

Efficacy of an Inhaled IL-13 Antibody Fragment in a Model of Chronic Asthma

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Abstract

Rationale: IL-13 is an important cytokine implicated in the pathogenesis of allergic asthma and is an attractive target for an inhaled therapeutic.

Objective: To investigate the efficacy of CDP7766, a nebulized inhaled anti-IL-13 monoclonal antibody Fab fragment, in a model of allergic asthma in cynomolgus macaques naturally sensitized to *Ascaris suum*.

Methods: CDP7766 was nebulized using a vibrating-membrane nebulizer on the basis of eFlow technology. The aerosol generated was analyzed to determine the particle size profile and the biophysical and functional properties of CDP7766. Nebulized CDP7766 (0.1–60 mg/animal, once daily for 5 d) was delivered via the inhaled route.

Measurements and Main Results: The investigational eFlow nebulizer used in this study generated a respirable aerosol of CDP7766 with no evidence of degradation, loss of potency, aggregation, or formation of particulates. Inhaled CDP7766 was well tolerated in the model (no adverse effects related to local irritation) and significantly inhibited BAL allergen-induced cytokine and chemokine upregulation (60 mg vs. vehicle: eotaxin-3, $P < 0.0008$; MIP [macrophage inflammatory protein]-1 β , IL-8, IFN- γ , $P \leq 0.01$). CDP7766 significantly inhibited the increase in pulmonary resistance stimulated by inhaled allergen, measured 15 minutes and 24 hours after allergen challenge.

Conclusion: Inhaled CDP7766 potently inhibited the function of IL-13 generated during the airway response to inhaled allergen in cynomolgus macaques, demonstrating the potential of inhaled anti-IL-13 therapeutics for the treatment of allergic asthma.

Keywords: allergen; *Ascaris suum*; therapeutics

IL-13, a multifunctional T helper-2 (Th2) cytokine, is involved in the pathogenesis of allergic asthma and, therefore, represents an important therapeutic target (1–7). After inhaled allergen challenge, increased local expression of IL-13 is observed in human (8) and nonhuman primate airways (9);

greater concentrations are also detected in patients with severe asthma under normal conditions (7) and during symptom exacerbation (10). IL-13 is produced in the lungs by lymphocytes (11, 12), submucosal mast cells (13), and eosinophils (14), and predominantly targets cells local to the

airways. In collaboration with other cytokines, IL-13 stimulates the airway epithelium, resulting in hyperplasia, mucus secretion, and proinflammatory cytokine generation (15–17). Fibroblast (18), mast cell (19), and airway smooth muscle (20) activation have also been reported.

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At a Glance Commentary

Scientific Knowledge on the

Subject: IL-13 is a key cytokine in the pathogenesis of allergic asthma. Variable results have been observed in studies evaluating systemic IL-13 antibodies; inhaled delivery may provide an alternative therapeutic approach in treating patients with asthma.

What This Study Adds to the

Field: In this study, a respirable aerosol of CDP7766 was generated using an investigational eFlow nebulizer, with no evidence of degradation or loss of potency. In a model of allergic asthma in cynomolgus macaques, inhaled CDP7766 inhibited IL-13 function, associated chemokine and cytokine upregulation, and the increase in pulmonary resistance stimulated by allergen challenge; there were no adverse effects related to local irritation. These preclinical data provide evidence to support further investigation of inhaled CDP7766 as a treatment for allergic asthma in humans.

In nonhuman primates, systemically administered IL-13–neutralizing monoclonal antibodies are effective at reducing allergen-induced airway inflammation (9, 21). However, clinical trials of systemic anti-IL-13 monoclonal antibodies have yielded mixed results. In patients with poorly controlled asthma, lebrikizumab significantly improved lung function in initial clinical studies (22, 23); however, a variable and weaker response was observed in pivotal phase III studies (24). Despite failing to meet the primary endpoint, lung function improvements were seen in phase II studies of the anti-IL-13 monoclonal antibody tralokinumab in patients with moderate-to-severe uncontrolled asthma (25, 26). These observations prompted evaluation of efficacy in predefined patient subpopulations in phase III studies (26).

Inhaled anti-IL-13 therapy is a novel approach that potentially offers several advantages over systemic administration; delivering a high dose directly to the target

organ may improve efficacy while also limiting systemic exposure and any associated side effects (27). Furthermore, inhaled delivery is convenient for patients, as it is consistent with their existing therapies and provides a simple, pain- and needle-free administration. As full-length monoclonal antibodies have relatively lower bioavailability, smaller forms, such as Fab fragments, may offer advantages because they can more effectively penetrate tissues to reach target receptors (28). It is important to note that Fab fragments lack an Fc region, rendering them unavailable for neonatal Fc Receptor (FcRn)-mediated recycling, resulting in a shorter half-life and lower systemic exposure (27).

To investigate the potential of inhaled administration of a selective IL-13 neutralizing antibody in patients with Th2-driven asthma, we developed CDP7766, a potent, biophysically stable monoclonal antibody Fab fragment with high affinity and specificity for IL-13 (29). CDP7766 acts by blocking IL-13 binding to IL-13R α 1 and resultant formation of the high-affinity IL-13:IL-13R α 1:IL-4R α signaling complex; CDP7766 also blocks IL-13 binding to IL-13R α 2 (29). Here we demonstrate the efficacy of inhaled CDP7766 in a model of allergic asthma in cynomolgus macaques. Some of the results of this study have been previously reported in the form of an abstract (30).

Methods

Identification of CDP7766 was previously described (29) (*see online supplement*).

CDP7766 was formulated in Dulbecco's phosphate-buffered saline (PBS), pH 6.5 (20 mg/ml). Laser diffraction and cascade impaction were used to measure aerosol particle size profile after nebulization with an investigational eFlow nebulizer system (PARI Pharma GmbH). The quantity of respirable nebulized CDP7766 delivered at the endotracheal tube in the *Ascaris suum* asthma model was determined by breath simulation (*see online supplement*).

Veterinary care of the animals was in accordance with the protocol, the testing facility's standard operating procedures, regulations outlined in First Rules of the Animal Welfare Act regulations (9CFR), the Public Health Service Policy on Humane Care and Use of Laboratory Animals, the Guide for the Care and Use of

Laboratory Animals, and the Charles River Laboratories Policy on Human Care. This was not a terminal study.

Sample size was determined by the contract research organization, on the basis of similarly designed studies and constraints of colony size. Thirty-two male/female (3–9 kg) cynomolgus macaques (*Macaca fascicularis*) naturally sensitized to *A. suum*, were randomly assigned to treatment groups. Animals entered treatment after screening, which evaluated untreated airway bronchoconstrictor and cellular inflammatory response to *A. suum* challenge. Animals were anesthetized with propofol (induction: $\sim$ 3 mg/kg intravenous; maintenance: 0.15–0.30 mg/kg/min intravenous infusion) before *A. suum* challenge on Days 1/2, and before BAL (performed before CDP7766 administration on Days –4/–6 and 24 hours after the last administration of *A. suum* [Day 3]). For both phases, animals received 15 breaths of *A. suum* antigen (Greer Laboratories) in PBS via an ultrasonic nebulizer. Doses were determined as the minimal dose previously shown to elicit a greater than 40% increase in pulmonary resistance. In the treatment phase, CDP7766 or vehicle was administered on Days –2, –1, 1, 2, and 3 over a $\sim$ 6-minute period. On Days 1/2, CDP7766 was administered 30 minutes before *A. suum* antigen. CDP7766 (3 ml nebulizer charge volume) was administered at concentrations of 20 mg/ml, 3.33 mg/ml, 0.33 mg/ml, or 0.033 mg/ml to deliver nominal output doses of 60 mg, 10 mg, 1 mg, and 0.1 mg, respectively. Peripheral blood samples were taken after CDP7766 administration (Day 3).

