ASTHMA

Lung-restricted inhibition of Janus kinase 1 is effective in rodent models of asthma

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Preclinical and clinical evidence indicates that a subset of asthma is driven by type 2 cytokines such as interleukin-4 (IL-4), IL-5, IL-9, and IL-13. Additional evidence predicts pathogenic roles for IL-6 and type I and type II interferons. Because each of these cytokines depends on Janus kinase 1 (JAK1) for signal transduction, and because many of the asthma-related effects of these cytokines manifest in the lung, we hypothesized that lung-restricted JAK1 inhibition may confer therapeutic benefit. To test this idea, we synthesized iJak-381, an inhalable small molecule specifically designed for local JAK1 inhibition in the lung. In pharmacodynamic models, iJak-381 suppressed signal transducer and activator of transcription 6 activation by IL-13. Furthermore, iJak-381 suppressed ovalbumin-induced lung inflammation in both murine and guinea pig asthma models and improved allergen-induced airway hyperresponsiveness in mice. In a model driven by human allergens, iJak-381 had a more potent suppressive effect on neutrophil-driven inflammation compared to systemic corticosteroid administration. The inhibitor iJak-381 reduced lung pathology, without affecting systemic Jak1 activity in rodents. Our data show that local inhibition of Jak1 in the lung can suppress lung inflammation without systemic Jak inhibition in rodents, suggesting that this strategy might be effective for treating asthma.

INTRODUCTION

Asthma affects more than 300 million people worldwide (*1*, *2*). Although a large majority of these patients is managed effectively with available drugs, there remains a segment of severely ill individuals in need of more efficacious, safe, and convenient treatments.

On the basis of preclinical studies and a large amount of clinical data, it has become clear that the pathophysiology of a major subset of patients with asthma is driven by multiple type 2 cytokines, including interleukin-13 (IL-13), IL-4, IL-5, IL-9, and thymic stromal lymphopoietin (TSLP) (*3*). Each of these cytokines mediates different aspects of asthma pathophysiology. Specifically, IL-4/13 signaling stimulates smooth muscle contraction, mucus release, and chemokine production in lung tissue (*4*), whereas IL-5 promotes eosinophil production in the bone marrow (*5*). Accordingly, several antibodies targeting IL-13 and/or IL-4 signaling, including dupilumab, tralokinumab, and lebrikizumab, and antibodies targeting IL-5 signaling, including mepolizumab, reslizumab, and benralizumab, have demonstrated efficacy in patients with uncontrolled persistent asthma with elevated type 2 cytokine activity (*4*). IL-9 acts on multiple cell types in the lung to exert pathogenic effects (*6*), and preclinical studies suggest that blockade of IL-9 may also be beneficial in asthma (*7*). Although a humanized IL-9 blocking antibody failed to improve lung function or reduce exacerbations in a clinical trial (*8*), this study did not use any pharmacodynamic (PD) markers to verify complete IL-9 blockade, and thus, it remains difficult to interpret this negative result. TSLP can activate mast cells and basophils and stimulates dendritic cells to elicit a T helper type 2 response (*9*). TSLP blockade resulted in the reduction of disease biomarkers in an allergen challenge study (*9*) and efficacy in a phase 2b clinical trial (*10*), suggesting that it, too, is a promising target for the treatment of asthma. Beyond the realm of type 2 cytokines, IL-6 (*11*, *12*) and type I (*13*) and type II (*14*) interferons (IFNs) have also been implicated in the pathogenesis of severe asthma, although it is presently unclear whether inhibiting activity of those cytokines would offer therapeutic benefit to patients with asthma.

IL-4, IL-13, IL-9, IL-6, and type I and type II IFNs signal through Janus kinase 1 (JAK1), whereas IL-5 and TSLP chiefly depend on JAK2 (*15*–*19*). The available clinical data on the biologics therefore provide a compelling rationale for use of a JAK1/2 inhibitor in asthma; hence, a molecule may not only target multiple disease-relevant mechanisms simultaneously, but could also be more convenient to use than an injectable biological agent. Others have previously demonstrated the utility of systemic JAK inhibition in a mouse model of asthma (*20*, *21*). Numerous JAK inhibitors have been developed, and several have become approved drugs for hematologic and immunologic conditions (*22*). However, these orally bioavailable compounds have also been associated with side effects considered unacceptable for an asthma therapy, such as increased risk of infection, anemia, neutropenia, increased serum cholesterol concentrations, and herpes zoster outbreak (*23*). Such a safety profile, which is generally common to all JAK inhibitors, has been an obstacle to the testing of any of these molecules in asthma.

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We therefore reasoned that lung-localized JAK inhibition through a molecule delivered by inhalation might be efficacious, yet safer than systemic JAK inhibition. However, the physicochemical properties consistent with oral bioavailability and favorable systemic pharmacokinetic (PK) properties are inconsistent with topical lung retention after inhalation, rendering all available JAK inhibitors unsuitable to test the effects of lung-restricted JAK inhibition. We therefore developed a JAK inhibitor with distinct physicochemical properties. In defining the selectivity requirements for this molecule, we focused on JAK1, because we deemed it most important to inhibit IL-4/13– and IL-9–driven biology, which occurs in the lung (*4*, *6*). Minimal selectivity for JAK1 over JAK2 is also likely required to prevent the development of pulmonary alveolar proteinosis as a consequence of granulocyte-macrophage colony-stimulating factor (GM-CSF) inhibition (*24*), which would be an intolerable side effect. Although reduced potency against JAK2 will also lead to lesser inhibition of IL-5 and TSLP, and thus may limit efficacy to some degree, we accepted this caveat in light of the stringent safety standards any marketable asthma drug must meet. Using this molecule, we demonstrate herein that lung-restricted Jak1 inhibition has therapeutic effects in preclinical models of asthma and combines favorably with corticosteroids.

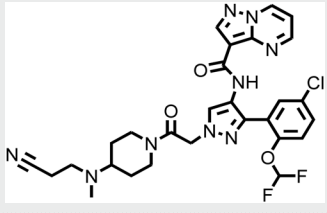
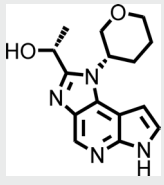
RESULTS

The JAK inhibitor iJak-381 is optimized for inhalation delivery

To test whether local inhibition of Jak1 had a therapeutic effect in asthma models, we developed iJak-381, a highly potent and selective JAK1 inhibitor of the pyrazolopyrimidine class (Table 1) (*25*). To achieve a lung-restricted exposure profile after inhalation, we optimized the inhibitor for properties that would facilitate both retention in the lung and minimization of systemic concentrations. Specifically, we designed a weakly basic, moderately soluble, and moderately permeable molecule. To prevent systemic exposure as a result of inadvertent swallowing of a portion of the inhaled dose, and/or as a result of permeability from the lung into the systemic circulation, we also designed the molecule to be rapidly cleared by the liver. Consistent with the stringent dosing constraints applied to inhalable therapies, iJak-381 showed a subnanomolar inhibitory constant (K_i) for JAK1 in the biochemical assay and was less potent against JAK2, tyrosine kinase 2 (TYK2), and JAK3. Because the potency of adenosine triphosphate (ATP)–competitive inhibitors in vivo depends on the concentration of ATP (*26*), we calculated expected concentration required for 50% inhibition (IC_{50}) at physiologic ATP concentrations for all JAK kinases and found iJak-381 to be moderately selective against JAK2 and highly selective against JAK3 and TYK2. The relevant properties of iJak-381 are summarized in Table 1, and methods to synthesize iJak-381 are published in a patent application. For control purposes, we used a previously published, orally bioavailable JAK1 inhibitor [oJak-989 (*27*), compound 21] with a similar selectivity profile (Table 1 and table S1).

