

Effects of Interleukin-13 Blockade on Allergen-induced Airway Responses in Mild Atopic Asthma

Gail M. Gauvreau¹, Louis-Philippe Boulet², Donald W. Cockcroft³, J. Mark FitzGerald⁴, Chris Carlsten⁴, Beth E. Davis³, Francine Deschesnes², MyLinh Duong¹, Billie L. Durn⁵, Karen J. Howie¹, Linda Hui⁴, Marion T. Kasaian⁵, Kieran J. Killian¹, Tara X. Strinich¹, Richard M. Watson¹, Nathalie Y⁴, Simon Zhou⁵, Donald Raible⁵, and Paul M. O'Byrne¹

¹Department of Medicine, McMaster University, Hamilton, Ontario; ²Institut de Cardiologie et de Pneumologie de l'Université Laval, Hôpital Laval, Quebec City, Quebec; ³Division of Respiratory Medicine, University of Saskatchewan, Saskatoon, Saskatchewan; ⁴Respiratory Medicine, University of British Columbia, Vancouver, British Columbia; and ⁵Clinical Research and Development, Pfizer (formerly Wyeth Research), Philadelphia, Pennsylvania

Rationale: Extensive evidence in animal models supports a role for IL-13 in the pathobiology of asthma. IMA-638 and IMA-026 are fully humanized IgG₁ antibodies that bind to different epitopes and neutralize IL-13 bioactivity.

Objectives: We hypothesized that anti-IL-13 treatment would inhibit allergen-induced late-phase asthmatic responses, airway hyperresponsiveness, and inflammation in subjects with asthma.

Methods: Fifty-six subjects with mild, atopic asthma were recruited for two double-blind, randomized, placebo-controlled, parallel group trials to compare IMA-638 and IMA-026 IL-13 antibody treatments with placebo treatment. Drug was administered on Days 1 and 8, and allergen challenges were performed on Days 14 and 35. The primary outcome variable was the late-phase area under the curve (AUC), and secondary outcome variables were the early- and late-phase maximum percent fall in FEV₁, early AUC, allergen-induced shift in airway hyperresponsiveness, and sputum eosinophils. **Measurements and Main Results:** The treatment difference with IMA-638 on Day 14 was $-19.1 \text{ FEV}_1 \times \text{hour}$ (95% confidence interval: $-36.2, -1.9$) for the allergen-induced early AUC and $-23.8 \text{ FEV}_1 \times \text{hour}$ (95% confidence interval: $-46.4, -1.2$) for the late AUC (both $P < 0.05$), but this effect was lost by Day 35. Treatment with IMA-026 did not attenuate the asthmatic responses on Day 14 or Day 35. There was no effect of either antibody on allergen-induced airway hyperresponsiveness or sputum eosinophils. The frequency of adverse events after administration of the IL-13 antibodies was similar to placebo.

Conclusions: IL-13 has a role in allergen-induced airway responses in humans. Further study is required to determine whether anti-IL-13 monoclonal antibodies will be beneficial clinically.

Clinical trial registered with www.clinicaltrials.gov (NCT 00410280 and NCT 00725582).

Keywords: anti-interleukin-13 therapy; mild asthma; allergen inhalation challenge; late asthmatic response; airway inflammation

AT A GLANCE COMMENTARY

Current Scientific Knowledge on the Subject

Extensive evidence in animal models supports a role for IL-13 in the pathobiology of asthma, and IL-13 blockade is being actively investigated as a therapy for the treatment of asthma.

What This Study Adds to the Field

The current study suggests that blocking binding of IL-13 to the IL-4 receptor α chain attenuates allergen-induced bronchoconstriction during the early and late asthmatic responses.

Asthma is a chronic inflammatory disease of the airways characterized by recurrent episodes of chest tightness and cough, with variable airflow obstruction, mucus hypersecretion, and airway hyperresponsiveness. There is extensive evidence to support a major role for IL-13 in the pathobiology of asthma. *In vitro*, IL-13 induces many cellular responses relevant to asthma, such as B-cell immunoglobulin E (IgE) production, generation of eosinophil chemoattractants, maturation of mucus-secreting goblet cells, production of extracellular matrix proteins and myofibroblast differentiation, and enhanced contractility of airway smooth muscle cells in response to cholinergic agonists (1–5). In animal models, administration of exogenous IL-13 induces airway inflammation and airway hyperresponsiveness changes, and neutralization of IL-13 effectively reduces these changes after allergen inhalation (6, 7). In human subjects with asthma, IL-13 is present in bronchoalveolar lavage fluid collected after segmental allergen challenge (8–10), implicating the cytokine in allergic airway responses. IL-13 is thought to mediate most of its effect through binding to receptors IL-13R α 1 and IL-4R α .