BAL supernatants were analyzed in triplicate for cytokine and chemokine concentrations using electrochemiluminescence immunoassay (Meso Scale Discovery, Inc.). Human Th1/Th2 cytokine and chemokine plates were used to determine specific concentrations (as per manufacturer's instructions). The cytokine and chemokine electrochemiluminescence assays were known *a priori* to detect most cynomolgus macaque homologs. Where cross-reactivity with the homologs was not known, reported data are for analytes showing greater than twofold increases in concentration after antigen challenge.

BAL samples were cytocentrifuged onto glass slides, methanol (100%) fixed,

Wright-Giemsa stained, and evaluated for cellular morphology and differential analysis by an operator blinded to treatment. Blood samples (1 ml) were analyzed (Bayer ADVIA 120 hematology analyzer).

Data were tested for distribution normality and, where appropriate, log₁₀ transformed before analysis using parametric statistics, as indicated (GraphPad Prism v5.01). A one-way ANOVA was performed, followed by Dunnett's test for multiple comparisons, as indicated.

Results

Aerosol Characteristics of Nebulized CDP7766

The configuration of the investigational eFlow nebulizer system used in this study generated a respirable aerosol of CDP7766 when formulated at 20 mg/ml in PBS. As shown in Table 1, the droplet size and dispersity were comparable when measured by laser diffraction and cascade impaction. The mass median diameter and mass median aerodynamic diameter of 3.2 μ m, geometric standard deviation of 1.5, and respirable fraction (particles < 5 μ m) of greater than 80% indicate that the eFlow configuration and formulation of CDP7766 were well matched to efficiently deliver therapeutic doses of CDP7766 to the terminal bronchioles. Breath simulation was performed using the experimental apparatus and ventilator parameters as used in the cynomolgus macaque model of asthma (modeled for a 6-kg cynomolgus macaque, tidal volume 67 ml, inhalation 0.96 s, exhalation 1.01 s, 31 breaths/min). Our *in vitro* data demonstrate that, using these specific ventilator parameters and apparatus configuration, the target dose would be delivered in ~6 minutes and that 40% of

the nebulizer aerosol dose would enter the endotracheal tube (Table 2).

Administration of Inhaled CDP7766 in a Model of Asthma in *A. suum*-Sensitive Cynomolgus Macaques

Cynomolgus macaques naturally sensitized to the nematode worm *A. suum* were treated with five consecutive daily doses of aerosolized CDP7766 at a nebulizer reservoir charge dose of 60 mg/d ($n = 8$), 10 mg/d ($n = 6$), 1 mg/d ($n = 6$), or 0.1 mg/d ($n = 4$), or vehicle (3 ml nebulized PBS, $n = 8$), on Days -2, -1, 1, 2, and 3 (Figure 1). Inhaled *A. suum* challenge was performed on Days 1 and 2 and BAL performed 24 hours after the final *A. suum* challenge (Figure 1). Five consecutive daily doses of inhaled aerosolized CDP7766 (0.1–60 mg/d) were well tolerated. CDP7766 was only detectable in the 10 mg/d and 60 mg/d groups (Figure 2). Peripheral venous blood samples were taken on Day 3, 24 hours after the second challenge with *A. suum*, and analyzed with an automated hematology analyzer. At all tested doses, CDP7766 had no effect on peripheral blood counts of white blood cells, red blood cells, platelets, lymphocytes, monocytes, eosinophils, or neutrophils (Table 3).

Effect of Inhaled CDP7766 on BAL Inflammatory Cells

Inhaled *A. suum* challenge stimulated a characteristic eosinophilic inflammatory response in the airways (Figure 3). Treatment with inhaled CDP7766 did not significantly reduce the absolute number of BAL total leukocytes, neutrophils, or eosinophils compared with vehicle during the treatment phase (Figures 3A–3C). However, when normalized with respect to pre-allergen exposure levels, the highest dose of inhaled CDP7766 (60 mg/d) modestly but significantly inhibited the *A. suum*-induced increase in the proportion of BAL eosinophils (% eosinophils after

allergen – % eosinophils before allergen: vehicle-treated group, $54.6 \pm 4.03\%$; CDP7766 60 mg/d group, $38 \pm 6.7\%$; $P < 0.05$). The eosinophilic response after *A. suum* challenge was greater in the treatment phase than in the screening phase (performed 4–6 weeks beforehand) (Figure 3C). When comparing the response to *A. suum* challenge in the treatment phase with the response in the screening phase, inhaled CDP7766 dose-dependently and significantly inhibited the increase in number of BAL eosinophils (Figure 3D).

Effect of Inhaled CDP7766 on BAL Cytokines and Chemokines

Multiplex analysis was performed using a human electrochemiluminescence immunoassay system (Meso Scale Discovery, Inc.) to determine the concentration of Th1 and Th2 cytokines and chemokines in BAL from cynomolgus macaques. The immunoassays with cross-reactivity for cynomolgus macaque homologs as indicated by the manufacturer are shown in Table 4; data are also presented for additional cytokines and chemokines where a twofold or greater increase in concentration was observed after *A. suum* challenge. *A. suum* challenge provoked a marked (>10-fold) and significant increase in the BAL concentration of IL-5 and the chemokines MIP (macrophage inflammatory protein)-1 β , macrophage-derived chemokine, eotaxin-1, and macrophage chemoattractant protein (MCP)-4 (Table 4). Additional cytokines and chemokines were also upregulated to a lesser extent after allergen challenge, as detailed in Table 4. CDP7766 (60 mg/d) significantly and robustly inhibited the *A. suum*-induced upregulation of all cytokines and chemokines tested except eotaxin-1, thymus- and activation-regulated chemokine (TARC), IFN- γ -inducible protein 10, MCP-1, IL-4, IL-2 and IL-1 β .

Table 1. Aerosol Particle Size Profiling after Nebulization of CDP7766 with the Investigational eFlow Nebulizer

	MMD (μ m)	MMAD (μ m)	GSD	RF (%)	TOR (mg/min)
Laser diffraction	3.3 (± 0.07)	NA	1.5 (± 0.01)	83.1 (± 1.4)	301 (± 2.0)
Cascade impaction	NA	3.2 (± 0.3)	1.6 (± 0.1)	83.1 (± 5.4)	NA

Definition of abbreviations: GSD = geometric standard deviation; MMAD = mass median aerodynamic diameter; MMD = mass median diameter; NA = not applicable; RF = respirable fraction; TOR = total output rate.

Laser diffraction and cascade impaction to measure the particle size distribution of a 20 mg/ml CDP7766 aerosol generated with the eFlow nebulizer. Data are shown as mean (SEM), $n = 3$ determinations.

Table 2. Determination of Delivered Dose after Nebulization of CDP7766 with the Investigational eFlow Nebulizer

Delivered Dose (%)	Exhalation Filter (%)	Residue (%)	Nebulization Time (min)	Delivery Rate (%/min)
40.4 (±8.0)	44.4 (±1.7)	13.7 (±4.1)	5.7 (±0.3)	7.1 (±1.4)

Breath simulation to determine and characterize the dose delivered at the endotracheal tube applying the same ventilator parameters as in the cynomolgus macaque model of asthma. Data are shown as mean (SEM), *n* = 3 determinations.

Interestingly, BAL IL-4 was upregulated after *A. suum* challenge and was inhibited by inhaled CDP7766 (60 mg/d, *P* = 0.08). As shown in Figure 4, CDP7766 was very effective at inhibiting upregulation of BAL IL-5 (*P* = 0.03), eotaxin-1 (*P* = 0.08), and eotaxin-3 (*P* = 0.0008) at doses as low as 0.1 mg/d.

Effect of Inhaled CDP7766 on Pulmonary Resistance

Inhaled *A. suum* challenge stimulated an immediate early pulmonary response, measured as an increase in pulmonary

resistance, peaking during the first 15 minutes after challenge. CDP7766 had no effect on the immediate increase in pulmonary resistance measured on Day 1 but dose-dependently and significantly inhibited the immediate increase in pulmonary resistance measured on Day 2 (Figure 5A).

