Autoimmune pathways in mice and humans are blocked by pharmacological stabilization of the TYK2 pseudokinase domain

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TYK2 is a nonreceptor tyrosine kinase involved in adaptive and innate immune responses. A deactivating coding variant has previously been shown to prevent receptor-stimulated activation of this kinase and provides high protection from several common autoimmune diseases but without immunodeficiency. An agent that recapitulates the phenotype of this deactivating coding variant may therefore represent an important advancement in the treatment of autoimmunity. BMS-986165 is a potent oral agent that similarly blocks receptor-stimulated activation of TYK2 allosterically and with high selectivity and potency afforded through optimized binding to a regulatory domain of the protein. Signaling and functional responses in human Tn17, Tn1, B cells, and myeloid cells integral to autoimmunity were blocked by BMS-986165, both in vitro and in vivo in a phase 1 clinical trial. BMS-986165 demonstrated robust efficacy, consistent with blockade of multiple autoimmune pathways, in murine models of lupus nephritis and inflammatory bowel disease, supporting its therapeutic potential for multiple immune-mediated diseases.

INTRODUCTION

Although recent advances in the treatment of autoimmune and chronic inflammatory diseases have provided important benefits to some patients, the unmet medical need remains high for many, with a particular need for better efficacy and driving remission in patients suffering from these debilitating diseases. Moreover, many treatments representing the current standard of care have safety concerns that either limit their chronic use (e.g., glucocorticoids) or are associated with considerable decline in host defense, which can result in serious infections or increased risk of malignancies.

Tyrosine kinase 2 (TYK2) is a nonreceptor tyrosine kinase that regulates signal transduction pathways downstream of the receptors for interleukin-23 (IL-23), IL-12, and type I interferons (IFNs) (1, 2). These cytokine/receptor axes drive the functions of T helper 17 (Th17), Th1, B, and myeloid cells critical in the pathobiology of autoimmune and chronic inflammatory diseases including systemic lupus erythematosus (SLE), lupus nephritis, Sjögren’s syndrome, Crohn’s disease, psoriasis, and systemic sclerosis. A coding variant that leads to the substitution of a proline residue with alanine at position 1104 of the catalytic domain of TYK2 has recently been shown to prevent receptor-mediated activation of TYK2 (3, 4). In a meta-analysis of genome-wide association studies, this deactivating P1104A variant was shown to provide protection from several autoimmune diseases including multiple sclerosis, Crohn’s disease, ulcerative colitis, ankylosing spondylitis, and psoriasis, with a gene dosage effect that was notably more than additive in the homozygous state (3). This same deactivating variant likely also provides protection from SLE, rheumatoid arthritis, type 1 diabetes (4, 5), and possibly, systemic sclerosis (6). Homozygosity for this variant was not associated with an increased risk for hospitalization due to mycobacterial, viral, or fungal infections (3, 5), suggesting that preventing TYK2 activation with novel therapeutics may strike an optimal balance between efficacy and safety.

TYK2—which is related to the Janus kinases JAK1, JAK2, and JAK3—is a complex protein with multiple domains (Fig. 1A) involved in both inter- and intramolecular interactions that mediate receptor-mediated activation of its catalytic domain. Its pseudokinase domain (also known as the JH2 domain) is evolutionarily related to a kinase but lacks catalytic activity. Instead, this domain plays a critical role in regulating the receptor-mediated activation of the adjacent catalytic domain through autoinhibitory interactions (7–9). We have reported small-molecule ligands that stabilize the TYK2 pseudokinase domain in a conformational state that inhibits receptor-mediated activation and activity of the catalytic domain by preventing release of autoinhibitory interactions between the pseudokinase and kinase domains (7). This stabilization results in blockade of downstream signal transduction and signal transducers and activators of transcription (STAT)—dependent gene transcription, recapitulating the mechanism provided by the P1104A coding variant shown to be protective from multiple autoimmune and chronic inflammatory disorders. Considering that targeting the pseudokinase domain also provides a path toward highly selective inhibitors of TYK2-mediated pathways, our drug discovery efforts focused on targeting the TYK2 pseudokinase domain to identify a potential therapeutic agent for the treatment of...
autoimmune and chronic inflammatory disorders. Here, we report the characterization of the highly selective agent BMS-986165 against autoimmune pathways in cells, in preclinical models of lupus nephritis and inflammatory bowel disease, and against SLE-like gene signature resulting from challenge with a type I IFN in a phase 1 trial with healthy volunteers.

**RESULTS**

**BMS-986165 binds with high selectivity to the pseudokinase domain of TYK2 and blocks both receptor-mediated activation of TYK2 and downstream signaling in human immune cells**

BMS-986165 was discovered through the efforts of our drug discovery program targeting the TYK2 pseudokinase domain to identify a potential therapeutic agent for human autoimmune diseases (Fig. 1, A and B). BMS-986165 was shown to compete with a fluorescent probe for binding to the vestigial adenosine 5′-triphosphate (ATP) binding site of human, recombinant TYK2 pseudokinase domain protein, with a median inhibitory concentration (IC₅₀) of 0.2 nM. Because this value likely represents the maximal limit of potency that can be measured in the assay (protein concentration of 0.5 nM) and, therefore, may underestimate the true affinity, the potency of the compound was evaluated at various concentrations of the fluorescent probe (i.e., Morrison titrations), with the results consistent with competitive binding and a dissociation constant (Kᵯ) of 0.02 nM determined for BMS-986165 (Fig. 1C and fig. S1).

BMS-986165 was highly selective for the TYK2 pseudokinase domain when profiled against a panel of 249 protein and lipid kinases and pseudokinases. Only two other kinases or pseudokinases (JAK1 pseudokinase domain, IC₅₀ = 1 nM and BMPR2 (bone morphogenetic protein receptor type II), IC₅₀ = 193 nM) other than the TYK2 pseudokinase domain were shown to bind BMS-986165 with IC₅₀ values under 200 nM, which represents a 1000-fold selectivity measure over the TYK2 pseudokinase domain IC₅₀ value and a 10,000-fold selectivity over the Kᵯ value. The dissociation constant for binding of BMS-986165 to the JAK1 pseudokinase domain was measured to be 0.33 nM, which represents a 17-fold selectivity for the TYK2 pseudokinase domain.

Previous pseudokinase domain binders have been shown to block receptor-mediated activation by stabilizing autoinhibitory interactions with the catalytic domain (7). Accordingly, we evaluated the impact of BMS-986165 on the phosphorylation of Tyr₁⁰⁵⁴ and Tyr₁⁰⁵₅ within the activation loop of the catalytic domain of TYK2 in T cells. Phosphorylation at these tyrosine residues is essential for inducing a catalytically active form of the kinase (10). As shown in Fig. 1D, BMS-986165 blocked the IFNα-stimulated phosphorylation of Tyr₁⁰⁵⁴/Tyr₁⁰⁵₅ in cells in a concentration-dependent manner, with an IC₅₀ value of 4 nM.