Consistent with their inhibitory activity in the biochemical assays, both compounds strongly inhibited IL-13–driven signal transducer and activator of transcription 6 phosphorylation (pSTAT6) in trifactor-dependent 1 cells (Fig. 1A). iJak-381 also inhibited erythropoietin (*28*) and IL-5 signaling (*29*) in the same cell line with ~6- to 10-fold selectivity,

Table 1. Chemical and PK properties of iJak-381 and oJak-989. K_i inhibitory constants were determined at concentrations of ATP matched to the Michaelis constant (K_m) of the respective kinase. Potencies and selectivities at 1 mM ATP were calculated as described in Materials and Methods. pK_a, acid dissociation constant; MDCK, Madin-Darby canine kidney cells; P_{app}, apparent permeability; A:B, apical to basolateral.

	iJak-381	oJak-989
Structure		
Molecular weight	612.03	286.33
Kinetic solubility (μM)	34.1	196
Calculated pK_a	6.4	4.5
MDCK permeability (P_{app} A:B) ($\times 10^{-6}$ cm/s)	2.7	7.6
Mouse hepatocyte clearance (% liver blood flow)	82	24
Mouse lung protein binding (%)	94.5	77.4
Mouse plasma protein binding (%)	98.3	45.2
K_i (nM) as determined at ATP K_m (JAK1/JAK2/JAK3/TYK2)	0.26/0.62/20.8/3.15	2.93/75.9/114/30.8
Calculated IC_{50} (nM) at 1 mM ATP (JAK1/JAK2/JAK3/TYK2)	8.52/53.4/5998/240	94.2/6563/32,873/2347
Calculated JAK1 selectivity at 1 mM ATP (JAK2/JAK3/TYK2)	6.3/704/28	70/349/25

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consistent with its lower potency for JAK2 inhibition (Table 1, Fig. 1B, and fig. S1A). Furthermore, iJak-381 also inhibited IL-4– or IL-13–driven pSTAT6 in immortalized human bronchial epithelial cells transformed with Ad12-SV40 (BEAS-2B; Fig. 1, C and D). IL-9 signaling was also potently inhibited by iJak-381 (Fig. 1E), whereas inhibition of IL-6 (Fig. 1F) and TSLP (fig. S1B) was less effective. Because iJak-381 also inhibited FYN in a broad kinase panel (table S1), we tested its ability to inhibit histamine release upon ligation of the high-affinity immunoglobulin E (IgE) receptor in laboratory of allergic diseases 2 cells. We found no inhibition even at a high concentration of 10 μM (fig. S1C). Last, to test more disease-relevant parameters, we assessed inhibition of mRNA transcription for STAT6-regulated genes, C-C motif chemokine 26 (*CCL26*) and periostin (*POSTN*) (*30*), in normal human bronchial epithelial (NHBE) cells in air-liquid interface (ALI) culture. These responses, which represent more distal readouts of IL-13 pathway activation in a physiological setting (*31*), were inhibited at similar potencies as the pSTAT6 readout itself (Fig. 1, G and H).

We next examined the localization of iJak-381 after oral (PO) administration. Consistent with our expectation, iJak-381 achieved very low systemic exposure (Fig. 2A). By comparison, oJak-989 achieved systemic exposures well above IC_{50} for at least 8 hours when dosed at 100 mg/kg (Fig. 2B). When dosed by intranasal (IN) drop delivery, iJak-381 had good lung retention with minimal plasma exposure, whereas an intranasal dose of oJak-989 resulted in rapid absorption to the systemic circulation and no appreciable lung retention (Fig. 2, C and D). To further improve lung delivery, we formulated iJak-381 as a dry powder and administered it using a dry powder inhalation (DPI) system (fig. S2). Using this method, we achieved sustained drug concentrations in the lung that were roughly 950× higher than the concentrations measured in the plasma (Fig. 2E). On the basis of these data, we concluded that iJak-381 was a suitable molecule to evaluate the effects of lung-localized JAK1 inhibition.

iJak-381 inhibits Jak1 signaling in vivo

We next developed a PD model to assess the compounds in vivo. Mice were dosed with compound 30 min before receiving an intravenous injection of IL-13, which results in Jak1-dependent pStat6 induction in the lung (Fig. 3, A and B). Fifteen minutes later, we

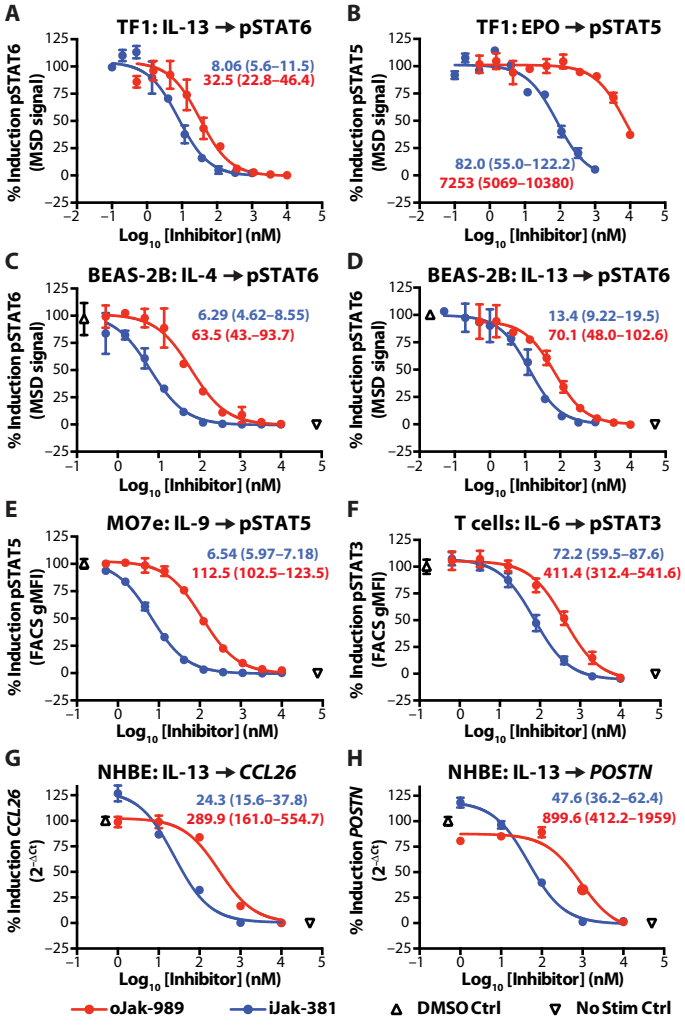

Fig. 1. Cell-based potency of iJak-381 and oJak-989. Mesoscale discovery (MSD) (**A** to **D**) or fluorescence-activated cell sorting (FACS) (**E** and **F**) analysis of pSTAT in response to cytokine stimulations. Stimulations, cell lines, and specific pSTAT readouts are indicated in the figure. TF1, trifactor-dependent 1; EPO, erythropoietin. (**G** and **H**) Quantitative reverse transcription polymerase chain reaction analysis of IL-13– or IL-4–induced *CCL26* and *POSTN* induction in NHBE cells cultured in ALI cultures and preincubated with inhibitors. Error bars represent SD of duplicate or triplicate measurements. Numbers in graphs indicate mean IC_{50} values in nanomolar and 95% confidence intervals for iJak-381 (blue) and oJak-989 (red). DMSO, dimethyl sulfoxide; gMFI, geometric mean fluorescence intensity.