Pulmonary resistance remained elevated when measured 24 hours after each challenge with *A. suum* (Figure 5B). It was not possible to take pulmonary function measurements at intermediate time points because of the need for animals to recover after *A. suum* challenge and anesthesia. Inhaled nebulized

CDP7766 had no effect on *A. suum*-induced increase in pulmonary resistance measured 24 hours after the first challenge (Figure 5B). However, CDP7766 dose-dependently and significantly inhibited *A. suum*-induced increase in pulmonary resistance measured 24 hours after the second *A. suum* challenge (Figure 5B).

Discussion

In this study, we demonstrated that neutralization of IL-13 using inhaled

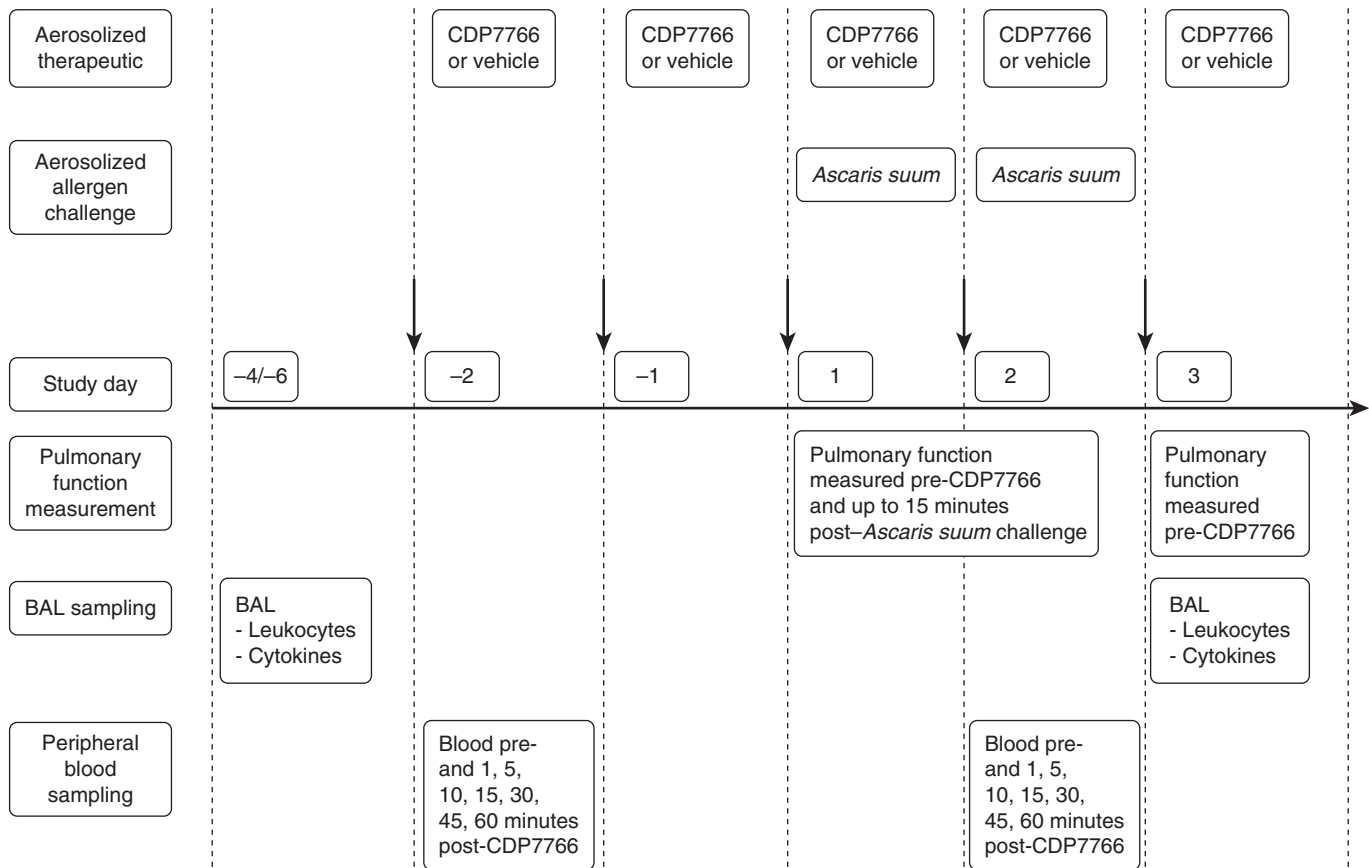


Figure 1. Protocol schematic for the treatment phase of the model of allergic asthma in cynomolgus macaques sensitized to *Ascaris suum*. The response of all animals (*N* = 32) to inhaled *A. suum* antigen was determined in a screening phase performed ~4 weeks before the treatment phase. In the treatment phase, animals were assigned to groups and received daily nebulized phosphate-buffered saline vehicle (*n* = 8), CDP7766 0.1 mg/d (*n* = 4), CDP7766 1 mg/d (*n* = 6), CDP7766 10 mg/d (*n* = 6), or CDP7766 60 mg/d (*n* = 8).

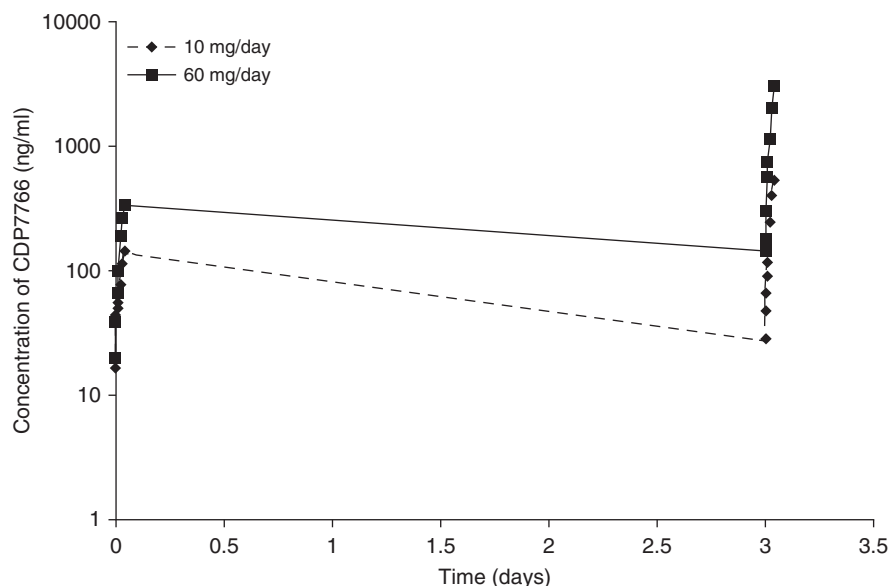


Figure 2. Geometric mean of CDP7766 plasma concentrations (ng/ml) in the 10 mg/d ($n = 6$) group and 60 mg/d group ($n = 8$).

CDP7766, a robust anti-IL-13 monoclonal antibody fragment, was effective at reducing the allergic airways response in a cynomolgus macaque model of asthma.

As previously reported by Lightwood and colleagues, CDP7766 was selected from a panel of antibody sequences to have biophysical properties optimal for the intended inhaled route of administration; specifically, high melting temperature ($>80^{\circ}\text{C}$) and resistance to shear-induced degradation and aggregation (29). Furthermore, the CDP7766 Fab fragment had high affinity for human IL-13 (dissociation constant, 12 pM) and potentially neutralized both human (wild type and R130Q variant) and cynomolgus macaque IL-13 in cell-based assays (29). After nebulization using a vibrating-membrane nebulizer on the basis of eFlow technology, CDP7766 retained binding affinity,

functional neutralization potency, and structural integrity. These data are consistent with previous reports that vibrating-membrane nebulizers are significantly less destructive for protein therapeutics than ultrasonic or jet-type nebulizers (31). Vibrating-membrane nebulizers are currently considered to be the most appropriate devices for the safe delivery of large amounts of active monoclonal antibody into the lungs (32, 33). Our data also confirm that the eFlow configuration used in this study efficiently generates an aerosol plume containing a high respirable fraction ($>80\%$) and particle size profile optimal for delivery to the terminal bronchioles. Data obtained using breath simulation suggest that 40% of the nebulizer dose will enter the endotracheal tube in the ventilated cynomolgus macaque model. There are

limited data on the effect of particle size on nebulized aerosol distribution in the bronchial tree in anesthetized ventilated nonhuman primates. Particle sizes of $\sim 3\ \mu\text{m}$ will deposit to the deep lung, but direct comparison between studies, and extrapolation to our results, is difficult given the different nebulizers and experimental configurations used (34, 35).

