis: Preclinical Characterization of INCB028050

Jordan S. Fridman, Peggy A. Scherle, Robert Collins, Timothy C. Burn, Yanlong Li, Jun Li, Maryanne B. Covington, Beth Thomas, Paul Collier, Margaret F. Favata, Xiaoming Wen, Jack Shi, Ryan McGee, Patrick J. Haley, Stacey Shepard, James D. Rodgers, Swamy Yeleswaram, Greg Hollis, Robert C. Newton, Brian Metcalf, Steven M. Friedman, and Kris Vaddi

Inhibiting signal transduction induced by inflammatory cytokines offers a new approach for the treatment of autoimmune diseases such as rheumatoid arthritis. Kinase inhibitors have shown promising oral disease-modifying antirheumatic drug potential with efficacy similar to anti-TNF biologics. Direct and indirect inhibition of the JAKs, with small molecule inhibitors like CP-690,550 and INCB018424 or neutralizing Abs, such as the anti-IL6 receptor Ab tocilizumab, have demonstrated rapid and sustained improvement in clinical measures of disease, consistent with their respective preclinical experiments. Therefore, it is of interest to identify optimized JAK inhibitors with unique profiles to maximize therapeutic opportunities. INCB028050 is a selective orally bioavailable JAK1/JAK2 inhibitor with nanomolar potency against JAK1 (5.9 nM) and JAK2 (5.7 nM). INCB028050 inhibits intracellular signaling of multiple proinflammatory cytokines including IL-6 and IL-23 at concentrations <50 nM. Significant efficacy, as assessed by improvements in clinical, histologic and radiographic signs of disease, was achieved in the rat adjuvant arthritis model with doses of INCB028050 providing partial and/or periodic inhibition of JAK1/JAK2 and no inhibition of JAK3. Diminution of inflammatory Th1 and Th17 associated cytokine mRNA levels was observed in the draining lymph nodes of treated rats. INCB028050 was also effective in multiple murine models of arthritis, with no evidence of suppression of humoral immunity or adverse hematologic effects. These data suggest that fractional inhibition of JAK1 and JAK2 is sufficient for significant activity in autoimmune disease models. Clinical evaluation of INCB028050 in RA is ongoing. The Journal of Immunology, 2010, 184: 5298–5307.

The pathogenic role of inflammatory cytokines in rheumatoid arthritis (RA) is well established (1). Multiple strategies have been pursued to affect their function using molecularly targeted biotherapeutics, such as those neutralizing TNF-α. More recently, Ab-mediated antagonism of IL-6 with tocilizumab has proven effective and bolsters the hypothesis that mitigating the presence and/or activity of cytokines can be efficacious (2). Preliminary clinical data with Abs targeted against IL-15 or IL-17 in RA are consistent with this concept (3, 4). Nonetheless, because of the complex etiology of RA, the potential redundancy of ILs, the inconvenience and risk of the dosing route, and limiting toxicities of current therapeutics, an unmet medical need clearly persists.

JAKs associate with various cytokine receptors and translate signals triggered by cytokine binding into intracellular responses...
IL-6 signaling, a JAK1/2-dependent process (2, 12). Therefore, we proposed to test the therapeutic hypothesis that selective inhibition of JAK1/2 could be efficacious using a number of cellular and animal models relevant to RA and other autoimmune disorders. INCBO28050, a potent and selective inhibitor of JAK1/2, is shown to inhibit signaling from pathogenic cytokines such as IL-6 and IL-23. Inhibition of JAK signaling prevents the production of chemokines and cytokines associated with uncontrolled RA (13). Finally, in three complementory rodent models of arthritis, we show that selective and fractional inhibition of JAK1/2 is both sufficient for efficacy and well tolerated.

Materials and Methods

Biochemical assays

Enzyme assays were performed using a homogeneous time-resolved fluorescence assay with recombimant epitope tagged kinase domains (JAK1, 837-1142; JAK2, 828-1132; JAK3, 718-1124; Tyk2, 873-1187) or full-length enzyme (cMET and Chk2) and peptide substrate. Each enzyme reaction was performed with or without test compound (1-point dilution), JAK, CMET, or Chk2 enzyme, 500 nM (100 nM for Chk2) peptide, ATP (at the Km specific for each kinase or 1 mM), and 2.0% DMSO in assay buffer. The calculated IC50 value is the compound concentration required for inhibition of 50% of the fluorescent signal. Additional kinase assays were performed at Cerep (Redmond, WA) using standard conditions at 200 nM. Enzymes tested included: Abl, Akt1, AtrA, Cdk2, Cdk2, Cdk4, Chk2, c-kit, EGFR, Epha4, ERK1, ERK2, FLT-1, HER2, IGF1R, IKG, IKK, JNK1, Lck, MEK1, p38α, p70S6K, PKA, PKCα, Src, and ZAP70.

Cellular assays

Human PBMCs were isolated by leukapheresis followed by Ficol-Hypaque centrifugation. For the determination of IL-6–induced MCP-1 production, PBMCs were plated at 3 × 103 cells per well in RPMI 1640 + 10% FCS in the presence or absence of various concentrations of INCBO28050. For the determination of IL-23–induced secretion of IL-17 and IL-22, PBMCs were maintained in RPMI 1640 medium supplemented with 10% FBS, 2 mM L-glutamine, 100 μg/ml streptomycin, and 100 μM penicillin. T cells were activated by culturing with anti-CD3 and anti-CD28 Abs. After 2 d, cells were washed and recultured with IL-23 (100 ng/ml), IL-23 (10 ng/ml) and various concentrations of INCBO28050. Cells were incubated for an additional 4 d at 37°C, then supernatants were collected and secreted IL-17 and IL-22 were measured by ELISA. The ability of INCBO28050 to inhibit IL-23–induced secretion of IL-17 and IL-22 is reported as the concentration required for 50% inhibition (IC50).

Phospho-STAT3 analysis

Isolated cells. For analysis of phospho-STAT3 in human PBMCs or PHA-stimulated T cells, cell extracts were prepared after 10–15 min preincubation with different concentrations of INCBO28050 and stimulation of cells for 15 min with IL-6 (100 ng/ml), IL-12 (20 ng/ml), or IL-23 (100 ng/ml). The extracts were then analyzed for phosphorylated STAT3 by using a phospho-STAT3 specific ELISA.