Consistent with the ability to block the receptor-mediated activation of TYK2, BMS-986165 showed equivalent cellular potency against TYK2-mediated phosphorylation of STAT proteins in cells. In primary human peripheral blood mononuclear cells (PBMCs), BMS-986165 inhibited IFNα-induced phosphorylation of STAT1 in CD3⁺ T cells (Fig. 1E) and CD19⁺ B cells (Fig. 1F) with equivalent potencies (IC₅₀ value of 3 nM). As detailed in Table 1, BMS-986165 showed similar potencies against the IFNα-induced phosphorylation of STAT2, STAT3, and STAT5 in the same cells and against the phosphorylation of STAT proteins using IFNβ to stimulate the response. IC₅₀ values in the 1 to 6 nM range were also observed against phosphorylation of STAT1, STAT3, and STAT5 in CD14⁺ monocytes and CD3⁻ CD19⁺ CD14⁺ natural killer (NK) cells when stimulated with either IFNα or IFNβ. Although the IFNα-stimulated phosphorylation of STAT1, STAT3, and STAT5 was also inhibited by JAK1 inhibitors such as tofacitinib, baricitinib, and upadacitinib at potencies consistent with those measured against other JAK1-mediated STAT phosphorylations (e.g., IL-2–stimulated pSTAT5), the JAK1 inhibitors were much less potent against IFNα-induced gene expression in PBMCs (table S1). The potency against IFNα-induced gene expression instead correlates with inhibition of STAT2 phosphorylation, which is inhibited far less potently by JAK1 inhibitors. The inhibition of STAT2 phosphorylation in this assay, therefore, likely reflects the TYK2 rather than JAK1 activity of these agents. The potent inhibition of STAT2 phosphorylation and gene expression by BMS-986165 indicates that TYK2 plays the dominant role over JAK1 in regulating pSTAT2-dependent type I IFN gene expression in PBMCs.

The observed potent inhibition of TYK2-mediated signaling by BMS-986165 in primary human cells is in contrast to its lack of potency against receptor-mediated pathways dependent on other JAK kinase family members but independent of TYK2. BMS-986165 showed an IC₅₀ of 592 nM against the JAK1- and JAK3-dependent IL-2–stimulated phosphorylation of STAT5 in peripheral blood T cells (Fig. 1G), representing over 100-fold less potent inhibition than was measured against the same endpoint in type I IFN–stimulated cells. Similarly, weak potency was also measured against other JAK1-regulated pathways, including IL-6–stimulated phosphorylation of STAT3 and IL-13–stimulated STAT6 phosphorylation (Table 2, 3, 11). Although BMS-986165 binds to the recombinantly expressed pseudokinase domain of JAK1, the weak functional activity against JAK1-dependent signaling is consistent with previous observations (7) and with other related compounds evaluated from the medicinal chemistry effort and showed a >500-fold shift when comparing the biochemical and cellular potencies (see fig. S2). Erythropoietin-induced, JAK2-mediated phosphorylation of STAT5A in TF-1 cells, an erythroleukemic cell line, was not inhibited at concentrations of BMS-986165 as high as 10,000 nM (fig. S3), indicating a >2000-fold selectivity for TYK2-mediated signaling. A similar lack of JAK2 functional activity was shown against thrombopoietin (TPO)–induced STAT3 and STAT5 phosphorylation in platelets (Table 2). The selectivity profile of BMS-986165 contrasts greatly to other JAK kinase inhibitors (table S1 and fig. S4).

**Inhibition of type I IFN–augmented B cell responses and monocyte differentiation to dendritic cells**

Type I IFNs contribute to autoimmune responses in diseases such as SLE, Sjögren’s syndrome, and systemic sclerosis by multiple mechanisms, including the enhancement of B cell responses to antigen receptor ligation and lowering the threshold for activation of B cells (12). As shown in Fig. 2A, BMS-986165 blocked IFNα-augmented CD86 expression on B cells stimulated through the B cell receptor (BCR) with an IC₅₀ of 4 nM. The compound at concentrations as high as 200 nM had no effect on the BCR-stimulated expression of CD86 in the absence of IFNα, demonstrating the functional selectivity of this agent for TYK2-dependent responses.

Type I IFNs have been shown to induce the differentiation of monocytes into antigen-presenting dendritic cells, and this is thought to be an important mechanism by which these cells drive the function of autoreactive B and T cells in lupus and other
Fig. 1. BMS-986165 binds to the pseudokinase domain of TYK2 and inhibits receptor-mediated TYK2 activation and downstream phosphorylation events in human immune cells. (A) Domain structure of TYK2. SH2, Src homology 2; Ferm, F for 4.1 protein, E for ezrin, R for radixin and M for moesin; MFI, median fluorescence intensity. (B) Chemical structure of BMS-986165. (C) Dependence of $K_{i,\text{app}}$ of BMS-986165 on fluorescent probe concentration for binding to the TYK2 pseudokinase domain in a homogeneous time resolved fluorescence (HTRF) assay. (D) Effect of BMS-986165 on IFN$\alpha$-induced phosphorylation of TYK2 at Tyr$^{1054}$/Tyr$^{1055}$ in kit225 T cells. Loading was normalized by coanalysis of tubulin levels. Effect of BMS-986165 on phosphorylation of STAT1 in CD3$^+$ T cells (E) and CD19$^+$ B cells (F) upon stimulation of human PBMCs with IFN$\alpha$. The data are representative of two independent experiments with different donors and with two independent replicates within this experiment. (G) Effect of BMS-986165 on the phosphorylation of STAT5 in CD3$^+$ T cells in human PBMCs stimulated with IL-2. The data are representative of three independent experiments with two independent replicates within this experiment.
autoimmune diseases (13). BMS-986165 potently inhibited IFNα-induced differentiation of monocytes to antigen-presenting cells as measured by CD80 expression (fig. S5), with concentrations as low as 5 nM providing ≥50% inhibition. Treatment of human PBMCs with BMS-986165 also blocked IFNα-stimulated interferon gamma-induced protein 10 (IP-10) production with an IC50 value of 6 nM (Fig. 2B and Table 2), further demonstrating the ability of BMS-986165 to block TYK2-dependent functional cellular responses driven by type I IFN stimulation.