IMA-638 and IMA-026 are fully humanized IgG₁ antibodies that bind to and potentially neutralize IL-13 bioactivity *in vitro* (11). They have similar pharmacokinetic (PK) characteristics in preclinical models (12), and have shown efficacy in sheep (IMA-638) (13) and cynomolgus monkey (IMA-638 and IMA-026) (11, 14) models of allergen challenge. IMA-638 is specific for the IL-13 epitope that binds IL-4R α , whereas IMA-026 is specific for the IL-13 epitope that binds IL-13R α 1 and IL-13R α 2. In the two studies reported here, IMA-638 and IMA-026 were evaluated in a human allergen inhalation challenge model. We hypothesized that anti-IL-13 treatment would inhibit allergen-induced late-phase asthmatic responses, airway hyperresponsiveness, and inflammation in subjects with mild

(Received in original form August 3, 2010; accepted in final form November 5, 2010)

Supported by Pfizer (formerly Wyeth Research) and the AllerGen Network of Centers of Excellence, Clinical Investigator Collaborative.

Author contributions: conception and design: G.M.G., L.P.B., D.W.C., J.M.F., P.M.O., D.R., B.D.; analysis and interpretation: C.C., B.E.D., F.D., M.D., K.J.H., L.H., M.T.K., K.J.K., T.X.S., R.M.W., N.Y., S.Z.; drafting the manuscript for important intellectual content: G.M.G., M.T.K., S.Z., D.R., P.M.O.

Role of the funding source: The study sponsor participated in the trial design, analyses, interpretation, and preparation of this article. The study sponsor was not involved in the decision to submit the article for publication.

Correspondence and requests for reprints should be addressed to Gail Gauvreau, Ph.D., HSC 3U26, McMaster University, 1200 Main St West, Hamilton, ON, L8N 3Z5 Canada. E-mail: gauvreau@mcmaster.ca

Am J Respir Crit Care Med Vol 183, pp 1007–1014, 2011

Originally Published in Press as DOI: 10.1164/rccm.201008-1210OC on November 5, 2010
Internet address: www.atsjournals.org

asthma. Some of the results of these studies have been previously reported in the form of an abstract (15).

METHODS

Subjects

Fifty-six subjects with mild atopic stable asthma were recruited for two clinical trials (NCT 00410280 and NCT 00725582). These subjects were nonsmoking, 18 to 60 years old, with body weight between 50 and 115 kg. FEV₁ was at least 70% of predicted and the provocative concentration of methacholine causing a 20% fall in FEV₁ (methacholine PC₂₀) was not more than 16 mg/ml (Table 1). Subjects had no other lung disease, no self-reported lower respiratory tract infection or worsening of asthma for 4 weeks before screening, and avoided exposure to sensitizing allergens apart from house dust mite. Subjects were not currently using inhaled corticosteroids (ICS) and used no asthma medication with the exception of infrequently inhaled β_2 -agonist, which was withheld for 8 hours before spirometry. Rigorous exercise and caffeinated beverages were avoided before laboratory visits.

Study Design

Two clinical trials were conducted at four study centers. Each trial was designed as double-blind, randomized, placebo-controlled, and with parallel groups, and each compared treatment with a humanized IL-13

antibody (either IMA-638 or IMA-026, 2 mg/kg) to treatment with placebo (Figure 1). The primary outcome variable was the late-phase asthmatic response, and the secondary outcome variables were the early-phase asthmatic response, airway hyperresponsiveness, sputum eosinophils, safety, and tolerability. The trials were performed from April 2007 to March 2008 (IMA-638) and from January 2009 to June 2009 (IMA-026). The randomized sequence of treatment was computer-generated, and treatment kit number was assigned on the day of dosing, using a centralized system. All subjects and trial staff remained blind to the random order until after locking the database, with the exception of the unblinded study nurse who prepared and administered the drug but performed no study assessments.

The study was approved by the ethics research board of the respective institutions, and signed informed consent was obtained from subjects. Screening of subjects occurred over 1–2 weeks with assessments of airway hyperresponsiveness, skin prick test, and safety labs (Figure 1). Responses to inhaled allergen challenge were also measured, and subjects who developed an early-phase asthmatic response (a fall in FEV₁ of at least 20% within 2 h after allergen challenge), followed by a late-phase asthmatic response (a fall in FEV₁ of at least 15% between 3 and 7 h after allergen challenge) were randomized to receive drug or placebo at a 1:1 allocation ratio. Each treatment period consisted of two subcutaneous doses of humanized IL-13 antibody administered 1 week apart. Allergen challenges were conducted 14 and 35 days after the first dose. Methacholine PC₂₀ was measured 24 hours before and 24 hours after allergen challenges. Sputum was collected after the methacholine challenge, and also 7 hours after allergen