Whole blood. Blood drawn from rats was collected into heparinized tubes and then aliquoted into microfuge tubes (0.3 ml per sample). In stimulation experiments, INCBO28050 at various concentrations was added for 10 min prior to stimulation with human IL-6 (100 ng/ml) for 15 min at 37°C. RBCs were lysed by using hypotonic conditions. WBCs were then quickly pelleted and lysed to make total cellular extracts. The extracts were analyzed for phosphorylated STAT3 by using a phospho-STAT3–specific ELISA. Blood from animals that were dosed with INCBO28050 was drawn at various times after INCBO28050 administration and processed as described above.

In vivo experiments

Animals were housed in a barrier facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. All of the procedures were conducted in accordance with the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals and with InCyte Animal Care and Use Committee guidelines. Animals were fed standard rodent chow and provided with water ad libitum.

Pharmacokinetics. Female rats (n = 6 per gender per group) were given a dose of 10 mg/kg INCBO28050 suspended in 0.5% methylcellulose and given by oral gavage at 10 ml/kg. The first three rats were bled at 0 (pre-dose), 2, 8, and 24 h, and the second three rats were bled 1, 4, and 12 h after dosing. EDTA was used as the anticoagulant, and samples were centrifuged to obtain plasma. An analytical method for the quantification of INCBO28050 has been developed and used to analyze samples from toxicology studies. The method combines a protein precipitation extraction with 10% methanol in acetonitrile and LC/MS/MS analysis. The method has demonstrated a linear assay range 1–5000 nM using 0.1 ml of study samples. Data were processed using Analyst 1.3.1 (Applied Biosystems, Foster City, CA). A standard curve was determined from peak area ratio versus concentration using a weighted linear regression (1/x2).

Rat adjuvant-induced arthritis. Adjuvant-induced arthritis was elicited in rats according to established methods. Lewis rats (150–200 g, female; Charles River Laboratories, Wilmington, MA) are injected at the base of the tail with 100 μl of an emulsion of CFA (10 mg/ml Mycobacterium butyricum in incomplete Freund’s adjuvant). Rats exhibited signs of inflammation within 2 wk of the injection of CFA. Each rat paw was scored following visual observation using a rating of 0–3, (0 = normal; 1 = redness and minimal swelling of digits; 2 = moderate swelling of the digits and/or paw; 3 = severe swelling of the digits and/or paw). Individual animal paw scores are combined and recorded as a sum of all four paws and groups means of these totals are reported. Percent inhibition in clinical score/severity is calculated using the following formula:

\[
\text{Vehicle – Treated (Vehicle × 100)}
\]

In addition, a plethysmometer (Stoelting, Wood Dale, IL) was used to measure paw volumes taken at baseline and study termination. At the termination of the experiment, paws were removed from euthanized rats for histologic analyses. Treatment was initiated when significant signs of disease were noted, and paws of animals were scored as 1 (normal) would be equivalent—usually occurring 2 wk after adjuvant injection. Graphs reflect endpoints collected only immediately prior to and after therapy was initiated (treatment day 0). Groups consisted of six animals, and statistical differences between treatment and vehicle controls were assessed using two-tailed Student t tests or ANOVA with a Dunnett’s test when appropriate.

Collagen-induced arthritis. DBA/1j mice (4–5-wk old males) were purchased from The Jackson Laboratory (Bar Harbor, ME). The model was established as described with minor modifications (14). Mice are im－
munized intradermally with 100 μl bovine type II collagen solution (Chondrex, Redmond, WA; IMB11) in CFA (Chondrex; 7001) in the base of the tail. Twenty-one days later, mice are reimmunized with 50 μl collagen solution in IFA (Condex; 7002). Mouse paws and ankles were measured weekly for clinical score severity, and groups of animals were scored as 0–3 (0 = normal; 1 = slight redness; 2 = moderate redness and swelling; 3 = moderate/severe redness and swelling). In the experiments performed in this study, treatment began when all animals had at least one affected paw and groups randomized to contain similar mean scores. Each group contained six animals. Anti-type II collagen Ab titers were determined using the Rheumera ELISA platform (no. 3000; Astarte Biologics, Redmond, WA) following the manufacturer’s instructions (n = 4 per group). Serum samples were diluted 1:100,000 and frozen prior to analysis. Two-tailed Student t tests were used to compare individual treatment groups to controls.

Anti-collagen Ab-induced arthritis. BALB/c mice (7–8-wk-old, female) were purchased from Charles River Laboratories. The model was initiated as described with minor modifications (15). Mice were injected with 200 μl arthrogenic anti-collagen Ab (ArthroMAB; Millipore, Bedford, MA). Two days later, mice were injected i.p. with LPS (Escherichia coli-derived, 25 μg) and treatment was initiated the following day (n = 5 per group). Scoring of mice was similar to that described above in the collagen-induced arthritis model. Differences in clinical scores at study termination (last day shown) were analyzed for significance using a Student two-sided t test. Hematologic parameters were measured using a Bayer Advia210 (Leverkusen, Germany). Two-tailed Student t tests were used to compare individual treatment groups to controls.

Delayed-type hypersensitivity. Experiments were performed as described previously (16). Dosing was initiated the evening preceding immune challenge and continued through termination (n = 8 per group). Two-tailed Student t tests were used to compare individual treatment groups.

The Journal of Immunology 5299

Downloaded from http://www.jimmunol.org/ by guest on November 15, 2017
Histology and microcomputed tomography imaging. Ankles from rats and mice were fixed in 10% neutral buffered formalin and sent to Bolder BioPATH (Boulder, Colorado) for analysis. Following incubation in 5% formic acid decalciﬁer (2–3 d for mice, 5–7 d for rats), tissues were processed for parafﬁn embedding and then stained with H&E (rats) or toluidine blue (mice). All tissues from all animals were examined microscopically by a board certiﬁed veterinary pathologist. Rat joints were given scores of 0–7 for inﬂammation and bone resorption. Measurements were taken from the dorsal skin surface (in ﬂexion angle) to ventral skin surface (across the tarsal joints) to semiquantitate the inﬂammatory edema. Corrections were made for any artiﬁcial tissue separation. Scoring of mice was on a scale of 0–5 for inﬂammation, pannus, cartilage damage, and bone resorption. A four-joint mean score was determined for each parameter as were summed parameters for each animal, and these values were used to determine group means. Statistical analysis of histopathologic parameters were done by comparing group data using the Student t test with signiﬁcance set at 5%.