### Table 1. Potency of BMS-986165 against type I IFN–induced signaling responses in human cells.

<table>
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<tr>
<th>Stimulus</th>
<th>Cells</th>
<th>Endpoint</th>
<th>BMS-986165 IC50 (nM)</th>
<th>IC50 range</th>
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<td>PBMC</td>
<td>STAT3 phosphorylation in CD3+ T cells</td>
<td>3</td>
<td>2–5</td>
<td>6</td>
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<td>PBMC</td>
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<td>1–3</td>
<td>6</td>
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<td>STAT1 phosphorylation in CD14+ monocytes</td>
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<td>STAT3 phosphorylation in CD14+ monocytes</td>
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<td>IFNβ</td>
<td>PBMC</td>
<td>STAT5 phosphorylation in CD14+ monocytes</td>
<td>1</td>
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**BMS-986165 blocks IL-12– and IL-23–dependent cellular functional responses in T cells**

Genetic deletion and deactivating mutations of TYK2 have demonstrated that this kinase plays a critical role in the signal transduction downstream of the receptors for the p40-containing cytokines IL-23 and IL-12, both of which are important in multiple autoimmune and inflammatory disorders (3, 14). The benefit of targeting these cytokines with antibodies against either p40 (e.g., ustekinumab) or IL-23 (e.g., guselkumab and risankizumab) has been clinically validated.
IL-23 is critical in the expansion and survival of pathogenic T_{H}17 cells and is a key driver in determining the pathogenicity of these cells, leading to the secretion of key proinflammatory cytokines such as IL-17, IL-21, and IL-22. BMS-986165 blocks IL-23–stimulated phosphorylation of STAT3 in CD161^{+}CD3^{+} T_{H}17 cells in human PBMCs in a concentration-dependent manner (Fig. 3A), with an IC_{50} value of 9 nM (Table 2). This potent activity against IL-23–dependent signaling in cells translated into functional inhibition of T_{H}17 function, with BMS-986165 also inhibiting IL-23–dependent production of pathogenic IL-17 from isolated CD4^{+} mouse T cells costimulated to produce IL-17, IL-21, and IL-22. BMS-986165 blocks IL-23–stimulated phosphorylation of STAT3 in CD161^{+}CD3^{+} T_{H}17 cells in human PBMCs with an IC_{50} value of 2 nM (Fig. 3B).

Similar to IL-23 as a critical driver of T_{H}17 pathobiology, IL-12 is essential for T_{H}1 development and drives the production of IFN\_\gamma, a major effector molecule in systemic autoimmune disorders such as SLE and lupus nephritis. In both human PBMCs, BMS-986165 was shown to inhibit IL-12–induced IFN\_\gamma production with an IC_{50} value of 11 nM (Fig. 3C and Table 2), and this potency against IL-12–induced IFN\_\gamma responses correlated with the ability of the agent to inhibit STAT4 phosphorylation in NK-92 cells, an activated NK cell line, stimulated with IL-12 (IC_{50} value of 5 nM). Phosphorylation of STAT1, STAT2, and STAT3 in plasmacytid dendritic cells stimulated by the type III IFN IL-29 was also inhibited by BMS-986165 (IC_{50} values of 22, 27, and 22 nM, respectively, Table 2). BMS-986165, as well as JAK1 inhibitors such as tofacitinib, also inhibited IL-10 signaling (IC_{50} values of 6 and 14 nM for BMS-986165 against pSTAT3 in T cells and monocytes, respectively; fig. S6 and Table 2), demonstrating the important roles of both TYK2 and JAK1 in these signaling pathways. Signaling induced by IL-22, an IL-10–related family member, is also inhibited by BMS-986165, although the potency of the agent against STAT3 phosphorylation is considerably weaker than against STAT1 phosphorylation (IC_{50} values of 158 and 23 nM, respectively; Table 2).

### In vivo pharmacodynamic activity against IL-12 and type I IFN–driven responses

Following on the findings detailed above showing that BMS-986165 potently and selectively blocked relevant functional pathways in immune cells, the agent was further evaluated in murine models dependent on these TYK2-mediated pathways. Activity against an IL-12–driven response in C57BL/6 mice represents a pharmacodynamic measure of TYK2 inhibition in vivo. In this model, mice were challenged with intraperitoneal injections of IL-12 and IL-18 to drive the production of IFN\_\gamma measured in the serum. Oral administration (PO) of BMS-986165 1 hour before IL-12 challenge inhibited IFN\_\gamma production in a dose-dependent manner (Fig. 4A), with pronounced reductions at doses of 1 and 10 mg/kg. Consistent with the in vitro potency of the compound, drug concentrations at the time of serum collection were measured to be 2.6 ± 0.6, 42 ± 10, and 769 ± 269 nM at doses of 0.1, 1, and 10 mg/kg, respectively, which represent 0.03×, 0.4×, and 7.7×, respectively, the in vitro IC_{50} of 100 nM (range, 62 to 123 nM; n = 3) measured in

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**Table 2. Potency of BMS-986165 in signaling and functional cellular assays.**

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Cells</th>
<th>Endpoint</th>
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<td>3–12</td>
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<td>NK-92 cells</td>
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<td>TPO</td>
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<td>TPO</td>
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<td>STAT5 phosphorylation</td>
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</table>
mouse whole blood (TYK2-dependent IFNα-induced pSTAT1; fig. S7), demonstrating the alignment between in vitro and in vivo potency.

Female NZB/W lupus-prone mice develop a disease closely resembling human SLE, including an elevated type I IFN–driven gene signature (18). To demonstrate the pharmacodynamic activity of BMS-986165 against type I IFN–driven gene expression, we administered the compound by oral gavage once daily for 2 days to NZB/W mice. Blood and tissues were collected, and the effect on canonical type I IFN–regulated genes (e.g., IFIT3, IFIT1, and MX1) was measured by quantitative polymerase chain reaction (PCR). BMS-986165 dose-dependently inhibited IFIT3 expression in both blood and kidney in this 2-day assay (Fig. 4B), with a dose as low as 15 mg/kg administered orally once daily (PO QD), providing suppression equivalent to that shown by an antibody which blocks the receptor for type I IFNs (anti-IFNAR). Similar suppression was obtained against the expression of type I IFN–regulated genes MX1 and IFIT1 (fig. S8A). Whole blood at the end of the study (24 hours after the last dose) was stimulated ex vivo with IFNα, and the induced phosphorylation of STAT1 was measured by flow cytometry. As shown in fig. S8B, BMS-986165 dose-dependently inhibited IFIT3 expression in both blood and kidney in this 2-day assay (Fig. 4B), with a dose as low as 15 mg/kg administered orally once daily (PO QD), providing suppression equivalent to that shown by an antibody which blocks the receptor for type I IFNs (anti-IFNAR). Similar suppression was obtained against the expression of type I IFN–regulated genes MX1 and IFIT1 (fig. S8A). Whole blood at the end of the study (24 hours after the last dose) was stimulated ex vivo with IFNα, and the induced phosphorylation of STAT1 was measured by flow cytometry. As shown in fig. S8B, BMS-986165 dose-dependently inhibited this phosphorylation with magnitudes consistent with the 26 ± 13, 87 ± 106, and 1826 ± 3307 nM trough drug concentrations measured in the blood at doses of 5, 15, and 45 mg/kg QD, respectively, at this time point (24 hours after dose), although the high drug variability in the drug exposures at the mid-dose results in a nonsignificant inhibition. A separate study showed robust inhibition in these ex vivo assays at earlier time points (fig. S9).