TABLE 1. SUBJECT DEMOGRAPHICS

IMA-638 (n = 14)						Placebo (n = 13)					
Subject	Sex	Age (yr)	% FEV ₁	M PC ₂₀	Allergen	Subject	Sex	Age (yr)	% FEV ₁	M PC ₂₀	Allergen
101	F	25	89.5	7.74	HDM (DP)	102	F	40	88.0	0.62	HDM (DP)
105	M	30	101.5	4.00	HDM (DP)	106	M	38	79.3	1.86	Grass
108	M	20	116.2	0.66	Grass	107	M	46	90.0	9.31	HDM (DP)
109	F	26	99.9	3.39	HDM (DP)	110	F	21	86.4	11.43	HDM (DP)
113	F	18	72.8	2.21	Cat	112	F	21	89.2	1.47	Cat
116	M	25	108.5	3.48	HDM (DF)	114	M	19	80.0	1.06	Grass
117	F	23	82.0	0.39	HDM (DP)	115	F	20	78.6	13.87	HDM (DP)
118	F	21	96.8	1.87	Cat	202	M	42	81.5	1.48	HDM (DF)
204	M	30	98.7	11.77	HDM (DP)	208	F	23	84.3	1.24	Cat
206	M	26	95.1	1.62	HDM (DP)	211	F	40	79.7	5.36	Cat
220	F	31	76.1	0.61	GRASS	214	F	19	86.8	2.03	HDM (DF)
223	M	27	101.0	1.26	Grass	218	F	44	111.8	6.92	Cat
301	F	43	82.5	0.08	HDM (DF)	303	M	47	97.5	1.17	Grass
302	M	21	81.2	0.13	Grass						
Mean		26.1	93.0	1.3*		Mean		32.3	87.1	2.7	
SEM		1.7	3.4			SEM		3.2	2.5		

IMA-026 (n = 14)						Placebo (n = 15)					
Subject	Sex	Age (yr)	% FEV ₁	M PC ₂₀	Allergen	Subject	Sex	Age (yr)	% FEV ₁	M PC ₂₀	Allergen
104	F	21	92	4.31	Cat	101	F	27	93	2.46	HDM (DP)
106	M	21	77	2.27	Grass	105	M	22	84	4.30	HDM (DP)
107	M	20	96	0.54	Ragweed	108	F	21	85	0.84	Cat
109	F	20	100	0.47	Cat	110	M	44	104	1.44	Cat
113	F	20	87	1.12	Cat	111	M	19	84	0.91	HDM (DP)
114	M	32	93	6.33	Cat	112	F	50	95	2.21	HDM (DP)
203	F	46	110	3.25	Cat	201	F	41	85	4.59	Cat
205	M	43	85	2.46	HDM (DF)	204	F	57	79	0.25	Cat
207	F	29	89	0.32	Tree	206	M	26	79	0.22	Cat
209	M	32	72	1.24	HDM (DF)	208	F	30	92	0.14	Cat
302	F	56	95	0.16	Cat	213	F	49	103	0.44	HDM (DF)
303	F	44	84	0.28	HDM (DF)	304	M	22	83	0.13	Grass
307	M	48	107	0.90	Tree	311	M	47	71	0.28	Grass
401	M	32	82	1.05	Cat	404	M	21	75	1.30	HDM (DF)
						410	M	26	83	1.54	Grass
Mean		33.1	90.6	1.1*		Mean		33.5	86.5	0.8	
SEM		3.3	2.8			SEM		13.0	9.6		

Definition of abbreviations: % FEV₁ = percent predicted forced expiratory volume in 1 second; DF = *Dermatophagoides farinae*; DP = *Dermatophagoides pteronyssinus*; F = female; HDM = house dust mite; M = male; M PC₂₀ = provocative concentration of methacholine causing a 20% fall in FEV₁.

Data are shown as means $\pm$ SEM, with the exception of M PC₂₀, which is shown as the geometric mean.

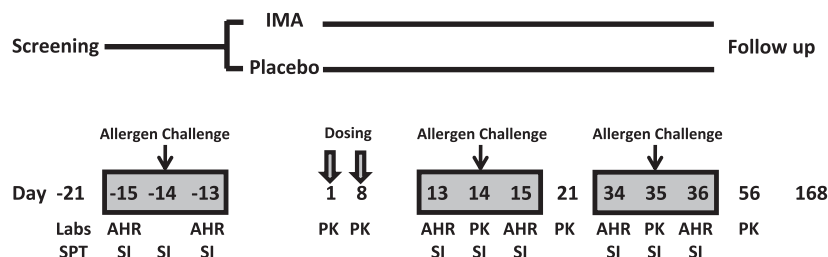


Figure 1. Study schematic. AHR = airway hyperresponsiveness; PK = pharmacokinetics; SI = sputum induction; SPT = skin prick test.

challenge. Blood was collected for pharmacokinetic analysis, eosinophils, IL-13, and IgE levels predosing and until 56 days (IMA-026) and 168 days (IMA-638) after the first dose.

Laboratory Procedures

Study medication. Drug was supplied in a lyophilized form and reconstituted with sterile water into a 100-mg/ml solution by the unblinded study nurse. Two doses of 2 mg/kg were administered subcutaneously approximately 1 week apart, based on the actual body weight of the subject. The total dose of 4 mg/kg was chosen on the basis of results of the phase I trials demonstrating safety and tolerability at doses up to 4 mg/kg. Placebo was the excipient in the antibody formulation.

Methacholine inhalation test. Methacholine inhalation challenge was performed as described by Cockcroft (16), using tidal breathing from a Wright nebulizer (Roxon, Montreal, PQ, Canada). The test was terminated when a fall in FEV₁ of at least 20% of the lowest postsaline value occurred, and the methacholine PC₂₀ was calculated.