Rat samples for microcomputed tomography (micro-CT) imaging were ﬁxed in 10% neutral buffered formalin and sent to MR Preclinical Services (Ann Arbor, MD). Micro-CT images were obtained of each sample using a GE RS-100 (General Electric, Waukesha, WI) small animal micro-CT scanner. After each acquisition, the images were isosurface rendered, and images of each sample were generated using EDS Beam and MicroView 2.1.2 software (General Electric).

Real-time PCR. Transcript levels of cytokines were evaluated using real-time PCR. Primers and probes were synthesized and puriﬁed by Biosearch Technologies (Novato, CA) with the 18S rRNA probe/primers being obtained from Applied Biosystems. Rat IFNγ transcript levels were analyzed using primers 5′-AACAGTAAAGCAAAAAAGGATGCA-3′ and 5′-TGGGTTTTGTCCTCACCAT-3′ and probe FAM-ACATGCAGGAGGTGGTGCCTTT-BHQ. Rat IL12A was detected with primers 5′-ATGAGTACCGCTGCCTTCAC-3′ and 5′-TTCCAT-3′ and probe FAM-TGCTGGATCTGT-3′. Rat IL22 transcript levels were assessed with primers 5′-GGACCTGCCAAGTGT-9′ and 5′-GGCAATGTGAGCACGAAGCT-9′ and probe FAM-ACATGCAGGAGGTGGTGCCTTT-BHQ. Rat samples for microcomputed tomography (micro-CT) imaging were ﬁxed in 10% neutral buffered formalin and sent to MR Preclinical Services (Ann Arbor, MD). Micro-CT images were obtained of each sample using a GE RS-100 (General Electric, Waukesha, WI) small animal micro-CT scanner. After each acquisition, the images were isosurface rendered, and images of each sample were generated using EDS Beam and MicroView 2.1.2 software (General Electric).

Real-time PCR. Transcript levels of cytokines were evaluated using real-time PCR. Primers and probes were synthesized and puriﬁed by Biosearch Technologies (Novato, CA) with the 18S rRNA probe/primers being obtained from Applied Biosystems. Rat IFNγ transcript levels were analyzed using primers 5′-AACAGTAAAGCAAAAAAGGATGCA-3′ and 5′-TGGGTTTTGTCCTCACCAT-3′ and probe FAM-ACATGCAGGAGGTGGTGCCTTT-BHQ. Rat IL12A was detected with primers 5′-ATGAGTACCGCTGCCTTCAC-3′ and 5′-TTCCAT-3′ and probe FAM-TGCTGGATCTGT-3′. Rat IL22 transcript levels were assessed with primers 5′-GGACCTGCCAAGTGT-9′ and 5′-GGCAATGTGAGCACGAAGCT-9′ and probe FAM-ACATGCAGGAGGTGGTGCCTTT-BHQ. Rat samples for microcomputed tomography (micro-CT) imaging were ﬁxed in 10% neutral buffered formalin and sent to MR Preclinical Services (Ann Arbor, MD). Micro-CT images were obtained of each sample using a GE RS-100 (General Electric, Waukesha, WI) small animal micro-CT scanner. After each acquisition, the images were isosurface rendered, and images of each sample were generated using EDS Beam and MicroView 2.1.2 software (General Electric).

Table I. Enzyme potency of INCB028050 (nM ± SD)

<table>
<thead>
<tr>
<th>Kinase</th>
<th>INCB028050</th>
<th>Potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>JAK1</td>
<td>59 ± 0.9</td>
<td>n = 4</td>
</tr>
<tr>
<td>JAK2</td>
<td>57 ± 1.7</td>
<td>n = 6</td>
</tr>
<tr>
<td>JAK3</td>
<td>&gt;400</td>
<td>n = 2</td>
</tr>
<tr>
<td>Tyk2</td>
<td>53</td>
<td>n = 2</td>
</tr>
<tr>
<td>cMET</td>
<td>&gt;10,000</td>
<td>n = 1</td>
</tr>
<tr>
<td>Chk2</td>
<td>&gt;1,000</td>
<td>n = 1</td>
</tr>
</tbody>
</table>

*Highest concentration tested.

Results

Biochemical and cellular activity of INCB028050

To characterize the biochemical potency and selectivity of INCB028050 within the JAK family of kinases, we assessed the ability of this compound to inhibit the enzymatic activity of the four JAK family members. Because INCB028050 is an ATP competitive kinase inhibitor, we were able to characterize the relative selectivity between JAKs (Table I) in a biologically relevant manner by performing these assays at ATP concentrations approximating those within cells. INCB028050 was a potent inhibitor of JAK1 and JAK2 with IC50 values of 5.9 and 5.7 nM, respectively. In an attempt to mitigate the potentially immunosuppressive effects of JAK3 inhibition, INCB028050 was designed using previously established structure-activity relationships to be JAK3 sparing (IC50 > 530 nM), as demonstrated by ~100-fold selectivity over the enzymatic IC50 values for JAK1 and JAK2. INCB028050 also demonstrated moderate (~10-fold) selectivity against Tyk2 (IC50 = 53 nM) and marked selectivity over the unrelated c-Met (IC50 > 10,000 nM) and Chk2 (IC50 > 1,000 nM) kinases. Moreover, when tested at a concentration ~100-fold the IC50 of JAK1 and JAK2 against a diverse panel of 28 kinases (see Materials and Methods for list), no signiﬁcant inhibition was observed (17). Two structurally related compounds (INCB027753 and INCB029843) were found to have JAK1/2 potencies exceeding 200 nM (the highest concentration tested; Supplemental Table I) and were used to support the conclusion that the cellular activities of INCB028050 described below were due to JAK1/2 inhibition.