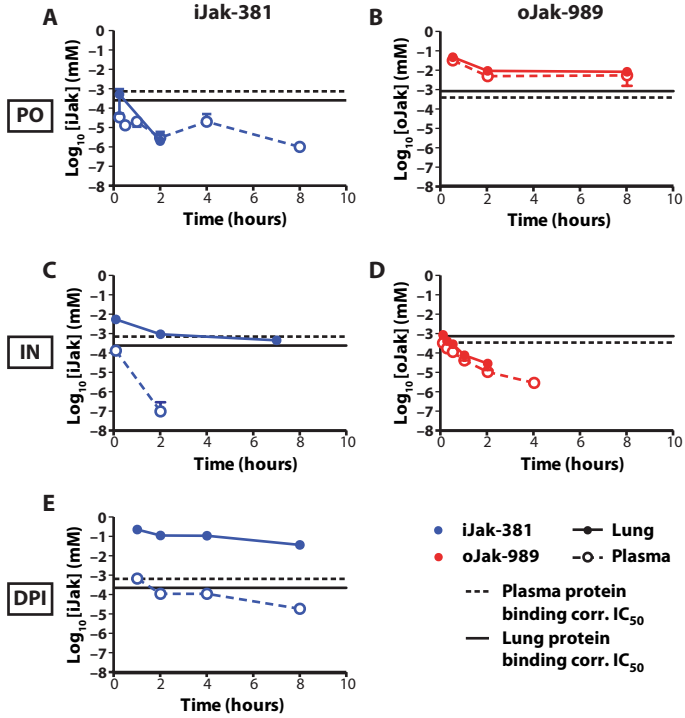

Fig. 2. PK profile of iJak-381 and oJak-989 with different delivery routes. Graphs show compound concentrations in the lung (solid circles) and plasma (open circles) upon oral delivery of iJak-381 (10 mg/kg) (**A**) or oJak-989 (100 mg/kg) (**B**), intranasal delivery of iJak-381 (0.3 mg/kg) (**C**) or oJak-989 (0.3 mg/kg) (**D**), and DPI administration of iJak-381 (10.7 mg/kg) (**E**). Black lines indicate IC_{50} values for the respective compound as determined in the IL-13–stimulated BEAS-2B cell-based assay and corrected for lung tissue binding (dashed) or plasma protein binding (solid). Mean and SD of three to six animals per group are shown.

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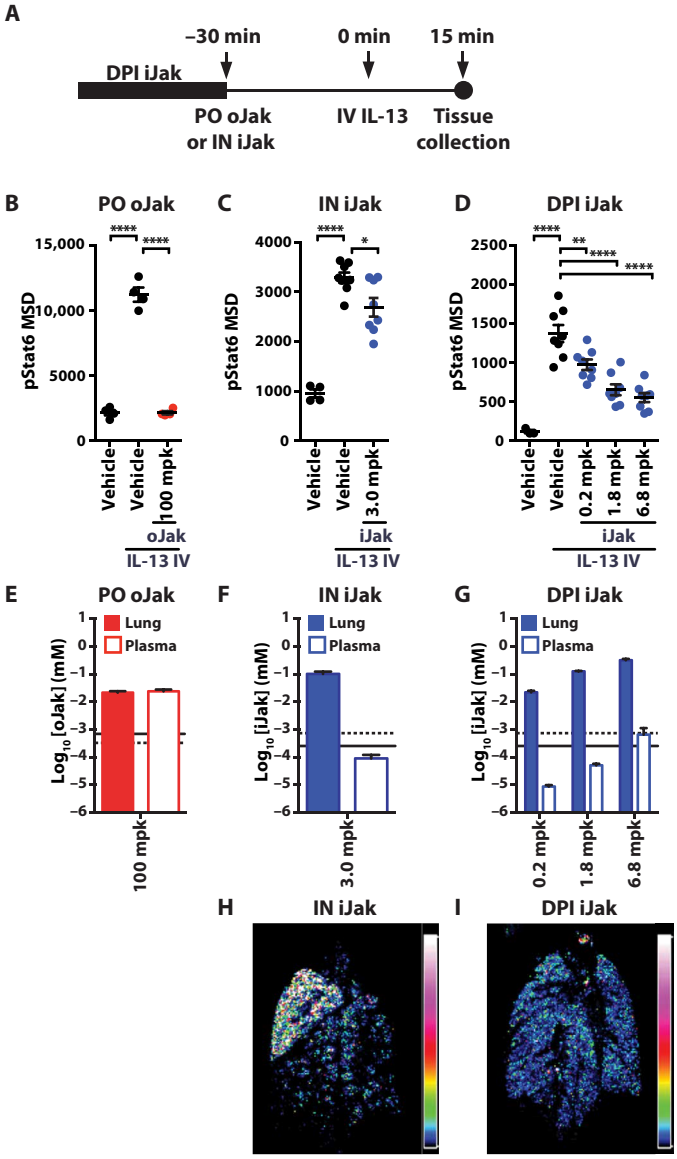

Fig. 3. PD model to assess Jak1 inhibition in the lung. (**A**) Design of IL-13 PD model. Animals received oJak-989 via oral delivery, iJak-381 via intranasal drop delivery, or a range of iJak-381 doses via DPI. Thirty minutes later, 3 μg of IL-13 was administered intravenously (IV). Fifteen minutes later, lungs and plasma were collected for pStat6 and PK analysis. (**B** to **D**) pStat6 MSD analysis of total lung homogenate samples. *$P < 0.05$, **$P < 0.01$, ****$P < 0.001$. mpk, milligrams per kilogram. (**E** to **G**) Total drug concentrations in lung (solid bars) and plasma (open bars) of oJak-989 (red) and iJak-381 (blue). Lines indicate IC_{50} values for the respective compound as determined in the IL-13–stimulated BEAS-2B cell-based assay and corrected for lung tissue binding (solid) or plasma protein binding (dashed). Error bars represent SEM of eight animals per group. (**H** and **I**) Representative MALDI-MS images from lungs collected directly after either IN or DPI delivery. Pixel intensity scale is representative of the detected relative drug abundance and demonstrates that drug is concentrated in a single lobe after IN administration but evenly distributed upon DPI.

harvested lungs and quantified pStat6 in total lung homogenates. Whereas PO oJak-989 administration quantitatively reduced pStat6 (Fig. 3B), IN iJak-381 initially only achieved a 26% reduction (Fig. 3C), despite achieving total lung exposures that were substantially above IC_{50} (Fig. 3, D and F). We reasoned that this result might be due to uneven tissue distribution of the intranasal drop dose of iJak-381, whereas systemically delivered IL-13 and its resulting pStat6 PD effect would be homogeneously distributed throughout the lung. To test this idea, we coadministered IL-13 and iJak-381 intranasally in the same formulation to ensure identical tissue distribution of IL-13 and iJak-381. Using this coformulation approach, iJak-381 was able to reduce pStat6 induction in the lung to a similar extent as the orally delivered oJak-989 (fig. S3). Having identified uneven compound distribution upon intranasal drop delivery as the likely cause of the limited PD effect, we sought to improve delivery of iJak-381 using a DPI administration system (fig. S2). Using this administration route, iJak-381 achieved dose-dependent pStat6 inhibition that was substantially and consistently greater than that of IN drop–dosed iJak-381 [Fig. 3, D and G; 26% reduction for IN (3.0 mg/kg) versus 57% reduction for DPI (1.8 mg/kg) ($P = 0.0114$) and versus 65% reduction for DPI (6.8 mg/kg) ($P = 0.0007$)]. Evaluation of local lung concentrations of iJak-381 by matrix-assisted laser desorption/ionization imaging–mass spectrometry (MALDI-MS) demonstrated unequivocally that a much more homogeneous distribution was achieved by DPI dosing compared to IN dosing (Fig. 3, H and I). We therefore used DPI dosing for all subsequent experiments with iJak-381.