Administration of a protein therapeutic directly to the airways has the potential to cause local side effects, such as cough (36), yet no such adverse effects were observed in the present study. CDP7766 was well tolerated by cynomolgus macaques when administered by nebulizer for 5 consecutive days at up to 60 mg/d.

No adverse changes in pulmonary resistance or BAL leukocyte counts were observed and, importantly, we found no increase in BAL neutrophil counts after administration of CDP7766 at any dose tested, compared with animals receiving vehicle. A previous report has documented a change in blood platelet counts after administration of intravenous anti-IL-13 monoclonal antibody in cynomolgus macaques (37). We did not observe any effect of CDP7766 at any dose on blood platelet counts or differential blood leukocyte counts measured 24 hours after *A. suum* challenge on Day 2.

Clinical studies indicate that systemic administration of anti-IL-13 monoclonal antibody in humans is generally well tolerated; however, increased eosinophil counts have been reported across clinical studies (22, 24, 25), highlighting a potential class effect of systemically administered anti-IL-13 therapy. In this study, CDP7766 was not associated with increased eosinophil number in cynomolgus macaques.

The effect of anti-IL-13 monoclonal antibody on BAL eosinophilia in

Table 3. Effect of Five Daily Doses of Inhaled CDP7766 on Peripheral Blood Hematology

	White Blood Cells ($\times 10^3/\mu\text{l}$)	Red Blood Cells ($\times 10^6/\mu\text{l}$)	Platelets ($\times 10^3/\mu\text{l}$)	Lymphocytes ($\times 10^3/\mu\text{l}$)	Neutrophils ($\times 10^3/\mu\text{l}$)	Eosinophils ($\times 10^3/\mu\text{l}$)	Monocytes ($\times 10^3/\mu\text{l}$)
Vehicle	9.76 (1.05)	5.55 (0.30)	351.80 (41.79)	2.96 (0.38)	5.67 (1.04)	0.67 (0.14)	0.352 (0.03)
CDP7766 0.1 mg/d	10.20 (1.19)	6.11 (0.35)	358.30 (39.60)	3.17 (0.56)	5.94 (1.56)	0.65 (0.03)	0.35 (0.04)
CDP7766 1 mg/d	10.35 (1.54)	5.88 (0.18)	373.80 (32.82)	3.98 (0.63)	4.97 (1.20)	0.97 (0.27)	0.31 (0.04)
CDP7766 10 mg/d	10.77 (1.11)	5.38 (0.22)	358.20 (43.32)	4.06 (0.51)	5.30 (0.78)	0.82 (0.14)	0.43 (0.06)
CDP7766 60 mg/d	9.34 (1.16)	5.26 (0.12)	334.30 (20.51)	2.99 (0.31)	4.62 (0.43)	1.28 (0.54)	0.343 (0.04)

Peripheral venous blood samples were taken on Day 3 in the treatment phase for automated hematological analysis. Data are shown as mean (SEM) (total, $N = 32$ animals: 0.1 mg/d, $n = 4$; 1 mg/d, $n = 6$; 10 mg/d, $n = 6$; 60 mg/d, $n = 8$).

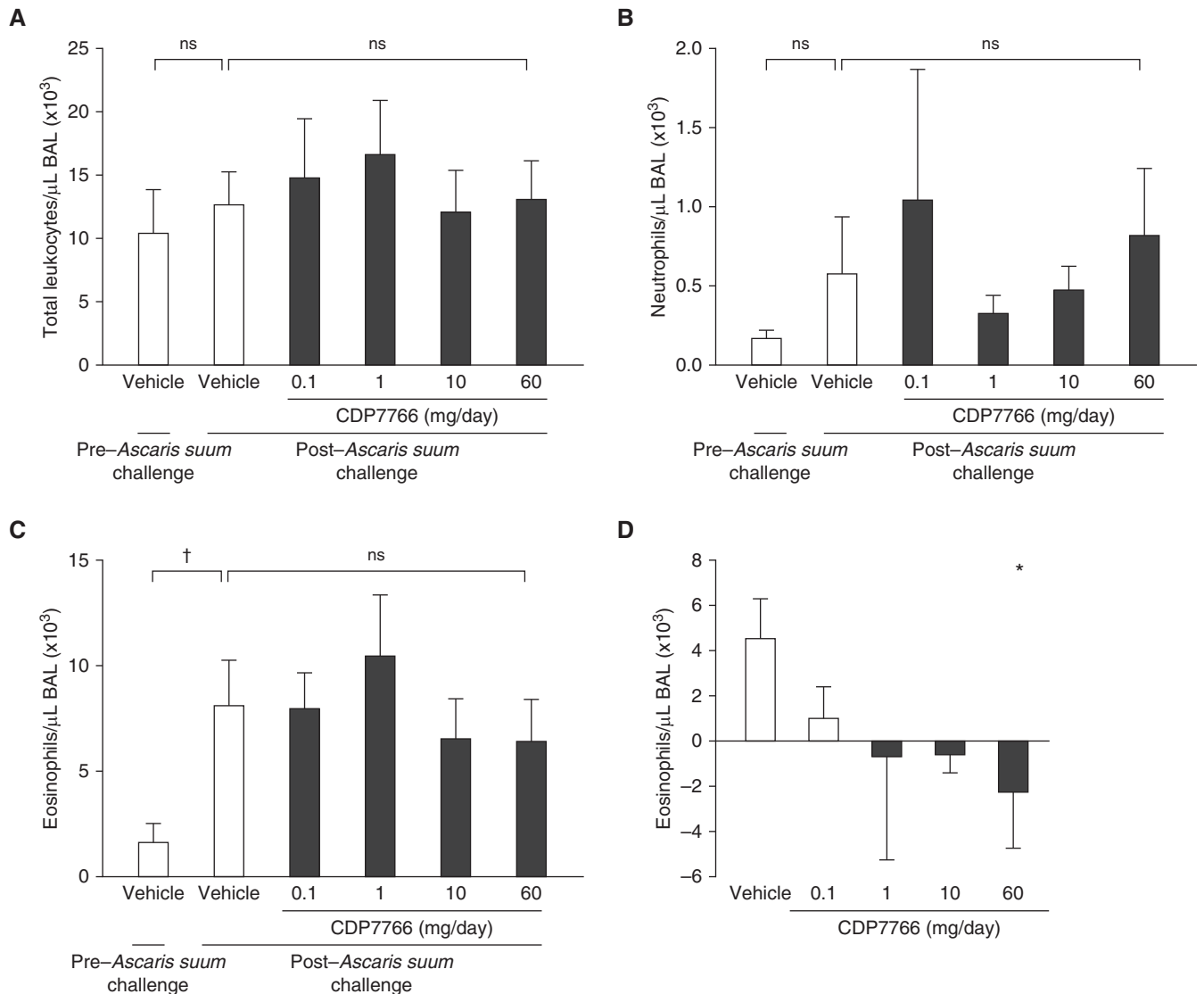


Figure 3. Effect of CDP7766 on BAL total leukocyte, neutrophil, and eosinophil numbers measured on Day 3. BAL total leukocytes were counted by hemocytometer and eosinophils were identified on cytopspins stained with Wright-Giemsa. (A) The effect of CDP7766 on the number of total leukocytes in the treatment phase 24 hours after challenge with *Ascaris suum* antigen on Day 2. (B) The effect of CDP7766 on the number of neutrophils in the treatment phase 24 hours after challenge with *A. suum* antigen on Day 2. (C) The effect of CDP7766 on the number of eosinophils in the treatment phase 24 hours after challenge with *A. suum* antigen on Day 2. (D) The effect of CDP7766 on the change in BAL eosinophil numbers between the screening phase and the treatment phase. Data are shown as mean (SEM), $n = 4-8$ (total $N = 32$). (A and B) One-way ANOVA with Dunnett's *post hoc* test as indicated; (C) $^{\dagger}P < 0.05$, one-way ANOVA with Dunnett's *post hoc* test as indicated; (D) $^*P < 0.05$, unpaired *t* test. ns = nonsignificant.