In cell-based assays, INCB028050 proved to be a potent inhibitor of JAK signaling and function. In PBMCs, INCB028050 inhibited IL-6–stimulated phosphorylation of the canonical substrate STAT3 (pSTAT3) and subsequent production of the chemokine MCP-1 with
IC$_{50}$ values of 44 nM and 40 nM, respectively (Fig. 1A, 1B). In isolated naive T-cells, INCB028050 also inhibited pSTAT3 stimulated by IL-23 (IC$_{50}$ = 20 nM; Fig. 1C). Importantly, this inhibition prevented the production of two pathogenic cytokines (IL-17 and IL-22) produced by Th17 cells—a subtype of helper T cells with demonstrable inflammatory and pathogenic properties—with an IC$_{50}$ value of ~50 nM. In stark contrast, the structurally similar but ineffective JAK1/2 inhibitors INCB027753 and INCB029843 had no significant effect in any of these assay systems when tested at concentrations up to 10 μM (Supplemental Table I).

**Pharmacodynamic and pharmacokinetic characterization of INCB028050**

Because plasma protein binding of kinase inhibitors can sometimes be significant and may therefore impact their in vivo effectiveness, we developed a whole blood assay (WBA) to better characterize what might be considered pharmacodynamically active plasma levels of INCB028050. Recombinant IL-6 was added to rat whole blood following preincubation with INCB028050 at various concentrations. The effects of selective JAK1/2 inhibition on IL-6 signaling were determined by analyzing the extent of phosphorylation of STAT3 by ELISA. INCB028050 inhibited IL-6–stimulated phosphorylation of STAT3 in whole blood with an IC$_{50}$ of 128 nM (Fig. 1D). Compared with results obtained in PBMCs (IC$_{50}$ = 44 nM), these data suggest that constituents in blood (e.g., plasma proteins) sequester nearly two thirds of the otherwise active drug.

Because of the essential role JAKs play in a normal physiology, we were interested to determine whether modest or intermittent inhibition of JAK1/2 would be effective. By integrating the WBA data with the favorable rat pharmacokinetics (Fig. 2), it is possible to calculate the approximate extent and duration of JAK1/2 inhibition in these experiments, recognizing there are limitations to such extrapolations. Making use of this modeling, an oral dose of 10 mg/kg is expected to inhibit JAK1/2 signaling (by ≥50%) in rats for ~8 h. To determine whether inhibition of JAK3 was likely to occur at the dose levels of INCB028050 used in these studies, we determined the effect of INCB028050 on the proliferation of the cell line shown elsewhere to depend on JAK3 activity—Ba/F3-TEL-JAK3 cells (18). We did so because some cytokines can signal through interactions between JAK1 and JAK3, and this may be dependent on context. INCB028050, at concentrations up to 10 μM, had no effect on the proliferation of Ba/F3-TEL-JAK3 cells (Supplemental Fig. 1). Because a dose of 10 mg/kg does not approach 10 μM, even at its peak (Fig. 2), the doses used in this study are not expected to inhibit JAK3 signaling.

**In vivo characterization of INCB028050 in a rat model of arthritis**

To assess the potential therapeutic utility of systemic JAK1/2 inhibition for the treatment of autoimmune arthritides, we used multiple rodent models of arthritis. Although related data have been published on a distinct compound (19), we evaluated the effects of a novel JAK3-sparing inhibitor in animals with established disease, a more strenuous measure of activity. Furthermore, we assessed the effects of INCB028050 on both cellular and humoral immunity. Initially, we used the rat adjuvant-induced model of arthritis (rAIA) beginning treatment in the therapeutic mode—that is, when rodents have established disease (see Materials and Methods). This model results in T cell-dependent inflammatory arthritis. A number of JAK-activating cytokines have been implicated in the pathobiology of this model, including IL-6 and multiple cytokines associated with Th17 cells (20). Although the IL-17 produced by pathogenic T cells does not necessarily signal through JAKs, the differentiation to and maintenance of IL-17–producing Th17 cells requires IL-6 and IL-23, both of which use JAK1 and/or JAK2 (21, 22).

We assessed the efficacy following daily oral administration of INCB028050 at doses of 1, 3, or 10 mg/kg based on its pharmacokinetic profile in this species. Disease severity was assessed periodically, scoring clinical signs of disease (see Materials and Methods for details). These doses were based on the PK/PD relationship established with the IL-6 WBA, with the goal of inhibiting JAK1/2 signaling by no more than 50% for half of the day. Relative to vehicle-treated animals, increasing doses of INCB028050 inhibited disease scores by 24% (p < 0.05), 57% (p < 0.01), and 81% (p < 0.01), respectively (Fig. 3A). Moreover, while the observed changes in clinical scores at study termination were impressive, suppression of clinical disease by INCB028050 was observed as early as 2 d after initiation of treatment. On the last day of treatment, paw volumes were measured by plethysmography as an objective endpoint. INCB028050 treatment, compared with vehicle, inhibited the increase in hind paw volumes during the 2 wk of treatment by 50% at a dose of 1 mg/kg and >95% at doses of 3 or 10 mg/kg (Fig. 3B). Because baseline paw volume measurements are taken on treatment day 0—in animals with significant signs of disease—it is possible to have >100% inhibition in animals showing marked improvement in swelling.

Blinded histologic analysis of the paws was performed on vehicle- or INCB028050-treated rats, and two specific parameters were evaluated—joint inflammation and ankle width (dorsal to ventral). Joint inflammation—a composite score of immune infiltrate, edema, and periarticular tissue appearance—was inhibited by 27% (p > 0.05), 64% (p < 0.05), and 82% (p < 0.05) at doses of 1, 3, and 10 mg/kg, respectively, compared with vehicle controls (Fig. 3C). This was consistent with respective decrements in ankle width of 19% (p > 0.05), 62% (p < 0.05), and 77% (p < 0.05) compared with vehicle controls (0%) and historical naive animals (100%; Fig. 3C).

We next evaluated the disease-modifying potential of INCB028050 by examining histologic evidence of bone resorption (Fig. 3C). Bone resorption was significant in vehicle-treated animals and coincided with severe joint destruction. Treatment of diseased animals with INCB028050 reduced bone resorption by 15% (p > 0.05), 61% (p < 0.05), and 67% (p < 0.05) with increasing dose level (1, 3, and 10 mg/kg). Radiographic analysis was conducted on a separate cohort of animals with similar clinical signs of disease treated with vehicle or INCB028050 (Fig. 3D). Compared with naive healthy rats, ankles from vehicle-treated mice exhibited massive joint destruction in vehicle-treated animals and coincided with severe joint destruction. Treatment of diseased animals with INCB028050 reduced bone resorption by 15% (p > 0.05), 61% (p < 0.05), and 67% (p < 0.05) with increasing dose level (1, 3, and 10 mg/kg). Radiographic analysis was conducted on a separate cohort of animals with similar clinical signs of disease treated with vehicle or INCB028050 (Fig. 3D). Compared with naive healthy rats, ankles from vehicle-treated mice exhibited massive joint destruction.