Because iJak-381 is not completely JAK1 selective and also inhibits JAK2-dependent cytokines to some extent, we next determined the effect of DPI-dosed iJak-381 on Gm-csf–induced pStat5 in vivo. Both iJak-381 and oJak-989 inhibited IL-13–induced pStat6 at therapeutic doses, and neither compound affected GM-CSF–induced pStat5, suggesting that they were selective for Jak1 in vivo (fig. S4). This result suggests that neither compound drives in vivo efficacy nor toxicity through inadvertent inhibition of Jak2.

To test iJak-381 in a more disease-relevant setting, we next tested its PD effect in the context of an antigen-driven disease model. In this experiment, ovalbumin (OVA)–immunized mice were challenged once with inhaled OVA, 1 hour after they had been dosed with test compounds. Lungs were harvested 2.5 hours later to assess pStat3 and pStat6 (Fig. 4A). Because multiple cytokines are up-regulated in response to antigen challenge, we observed induction of both pStat6—presumably due to Il-4/13 signaling—and pStat3, which is known to be induced by many Jak1-dependent cytokines (Fig. 4, B and C). iJak-381 reduced both pStat6 ($P < 0.0001$) and pStat3 ($P = 0.03$) induction in the lung by ~80%. As seen previously, oJak-989 concentrations were comparable in lung and plasma, whereas DPI-administered iJak-381 resulted in high lung and low plasma concentrations (Fig. 4, D and E).

iJak-381 inhibits OVA-induced lung pathology in mice

To fully understand the ability of iJak-381 to confer therapeutic benefit in an asthma model, we administered OVA challenges to immunized mice daily for 7 days to induce robust eosinophil recruitment to the lung. Before each challenge, animals received either iJak-381 through DPI or oJak-989 orally (Fig. 5A). Twenty-four hours after the final OVA challenge, lungs were lavaged, and eosinophil recruitment was quantified. OVA challenge increased total bronchoalveolar lavage (BAL) cells and eosinophils, and both PO oJak-989 and DPI iJak-381 reduced pulmonary inflammatory cell influx (Fig. 5, B and C). Consistent with a therapeutic effect, expression of Il-13–dependent genes (*Nos2*, *Clca1*, *Ccl11*, and *Muc5ac*) in the lung was also reduced (Fig. 5, D to G). This therapeutic effect of iJak-381 was confirmed, and dose dependence was established, in a separate study in the same model (fig. S5).

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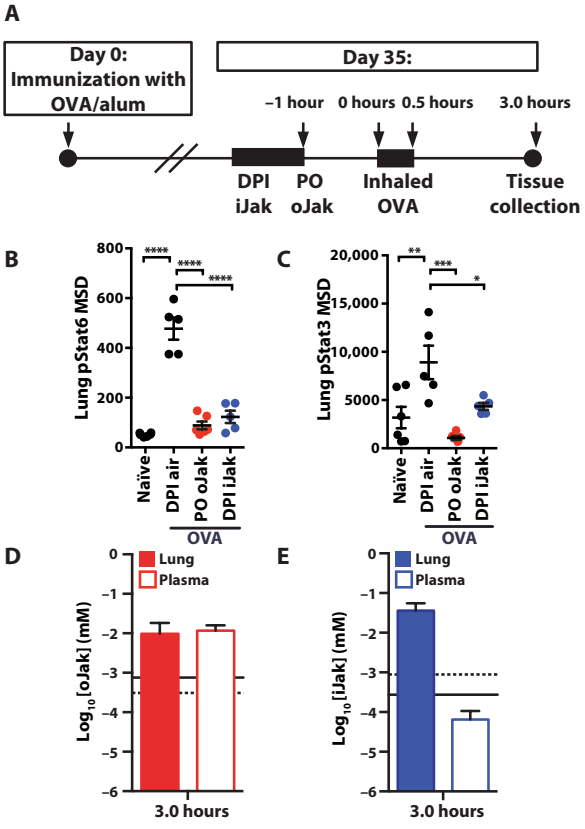

Fig. 4. Disease-relevant PD model. (**A**) Design of OVA pStat induction model. Animals were immunized with OVA/alum on day 0. Thirty-five days later, animals received either oJak-989 (100 mg/kg) via oral delivery or iJak-381 (10.7 mg/kg) via DPI. One hour after drug delivery, animals were challenged with inhaled OVA for 30 min. Two and a half hours after the end of the OVA challenge, lungs and plasma were collected. (**B** and **C**) pStat6 (B) and pStat3 (C) MSD analysis of total lung homogenate samples. *$P < 0.05$, **$P < 0.05$, ***$P < 0.005$, ****$P < 0.001$. (**D** and **E**) Total drug concentrations in lung (solid bars) and plasma (open bars) of oJak-989 (D) and iJak-381 (E). Lines indicate IC_{50} values for the respective compound as determined in the IL-13–stimulated BEAS-2B cell-based assay and corrected for lung tissue binding (solid) or plasma protein binding (dashed). Error bars represent SEM; $n = 5$ to 6 animals per group.

To determine whether iJak-381 had systemic effects, we chose to quantify splenic natural killer (NK) cells. NK cells have been identified as the most sensitive readout for systemic JAK inhibition (*32*, *33*) and can thus serve as a suitable proxy for systemic JAK-dependent effects. Oral administration of oJak-989 led to a substantial reduction in splenic total cell count (Fig. 5H) and the percentage of NK cells (Fig. 5I). In contrast, DPI iJak-381 did not affect total splenocyte numbers or splenic NK cell percentage (Fig. 5, H and I). There was also no effect on any peripheral cells based on complete blood count analysis of naïve animals treated with high doses of iJak-381 (fig. S6). Together, these observations are consistent with a lung-restricted effect of this inhibitor. When comparing the local reduction of eosinophils in the lung to the reduction of NK cells in the spleen across multiple studies, we observed a direct correlation after PO oJak-989 treatment. However, with DPI iJak-381 treatment, only eosinophils in the lung were reduced, with no correlative effect on peripheral NK cells (Fig. 5J; slopes are different with $P < 0.0001$). We observed a similar effect when assessing NK cells in the blood (Fig. 5K; slopes are different with $P < 0.0001$). Treatment with iJak-381 thus inhibits Jak1 within the lung without causing systemic Jak1 inhibition.

Last, we measured the effect of iJak-381 treatment on methacholine-induced airway resistance. Consistent with the reduced cellular infiltration and lung gene expression, we observed reduced airway resistance upon treatment of mice with iJak-381 (Fig. 5, L and M)

iJak-381 confers therapeutic efficacy in an allergen-driven mouse model

A limitation of the widely used OVA mouse model is that it does not recapitulate the neutrophilic component seen in human patients with asthma (*34*). We therefore tested iJak-381 in a variation of a recently described asthma model driven by three common human allergens, namely, *Aspergillus*, *Alternaria*, and house dust mite (*Dermatophagoides farinae*) (*35*). We will refer to this model as the "AAH model." To model a therapeutic scenario, we only treated mice with oJak-989 or iJak-381 from days 9 to 14 of this 2-week study (Fig. 6A). As in the OVA experiment, both molecules had a strong effect on BAL cellularity (Fig. 6B), which was mainly driven by eosinophils (Fig. 6C). In contrast to the OVA model, there is a detectable neutrophilic component in allergen-treated mice, which was reduced by both oJak-989 and iJak-381 (Fig. 6D). Consistent with decreases in eosinophils and neutrophils, we also observed reductions in Ccl11 and C-X-C motif ligand 1 (Cxcl1) in BALF, respectively (Fig. 6, E and F). We note that these chemokines were analyzed 24 hours after the final dose of iJak-381 or oJak-989, when drug concentrations are low (fig. S7). As seen previously, spleen cellularity and the percentage of NK cells were not affected by iJak-381, confirming its lung-localized effect (Fig. 6, G and H). Because iJak-381 strongly suppressed neutrophils, which are not part of type 2 cytokine–driven disease, we therefore conclude that this inhibitor has activity beyond inhibition of type 2 cytokine pathways.