**Efficacy in murine models of inflammatory bowel disease**

Administration of an agonistic anti-CD40 monoclonal antibody (mAb) to T and B cell–deficient severe combined immunodeficient (SCID) mice leads to both a systemic wasting disease driven by IL-12–dependent mechanisms and an innate lymphoid cell–dependent colitis, which requires IL-23 (19). Prophylactic treatment with BMS-986165 in this anti-CD40 antibody–induced colitis model in SCID mice dose-dependently inhibited the IL-12–dependent weight loss as shown in Fig. 5A, with all doses providing protection. Essentially complete protection from weight loss was observed at the high dose of 50 mg/kg twice daily BID PO, equivalent to that obtained with a blocking anti-IFNAR antibody–treated mice were unresponsive to this ex vivo IFNα stimulation, indicating achievement of complete blockade. These results demonstrate that a pharmacodynamic response (inhibition of type I IFN–inducible gene expression) with BMS-986165 is as effective as a blocking anti-IFNAR antibody. Furthermore, this pharmacodynamic response can be achieved at doses resulting in circulating drug concentrations that do not achieve continuous, complete inhibition of TYK2-dependent pathways in mice (Fig. 4C). In an analogous pharmacodynamic measure of the impact on IL-12–dependent T_{H}1 cells, 2 weeks of treatment with BMS-986165 at 30 mg/kg PO QD were shown to reduce IFNγ+ T_{H}1 cells in the spleens of NZB/W mice (Fig. 4D). Although the 10 mg/kg dose did not have an impact in this short-duration study, a longer-duration efficacy study showed that this dose was effective at reducing IFNγ+ T_{H}1 cells (fig. S10).
Effect of BMS-986165 on IL-12–induced production of IFN-γ in human PBMCs. The data are representative of eight independent experiments with means ± SEM shown for three independent replicates within this experiment.

Fig. 3. BMS-986165 blocks functional responses in primary immune cells driven by IL-23 and IL-12. (A) Effect of BMS-986165 on IL-23–induced STAT3 phosphorylation in the CD161+ CD3+ T17 population in human PBMCs (representative flow cytometry dot plot and phospho-STAT3 histogram shown). The data are representative of three independent experiments with two independent replicates within the experiment. (B) Effect of BMS-986165 on IL-17A production from CD4+ mouse Tq17 cells induced upon coculture with IL-23, PGE2, anti-CD3, and anti-CD28. The data represent means ± SEM shown for three independent replicates within this experiment. (C) Effect of BMS-986165 on IL-12–induced production of IFNγ in human PBMCs. The data are representative of eight independent experiments with means ± SEM shown for three independent replicates within this experiment.

anti-p40 antibody comparator. Histological evaluations of the colons from vehicle control mice showed prominent epithelial hyperplasia and damage in medial and distal sections. A reduction of goblet cells was also found, and most of the vehicle control animals had crypt abscesses and marked infiltrate extending from lamina propria into mucosa. Treatment with BMS-986165 dose-dependently protected mice from histologically evident colitis, with the 15 and 50 mg/kg BID dose groups providing protection equivalent to the anti-p40 antibody–treated mice as shown in Fig. 5B (see representative images in Fig. 5C). Pharmacokinetic (PK) measurements on study animals showed that drug concentrations remained above the mouse whole blood IC50 value of 100 nM (IFNα–induced phospho-STAT1), whereas the 25 and 50 mg/kg BID doses provided continuous daily coverage (trough drug concentrations of 67 ± 19, 163 ± 119, 223 ± 155 nM, respectively).

Efficacy in NZB/W lupus-prone mice

Type I IFN–dependent pathobiology is important in driving nephritis in NZB/W F1 lupus-prone mice, as evidenced by the fact that mice deficient in the receptor for type I IFNs (IFNAR−/− mice) protected from severe disease in a similar strain (New Zealand Mixed 2328 mice) (22). IL-12–driven Tq1 and IFNγ mechanisms are also important in the pathobiology in NZB/W mice. Protection from nephritis in these mice has been demonstrated with treatment of either an anti-p40 antibody or soluble IFNγ receptors (23, 24). Because the blockade of both type I IFN and IL-12/p40 pathways has been clinically validated in trials of human SLE (25, 26), the use of this model would be expected to be predictive of the utility of BMS-986165 in SLE.

As shown in Fig. 6A, after initiation of dosing with BMS-986165 at 26 weeks of age, the compound dose-dependently inhibited the increase in severe proteinuria, a measure of the underlying nephritis, which progressively increased in vehicle control mice over the subsequent 16 weeks. Both the 10 and 30 mg/kg QD doses were effective at blocking the progression of severe proteinuria. Histological evaluation of the kidneys from vehicle control mice (Fig. 6C) showed advanced nephritis, with mesangial hypertrophy of the glomeruli, prominent cellular casts/crescents, and capsular fibrosis. Tubular epithelial cells were frequently damaged, and protein casts were numerous. In addition, there was a prominent mononuclear cell infiltrate present in the interstitium of many of the kidneys examined. In contrast, treatment with BMS-986165 at 10 and 30 mg/kg provided considerable protection of tubulointerstitial and glomerular nephritis (Fig. 6B; see representative images in Fig. 6C), as well as the inflammatory cell infiltration (Fig. 6D), with protection at least as good as prednisolone. The blocking anti-IFNAR antibody, which does not have the combined benefit of inhibiting both type I IFN– and IL-12–dependent mechanisms with BMS-986165, did not provide statistically significant protection from nephritis. Immunoglobulin G (IgG) immune
complex deposition, critical in driving disease pathobiology in both this murine model and human lupus nephritis, was prominent in the capillaries of the glomeruli of vehicle control mice. Treatment with BMS-986165 reduced immune complex deposits in a dose-dependent manner, to the same degree as the blocking anti-IFNAR antibody (10 mg/kg, sc) was included as a comparator. The data represent means ± SEM of n = 7 per group, mpk, mg/kg. (B) Effect on elevated expression of IFIT3 in blood and kidney from NZB/W mice after 2 days of treatment with BMS-986165 (PO BID) or a blocking anti-IFNAR antibody (0.5 mg per mouse, SC), with drug PK measurements (C) being obtained in separate littermate-control mice. The gene expression represents mean ± SEM of n = 5 per group and P values from one-way ANOVA with Dunnett’s posttest. (D) Impact on IFNγ-producing splenic T cells after 2 weeks of treatment with BMS-986165 (PO QD) of NZB/W lupus-prone mice, with a representative dot plot of IFNγ and IL-17 intracellular staining of live cells from a vehicle control mouse, and the results showing the dose-responsive effect of BMS-986165 on the number of IFNγ-positive cells. The results represent means ± SEM of n = 6 to 7 per group.