Allergen inhalation challenge. Allergen inhalation was performed as described by O'Byrne and colleagues (17). The concentration of allergen extract for inhalation was determined from a formula described by Cockcroft and coworkers (18). During a screening period, doubling concentrations of allergen were given until a fall of at least 20% in FEV₁ at 10 minutes after allergen challenge was reached. The FEV₁ was then measured at regular intervals until 7 hours after allergen inhalation. Subjects with an early- and late-phase asthmatic response were enrolled. The same dose of allergen was to be administered for the two postdosing allergen challenges on Day 14 and Day 35.

Sputum analysis. Sputum was induced and processed according to the method described by Pizzichini and coworkers (19). The total cell count was determined with a Neubauer hemocytometer chamber (Hausser Scientific, Blue Bell, PA) and expressed as the number of cells per milliliter of sputum. Cells were prepared on glass slides for differential counts and stained with Diff-Quik (American Scientific Products, McGaw Park, IL). One of the investigative sites was designated to perform centralized inflammatory cell counting for all of the sites. All sputum samples were used regardless of squamous cell contamination; however, the cell percentage was based only on the inflammatory cells and airway epithelial cells.

Peripheral blood analyses. Blood samples were collected to measure total IgE, and blood eosinophils. Serum samples were analyzed for IMA-638 and IMA-026 levels and anti-IMA-638 and anti-IMA-026 antibodies by validated ELISA. Serum maximum concentration (C_{max}) and the time to attain C_{max} (t_{max}) were determined directly from the observed data. Last-dose PK parameters were derived from the serum concentration versus time data by a noncompartmental PK analysis method implemented in WinNonlin (version 5; Pharsight, Mountain View, CA). Serum samples were analyzed for IL-13 levels by a validated immunoassay (20).

Statistical Analysis

No formal sample size calculations were performed because this is a proof-of-concept study. The sample size was largely determined on the basis of feasibility and previous experience showing that 15 subjects per treatment arm are sufficient to evaluate efficacy of drug on late-phase bronchoconstriction (21). Data were analyzed from the intent-to-treat population and are presented as means $\pm$ standard error of the mean (SEM) or standard deviation (SD) as indicated. Methacholine

PC₂₀ was calculated by linear interpolation of the log dose-response curves and log-transformed to fit a normal distribution before statistical analysis, and is presented as geometric mean $\pm$ range. The primary analysis was the treatment comparison of the area under the curve (AUC) for the late-phase asthmatic response between active drug and placebo. Secondary analyses were conducted on the maximum percent fall of the early- and late-phase asthmatic responses, the AUC for the early response, the allergen-induced shift in methacholine PC₂₀, and sputum eosinophil counts. The differences from pre- to postallergen challenge were computed, and drug and placebo were compared by analysis of covariance with predose data (collected during the screening period) as the baseline covariate, and treatment as a factor.

RESULTS

Twenty-seven subjects were enrolled into the IMA-638 study (IMA-638, $n = 14$; placebo, $n = 13$) and all completed the study. Twenty-nine subjects were enrolled into the IMA-026 study (IMA-026, $n = 14$; placebo, $n = 15$) (Table 1). Two subjects randomized to placebo did not complete the IMA-026 study; one was withdrawn on Day 1 with urticaria, and one was withdrawn on Day 15 because of an asthma exacerbation.

IMA-638 and IMA-026 Pharmacokinetics

The serum concentration-time profiles of IMA-638 and IMA-026 in these allergen challenge studies were consistent with their respective single-dose pharmacokinetics. The terminal elimination half-life for IMA-638 and IMA-026 after the last and second dose was 25.1 days (± 4.7 SD) and 26.1 days (± 6.3 SD), respectively (Figure 2). The C_{max} after the second dose was 30.5 $\mu\text{g/ml}$ (± 6.4 SD) and 28.3 $\mu\text{g/ml}$ (± 5.0 SD), and t_{max} after the second dose was 8.5 days (± 4.4 SD) and 8.5 days (± 3.5 SD), for IMA-638 and IMA-026, respectively, consistent with the respective t_{max} observed in single ascending dose studies. After the two subcutaneous doses on Days 1 and 8, the highest drug concentration was observed approximately on Day 14 (8 d after the second dose), on the day of the first allergen challenge. Overall the 2×2 mg/kg PK profile of the two IMA antibodies was similar in both studies.

Allergen-induced Asthmatic Responses

Eleven subjects did not receive the highest dose of allergen as determined during the screening challenge, mainly for safety reasons. Administering lower doses of allergen to these subjects did not influence the measures of efficacy because this occurred in a manner resulting in an overall smaller challenge in the placebo groups.