iJak-381 combines favorably with corticosteroids

Although inhaled and orally dosed corticosteroids are a mainstay of asthma treatment in humans, their side effect profile precludes continuous administration of high doses (*36*). We therefore tested whether Jak1 inhibition provides additional therapeutic benefit in combination with systemically delivered dexamethasone in the AAH model (*35*). Both iJak-381 and dexamethasone reduced total BAL cells and eosinophils (Fig. 7, A to C). iJak-381 caused substantial neutrophil and Cxcl1 reduction (Fig. 7, D and E), again with no appreciable effect on spleen cellularity (Fig. 7F). Even when combined with the low dose of dexamethasone, spleen cellularity was not suppressed beyond the cellularity seen in naïve mice. Although high-dose dexamethasone administration also resulted in neutrophil and Cxcl1 reduction, this treatment concomitantly reduced spleen cellularity compared to naïve mice, illustrating the general immune-suppressive effect of systemic corticosteroid treatment. Our data therefore suggest that an inhaled JAK inhibitor may provide additional efficacy through neutrophil reduction, which is considered a high priority in the clinic (*37*).

iJak-381 inhibits OVA-induced lung inflammation in guinea pigs

Last, we sought to test the efficacy of iJak-381 in a second species to increase confidence in this therapeutic approach. Guinea pigs are an excellent model to study lung inflammation; their lung architecture

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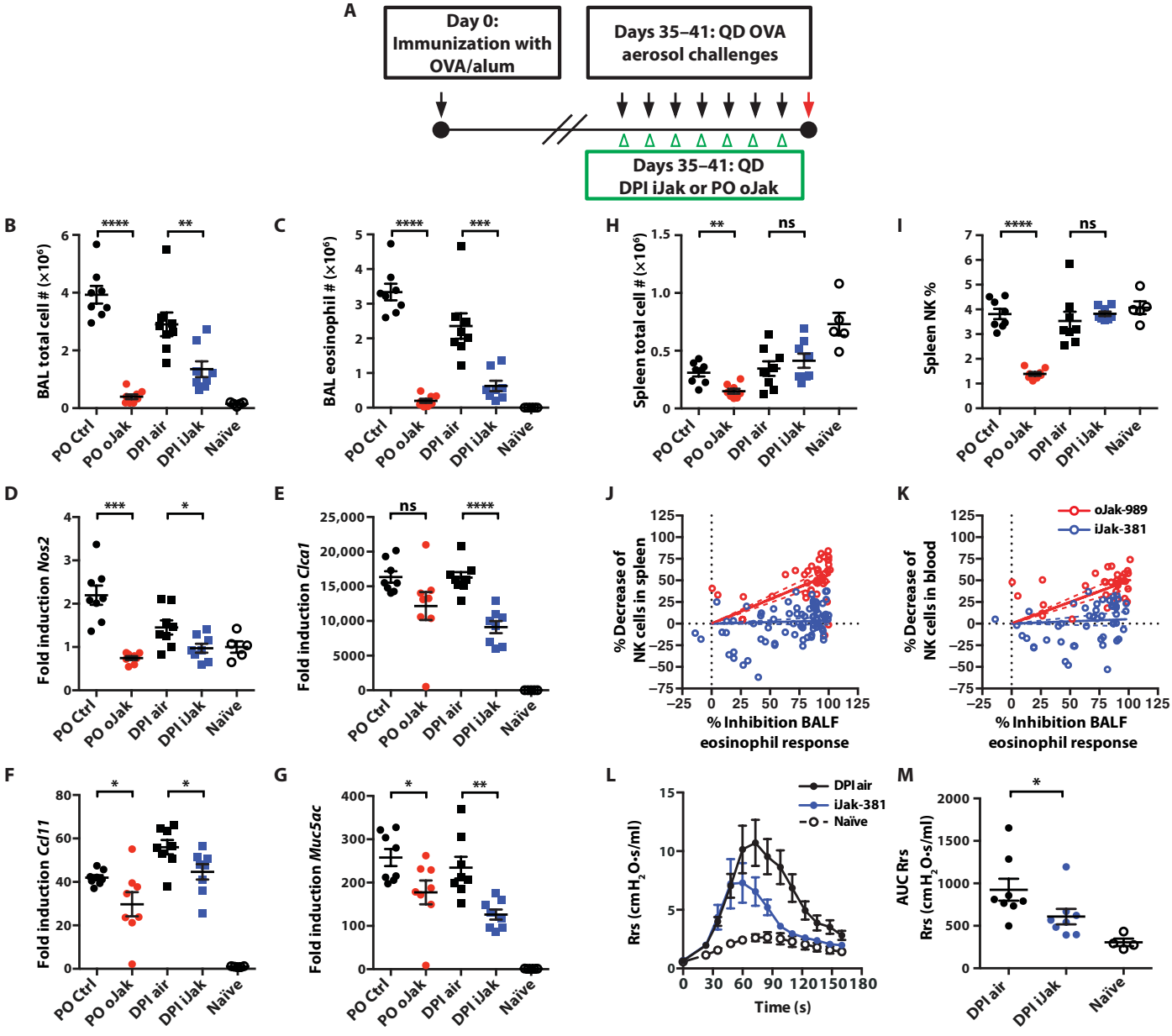

Fig. 5. Efficacy of iJak-381 and oJak-989 in a murine model of eosinophilic asthma. (**A**) Design of mouse OVA asthma model. Animals received either oJak-989 (100 mg/kg) or vehicle via oral delivery or iJak-381 (8.6 mg/kg; dry powder) or air only via inhalation 1 hour before each OVA challenge. An unimmunized, unchallenged group was also included (naïve). Twenty-four hours after the final challenge, BAL fluid (BALF), lungs, spleens, and plasma were collected (red arrow). QD, quaque die. (**B** and **C**) Analysis of total cell counts (B) and eosinophils (C) from BALF. (**D** to **G**) Fluidigm analysis of gene expression in the lung relative to the expression measured in naïve animals. (**H** and **I**) Total spleen cell count and the percentage of NK cells as determined by FACS analysis. (**J** and **K**) Decrease of NK cells in spleen (J) and blood (K) plotted against inhibition of BAL eosinophil response with linear regression of results with *y*-axis intercept constrained to the origin (combined results from four studies). (**L** and **M**) Resistance of the respiratory system (Rrs) measurements (L) and area under the curve (AUC) thereof (M) of OVA-challenged mice challenged with methacholine (70 mg/ml). Error bars represent SEM; $n = 5$ to 8 animals per group. ns, not significant. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$, $^{****}P < 0.001$.

is much more similar to humans than that of mice, and they mount a robust response to inhaled challenges (*38*, *39*). In our guinea pig model, animals received two OVA immunizations, followed by a single inhaled OVA challenge (Fig. 8A) (*40*). This led to profound inflammation as judged by histological analysis of the lung (Fig. 8B). Affected lungs were characterized by widespread alveolar inflammatory cell infiltrate composed predominantly of eosinophils with lesser numbers of neutrophils and increased alveolar macrophages. Alveolar septa were thickened because of increased numbers of mononuclear inflammatory cells and alveolar epithelial cells. Bronchioles had areas of thickened epithelium and mixed eosinophil/mononuclear inflammatory cell infiltrate present in the lumen and mucosal and submucosal layers. DPI iJak-381 administration resulted in a dose-dependent reduction of inflammation, with very few remaining tissue