**FIGURE 2.** Oral pharmacokinetics of INCB028050 in rats. Female rats were dosed orally with 10 mg/kg INCB028050 in methocellulose (0.5%), and anticoagulated blood samples were harvested prior to and 1, 2, 4, 8, 12, and 24 h after dosing. Six animals were dosed, using three animals for each time point. Blood samples were centrifuged and individual plasma samples were analyzed for compound content using LC/MS/MS with average concentration shown on the graph. Error bars represent SE. For reference, a hashed horizontal line was inserted at the whole blood IC$_{50}$ for inhibition of IL-6–stimulated STAT3 phosphorylation.

[Supplementary Figure 1](#)
destruction (Fig. 3, compare upper to middle). Consistent with the observed histologic effects, orally administered INCB028050 (10 mg/kg, daily for 2 wk) resulted in radiographic improvements with restoration of the normal architecture and appearance to the ankle and tarsals (Fig. 3, compare middle to bottom). These data support the potential for JAK1/2 inhibitors such as INCB028050 to have disease-modifying antirheumatic drug (DMARD) potential, although clinical translation of such findings is by no means assured.

Finally, to determine the extent of JAK1/2 inhibition associated with the efficacy observed above, we determined the effects of the compound on JAK/STAT signaling in peripheral blood samples collected at various times prior to (24 h) or immediately after (1 and 4 h) the last oral dose (treatment day 14). The quantity of pSTAT3 is elevated 2–3-fold in the peripheral blood of arthritic rats, consistent with a robust and ongoing inflammatory reaction (data not shown). Levels of pSTAT3 were reduced by INCB028050 in a dose- and

FIGURE 3. Anti-inflammatory and DMARD activity of once daily INCB028050 in rats with established disease in the adjuvant arthritis model. Rats with demonstrable signs of inflammation were orally administered vehicle or increasing doses of INCB028050 (1, 3, or 10 mg/kg) once daily for 2 wk (n = 6 per group; see Materials and Methods for details). Improvements in clinical signs of disease (A) were noted as early as 2 d after initiating treatment and were maintained in a dose-dependent manner. Plethysmography (B) was used at study termination to measure changes in paw volumes, comparing compound-treated groups to vehicle-treated animals (no inhibition) and naive controls (100% inhibition). Blinded analysis of histologic sections of ankles and paws was consistent with clinical observations illustrating dose-related improvements in joint inflammation (C, left), ankle width (C, middle) and bone resorption (C, right). Data are presented as mean ± SE. Micro-CT imaging (D) of representative ankles from similarly treated animals showed clear evidence that INCB028050 (10 mg/kg/d) normalized the joint architecture and prevented bone destruction (white arrows).
Suppression of phosphorylated STAT3 in the peripheral blood of rAIA animals treated orally with INCB028050 (n = 3)

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>% Inhibition at 1 h</th>
<th>% Inhibition at 4 h</th>
<th>% Inhibition at 24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>55 ± 6</td>
<td>0.1 ± 37</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>13 ± 37</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>100</td>
<td>84 ± 18</td>
<td>0</td>
</tr>
</tbody>
</table>

Extrapolated from Fig. 1.

Table III. Sustained modest inhibition of JAK1/2 is efficacious in the rAIA model (n = 6, ± SD)

<table>
<thead>
<tr>
<th>Dose (mg/kg/d)</th>
<th>INCB028050 Plasma Concentration (nM)</th>
<th>pSTAT3 Inhibition</th>
<th>Clinical Score (%) Inhibition</th>
<th>Paw Swelling (%) Inhibition</th>
<th>Histologic Improvement (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.18</td>
<td>13 ± 2</td>
<td>14</td>
<td>25 ± 42</td>
<td>63 ± 15</td>
<td>29 ± 15</td>
</tr>
<tr>
<td>0.6</td>
<td>53 ± 8</td>
<td>36</td>
<td>50 ± 34</td>
<td>96 ± 36</td>
<td>40 ± 8</td>
</tr>
<tr>
<td>1.8</td>
<td>181 ± 19</td>
<td>62</td>
<td>90 ± 18</td>
<td>≥100</td>
<td>60 ± 10</td>
</tr>
</tbody>
</table>

aBased on a body weight of 200 g.

bExtrapolated from Fig. 1D.

cytokines were found to be elevated during the inflammatory phase of the rAIA model (day 14, Table IV). Observed increases ranged from an ~6-fold increase in expression of the Th1 cytokines IL-12 and IFNγ to a >100-fold increase in expression of the Th17 cytokines IL-17 and IL-22. Treatment with INCB028050 reduced the expression of these pathogenic cytokines by 55 to ~80%. These data are consistent with the in vitro cellular data showing that selective JAK1/2 inhibition mitigates signaling from multiple inflammatory cytokines as well as the production of inflammatory factors in response to cytokine stimulation (Fig. 1).

Effects of INCB028050 in murine models of humoral and cellular autoimmunity

The therapeutic potential of INCB028050 was also explored in mouse models of arthritis, which are largely driven by humoral mechanisms. The collagen-induced arthritis (CIA) model has been extensively used in the evaluation of novel therapeutics and recapitulates many of the clinical and histologic features of human RA. This complex model requires humoral and cellular immune responses for a robust inflammatory response. A number of cytokines have been implicated as major regulators in mouse CIA, including IL-6, IL-23, IL-17, TNF-α, and IL-18 (23–27). Indeed, circulating plasma levels of IL-6 correlate with disease severity in the CIA model, and genetic or biologic antagonism of IL-6 markedly suppresses or eliminates disease incidence and severity, at least in part, by suppressing a robust humoral response (23, 27). Similar experiments have demonstrated an important role for Th17 cells and associated cytokines (e.g., IL-17, IL-21, and IL-23) (20, 24–26).