IMA-638 significantly attenuated the early maximum percent fall in FEV₁ and the early-phase AUC (AUC_{0-2h}) during the early-phase asthmatic response on Day 14 ($P < 0.05$), but this effect was lost on Day 35. In addition, IMA-638 significantly inhibited the late-phase AUC (AUC_{3-7h}) measured on Day 14 ($P < 0.05$). The maximum percent fall in FEV₁ during the late-phase asthmatic response was reduced by IMA-638 on Day 14, but this

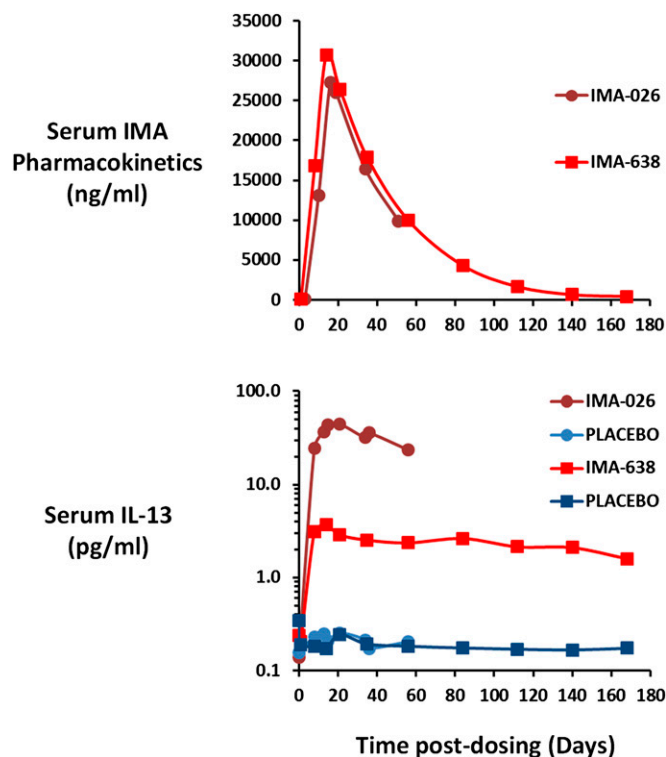


Figure 2. Mean serum pharmacokinetics (PK) and IL-13 levels measured before and after dosing with IMA-026, IMA-638, and placebo.

was not significantly different from placebo ($P = 0.09$). The treatment difference is shown in Table 2. The observed differences between IMA-638 and placebo on Day 14 are not due to lower allergen doses because only subjects in the placebo group did not receive full doses of allergen. Had these subjects received full doses of allergen, the treatment difference between IMA-638 and placebo could have been even greater. Even though IMA-026 attenuated the late-phase asthmatic response compared with placebo at the final time points, these changes did not reach statistical significance. IMA-026 did not inhibit the early-phase asthmatic response (Figure 3 and Table 2).

TABLE 2. EARLY- AND LATE-PHASE ASTHMATIC RESPONSES

Phase	Units	Day	IMA-638	Placebo	Treatment Difference	P Value	95% Confidence Interval
Early	Maximum % fall	14	-20.4	-30.2	-9.8*	0.04	-19.16, -0.35
		35	-22.8	-26.8	-4.1	0.39	-13.5, 5.4
	AUC _{0-2h} (FEV ₁ × h)	14	21.5	40.6	-19.1*	0.03	-36.2, -1.9
		35	25.8	38.9	-13.2	0.13	-30.3, 4.0
Late	Maximum % fall	14	-12.8	-19.3	-6.4	0.09	-14.0, 1.2
		35	-11.3	-15.6	-4.2	0.27	-11.8, 3.4
	AUC _{3-7h} (FEV ₁ × h)	14	25.5	49.3	-23.8*	0.04	-46.4, -1.2
		35	21.9	39.1	-17.2	0.13	-39.7, 5.4
Phase	Units	Day	IMA-026	Placebo	Treatment Difference	P Value	95% Confidence Interval
Early	Maximum % fall in FEV ₁	14	-29.8	-27.5	2.4	0.65	-8.1, 12.9
		35	-30.2	-30.6	-0.4	0.94	-10.8, 10.0
	AUC _{0-2h} (FEV ₁ × h)	14	40.6	39.0	1.5	0.88	-18.3, 21.3
		35	41.4	44.1	-2.6	0.79	-22.2, 17.0
Late	Maximum % fall in FEV ₁	14	-16.4	-20.9	-4.5	0.31	-13.4, 4.4
		35	-15.8	-22.1	-6.3	0.16	-15.1, 2.5
	AUC _{3-7h} (FEV ₁ × h)	14	37.3	46.2	-8.8	0.52	-36.3, 18.7
		35	34.7	51.4	-16.8	0.22	-44.0, 10.5

Definition of abbreviations: AUC = area under the curve; AUC_{0-2h}, AUC_{3-7h} = early- and late-phase AUC measurements, respectively.

* $P < 0.05$.

Airway Hyperresponsiveness

The PC₂₀ values for most subjects decreased after each allergen challenge; however, there was no difference between the treatment groups in terms of PC₂₀ values or the change from preallergen challenge to postallergen challenge for any of the time points examined in either study (Figure 4). Furthermore, there were no statistically significant treatment differences in the log₂ PC₂₀ values of IMA-638 or IMA-026 on allergen-induced airway hyperresponsiveness.

Sputum Inflammatory Cells

Sputum eosinophils increased significantly both 7 and 24 hours after allergen challenge with placebo treatment ($P < 0.0001$). There was no effect of either IMA-638 or IMA-026 on sputum eosinophils (Figure 5) or any other sputum cell types after allergen challenge ($P > 0.05$).