Reduction of type I IFN–dependent gene signature in whole blood from patients with lupus and pharmacodynamic response against type I IFN–dependent gene expression in humans

An elevated type I IFN–driven transcriptional signature is evident in the blood of most of the patients with lupus and has been correlated with disease activity (27). Moreover, blockade of type I IFN receptor (IFNAR) activity with an anti-IFNAR antibody has been shown to reduce disease activity in patients with SLE (25). To demonstrate the potential of BMS-986165 to reduce type I IFN–driven responses in SLE, whole blood from 31 patients with lupus was treated for 5 hours with either BMS-986165 or a blocking anti-IFNAR antibody, and the effect on type I IFN–regulated genes was measured by
quantitative PCR. In this ex vivo assay, BMS-986165 inhibited expression of type I IFN–regulated genes representative of the IFN signature elevated in patients with SLE (28), as shown in Fig. 7A. The inhibition by BMS-986165 was as effective as that achieved with the blocking anti-IFNAR antibody, and the magnitude of the impact on these genes was comparable between these two agents.
Fig. 6. Treatment with BMS-986165 (PO QD) provides protection from nephritis in NZB/W lupus-prone mice. Dosing was initiated at 26 weeks of age, and a blocking anti-IFNAR antibody (0.5 mg per mouse, subcutaneously twice weekly) was included as a comparator (n = 15 mice per group). (A) Percentage of mice with severe proteinuria (≥300 mg/dl) over the 16-week course of treatment. (B) Total nephritis histology scores (tubulointerstitial and glomerular nephritis) of kidneys at the end of the study. n.s., not significant. (C) Representative images of H&E-stained kidney sections from mice treated with vehicle, BMS-986165 (30 mg/kg), prednisolone, or anti-IFNAR antibody. (D) Mononuclear cellular infiltration in kidneys at the end of the study. (E) IgG immune complex deposition in kidneys at the end of the study. (F) Anti-dsDNA titers in serum from mice at the end of the study. The results represent means ± SEM, P values from one-way ANOVA with Dunnett’s posttest.
Fig. 7. BMS-986165 reduces the elevated expression of type I IFN–regulated genes both ex vivo in blood from patients with lupus and in a phase 1 study of normal healthy volunteers challenged with IFNα2A. (A) Type I IFN–dependent gene expression in whole blood from patients with lupus was measured after incubation with either BMS-986165 (500 nM) or anti-IFNAR antibody (135 μg/ml) for 5 hours. Results are normalized to untreated blood for each patient, and the data represent means ± SD (n = 31); # P < 0.0001 by pairwise nonparametric comparison. (B) On day 13 of a multiple ascending dose study in healthy volunteers, 2 hours after the morning dose of BMS-986165, IFNα2A was administered by subcutaneous injection to stimulate expression of IFN-responsive genes. Blood was collected at various times over the following 24 hours, and the expression of CXCL10, ISG20, and IFI27 was measured by quantitative PCR. The induction was normalized to samples from the same individual collected before challenge with IFNα2A, and the results represent geometric means ± SEM. The x axis denotes time after challenge with IFNα2A.
The minimal impact of either BMS-986165 or the blocking anti-IFNAR on LBALS3BP is likely the result of the unusually long mRNA half-life of this gene (29).

On the basis of the compelling preclinical in vitro and in vivo profile detailed above, BMS-986165 has progressed into clinical development for the treatment of autoimmune and inflammatory disorders such as SLE and psoriasis. In a phase 1 trial of BMS-986165, healthy volunteers were administered a type I IFN (IFNα2A, Roferon-A) on day 13 of the multiple ascending dose portion of the study, and IFN-regulated gene expression was monitored.

As shown in Fig. 7B, induction of the type I IFN–inducible genes CXCL10, ISG20, and IFI27 was evident 3 hours after challenge. Whereas expression of CXCL10 and ISG20 peaked between 6 and 9 hours after challenge, after which the expression fell back toward baseline, IFI27 expression continued to rise throughout the 24-hour interval after challenge, consistent with the long half-life of mRNA for this gene (29). BMS-986165 treatment inhibited these responses in a dose-dependent manner, with the 12-mg BID dose providing 97, 88, and 99% inhibition of peak gene expression of CXCL10, ISG20, and IFI27, respectively. These genes are representative of the type I IFN gene signature elevated in patients with SLE, Sjögren’s syndrome, and systemic sclerosis (28, 30–32). These results demonstrate that BMS-986165 treatment can potently and effectively suppress TYK2-dependent functional responses in humans. Treatment with BMS-986165 was well tolerated. Adverse events (AEs) in the trial were mild to moderate in severity, there were no severe AEs related to BMS-986165, and the frequency of nonserious AEs was similar in the active (75%) and placebo (76%) groups. No events of anemia, neutropenia, and thrombocytopenia were observed. In addition, no changes were observed in mean hemoglobin, platelets, neutrophil counts, lymphocytes, T cells, NK cells, or B cells (see fig. S12).

DISCUSSION
TYK2 regulates the signaling and functional responses downstream of the receptors for IL-23, IL-12, and type I IFNs, each of which has been shown to be critical in the pathobiology of multiple autoimmune and chronic inflammatory diseases. In most of these disorders, more than one of these pathways is important in driving the underlying pathobiology, suggesting that BMS-986165, as a selective inhibitor of TYK2-mediated signaling, represents an intriguing approach to improved treatment for these debilitating diseases. Phase 2 clinical trials with a blocking anti-IFNAR antibody (anifrolumab) or an antibody blocking both IL-12 and IL-23 (ustekinumab and anti-p40) have each shown clinical efficacy in patients with SLE (25, 26). This is consistent with the importance of these pathways in lupus-prone NZB/W F1 mice in addition to the elevated gene signatures associated with IFNα and IFNβ as well as IL-12–dependent IFNy in patients with SLE (33). The glucocorticoid-equivalent efficacy of BMS-986165 in NZB/W lupus-prone mice is particularly compelling, and coupled with the impact of BMS-986165 treatment on the type I IFN–regulated gene signature both ex vivo with SLE blood and in vivo after IFNα2A challenge in human volunteers, these results provide a compelling rationale for evaluating this agent in a phase 2 trial in patients with SLE. Because type III IFNs may also contribute to the underlying pathobiology in lupus (34, 35), the impact of BMS-986165 on IL-29–induced signaling may represent another mechanism by which BMS-986165 treatment may benefit patients with SLE. However, TYK2 has been shown not to be critical for antiviral responses to type III IFNs (36).