In the murine CIA model, we assessed the effectiveness of INCB028050 in animals with established disease (i.e., therapeutic mode; Fig. 4A). Doses of 1, 3, or 10 mg/kg were administered twice daily for 15 d in animals with demonstrable signs of inflammation. This dosing schedule was used because the t1/2 of INCB028050 in mice is approximately one third that observed in rats (data not shown). Improvements in clinical signs of disease were noted as early as 4 d after initiation of dosing, and a dose-dependent reduction of clinical scores compared with vehicle controls—19% (p = 0.55), 67% (p < 0.0001), and 61% (p < 0.0001), respectively—was seen at study termination. This finding was not surprising based on the potent ability of INCB028050 to inhibit IL-6 and IL-23 signaling and function (Fig. 1), both of which are essential in the development of disease in the CIA model. However, because of the supportive effects of some JAK-activating cytokines on B cell proliferation and function, it was possible that the observed efficacy of INCB028050 in this model was due to suppression of a humoral response to collagen. To assess this experimentally, we determined the levels of anti-collagen Abs in serum samples from a separate cohort of control and treated mice. INCB028050 treatment administered at the doses used in this study in a therapeutic model did not suppress the production of anti-collagen Abs in these experiments (Fig. 4B; p = 0.4), indicating that the effects are not due to direct suppression of Ab responses. However, because INCB028050 was administered in therapeutic mode in these studies, we cannot rule out the possibility that earlier administration (preventative mode)
might have affected anti-collagen Ab titers. Paws from the vehicle control group and the 10 mg/kg INCB028050 group were analyzed for histologic signs of damage. INCB028050 treatment improved a composite score of joint damage by 47% (Fig. 4C; n = 6; p < 0.01).

In addition, each individual parameter was significantly improved (p < 0.01, t test): inflammation (43%), pannus (53%), cartilage damage (41%), and bone damage (53%). Representative histologic sections are shown (Fig. 4D) with arrows pointing to affected joints.

We also used an alternative model of arthritis that is dependent on Abs, but circumvents the need for the generation of humoral immune response—the collagen Ab-induced arthritis (CAIA) model (15). Arthritis in CAIA is induced by direct injection of a mixture of mAbs against various epitopes of type II collagen. Localization of collagen-specific Abs in the joints and subsequent local immune activation results in significant infiltration of polymorphonuclear and mononuclear cells leading to synovitis, pannus formation, and cartilage and bone destruction. Therefore, the CAIA model is suitable for measuring the effectiveness of therapeutic intervention subsequent to immune priming to auto-Ag. In our studies, we used dinitrofluorobenzene as the hapten, applying it ventrally during the sensitization phase and to the ears (pinna) during the challenge phase. Because a twice-daily dose of 10 mg/kg INCB028050 proved efficacious in both the CIA and CAIA models, we investigated the effects of this dose on inflammation and immune activation in mouse ears (Fig. 6). INCB028050, compared with vehicle, inhibited the DTH response by 48% (p < 0.00001, t test). These data demonstrate that INCB028050 modulates cellular immunity, likely accounting for at least some of the observed in vivo activity.

Hematological impact of selective JAK1/2 inhibition

Whereas INCB028050 was clearly efficacious, it should also be recognized that JAK signaling is central to a number of fundamental processes, including the generation of RBCs (29, 30). As such, continuous absolute inhibition of JAK signaling is expected to be deleterious, as suggested by various knockout studies in mice and the reduced hemoglobin levels observed in nonhuman primates receiving the broad spectrum JAK inhibitor CP-690,550 (6, 9).

Therefore, at the termination of the CAIA study, blood samples were analyzed to determine whether efficacious levels of JAK1/2 inhibition adversely affected hemoglobin, RBCs, total WBCs, or absolute neutrophil or lymphocyte counts (Fig. 7). No significant

Table IV. Oral administration of INCB028050 (10 mg/kg) reduces elevated cytokine mRNA levels in draining lymph nodes from rats with AIA

<table>
<thead>
<tr>
<th>Expression</th>
<th>Relative to Control (d 3)</th>
<th>Expression Relative to Control (d 14)</th>
<th>% Inhibition by INCB028050 (d 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-γ</td>
<td>1.1</td>
<td>7.0</td>
<td>65</td>
</tr>
<tr>
<td>IL-12a</td>
<td>2.1</td>
<td>4.4</td>
<td>76</td>
</tr>
<tr>
<td>IL-17</td>
<td>1.6</td>
<td>244.7</td>
<td>55</td>
</tr>
<tr>
<td>IL-21</td>
<td>1.3</td>
<td>4.5</td>
<td>75</td>
</tr>
<tr>
<td>IL-22</td>
<td>8.3</td>
<td>330.1</td>
<td>79</td>
</tr>
</tbody>
</table>

FIGURE 4. INCB028050 improves clinical and histologic signs of disease in the murine CIA model. DBA/1 male mice with clinical signs of joint inflammation were randomized to receive twice daily oral doses of vehicle or INCB028050 (1, 3, or 10 mg/kg; n = 8 per group). Treatment with the selective JAK1/2 inhibitor resulted in marked improvement in clinical signs of disease (A, p < 0.0001) for 3 and 10 mg/kg doses) without suppression of humoral immunity (p > 0.05), as demonstrated by a lack of suppression of anti-collagen Ab titers from serum (B, n = 5).

Paws from the vehicle and 10 mg/kg dose groups were processed for histologic assessment of joint inflammation, pannus formation, and bone and cartilage destruction, and a composite score was determined (C, n = 6).

INCB028050 inhibited histologic signs of disease by 47% (p < 0.01). Examples of front and hind paws from both groups with representative signs of damage are shown (D). Arrows point to affected joints and W identifies the wrist. Original magnification ×16.
differences were noted in any parameter, which is consistent with the hypothesis that periodic incomplete inhibition of JAK1 and JAK2 can be efficacious in the absence of suppressing normal bone marrow function \( (p > 0.05, t \text{ test}) \). Recognizing that any effect on hemoglobin and/or RBCs can be underestimated owing to the duration of treatment, we also analyzed reticulocytes, which are temporally more responsive, and found a similar lack of effect \( (p > 0.05, t \text{ test}) \), consistent with a lack of myelosuppression at these otherwise efficacious and well-tolerated doses.