IL-13 Levels in Serum

Total serum IL-13 (free and IMA-bound) concentration-time profiles are shown in Figure 2. IL-13 concentrations increased after IMA-638 and IMA-026, but not placebo, administration. This increase in IL-13 is most likely due to an increase in bound (although inactive) IL-13. Because the IL-13 assay detects both free IL-13 and IL-13 bound to the IMA antibodies, the free IL-13 levels are much lower in the anti-IL-13-treated subjects than in the placebo subjects. The observed elevation of IL-13 levels during IMA-026 treatment was much higher than with IMA-638 treatment.

Eosinophils, Total and Specific IgE in Blood

There was no effect of IMA-638 or IMA-026 on the levels of eosinophils or total IgE measured in peripheral blood at any time point from Day 8 to the final postdose evaluation.

Safety

Hematology, chemistry, and urinalysis were similar across the treatment groups. There was no clinically significant change in safety labs, electrocardiograms, or vital signs after treatment of IMA-638 or IMA-026. Sixteen treatment-emergent adverse events (AEs) were reported by 7 subjects (50%) dosed with IMA-638, and 15 AEs were reported in 8 subjects (62%) dosed with the corresponding placebo treatment. Fifteen AEs were

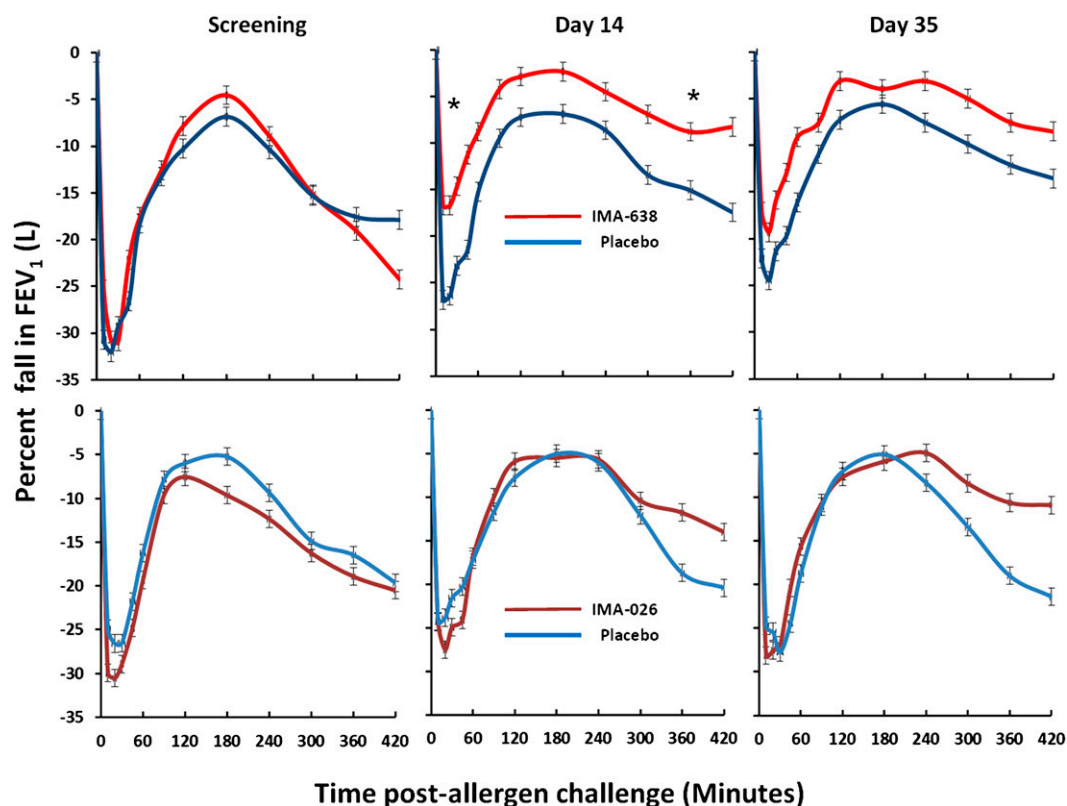


Figure 3. The allergen-induced maximum percent fall in FEV₁ shown during the screening period, and again at 14 and 35 days postdosing with IMA-026, IMA-638, and placebo. Data are shown as means ± SEM. **P* < 0.05.

reported by 9 subjects (60%) dosed with IMA-026, and 11 AEs were reported in 5 subjects (36%) dosed with the corresponding placebo treatment. Upper airway infections, injection site erythema and irritation, and pharyngolaryngeal pain were the most commonly reported AEs, with no apparent difference in

frequencies between active drug and placebo. All AEs were considered mild in severity and of short duration. There were no serious adverse events reported in either study. All serum samples collected had negative anti-IMA serum titers (<1.40 units) except for one subject receiving IMA-026, who had