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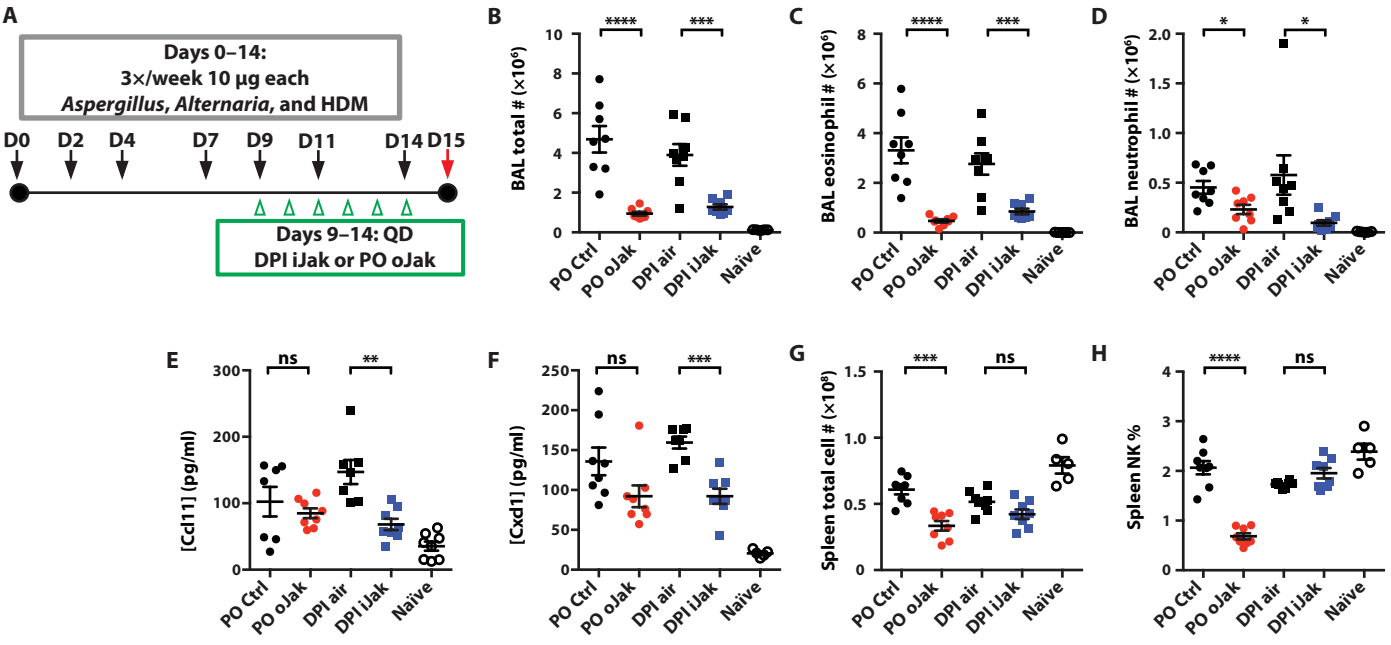

Fig. 6. Efficacy of iJak-381 in an allergen-driven asthma model. (**A**) Design of the AAH model. Animals were challenged three times per week with human allergens as indicated. From days 9 to 14, animals received either oJak-989 (100 mg/kg) via oral delivery or iJak-381 (9.9 mg/kg) via DPI 1 hour before the allergen challenge. Control animals received allergens, but either received vehicle PO or were placed on towers containing only air. An unimmunized, unchallenged group was also included (naïve). Twenty-four hours after the final challenge, BALF, lungs, spleens, and plasma were collected (red arrow). HDM, house dust mite. (**B** to **D**) Analysis of total cell counts, eosinophils, and neutrophils from BALF. (**E** and **F**) Enzyme-linked immunosorbent assay (ELISA) analysis of Ccl11 and Cxcl1 in BALF harvested on day 15. (**G** and **H**) Total spleen cell and splenic NK cell percentage as determined by FACS analysis. **P* < 0.05, ***P* < 0.01, ****P* < 0.005, *****P* < 0.001. Error bars represent SEM; *n* = 5 to 8 animals per group.

infiltrates (Fig. 8, B and C). We observed lung-restricted exposure of iJak-381 in guinea pigs (Fig. 8D), similar to what had been seen in DPI iJak-381–treated mice.

DISCUSSION

Numerous systemic JAK inhibitors have been developed (*41*, *42*); however, their effects in human asthma have not been thoroughly investigated. Using iJak-381, a JAK1 inhibitor specifically designed for lung-restricted pharmacological activity, we demonstrated that local and selective JAK1 inhibition in the lung exerts therapeutic effect in three preclinical asthma models in two different species. In the lungs of mice, treatment with iJak-381 inhibited cytokine signaling and normalized antigen-driven influx of both eosinophils and neutrophils in both preventive and therapeutic settings. iJak-381 had a stronger effect on neutrophils than orally administered dexamethasone, suggesting that topical JAK inhibition in the lung will provide added benefit to the current human standard of care. iJak-381 also improved lung function, which is consistent with its effect on cellularity and inflammatory gene expression in the lung. In guinea pigs, whose lung anatomy is more similar to humans than that of the mouse, iJak-381 dose dependently reversed lung pathology. Our data therefore suggest that topical JAK1 inhibition might have therapeutic effects in patients with asthma.

Inhalation provides an excellent route to administer highly soluble molecules directly to the bloodstream (*43*), and thus, it is of paramount importance to verify that the effects of any inhaled compound are not due to systemic distribution of the compound via the circulation. In this study, we provide direct evidence for a lung-restricted effect of iJak-381, as we demonstrate the specific absence of systemic PD effects. This lung-restricted pharmacology was achieved through particular physicochemical properties of iJak-381, which confer lung retention and would render it unsuitable for oral dosing. Because our molecule relies solely on the inhalation process for even distribution within the lung and cannot use the circulation as a secondary distribution vehicle, the particular mode of administration became paramount. We provide evidence to show that dosing by DPI offers superior compound distribution within the lung compared to intranasal drop dosing. Thus, although DPI dosing is labor intensive and costly, our data suggest that it is the best way to achieve uniform lung distribution upon inhalation, which appears to be important for iJak-381.

PK analysis revealed that lung exposure of iJak-381 administered by DPI was roughly 950× higher than corresponding plasma exposure. Although these data unequivocally demonstrate lung retention of iJak-381, the measurements of iJak-381 concentrations cannot differentiate free, intracellular iJak-381 from iJak-381 residing in the luminal space of the airways or bound to proteins or tissue. Both the PD and efficacy effects we observed are lower than one might anticipate given the high total lung concentrations of iJak-381, suggesting that only a fraction of the total compound delivered to the lung is freely available to inhibit cytosolic Jak1. These observations would be consistent with a model in which lung retention is achieved through slow dissolution and/or release from lung tissue, providing a reservoir of compound that is absorbed slowly, yet cleared rapidly once it reaches the systemic circulation. This hypothesis theoretically explains not only why such an apparent excess of compound is required to achieve a PD effect but also why the lung drug concentrations remain