**Discussion**

INCB028050 is a potent, selective, orally bioavailable inhibitor of JAK1 and JAK2 with demonstrable selectivity against JAK3 and a broad panel of unrelated kinases. In cell-based assays relevant to autoimmune diseases such as RA and psoriasis, INCB028050 inhibited JAK signaling and function initiated by two clinically validated therapeutic targets (IL-6 and IL-23) at concentrations \( \leq 60 \text{nM} \) and did not significantly affect JAK3-dependent proliferation at concentrations up to \( 10 \mu\text{M} \) (Supplemental Fig. 1). Structurally similar compounds lacking JAK1/2 inhibitory activity were inactive in these cellular cytokine assays (Supplemental Table I) consistent with a central role for JAK1/2 in these models systems and the selectivity of INCB028050. However, we recognize that the absence of observable off-target activity with INCB028050 does not exclude the possibility that this compound impacts an untested enzyme, receptor, channel or transporter.

In vivo, INCB028050 was efficacious in the rAIA model when administered orally or by continuous infusion. In this aggressive, T cell-driven arthritis model periodic and/or fractional inhibition of JAK1/2 signaling by INCB028050 appears sufficient to achieve remarkable efficacy in animals with active signs of disease regardless of what endpoint is used, including radiographic improvements associated with DMARD activity. This conclusion is based on the oral efficacy of INCB028050 (Fig. 3) and the associated pharmacodynamic data \( (p\text{STAT3 inhibition}; \text{Table II}) \), which takes into account any potential accumulation of drug during the experiment as well as any potentially active metabolites of INCB028050 produced during its elimination, although no data for either exist at this time \( (\text{data not shown}) \). Similarly, continuous infusion of INCB028050 is efficacious at steady-state plasma drug levels below the whole blood assay IC50 for JAK1/2 signaling \( (\text{Table III}; \text{Fig. 1D}) \). Plasma levels achieved by either route of administration were well below those expected to inhibit JAK3 based both on enzyme and cellular potency \( (\text{Table I}; \text{Supplemental Fig. 1}) \) supporting the hypothesis that JAK1/2 inhibition is sufficient for efficacy in this destructive preclinical model of arthritis. Moreover, these efficacy data were at least as impressive as those associated with selective inhibition of IL-17, using a receptor IgG1 Fc fusion protein, consistent with the ability of INCB028050 to affect signaling from multiple inflammatory cytokines.

**FIGURE 5.** INCB028050 is efficacious and well tolerated independently of effects on humoral immunity. Oral doses of INCB028050 (1 or 10 mg/kg, twice per day) proved effective at improving clinical \( (A; 10 \text{ mg/kg}, 56\% \text{ inhibition, } p < 0.05) \) and histologic \( (B, C; 10 \text{ mg/kg}) \) signs of disease in mice inoculated with a mixture of arthrogenic mAbs against collagen \( (n = 5 \text{ per group}) \). Arrows point to areas of significant inflammation and/or bone destruction \( (B) \). Vehicle sections are representative of the entire group, and sections from INCB028050-treated mice represent the majority of the treatment group. Original magnification \( \times 16 \).

**FIGURE 6.** Suppression of delayed-type hypersensitivity by INCB028050. Animals were sensitized on the abdomen and subsequently challenged on the pinna with dinitrofluorobenzene \( (n = 8 \text{ per group}) \). INCB028050 (10 mg/kg, by mouth, twice per day) was administered just prior to and during the challenge phase. Ear measurements \( (A) \) were made and swelling is shown in units \( (1 \text{ U} = 0.0001 \text{ inch}) \). Treatment with INCB028050 resulted in a 48% suppression in ear swelling compared with vehicle controls \( (p < 0.00001, t \text{ test}) \).

**FIGURE 7.** Lack of effect on hematologic parameters. Terminal blood samples from the mouse CAIA experiment were analyzed for changes in hematologic parameters \( (n = 5 \text{ per group for treated mice and } n = 2 \text{ for naives}) \). No significant effects were observed \( (p > 0.05, t \text{ test}) \).
cytokines that signal through JAK1 and/or JAK2 (31). However, because a direct comparison of these modalities was not performed, quantitative comparisons are difficult.

In the murine CIA model, dependent on both B and T cells, oral INCB028050 was also efficacious. Interestingly, although IL-6 is reported to be important in both murine CIA and in RA patients, the effectiveness of INCB028050 in this setting may be due, at least in part, to its ability to affect IL-6 signaling to such a degree that it is anti-inflammatory without being immune suppressive, as assessed by quashing an established humoral response. This hypothesis is based on reports that anti-collagen Ab titers were suppressed in both the IL-6 knockout mice and those treated with anti-IL-6R Abs (23, 27). This implies that some degree of IL-6 signaling is central to mounting and/or maintaining a robust humoral response and that complete abolition of IL-6 signaling, whether through genetic recombination or Ab sequestration, significantly antagonizes humoral immunity. In contrast, INCB028050 did not suppress an ongoing humoral response to collagen, but was efficacious when administered in the therapeutic mode at doses affecting but not eradicating IL-6 signaling. Conceivably, the ability of INCB028050 to affect the IL-17/23 axis may have also contributed to the impressive responses in the absence of humoral suppression (Fig. 1; Table IV). Mice with genetic disruption of IL-23p19, but not IL-12p35, are resistant to CIA, and this correlates with reduced production of IL-17, presumably from Th17 cells (25). In agreement with this finding, mice lacking IL-17 are also resistant to CIA but, similar to the IL-6 knockout mice, have a suppressed humoral response to Ag (26). Nonetheless, neutralization of IL-17 in the therapeutic mode also suppresses the incidence and severity of disease, but does not affect anti-collagen Ab titers (24). The differences in effects on humoral responses likely reflect the ability of IL-17 to contribute to a humoral response during the immune education phase, although this effect is not necessary for IL-17 antagonism to be effective. More exhaustive studies with JAK inhibitors during the various phases of humoral immunity will be required to definitively describe the effects of these kinases and INCB028050 on this biology. However, the ability of INCB028050 to inhibit a DTH response clearly demonstrates its capacity to modulate cellular immunity in a hypersensitivity setting and suggests that at least part of the observed efficacy with INCB028050 is due to this immune modulating capability.