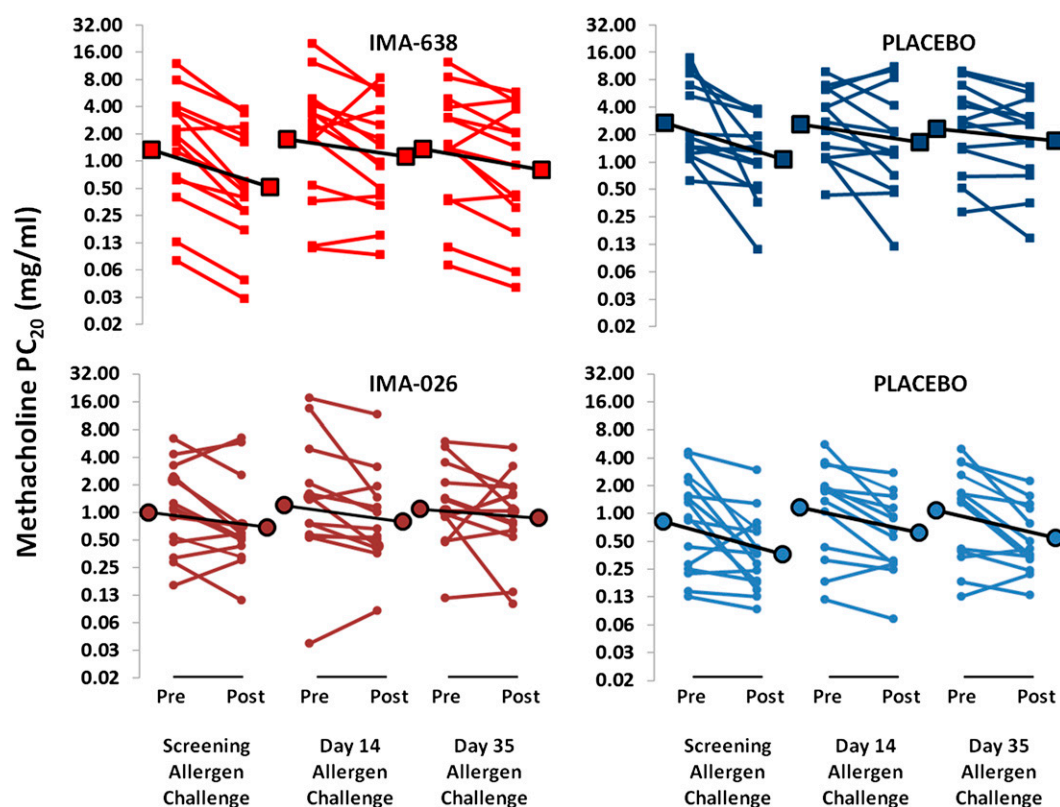


Figure 4. Airway hyperresponsiveness measured as methacholine PC₂₀ (provocative concentration of methacholine causing a 20% fall in FEV₁) before and after allergen challenges during the screening period, and again at 14 and 35 days postdosing with IMA-026, IMA-638, and placebo. Individual and group mean data are shown.

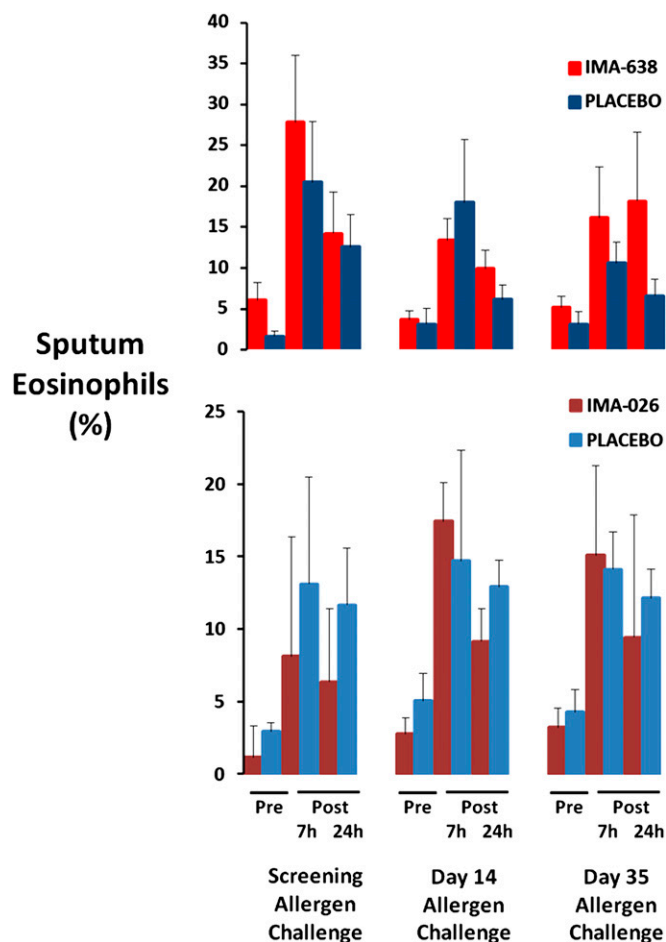


Figure 5. Allergen-induced sputum eosinophils during the screening period, and again at 14 and 35 days postdosing with IMA-026, IMA-638, and placebo. Data are shown as means $\pm$ SEM.

a single positive serum titer on Day 21 of 1.85 units. Subsequent analysis showed no neutralizing antibodies present.