cynomolgus macaques naturally sensitized to *A. suum* is variable. Some studies demonstrate inhibition (9, 21) and others show inconsistent or nonsignificant effects (37, 38). In the present study, we observed no significant effect of CDP7766 on BAL absolute total leukocyte or eosinophil counts in the treatment phase. However, CDP7766 significantly inhibited the increase in eosinophilia when comparing the response in the treatment and screening phases. CDP7766 modestly but significantly

reduced the percentage ($\sim 30\%$) of eosinophils in BAL in the treatment phase. Together, these results suggest that BAL or sputum eosinophil counts are unlikely to be of use as a robust and sensitive biomarker of IL-13 activity in allergen challenge studies in humans. Our results indicate that, in addition to other effects, local neutralization of IL-13 may be beneficial in reversing the chronic increase in airway eosinophils that characterizes allergic asthma.

Anti-IL-13 monoclonal antibody administered systemically in sensitized cynomolgus macaques has been shown to inhibit *A. suum*-induced upregulation of BAL eotaxin and RANTES (regulated upon activation, normal T cell expressed and secreted) (9, 21). In the present study, we have extended these observations and measured a panel of Th1 and Th2 cytokines and chemokines in BAL after *A. suum* challenge, with the aim of determining which were upregulated and sensitive to

Table 4. BAL Chemokine and Cytokine Upregulation after *Ascaris suum* Challenge and the Effect of Treatment with 60 mg/d Inhaled CDP7766

BAL Analyte	Analyte pg/ml before Allergen Challenge	Analyte pg/ml after Allergen Challenge	Fold Increase in Analyte after <i>Ascaris suum</i> Challenge	Analyte pg/ml after Allergen Challenge CDP7766 60 mg/d	Effect of CDP7766 60 mg/d (% <i>inhib</i>)	Cynomolgus Macaque Analyte Cross-Reactivity Confirmed
IL-5	0.1 (0.03)	301.1 (107.0)	3,367.0 ($P < 0.0001$)	66.6 (42.8)	78 ($P = 0.03$)	
MIP-1 β	1.4 (1.0)	188.9 (141.8)	139.23 ($P = 0.03$)	4.1 (2.0)	99 ($P = 0.01$)	Y
MDC	135.1 (39.5)	7,205.3 (2,329)	30.2 ($P = 0.002$)	1,396.6 (689.2)	82 ($P = 0.03$)	
Eotaxin-1	9.3 (2.6)	261.5 (127.4)	28.2 ($P = 0.0005$)	28.5 (5.4)	92 ($P = 0.08$)	
MCP-4	20.7 (9.8)	318.6 (172.2)	15.4 ($P = 0.08$)	10.9 (1.4)	103 ($P = 0.03$)	
Eotaxin-3	4.8 (0.8)	43.5 (16.2)	9.1 ($P = 0.001$)	7.8 (1.2)	92 ($P = 0.0008$)	Y
IL-8	0.4 (0.2)	3.1 (1.2)	7.6 ($P = 0.02$)	0.7 (0.2)	90 ($P = 0.008$)	Y
TARC	6.2 (1.4)	19.5 (6.8)	3.2 ($P = 0.03$)	18.3 (5.2)	9 ($P = 0.75$)	
IFN- γ	0.3 (0.1)	1.9 (0.5)	6.6 ($P = 0.02$)	0.6 (0.1)	83 ($P = 0.01$)	Y
IP-10	40.6 (7.7)	151.8 (55.8)	3.7 ($P = 0.07$)	86.4 (19.0)	59 ($P = 0.6$)	Y
IL-12p70	1.0 (0.3)	3.1 (0.7)	3.0 ($P = 0.04$)	1.2 (0.3)	92 ($P = 0.05$)	Y
MCP-1	611.5 (160.8)	1,830.0 (748.1)	3.0 ($P = 0.13$)	1,904.6 (847.0)	-6 ($P = 0.84$)	Y
IL-4	0.5 (0.1)	1.1 (0.2)	2.2 ($P = 0.07$)	0.6 (0.1)	92 ($P = 0.08$)	Y
IL-2	0.7 (0.2)	1.1 (0.3)	1.5 ($P = 0.27$)	0.5 (0.2)	157 ($P = 0.27$)	Y
IL-1 β	1.5 (0.5)	2.3 (0.4)	1.6 ($P = 0.13$)	2.0 (0.5)	43 ($P = 0.44$)	Y

Definition of abbreviations: *inhib* = inhibition; IP-10 = IFN- γ -inducible protein 10; MCP = macrophage chemoattractant protein; MDC = macrophage-derived chemokine; MIP-1 β = macrophage inflammatory protein-1 β ; TARC = thymus- and activation-regulated chemokine; Y = yes. Data are shown as fold increase in analyte after *Ascaris suum* challenge, percentage inhibition after treatment with 60 mg/d inhaled CDP7766, and analyte in pg/ml. Data are shown as mean (SEM), $n = 8$. t test two-tailed (vehicle group and CDP7766 60 mg/d group) and paired t test one-tailed (vehicle group before and after administration with *A. suum*).

inhaled anti-IL-13 treatment. IL-5, eotaxin-1, eotaxin-3, macrophage-derived chemokine, IL-13, and MIP-1 β were most strongly upregulated in BAL after inhaled *A. suum* challenge. In addition, non-Th2 cytokines such as IL-8 and IFN- γ were also upregulated. Our data are in broad

agreement with a study investigating the changes in BAL cytokine and chemokine protein and mRNA transcripts after inhalation of house dust mite antigen in sensitized cynomolgus macaques (39). With the exception of TARC, IFN- γ -inducible protein 10, MCP-1, and IL-1 β , CDP7766

dosed at 60 mg/d robustly reduced ($\geq 78\%$) the concentration of all cytokines and chemokines that were upregulated after *A. suum* challenge. TARC is reported to be upregulated by IL-13 in combination with TNF (tumor necrosis factor) in airway smooth muscle (40), so it is unclear why, in