Similarly, in psoriasis and related diseases such as psoriatic arthritis, the IL-23/T H 17 axis is a particularly important driver of disease pathobiology, with both anti-p40 and anti–IL-23 antibodies robustly reducing disease activity (15, 16). Type I IFN–regulated genes are also up-regulated in psoriatic plaques and may be important drivers of the disease (37–39). In systemic sclerosis, a type I IFN–regulated gene signature in both skin and blood is elevated and correlated with serum antinuclear antibodies (40, 41). A role for the IL-23/T H 17 axis in systemic sclerosis is also evidenced by elevated serum concentrations of IL-23 and IL-17 and increased peripheral blood T H 17 cell numbers, and genome-wide association studies show that IL-23R and STAT4 single nucleotide polymorphisms are linked with the disease (42–45). In each of these disorders, as well as others such as Crohn’s disease and ulcerative colitis, the deactivating coding variant of TYK2 provides protection (3) and represents potential disease indications for treatment with BMS-986165. BMS-986165 was shown to be efficacious in a recently completed phase 2 double-blind trial in moderate-to-severe psoriasis (46).

Targeting of the pseudokinase domain of TYK2 rather than the active site of the catalytic domain also provides a unique approach with potential advantages. As detailed above, BMS-986165 acts allosterically to block the receptor-mediated activation of the kinase, a mechanism analogous to that of the TYK2-deactivating P1104A coding variant that protects from multiple autoimmune diseases. In addition, targeting the pseudokinase of TYK2 also provided a path toward the identification of a highly selective inhibitor of TYK2-regulated pathways that allows for interrogation of a wide dose range in clinical trials without concern for off-target toxicities due to other kinase activities, particularly the related JAK kinases. Traditional active site-directed JAK kinase inhibitors such as tofacitinib and baricitinib show little selectivity within the family (tofacitinib is a JAK1/JAK3 inhibitor with little selectivity over JAK2, baricitinib is a JAK1/JAK2 dual inhibitor) (47). This is a result of the highly conserved nature of amino acid residues within the active site of this family of kinases, and no clinical agents selective for the active site of TYK2 have been reported despite considerable medicinal chemistry efforts. Moreover, the JAK kinases JAK1, JAK2, and JAK3 regulate signal transduction for more than 25 receptors of cytokines and growth factors (11), and pan-JAK inhibitors have narrow therapeutic margins due to risks of infection, anemia, and leukopenia (47). Although BMS-986165 does bind to the pseudokinase domain of JAK1, the weak functional activity against JAK1-dependent signaling is consistent with previous observations (7), and either reflects a unique mechanistic attribute of TYK2, or the apparent binding to the JAK1 pseudokinase domain represents an artifact overestimation of the affinity for the endogenous JAK1 full-length enzyme in cells. The latter is more likely as an examination of the relationship between this binding assay, and JAK1 functional activity in cells shows a correlation but with a >500-fold shift when comparing the biochemical and cellular potencies. No functional activity with BMS-986165 against JAK2-dependent signaling is evident. Consistent with the preclinical assessment of TYK2 functional selectivity, analysis of laboratory values from the phase 1 study did not demonstrate effects on hematologic parameters that have been observed with nonspecific inhibitors of the JAK kinases, either in the 2-week multiple ascending dose trial in normal healthy individuals or in a recently completed 12-week psoriasis trial (46). Dyslipidemia, which is common...
to JAK1 inhibitors through effects on IL-6 (47), was not observed with BMS-986165 treatment (46). Therefore, BMS-986165 should be considered a different class of agent compared to nonselective JAK inhibitors because it allows an evaluation of the efficacy and safety of selective, robust, and continuous inhibition of TYK2 in patients with autoimmunity without undesirable hematologic and lipid effects.

This study has several limitations. First, mouse models of autoimmunity do not necessarily reflect the disease pathobiology in humans. Therefore, the efficacy observed with BMS-986165 in the colitis and lupus models used in the present report may not be recapitulated in patients. It is encouraging, however, that the benefit of blocking IL-12 and IL-23 with antibodies against p40 (e.g., ustekinumab) has been clinically validated in disorders such as Crohn’s disease (15) and SLE (26), and BMS-986165 has been shown to be efficacious against severe psoriasis in a phase 2 trial (46). Although blockade of the type I IFN pathway with a blocking anti-IFNAR antibody (anifrolumab) has been reported to provide clinical benefit in a phase 2 trial in SLE (25), it was recently announced that a phase 3 trial with this agent did not meet its primary endpoints (NCT02446899). Similarly, although BMS-986165 treatment effectively inhibited gene expression induced by type I IFN challenge in healthy volunteers, it is not yet clear whether these responses are reflective of the proinflammatory mechanisms of type I IFN in lupus and other autoimmune disorders. Last, although the apparent lack of heightened risk of serious infection or malignancies in individuals homozygous for the TYK2-deactivating P1104A coding variant is encouraging, this will need to be carefully evaluated in clinical trials with BMS-986165 because these Tyk2-regulated pathways are important in immunity toward both mycobacterial and viral pathogens. A recent report showed that tuberculosis in endemic areas of the world is more frequent in patients with the P1104A allele (48). Individuals with a complete loss of protein resulting from five different null mutations of Tyk2 have also been shown to have an immunodeficient phenotype (14), although this likely reflects additional scaffolding functions of Tyk2 that are lost with the null mutations and would not be recapitulated with the P1104A coding variant or, presumably, BMS-986165 treatment. Moreover, BMS-986165 demonstrates efficacy at least as good as biologics (anti-p40 or anti-IFNAR antibodies) across multiple murine models of autoimmunem and chronic inflammatory diseases at doses that do not require complete and continuous blockade of TYK2.

In summary, the present results demonstrate the therapeutic potential of targeting TYK2, especially with an agent that acts allosterically through the pseudokinase domain to prevent receptor-mediated activation of TYK2, in the treatment of multiple autoimmune diseases, and support the continued clinical evaluation of this agent. BMS-986165 is currently under evaluation in clinical trials in patients with Crohn’s disease, SLE, and psoriasis.