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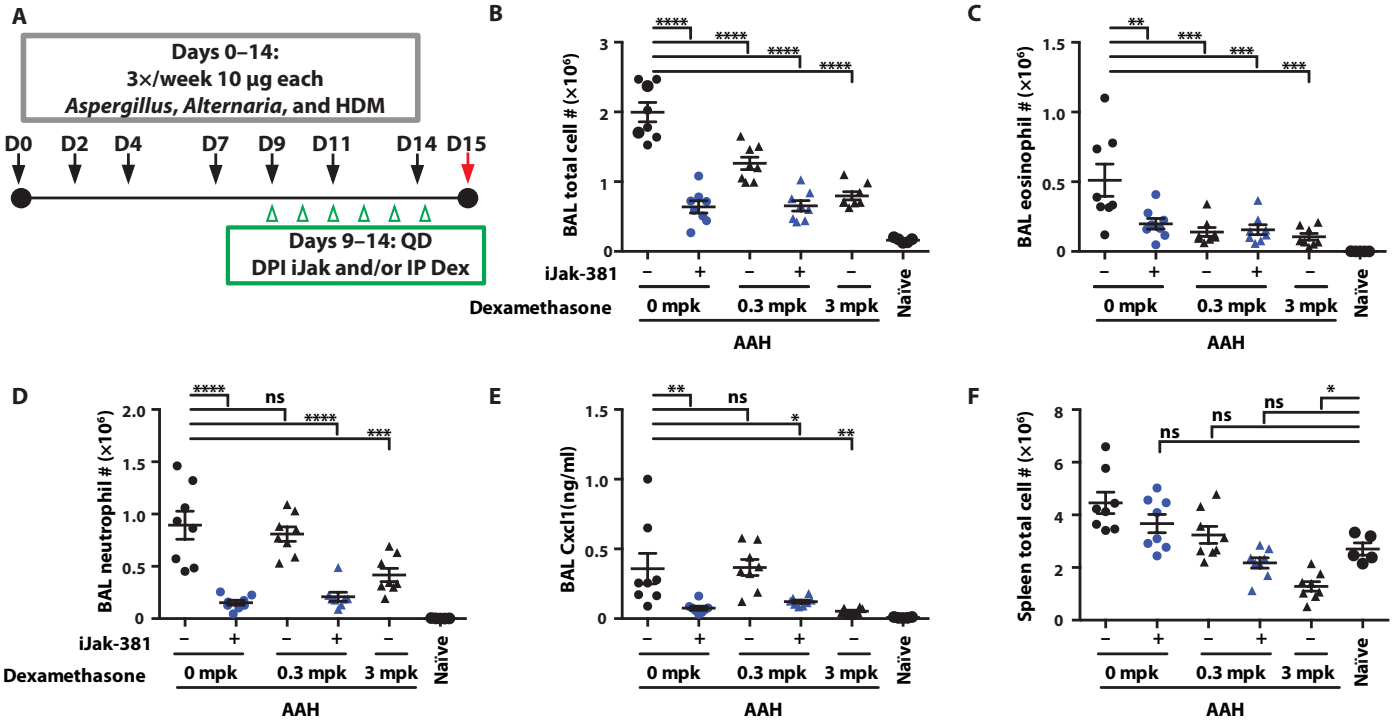

Fig. 7. Combination of iJak-381 with dexamethasone. (**A**) Experimental design is similar to the experiment shown in Fig. 6. From days 9 to 14, animals received either iJak-381 (9.2 mg/kg) via DPI or time-matched tower control treatment 1 hour before the allergen challenge and/or dexamethasone (Dex; 0.3 or 3.0 mg/kg) via intraperitoneal (IP) injection 30 min before allergen challenge. An unimmunized, unchallenged group was also included (naïve). Twenty-four hours after the final challenge, BALF, lungs, spleens, and plasma were collected (red arrow). (**B**) Analysis of cell counts, (**C**) eosinophils, and (**D**) neutrophils from BALF. (**E**) ELISA analysis of Cxcl1 in BALF harvested on day 15. (**F**) Spleen cell counts of experimental animals. *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$, ****$P < 0.001$. Error bars represent SEM; $n = 5$ to 8 animals per group.

high over an extended period of time. We recognize that lung retention may be different in higher species, particularly because mice do not cough and have a simpler lung architecture than humans, both of which may influence the lung retention of iJak-381. In that context, it was important to observe both lung retention and efficacy of iJak-381 in the guinea pig, a species that can cough and whose lung anatomy much more closely models the human lung (*38*, *39*).

One feature of systemic JAK inhibitors is that they achieve therapeutic effects, as well as systemic side effects, at exposures that do not fully inhibit enzyme activity over the entire dosing period (*44*, *45*). Thus, it is a relevant question whether the low systemic concentrations of iJak-381 could nevertheless still lead to systemic side effects and even contribute to efficacy. One of the most sensitive markers of systemic JAK inhibition is the number of NK cells in peripheral tissues (*32*, *33*), and we therefore used this marker as a sentinel for systemic JAK inhibition. iJak-381 showed efficacy as measured by reduction of BAL cellularity and eosinophil counts in the lung while not appreciably affecting splenic NK cell percentages or spleen cellularity. Thus, we conclude that the systemic exposure does not contribute to efficacy of iJak-381 in our animal models.

The OVA models we used generate a highly eosinophilic asthma phenotype, and eosinophil production is strongly dependent on Il-5. iJak-381 does not potently suppress Il-5, nor does it reach the bone marrow, where Il-5 signals to drive eosinophil production. Instead, suppression of eosinophilia by iJak-381 would be consistent with inhibition of Il-13 signaling (*46*), which results in lower eotaxin expression, leading to reduced recruitment of eosinophils to the lung. Furthermore, because of the generally lower amount of inflammation in iJak-381–treated animals, production of Il-5 may also be reduced.

Inhibition of type 2 cytokines would not explain the suppression of neutrophil influx observed in the allergen-driven asthma model. We currently do not know which Jak-dependent pathway drives neutrophil influx in this model. In human asthma, there is emerging evidence that other nontype 2 cytokines that signal via JAK kinases may contribute to the pathogenesis and severity of asthma. Severe asthmatics with elevated plasma IL-6 concentrations have been shown to have increased neutrophilic inflammation, worse lung function, and increased exacerbation frequency as compared to those with IL-6 concentrations in the normal range (*12*). A subset of patients with asthma also displays a type I IFN–responsive gene signature metric (ISM) in lung tissue and peripheral blood in the absence of evidence of viral infection, and this ISM is associated with increased bacterial and fungal burden in the airways, increased exacerbation frequency, and decreased clinical benefit from omalizumab (anti-IgE) treatment (*13*). Another study has provided evidence for increased IFN-γ in the airways of a subset of severe asthmatics and potential mechanistic contribution of IFN-γ to a preclinical asthma model (*14*). Because iJak-381 will inhibit signaling by IL-6 and IFNs, we hypothesize that iJak-381 will confer therapeutic benefit through these mechanisms, in addition to its effects on type 2 cytokine signaling.

There are several limitations to this study which may serve as subjects for further investigation or alternative interpretation. First, none of our preclinical models of asthma truly recapitulate the complex biology of the human disease, nor do they allow for the assessment