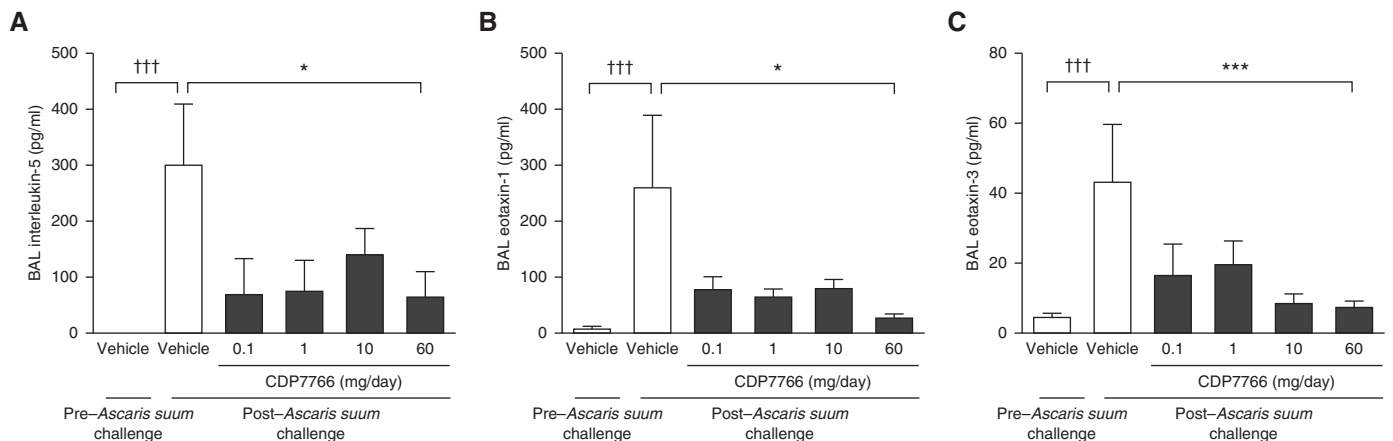


Figure 4. Effect of inhaled CDP7766 on BAL (A) IL-5, (B) eotaxin-1, and (C) eotaxin-3. BAL was sampled 24 hours after administration of *Ascaris suum* on Day 2 and analytes measured by electrochemiluminescence immunoassay. Data are shown for all CDP7766 dose groups as mean (SEM), $n = 4-8$ (total $N = 32$). $*P < 0.05$, $***P < 0.001$, vehicle after *A. suum* challenge versus CDP7766 all doses; $^{+++}P < 0.001$, vehicle before *A. suum* challenge versus vehicle after *A. suum* challenge, one-way ANOVA with Dunnett's test as indicated.

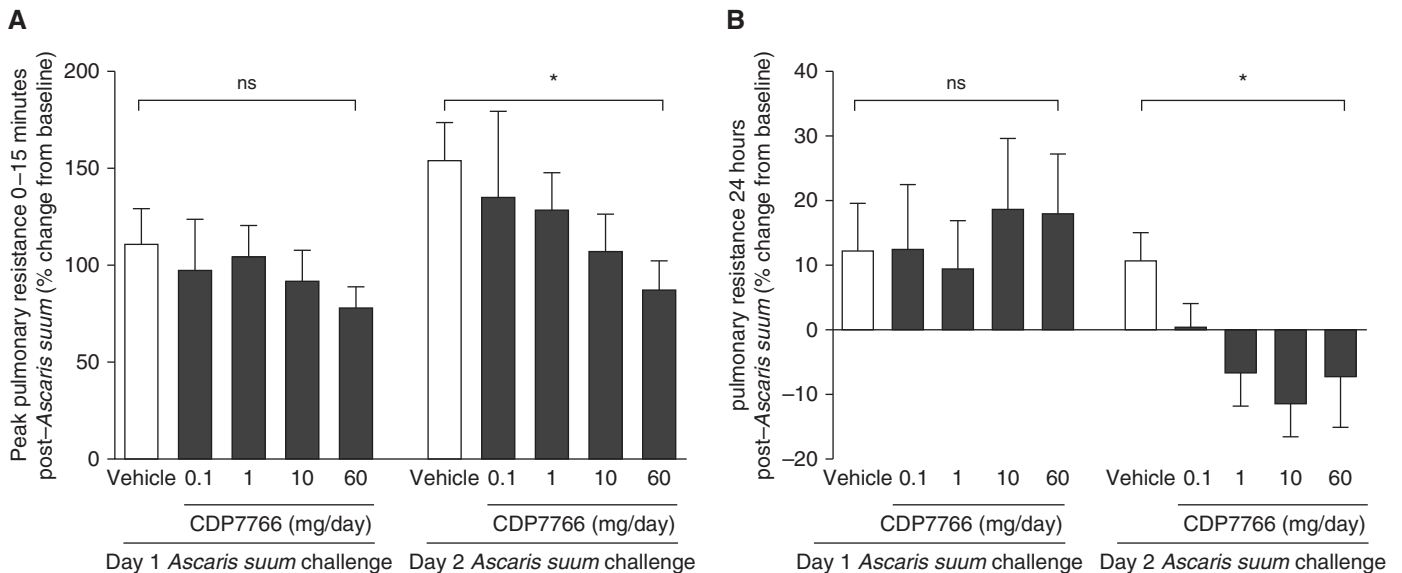


Figure 5. Effect of inhaled CDP7766 on bronchoconstriction stimulated by *Ascaris suum*. Pulmonary resistance was measured (A) 0–15 minutes and (B) 24 hours after each administration of *A. suum*. Data are shown as the percentage change from baseline pulmonary resistance measured before each administration of *A. suum*. Data are shown as mean (SEM), $n = 4–8$ (total $N = 32$). * $P < 0.05$, unpaired t test as indicated. ns = non-significant.

our study, anti-IL-13 treatment failed to prevent upregulation. It is unlikely that airway smooth muscle was not accessible to the inhaled Fab fragment, as CDP7766 potentially inhibited other mediators upregulated by IL-13 in airway smooth muscle, including IL-5 and eotaxin (20, 41). Interestingly, CDP7766 inhibited BAL IL-4 upregulation, although not reaching statistical significance, indicating a potential role for IL-13 in driving pathology through IL-4. This is a novel finding requiring additional study and suggests that therapeutic IL-13 neutralization, in the airways at least, may inhibit IL-4-driven airway pathology. The mechanism for IL-13 control of IL-4 production is unclear; one hypothesis is that IL-13 stimulates eotaxin-1 release from airway epithelial cells (42), which then in turn stimulates IL-4 release from airway eosinophils (43). As key drivers of Th2-mediated inflammation, the therapeutic benefits of directly inhibiting both IL-5 with mepolizumab (44) and IL-4 with dupilumab (45) have previously been demonstrated in patients with uncontrolled moderate-to-severe asthma. However, our results suggest that indirect inhibition of IL-5 and IL-4 with inhaled CDP7766 may provide clinical benefits beyond those expected with inhibition of IL-13 alone.

We investigated the effect of inhaled anti-IL-13 treatment on pulmonary

function after inhaled allergen challenge. Inhaled *A. suum* stimulated an increase in pulmonary resistance measured within 15 minutes of challenge (~120% increase over baseline), which remained elevated 24 hours later (~11% increase over baseline). These results are similar to those previously reported where the “early-phase” (0–15 min) and “late-phase” (6 and 24 h) airway responses to allergen have been studied in sensitized cynomolgus macaques after challenge with *A. suum* (46). In our study, it was not possible to repeatedly measure pulmonary resistance at intermediate time points, so we were unable to determine if the airway response to *A. suum* followed a classic biphasic curve, as observed in human patients after inhaled allergen challenge. We found that inhaled CDP7766 significantly reduced the allergen-induced increase in pulmonary resistance when measured in the first 15 minutes after *A. suum* challenge and when measured after 24 hours. Although there was no significant effect on the “early” or “late” increase in pulmonary resistance after *A. suum* challenge on Day 1, CDP7766 effectively inhibited the increase in pulmonary resistance after *A. suum* challenge on Day 2. The mechanism for this is not understood, but it may reflect a switch in the inflammatory mediators driving the pulmonary response to allergen between the first and second

administration of *A. suum* or a switch in response of the airway smooth muscle to the inflammatory milieu. Our data are consistent with previous observations in sheep (47) and humans (48) that neutralization of IL-13, or antagonism of IL-4R α (49), reduces the magnitude of the allergen-induced pulmonary response.

The role of IL-13 in mediating airway hyperresponsiveness to inhaled nonspecific spasmogens has been investigated in preclinical and clinical studies. In clinical studies in humans with mild allergic asthma, anti-IL-13 monoclonal antibody treatment did not affect airway hyperresponsiveness to the inhaled spasmogen methacholine (48, 50). The IL-4R α antagonist pitrakinra dose-dependently inhibited airway hyperresponsiveness to methacholine when administered systemically or by inhalation in cynomolgus macaques but had no effect on airway hyperresponsiveness to methacholine in human patients with mild allergic asthma (49). Clearly there are significant differences observed between species with respect to the effect of IL-13 neutralization on airway hyperresponsiveness to inhaled spasmogens. However, our data in the present study, and previously published data, demonstrate that IL-13 is an important mediator of allergen-induced pulmonary responses across species,

including in humans with allergic asthma. Furthermore, local neutralization of IL-13 is sufficient to inhibit the pulmonary response to allergen.