The ability of INCB028050 to affect joint inflammation independently from suppressing an established humoral response was also addressed in the CAIA model, in which joint inflammation is induced by systemic administration of a mixture of anti-collagen II Abs (15). This model induces a polymorphonuclear and mononuclear cell infiltrate and a robust inflammatory response, resulting in pannus formation and joint destruction. Interestingly, Kagari et al. (32) showed that IL-6 knockout mice were susceptible to CAIA, which is consistent with a dominant role for IL-6 in supporting a humoral response to autoantigen. In this model, INCB028050 suppressed both clinical and histologic signs of disease consistent with its ability to affect JAK activating pathogenic cytokines including, but not limited to, IL-6.

Because genetic data indicate that complete ablation of JAK1 or JAK2 in mice is lethal, and because decades of research define the effect of INCB028050 on both inflammatory and humoral responses, we addressed the effect of INCB028050 on the ability of JAK inhibitors during the various phases of humoral immunity will be required to definitively describe the effects of these kinases and INCB028050 on this biology. However, the ability of INCB028050 to inhibit a DTH response clearly demonstrates its capacity to modulate cellular immunity in a hypersensitivity setting and suggests that at least part of the observed efficacy with INCB028050 is due to this immune modulating capability.

The ability of INCB028050 to affect joint inflammation independently from suppressing an established humoral response was also addressed in the CAIA model, in which joint inflammation is induced by systemic administration of a mixture of anti-collagen II Abs (15). This model induces a polymorphonuclear and mononuclear cell infiltrate and a robust inflammatory response, resulting in pannus formation and joint destruction. Interestingly, Kagari et al. (32) showed that IL-6 knockout mice were susceptible to CAIA, which is consistent with a dominant role for IL-6 in supporting a humoral response to autoantigen. In this model, INCB028050 suppressed both clinical and histologic signs of disease consistent with its ability to affect JAK activating pathogenic cytokines including, but not limited to, IL-6.

Because genetic data indicate that complete ablation of JAK1 or JAK2 in mice is lethal, and because decades of research define a fundamental role for these kinases in the signaling pathways for numerous important cytokines, it is unlikely that unremitting inhibition of their activity would be tolerated (reviewed in Ref. 6). In this study, we have demonstrated that a selective JAK1/2 inhibitor (INCB028050) is efficacious in three rodent models of arthritis without complete or constant inhibition of JAK signaling. Effective treatment paradigms in these models have been well tolerated as exemplified by the lack of effects of INCB028050 on a number of hematologic parameters and body weight (Fig. 3E and data not shown). Moreover, a related compound, INCB018424, has been well tolerated in RA and cancer patients suggesting that this mechanism is a viable therapeutic option for patients suffering from RA or other diseases in which JAK signaling is dysregulated (33, 34). These results warrant further clinical evaluation of selective JAK1/2 inhibitors such as INCB028050.

Disclosures
All authors have been or are currently employees of the Incyte Corporation and own shares and/or participate in the company stock option plan.

References

Downloaded from http://www.jimmunol.org/ by guest on November 15, 2017

Downloaded from http://www.jimmunol.org/ by guest on November 15, 2017
cytokine IL-23 is composed of IL-12Rbeta1 and a novel cytokine receptor subunit, IL-23R.


Supplemental Figure 1. Lack of effect of INCB028050 on JAK3-driven cellular proliferation. Ba/F3-TEL-JAK3 cells were treated with DMSO (control) or INCB028050 at concentrations up to 10 μM. No effect on proliferation was observed during the 3 day treatment using the Cell-Titer Glo® (Promega) assay kit.

Supplemental Figure 2. INCB028050 reduces elevated cytokine mRNA levels in the rAIA model. Draining lymph nodes were collected on treatment day 14 from vehicle or INCB028050 (10 mg/kg, BID) treated rAIA rats with clinical scores similar to those described in Table III. These were compared to samples from naïve, syngenic, age matched rats and showed elevated Th1 and Th17 associated cytokine mRNA on treatment day 14 and that INCB028050 reduced these toward normal. Individual sample amplification curves are shown in duplicate and quantified in Table IV (n=4/group).
Supplemental Table I. Structural analogues of INCB028050 that lack potency against JAK1 and JAK2 do not affect IL-6 or IL-23 signaling or function in PBMCs or T-cells, respectively (n ≥ 2).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Assay</th>
<th>IC50 (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>INCB027753</td>
<td>JAK1 enzyme (1mM ATP)</td>
<td>&gt; 0.2</td>
</tr>
<tr>
<td></td>
<td>JAK2 enzyme (1mM ATP)</td>
<td>&gt; 0.2</td>
</tr>
<tr>
<td></td>
<td>IL-6 stimulated pSTAT3</td>
<td>&gt; 10</td>
</tr>
<tr>
<td></td>
<td>IL-6 stimulated MCP-1</td>
<td>&gt; 10</td>
</tr>
<tr>
<td></td>
<td>IL-23 stimulated pSTAT3</td>
<td>&gt; 10</td>
</tr>
<tr>
<td></td>
<td>IL-23 stimulated IL-17</td>
<td>&gt; 10</td>
</tr>
<tr>
<td></td>
<td>IL-23 stimulated IL-22</td>
<td>&gt; 10</td>
</tr>
<tr>
<td>INCB029843</td>
<td>JAK1 enzyme (1mM ATP)</td>
<td>&gt; 0.2</td>
</tr>
<tr>
<td></td>
<td>JAK2 enzyme (1mM ATP)</td>
<td>&gt; 0.2</td>
</tr>
<tr>
<td></td>
<td>IL-6 stimulated pSTAT3</td>
<td>&gt; 10</td>
</tr>
<tr>
<td></td>
<td>IL-6 stimulated MCP-1</td>
<td>&gt; 10</td>
</tr>
<tr>
<td></td>
<td>IL-23 stimulated pSTAT3</td>
<td>&gt; 10</td>
</tr>
<tr>
<td></td>
<td>IL-23 stimulated IL-17</td>
<td>&gt; 10</td>
</tr>
<tr>
<td></td>
<td>IL-23 stimulated IL-22</td>
<td>&gt; 10</td>
</tr>
</tbody>
</table>