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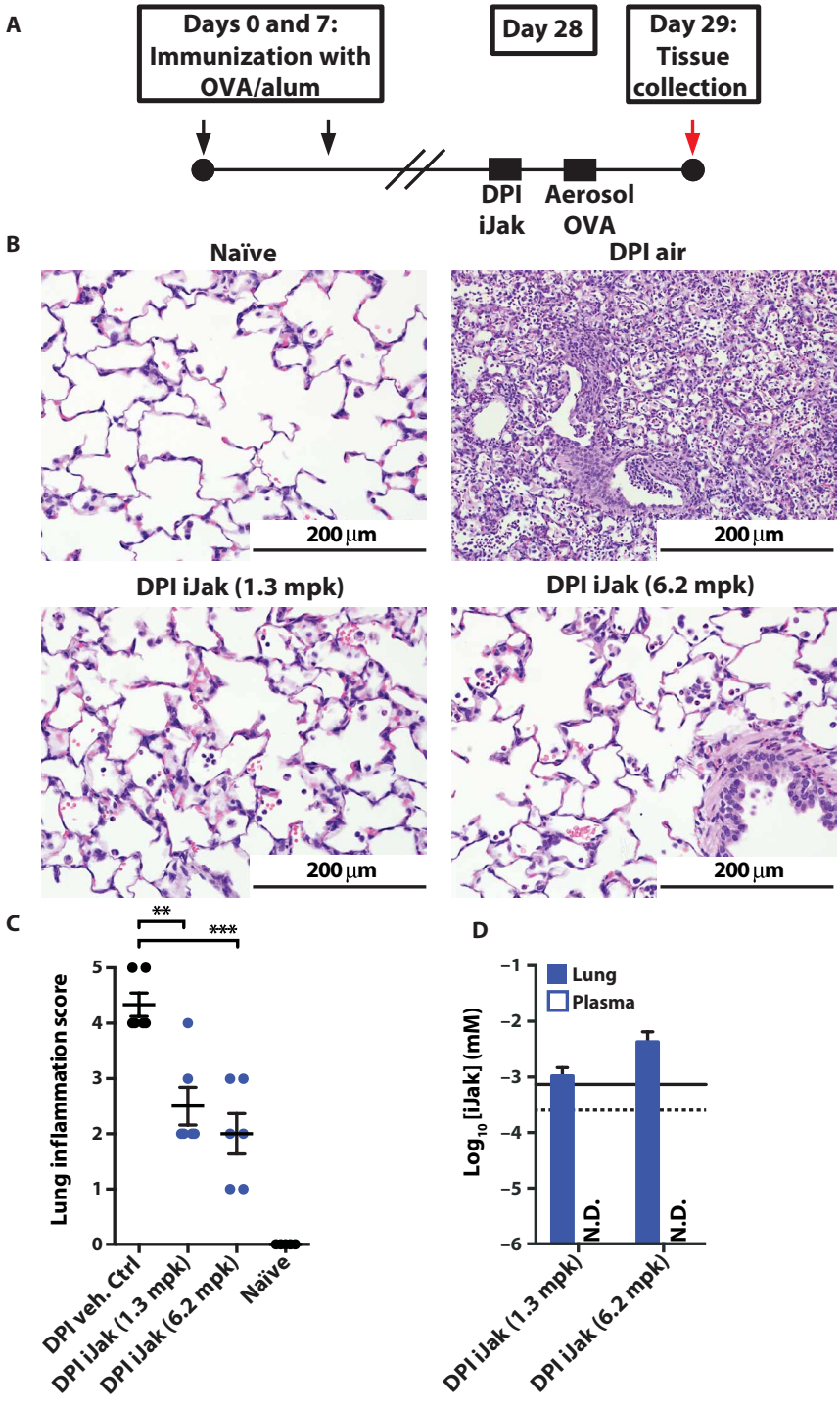

Fig. 8. Efficacy of iJak-381 in a guinea pig asthma model. (**A**) Design of guinea pig OVA asthma model. Animals were immunized twice with OVA (days 0 and 7). On day 28, animals received a single OVA challenge. One hour before the OVA challenge, animals received either air or iJak-381 at the indicated dose via DPI. Twenty-four hours after the final challenge, lungs, spleens, and plasma were collected (red arrow). (**B**) Representative images of hematoxylin and eosin–stained formalin-fixed lung sections. (**C**) Inflammation severity scores of lung sections. Histologic sections were subjectively scored on a scale of 0 to 5 for severity and distribution of inflammatory cell infiltrate: 0, normal; 1, minimal; 2, mild; 3, moderate; 4, marked; and 5, severe. $^{**}P < 0.01$, $^{***}P < 0.005$. (**D**) Drug concentrations in lung tissue (solid bars) and plasma [not detectable (N.D.)] 24 hours after aerosol challenge. Lines indicate IC_{50} values for iJak-381 as determined in the IL-13–stimulated BEAS-2B cell-based assay and corrected for lung tissue binding (solid) or plasma protein binding (dashed). Mouse blood and lung tissue binding numbers were used for the calculations. Error bars represent SEM; $n = 4$ to 6 animals per group.

of the two major clinical endpoints, forced expiratory volume in 1 s and exacerbation rate. Although our models depend on biological pathways that have been validated in the clinic, only a clinical trial will tell whether pulmonary JAK1 inhibition will confer therapeutic benefit to human patients. Similarly, although the therapeutic effect in preclinical models is dose dependent, it would be difficult to estimate a human efficacious dose based on this model data and in the absence of human PK data. Last, although the effects of iJak-381 appeared to be restricted to the lung, and although we have not observed any obvious toxic effects of iJak-381 inhalation in preclinical species, this study does not formally assess the risks of pulmonary JAK1 inhibition, particularly with regard to bronchopulmonary infections.

In summary, our results suggest that lung-restricted inhibition of JAK1 is sufficient to suppress asthma-related inflammation. Our data indicate that inhaled treatment has the potential to be highly efficacious and to combine well with corticosteroids, which represent the standard of care in asthma, but do not have strong effects on neutrophilic inflammation. Furthermore, our data also predict that this approach will result in minimal systemic side effects due to lung-restricted exposure. Clinical evaluation of this concept is therefore warranted.

MATERIALS AND METHODS

Study design

The overall goal of this study was to determine whether lung-restricted Jak1 inhibition was sufficient to confer therapeutic benefit in preclinical models of human asthma. To this end, we developed a Jak1 inhibitor with lung-restricted activity when dosed by DPI and tested its ability to suppress pulmonary inflammation in three different experimental asthma models in two preclinical species. We measured parameters of pulmonary and systemic Jak1 inhibition to demonstrate a lung-restricted effect. Sample sizes were determined on the basis of previous experience and technical feasibility and are indicated in each figure legend. Standard measurements were used to assess inflammation, airway hyperreactivity, and gene expression. Histology analysis was carried out by a professional histopathologist who was blinded to the sample identity.

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Statistical analysis

Selected figures were graphed for the report, and statistical analysis was performed, using PRISM v6.0 software (GraphPad). Comparison of single treatments against control treatments was performed using two-tailed Student's *t* test with Welch's correction (Figs. 3, B and C, 5, and 6). Analysis of statistical differences comparing multiple treatments to each other or to a control treatment was performed via ordinary one-way analysis of variance (ANOVA) with Tukey's multiple comparison test with a single pooled variance (Figs. 3D, 4, 7, and 8). Analysis of NK cell response to BAL eosinophil response was performed by a linear regression analysis of the data with a line through the origin. Each linear regression was then analyzed using an extra sum of pairs *F* test to determine that the slopes were significantly different.

SUPPLEMENTARY MATERIALS

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Methods

Fig. S1. Cell-based potency of inhibitors for IL-5–, TSLP-, and IgE-induced signals.

Fig. S2. DPI system.

Fig. S3. Co-dose PD model.

Fig. S4. IL-13 and Gm-csf PD models.

Fig. S5. Dose-response curve of iJak-381 in asthma model.

Fig. S6. Complete blood count analysis of iJak-treated animals.

Fig. S7. Drug concentrations in AAH model.

Table S1. Biochemical selectivity of iJak-381 and oJak-989.

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Lung-restricted inhibition of Janus kinase 1 is effective in rodent models of asthma

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Janus goes local in asthma

In asthma, swelling of the airways and increased mucus production cause breathing difficulties that can be life threatening. Although current treatments are effective in most cases, alternative strategies are needed for patients who do not respond to available drugs. Oral administration of Janus kinase 1 (JAK1) inhibitors has been shown to be effective in a mouse model of asthma; however, systemic side effects preclude their use in patients. Now, Dengler *et al*. have developed an inhalable JAK1 inhibitor specifically targeting JAK1 in the lung. The inhibitor exerted therapeutic effects in multiple asthma models in rodents. The results suggest that local JAK1 inhibition might be sufficient for treating asthma.

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