This study had several limitations; although we believe that the model of allergic asthma used was the most appropriate preclinical model available, further validation in human studies is required to determine the therapeutic potential of CDP7766. The study was also limited by small sample size; however, this did not preclude statistically significant observations. In addition, the duration of treatment in this study was relatively short (5 d);

therefore, we cannot comment on potential longer-term therapeutic effects of CDP7766.

In conclusion, we have demonstrated that inhalation of an anti-IL-13 monoclonal antibody Fab fragment, CDP7766, using the investigational eFlow nebulizer, is efficacious in a model of asthma in cynomolgus macaques, potentially inhibiting allergen-induced lung inflammation and pulmonary responses. To our knowledge, these findings are the first detailing of an inhaled anti-IL-13 monoclonal antibody, with the data providing evidence to support further investigation of inhaled anti-IL-13 therapeutics in the treatment of

chronic inflammatory lung diseases such as asthma. Consequently, a trial has recently been completed, in which the dry powder formulation of CDP7766 (VR942) was evaluated for the treatment of asthma (NCT02473939). ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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References

- Bagnasco D, Ferrando M, Varricchi G, Passalacqua G, Canonica GW. A Critical evaluation of anti-IL-13 and anti-IL-4 strategies in severe asthma. *Int Arch Allergy Immunol* 2016;170:122–131.
- Durham AL, Caramori G, Chung KF, Adcock IM. Targeted anti-inflammatory therapeutics in asthma and chronic obstructive lung disease. *Transl Res* 2016;167:192–203.
- Kau AL, Korenblat PE. Anti-interleukin 4 and 13 for asthma treatment in the era of endotypes. *Curr Opin Allergy Clin Immunol* 2014;14:570–575.
- Doucet C, Brouty-Boyé D, Pottin-Clémenceau C, Canonica GW, Jasmin C, Azzarone B. IL (IL) 4 and IL-13 act on human lung fibroblasts: implication in asthma. *J Clin Invest* 1998;101:2129–2139.
- Richter A, Puddicombe SM, Lordan JL, Bucchieri F, Wilson SJ, Djukanovic R, et al. The contribution of interleukin (IL)-4 and IL-13 to the epithelial-mesenchymal trophic unit in asthma. *Am J Respir Cell Mol Biol* 2001;25:385–391.
- Zhu Z, Homer RJ, Wang Z, Chen Q, Geba GP, Wang J, et al. Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. *J Clin Invest* 1999;103:779–788.
- Saha SK, Berry MA, Parker D, Siddiqui S, Morgan A, May R, et al. Increased sputum and bronchial biopsy IL-13 expression in severe asthma. *J Allergy Clin Immunol* 2008;121:685–691.
- Huang SK, Xiao HQ, Kleine-Tebbe J, Paciotti G, Marsh DG, Lichtenstein LM, et al. IL-13 expression at the sites of allergen challenge in patients with asthma. *J Immunol* 1995;155:2688–2694.
- Bree A, Schlerman FJ, Wadanoli M, Tchistiakova L, Marquette K, Tan XY, et al. IL-13 blockade reduces lung inflammation after *Ascaris suum* challenge in cynomolgus monkeys. *J Allergy Clin Immunol* 2007;119:1251–1257.
- Massier E, Nieves A, Lorec AM, Dupuy P, Pinet D, Pinet C, et al. T-cell activation during exacerbations: a longitudinal study in refractory asthma. *Allergy* 2008;63:1202–1210.
- Boniface S, Koscher V, Mamessier E, El Biase M, Dupuy P, Lorec AM, et al. Assessment of T lymphocyte cytokine production in induced sputum from asthmatics: a flow cytometry study. *Clin Exp Allergy* 2003;33:1238–1243.
- Halim TY, Krauss RH, Sun AC, Takei F. Lung natural helper cells are a critical source of Th2 cell-type cytokines in protease allergen-induced airway inflammation. *Immunity* 2012;36:451–463.
- Brightling CE, Symon FA, Holgate ST, Wardlaw AJ, Pavord ID, Bradding P. IL-4 and -13 expression is co-localized to mast cells within the airway smooth muscle in asthma. *Clin Exp Allergy* 2003;33:1711–1716.
- Berry MA, Parker D, Neale N, Woodman L, Morgan A, Monk P, et al. Sputum and bronchial submucosal IL-13 expression in asthma and eosinophilic bronchitis. *J Allergy Clin Immunol* 2004;114:1106–1109.
- Malavia NK, Mih JD, Raub CB, Dinh BT, George SC. IL-13 induces a bronchial epithelial phenotype that is profibrotic. *Respir Res* 2008;9:27.
- Atherton HC, Jones G, Danahay H. IL-13-induced changes in the goblet cell density of human bronchial epithelial cell cultures: MAP kinase and phosphatidylinositol 3-kinase regulation. *Am J Physiol Lung Cell Mol Physiol* 2003;285:L730–L739.
- Shim JJ, Dabbagh K, Ueki IF, Dao-Pick T, Burgel PR, Takeyama K, et al. IL-13 induces mucin production by stimulating epidermal growth factor receptors and by activating neutrophils. *Am J Physiol Lung Cell Mol Physiol* 2001;280:L134–L140.
- Wenzel SE, Trudeau JB, Barnes S, Zhou X, Cundall M, Westcott JY, et al. TGF-beta and IL-13 synergistically increase eotaxin-1 production in human airway fibroblasts. *J Immunol* 2002;169:4613–4619.
- Kaur D, Hollins F, Woodman L, Yang W, Monk P, May R, et al. Mast cells express IL-13R alpha 1: IL-13 promotes human lung mast cell proliferation and Fc epsilon RI expression. *Allergy* 2006;61:1047–1053.
- Moore PE, Church TL, Chism DD, Panettieri RA Jr, Shore SA. IL-13 and IL-4 cause eotaxin release in human airway smooth muscle cells: a role for ERK. *Am J Physiol Lung Cell Mol Physiol* 2002;282:L847–L853.
- Kasaian MT, Tan XY, Jin M, Fitz L, Marquette K, Wood N, et al. IL-13 neutralization by two distinct receptor blocking mechanisms reduces immunoglobulin E responses and lung inflammation in cynomolgus monkeys. *J Pharmacol Exp Ther* 2008;325:882–892.
- Corren J, Lemanske RF, Hanania NA, Korenblat PE, Parsey MV, Arron JR, et al. Lebrikizumab treatment in adults with asthma. *N Engl J Med* 2011;365:1088–1098.
- Hanania NA, Noonan M, Corren J, Korenblat P, Zheng Y, Fischer SK, et al. Lebrikizumab in moderate-to-severe asthma: pooled data from two randomised placebo-controlled studies. *Thorax* 2015;70:748–756.
- Hanania NA, Korenblat P, Chapman KR, Bateman ED, Kopecky P, Paggiaro P, et al. Efficacy and safety of lebrikizumab in patients with uncontrolled asthma (LAVOLTA I and LAVOLTA II): replicate, phase 3, randomised, double-blind, placebo-controlled trials. *Lancet Respir Med* 2016;4:781–796.
- Piper E, Brightling C, Niven R, Oh C, Faggioni R, Poon K, et al. A phase II placebo-controlled study of tralokinumab in moderate-to-severe asthma. *Eur Respir J* 2013;41:330–338.
- Brightling CE, Chanez P, Leigh R, O'Byrne PM, Korn S, She D, et al. Efficacy and safety of tralokinumab in patients with severe uncontrolled asthma: a randomised, double-blind, placebo-controlled, phase 2b trial. *Lancet Respir Med* 2015;3:692–701.
- Jevševar S, Kusterle M, Kenig M. PEGylation of antibody fragments for half-life extension. *Methods Mol Biol* 2012;901:233–246.