**MATERIALS AND METHODS**

**Study design**

The objective of this study was to characterize and evaluate the impact of BMS-986165 in both preclinical and clinical settings. Binding potency of BMS-986165 to the TYK2 pseudokinase domain was measured by competition with a fluorescent probe for binding to the vestigial ATP binding site of human, recombinant TYK2 pseudokinase domain protein, with selectivity measured using a panel of 249 protein and lipid kinases and pseudokinases. In vitro signaling and functional endpoints were studied using primary human immune cells to evaluate the impact of TYK2 pathway blockade on responses induced by IL-23, IL-12, and type I IFNs, as well as selectivity over pathways dependent on other JAK kinases. Next, in vivo pharmacodynamic responses against these pathways in mice were evaluated using a mouse model in which IFNγ production was induced after challenge with IL-12 or against the type I IFN–dependent gene signature in NZB/W lupus-prone mice. Murine models of inflammatory bowel disease, including colitis in SCID mice induced by an agonistic anti-CD40 antibody as well as a colitis model induced by transfer of CD4+ CD45RBhigh T cells to SCID mice, were used to evaluate the efficacy of BMS-986165 compared to a blocking anti-p40 antibody. The efficacy of BMS-986165 compared to a blocking anti-IFNAR antibody against lupus nephritis was assessed in NZB/W lupus-prone mice by measuring proteinuria and anti-dsDNA titers along with histological evaluations of nephritis, inflammatory infiltration, and immune complex deposition. To demonstrate the potential of BMS-986165 to reduce type I IFN–driven responses in SLE, whole blood from patients with lupus was treated for 5 hours with either BMS-986165 or a blocking anti-IFNAR antibody, and the effect on type I IFN–regulated genes was measured by quantitative PCR. BMS-986165 was then evaluated in a phase 1 trial in healthy volunteers (NCT02534636) during which participants were administered a type I IFN (IFNα2A, Roferon-A) on day 13 of the multiple ascending dose portion of the study, and IFN-regulated gene expression was measured. In addition to these pharmacodynamic responses meant to recapitulate the type I IFN–regulated gene signature present in most of the patients with lupus, the selectivity for other JAK kinase–mediated pharmacology was determined by measuring hematologic endpoints such as hemoglobin, platelets, neutrophil counts, lymphocytes, T cells, NK cells, or B cells. Mice were randomized into treatment groups, and for histological scoring, observers were blinded to treatment group. Primary data are reported in data file S1.

**BMS-986165**

BMS-986165 (6-cyclopropaneamido-4-((2-methoxy-3-(1-methyl-1H-1,2,4-triazol-3-yl)phenyl)amino)-N-(4H3)methylpyridazine-3-carboxamide) was synthesized as previously reported (49).

**Mice and ethics statement**

All animal procedures were conducted with the approval of the Bristol-Myers Squibb Animal Care and Use Committee. Mice were housed under a 12-hour/12-hour light/dark cycle and provided standard access to rodent chow diet and fresh drinking water ad libitum.

**IL-12–induced serum IFNγ production in mice**

Female C57BL/6 mice (Charles River Laboratories), age 8 to 10 weeks, were dosed by oral gavage with BMS-986165 in vehicle [EtOH (ethanol):TPGS (D-α-tocopherol polyethylene):PEG300 (polyethylene glycol 300), 5:5:90]. One hour later, mice were treated intraperitoneally with 0.01 μg per mouse of recombinant murine IL-12 (R&D Systems). One hour after IL-12 administration, the mice were treated intraperitoneally with 1 μg per mouse of recombinant murine IL-18 (R&D Systems). Three hours later, blood was collected onto PK bioanalysis cards (PerkinElmer) and into serum separator tubes [Becton Dickinson (BD)], the latter for determination of IFNγ concentrations by enzyme-linked immunosorbent assay (Life Technologies).

**Pharmacodynamic responses in NZB/W mice**

Female NZB/W mice (the Jackson laboratory), at 6 months of age when a robust IFN-dependent gene signature is typically evident and...
animals begin to display severe proteinuria, were dosed by oral gavage with BMS-986165 in vehicle (EtOH:TPGS:PEG300, 5:5:90) once daily for 2 days. Blood was collected for measurements of IFN-dependent gene transcription. A 0.5-cm piece of spleen, liver, and left kidney was also collected for analysis of the IFN-dependent gene expression by quantitative PCR, normalizing the expression to peptidylprolyl isomerase A (PPIA) as a housekeeping gene.

To determine effects on T<sub>11</sub> cells in female NZB/W mice (age, 14 weeks; the Jackson laboratory), BMS-986165 was administered by oral gavage in vehicle (EtOH:TPGS:PEG300, 5:5:90) once daily for 2 weeks. Spleens were excised postmortem at study completion and processed individually into single-cell suspension using the gentleMACS Dissociator (Miltenyi Biotec). After lysing red blood cells, cells were stimulated for 4.5 hours at 37°C with phorbol 12-myristate 13-acetate (Sigma) and ionomycin (Sigma), with monoesin (eBioscience) added after 30 min of incubation. Cells were washed and stained with LIVE/DEAD Fixable Aqua Dead Cell (L34957, Invitrogen), fixed/permeabilized (BD), and stained with Rat Anti-Mouse CD3 fluorescein isothiocyanate (FITC) (555274, BD), anti-mouse IFNγ PE-Cyanine7 (25-7311-82 eBioscience), and rat anti-mouse IL-17A PerCP-Cyanine5.5 (45-7177-82, eBioscience). After gating on single live cells, the number of IFNγ<sup>+</sup> cells as a percentage of live cells was quantitated by flow cytometry using the FACSCanto II.

### Efficacy studies in NZB/W lupus-prone mice

Baseline body weight, proteinuria, and serum dsDNA titers were determined for female NZB/W mice, age 26 weeks (the Jackson laboratory), before their randomization into treatment groups, each with n = 15. Mice were dosed by oral gavage, once daily, for 16 weeks and included the following treatment groups: BMS-986165 at 3, 10, and 30 mg/kg in vehicle (EtOH:TPGS:PEG300, 5:5:90), vehicle alone, or prednisolone (Sigma) at 1 mg/kg. Mouse anti-IFN receptor antibody (anti-IFNAR; MR1-5A3, BioXCell) was dosed at 0.5 mg per mouse (n = 10), subcutaneously, twice a week for the duration of the study. Mice were routinely monitored for overall health, and body weight, proteinuria, and dsDNA titers were measured every 3 weeks, with the last measurement at week 15. Interim blood samples were drawn for PK measurements and for ex vivo target engagement assessment. The study was terminated at a point (based on severe proteinuria score and general observations of the health and appearance of the mice), which historically indicated that deaths in the vehicle control group were imminent. At the end of the study (week 16), body weights were measured, and terminal blood samples were drawn for PK, dsDNA titers, and cytokine assay. Kidneys and spleens were processed for histology (see Supplementary Materials and Methods) and gene expression analyses.