28. Li Z, Krippendorff BF, Sharma S, Walz AC, Lavé T, Shah DK. Influence of molecular size on tissue distribution of antibody fragments. *MAbs* 2016;8:113–119.
29. Lightwood D, O'Dowd V, Carrington B, Veverka V, Carr MD, Tservistas M, et al. The discovery, engineering and characterisation of a highly potent anti-human IL-13 fab fragment designed for administration by inhalation. *J Mol Biol* 2013;425:577–593.
30. Gozzard N, Lightwood D, Tservistas M, Zehentleitner M, Sarkar K, Turner A, et al. Novel inhaled delivery of anti-IL-13 mAb (Fab fragment): preclinical efficacy in allergic asthma [abstract]. *Allergy Immunol* 2017;50:PA4019.
31. Maillet A, Congy-Jolivet N, Le Guellec S, Vecellio L, Hamard S, Courty Y, et al. Aerodynamical, immunological and pharmacological properties of the anticancer antibody cetuximab following nebulization. *Pharm Res* 2008;25:1318–1326.
32. Respaud R, Marchand D, Parent C, Pelat T, Thullier P, Tournamille JF, et al. Effect of formulation on the stability and aerosol performance of a nebulized antibody. *MAbs* 2014;6:1347–1355.
33. Respaud R, Vecellio L, Diot P, Heuzé-Vourc'h N. Nebulization as a delivery method for mAbs in respiratory diseases. *Expert Opin Drug Deliv* 2015;12:1027–1039.
34. Cheng YS, Irshad H, Kuehl P, Holmes TD, Sherwood R, Hobbs CH. Lung deposition of droplet aerosols in monkeys. *Inhal Toxicol* 2008;20:1029–1036.
35. Dubus JC, Vecellio L, De Monte M, Fink JB, Grimbert D, Montharu J, et al. Aerosol deposition in neonatal ventilation. *Pediatr Res* 2005;58:10–14.
36. Quattrin T, Bélanger A, Bohannon NJ, Schwartz SL; Exubera Phase III Study Group. Efficacy and safety of inhaled insulin (Exubera) compared with subcutaneous insulin therapy in patients with type 1 diabetes: results of a 6-month, randomized, comparative trial. *Diabetes Care* 2004;27:2622–2627.
37. Martin PL, Fisher D, Glass W, O'Neil K, Das A, Martin EC, et al. Preclinical safety and pharmacology of an anti-human interleukin-13 monoclonal antibody in normal macaques and in macaques with allergic asthma. *Int J Toxicol* 2008;27:351–358.
38. Tomkinson A, Tepper J, Morton M, Bowden A, Stevens L, Harris P, et al. Inhaled vs subcutaneous effects of a dual IL-4/IL-13 antagonist in a monkey model of asthma. *Allergy* 2010;65:69–77.
39. Ayanoglu G, Desai B, Fick RB Jr, Grein J, de Waal Malefyt R, Mattson J, et al. Modelling asthma in macaques: longitudinal changes in cellular and molecular markers. *Eur Respir J* 2011;37:541–552.
40. Faffe DS, Whitehead T, Moore PE, Baraldo S, Flynt L, Bourgeois K, et al. IL-13 and IL-4 promote TARC release in human airway smooth muscle cells: role of IL-4 receptor genotype. *Am J Physiol Lung Cell Mol Physiol* 2003;285:L907–L914.
41. Grunstein MM, Hakonarson H, Leiter J, Chen M, Whelan R, Grunstein JS, et al. IL-13-dependent autocrine signaling mediates altered responsiveness of IgE-sensitized airway smooth muscle. *Am J Physiol Lung Cell Mol Physiol* 2002;282:L520–L528.
42. Li L, Xia Y, Nguyen A, Lai YH, Feng L, Mosmann TR, et al. Effects of Th2 cytokines on chemokine expression in the lung: IL-13 potently induces eotaxin expression by airway epithelial cells. *J Immunol* 1999;162:2477–2487.
43. Spencer LA, Melo RC, Perez SA, Bafford SP, Dvorak AM, Weller PF. Cytokine receptor-mediated trafficking of preformed IL-4 in eosinophils identifies an innate immune mechanism of cytokine secretion. *Proc Natl Acad Sci USA* 2006;103:3333–3338.
44. Ortega HG, Liu MC, Pavord ID, Brusselle GG, FitzGerald JM, Chetta A, et al.; MENSA Investigators. Mepolizumab treatment in patients with severe eosinophilic asthma. *N Engl J Med* 2014;371:1198–1207.
45. Wenzel S, Castro M, Corren J, Maspero J, Wang L, Zhang B, et al. Dupilumab efficacy and safety in adults with uncontrolled persistent asthma despite use of medium-to-high-dose inhaled corticosteroids plus a long-acting β_2 agonist: a randomised double-blind placebo-controlled pivotal phase 2b dose-ranging trial. *Lancet* 2016;388:31–44.
46. Gundel RH, Wegner CD, Torcellini CA, Clarke CC, Haynes N, Rothlein R, et al. Endothelial leukocyte adhesion molecule-1 mediates antigen-induced acute airway inflammation and late-phase airway obstruction in monkeys. *J Clin Invest* 1991;88:1407–1411.
47. Kasaian MT, Donaldson DD, Tchistiakova L, Marquette K, Tan XY, Ahmed A, et al. Efficacy of IL-13 neutralization in a sheep model of experimental asthma. *Am J Respir Cell Mol Biol* 2007;36:368–376.
48. Gauvreau GM, Boulet LP, Cockcroft DW, Fitzgerald JM, Carlsten C, Davis BE, et al. Effects of interleukin-13 blockade on allergen-induced airway responses in mild atopic asthma. *Am J Respir Crit Care Med* 2011;183:1007–1014.
49. Wenzel S, Wilbraham D, Fuller R, Getz EB, Longphre M. Effect of an interleukin-4 variant on late phase asthmatic response to allergen challenge in asthmatic patients: results of two phase 2a studies. *Lancet* 2007;370:1422–1431.
50. Luo J, Liu D, Liu CT. The efficacy and safety of anti-interleukin 13, a monoclonal antibody, in adult patients with asthma: a systematic review and meta-analysis. *Medicine (Baltimore)* 2016;95:e2556.

Online Data Supplement

Efficacy of an inhaled interleukin-13 antibody fragment in a model of chronic asthma

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Supplementary Materials and Methods

CDP7766 identification

CDP7766 was discovered as described in detail by Lightwood *et al.* (34). CDP7766 is a 50 kDA humanized immunoglobulin-G1 monoclonal antibody Fab fragment. CDP7766 potently neutralized human and cynomolgus macaque interleukin-13 in a HEK-BLUE Signal Transducer and Activator of Transcription-6 cell-based assay (human interleukin-13 CDP7766 IC₅₀ 0.611 ± 0.039 ng/mL; cynomolgus macaque interleukin-13 CDP7766 IC₅₀ 3.187 ± 0.544 ng/mL).

Breath simulation to determine quantity of respirable nebulized CDP7766 for delivery

The breath simulation was configured to replicate the ventilator apparatus used in *in vivo* studies. The nebulizer was placed directly into the inhalation arm of the ventilation circuit as close as possible to the Y-piece. A polypropylene inhalation filter and an inflatable test lung were placed at the outlet of the Y-piece where the endotracheal tube would be placed for *in vivo* studies. During nebulization, the aerosol that was collected on the inhalation filter represented the delivered dose. A polypropylene exhalation filter was placed in the exhalation arm of the ventilator circuit to collect the aerosol that did not enter the endotracheal tube and was lost during the exhalation phase. As CDP7766 could not be recovered directly from the polypropylene filters, albuterol was used as a marker for the determination of the delivered dose. CDP7766 was spiked with albuterol to a concentration of 250 µg/mL and filter extracts were analyzed for albuterol content by reverse phase HPLC (Waters 2695 Separation Module, Detector PDA 2996). The residue left in the nebulizer device as well as the nebulization time required to nebulize the fill volume was also determined.