### Anti-CD40–driven colitis in SCID mice

The efficacy of BMS-986165 was compared with that of the anti-p40 antibody (7016-7123-M013.3, mAb clone CD17.8, eBioscience) in a p40-dependent model of colitis using B6.CB17-Prkdc<sup>scid</sup>/SzJ (stock no. 001913) mice obtained from the Jackson laboratory. On days 1 and 2, mice (n = 5 per group) were injected subcutaneously with either anti-p40 antibody (10 mg/kg) or phosphate-buffered saline (PBS) alone. Starting on day 0 and continuing daily through day 5, additional groups of mice (n = 10 per group) were dosed with 0 (vehicle control), 5, 15, or 50 mg/kg PO BID BMS-986165 in an aqueous suspension vehicle containing 0.5% Methocel (A4M) and 0.1% TWEEN 80 with final particle size typically ~200 to 300 nm (d50).

In addition, on day 0, colitis was induced in all six groups with a single intraperitoneal injection of 100 μg of FGK4.5 anti-CD40 mAb (clone EB0016-2, BioXCell) in PBS. On a daily basis, mice were weighed and monitored for signs of colitis including body weight loss and the accompanying loose stools and diarrhea. On day 6, all animals were euthanized. Intestine sections were fixed in formalin or added to RNAlater for histological evaluations or cytokine profiling via reverse transcription PCR (RT-PCR), respectively. Terminal blood was collected for measuring circulating cytokine concentrations.

### T cell transfer model of colitis

To evaluate BMS-986165 in the CD45RB<sup>high</sup> T cell transfer model of colitis, donor cells were obtained from male BALB/c mice (Harlan Laboratories), and C.B-17-igh-ib-Lcr-Tac-Prkdcsid mice (Taconic Biosciences) were used as the recipients. Splenic CD4<sup>+</sup> cells from BALB/c donors were enriched by red cell lysis, stained with anti-mouse CD4 eFluor 450 Ab (48-0042-82, eBioscience) and negatively selected using the Rapid CD4 Negative Selection Kit (19852A, STEMCELL). Isolated CD4<sup>+</sup> cells were further stained with CD45RB FITC (553100, BD Biosciences) and CD62L PE (12-0621-82, eBioscience) mAbs and sorted for CD4<sup>+</sup>CD45RB<sup>high</sup>CD62L<sup>high</sup> cells using the Beckman Coulter FACS sorter (BD Influx) to obtain a 98.8% pure population. On day 0, all recipients were intraperitoneally injected with 250,000 CD4<sup>+</sup>CD45RB<sup>high</sup>CD62L<sup>high</sup> T cells. In addition, on day 0 and continuing for the duration of the study, recipients (n = 9 per group) were dosed with 0 (vehicle control), 5, 15, or 50 mg/kg PO BID BMS-986165 in an aqueous suspension vehicle containing 0.5% Methocel (A4M) and 0.1% TWEEN 80 with final particle size typically ~200 to 300 nm (d50), and either anti-p40 antibody (10 mg/kg) or PBS (n = 7 per group) by subcutaneous injection twice weekly. All mice were observed and weighed once in week 1, twice in weeks 2 and 3, and three times in weeks 4 through 7. Clinical signs of colitis were noted including the body weight loss and were accompanied by the appearance of loose stools and diarrhea. On day 48, animals were euthanized, and intestine sections were fixed in formalin or added to RNAlater for histological evaluations (see Supplementary Materials and Methods) or cytokine profiling via RT-PCR, respectively. Terminal blood was collected for measuring circulating cytokine concentrations.

### Effect of BMS-986165 treatment on type I IFN gene expression in healthy volunteers challenged with IFNa2A

The clinical study, “Randomized, double-blind, placebo-controlled, single and multiple ascending dose study to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of BMS-986165 in healthy subjects” (NCT02534636), was approved by the Alfred Hospital Ethics Committee, The Alfred Hospital, Commercial Road, Prahan, Victoria 3181, Australia. The study complied with all relevant ethical regulations, and all subjects in the study provided written informed consent. On day 13 of the multiple ascending dose portion of the study in healthy volunteers, IFNa2A (Roferon, 3 million IU) was administered by subcutaneous injection. Blood was collected before and after administration of IFNa2A, and the expression of CXCL10, ISG20, and IFI27 was measured by quantitative RT-PCR. Gene expression was normalized to housekeeping genes (B2M, ATP5B, and RPL37A), and the induction was expressed as fold change relative to the predose sample on day 1.

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Author contributions: L.C., A.Z.-F., J.S., D.B., J.P., Q.Z., and J.R.B. designed the human and mouse cellular experiments, and L.C., A.Z.-F., J.S., D.B., and J.P. performed the experiments and data analysis. K.M.G., K.W.M., L.C., and J.R.B. designed the in vivo pharmacodynamic studies in mice, and K.M.G. with L.C. performed the experiments and data analysis. J.H. designed, conducted, and performed data analysis on the PBMC gene expression experiment. C.C. designed, performed, and analyzed the biochemical K determinations. Y.Z., K.W.M., A.C., L.M.S.-C., and J.R.B. designed the lupus mouse study. Y.Z. performed the experiment and data analysis, and K.Z. performed the quantitative PCR analyses. X.Y., J.H.X., K.W.M., K.M.G., L.M.S.-C., J.R.B., and A.C. designed the colitis studies in mice, and X.Y. performed the experiment and data analysis. E.M.H. performed and analyzed all histopathology evaluations. C.D. performed all PK measures, and A.C. and C.D. analyzed the experiments. M.D.C. and J.A.C. designed the in vitro lupus blood study, and M.D.C. performed the experiment and data analysis. I.M.C. and S.M.R. designed and implemented the type I IFN pharmacodynamic experiment as part of the phase 1 trial. S.L. designed, performed, and analyzed the biochemical, cellular, and whole blood potency determinations. R.M.M., S.T.W., and D.S.W. led the medicinal chemistry effort that designed and implemented the type I IFN pharmacodynamic experiment as part of the phase 1 trial. R.M.M., S.T.W., and D.S.W. are inventors on U.S. patent 9,055,748 (2016).


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Autoimmune pathways in mice and humans are blocked by pharmacological stabilization of the TYK2 pseudokinase domain


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Taming cytokine signaling through TYK2 inhibition

Targeting Janus kinases can interrupt cytokine signaling in autoimmune disease, but the current inhibitors are not specific. Burke et al. investigated inhibiting a related kinase, TYK2. The inhibitor, BMS-986165, was selective and able to prevent human cells from responding to IL-12, IL-23, or type I IFN. BMS-986165 prevented disease in mouse models of colitis or systemic lupus erythematosus. BMS-986165 treatment of cells from patients with lupus resulted in diminished IFN signature. The drug was well tolerated by healthy volunteers during a phase 1 trial and dampened responses to an in vivo IFN challenge. The drug has already shown promise in a separate phase 2 study of patients with psoriasis and could be broadly applied to other autoimmune diseases.