DISCUSSION

Our investigations compared the efficacy of two different anti-IL-13 monoclonal antibodies, IMA-638 and IMA-026, in an allergen challenge model of human allergic asthma. These antibodies target different epitopes of human IL-13 with similar affinity constants, and both are potent neutralizers of IL-13 bioactivity *in vitro* (11). IMA-638 treatment significantly reduced both early and late asthmatic responses after allergen challenge, to a similar degree as leukotriene antagonists (22). This is in contrast to ICS, which do not suppress early asthmatic responses but completely abolish late asthmatic responses (22). IMA-026 appeared to reduce the late-phase asthmatic response, but the reduction did not reach statistical significance. These findings demonstrate the potential utility of targeting IL-13 for the treatment of allergen-induced airway responses.

IMA-638 and IMA-026 are directed toward distinct, non-overlapping epitopes of human IL-13, and differentially inhibit the binding of IL-13 to IL-13R α 1 and IL-4R α chains of the receptor. IMA-638 blocks binding of IL-13 to IL-4R α , but allows IL-13 interaction with IL-13R α 1, whereas IMA-026 prevents the interaction of IL-13 with IL-13R α 1. In the current study, IMA-638 and IMA-026 displayed comparable PK char-

acteristics, with a long circulating half-life of 25–26 days, but much higher titers of IL-13 (free plus bound) were detected in the circulation after dosing with IMA-026 as compared with IMA-638. This observation could be explained by differences in clearance of IL-13 complexed to IMA-638 and IMA-026. Mechanisms for the clearance of IL-13 *in vivo* are not well understood, but may involve interaction with IL-13R α 2 (23), which binds IL-13 with high affinity and internalizes the cytokine (24). IL-13R α 2 is thought to act primarily as a natural antagonist and decoy (25), with potential signaling activity under some conditions (26). Humans lack the soluble form of IL-13R α 2, but inducibly express cell surface IL-13R α 2 on a range of cell types (27, 28). If IL-13R α 2 has a role in mediating cytokine clearance, this could account for the observed difference in circulating IL-13 concentrations.

IMA-638 reduced both early- and late-phase asthmatic responses. The late-phase asthmatic response involves leukocyte influx to the lung, along with increased vascular permeability (29). In human allergen challenge studies, an IL-4 variant also showed reduced late-phase asthmatic responses (30), consistent with a role for IL-4 and IL-13 in the late-phase reaction. In contrast, the early-phase asthmatic response to allergen challenge is due to the rapid release of preformed mediators, including histamine, prostaglandins, and leukotrienes (29). An effect of IMA-638 on early-phase bronchoconstriction had been seen preclinically in a sheep model (13), but the mechanism for this effect is not clear. IL-13 could play a role in early-phase bronchoconstriction by directly inducing smooth muscle contractility (31), enhancing the contractile response to leukotrienes (32), modulating the production of leukotrienes or expression of leukotriene receptors (33), or priming mast cells for increased Fc ϵ R1 and histamine release (34). An effect of IMA-638 but not IMA-026 suggests that blocking signaling through IL-4R α may be more effective on allergen-induced early- and late-phase bronchoconstriction than in blocking signaling through IL-13R α 1. Although IMA-638 blocks IL-13 interaction with IL-4R α , both antibodies were directed against IL-13 cytokine and not IL-13R α 1/IL-4R shared with IL-4, and have no neutralization activity against IL-4 (11).

The effect of IMA-638 was limited to the inhibition of the early- and late-phase asthmatic FEV₁ response. There was no effect of either anti-IL-13 antibody on airway hyperresponsiveness (AHR) to methacholine, blood or sputum eosinophils, or total IgE. The lack of an effect on AHR was surprising, based on the extensive preclinical literature demonstrating that IL-13 antagonists attenuate allergen-induced AHR in animal models (6, 7). It was also surprising that neither anti-IL-13 antibody inhibited allergen-induced airway inflammation, as shown previously in cynomolgus monkeys (11, 14). Although the number of sputum samples available for assessment was low, we observed no patterns in blood or airways to indicate efficacy. It is not known, however, what effect long-term IL-13 antagonism would have on AHR or inflammation in chronic asthma. These results also highlight a lack of correlation that can exist between animal models of asthma and human asthma.

In other studies, in which patients with uncontrolled asthma, who were receiving ICS or ICS and long-acting inhaled β_2 -agonists (LABAs) were treated with IMA-638 (35) or AMG 317, an IL-4R antagonist (36) for 12 weeks, there was no effect on symptoms, lung function, or time to exacerbation compared with placebo (35, 36). The differences in the results between the data reported here and the 12-week chronic asthma studies might reflect differences in the patient populations (i.e., subjects with stable mild asthma receiving only short-acting inhaled β_2 -agonists in the current studies, and subjects with uncontrolled moderate-severe asthma receiving ICS or ICS/LABAs in the

chronic asthma studies). Allergen inhalation challenge is a clinical model of a mild asthma exacerbation, so another possibility is that IMA-638 might have had an effect on a different end point such as asthma exacerbations, similar to what has been reported for the anti-IL-5 antibody mepolizumab (37, 38), and that would require a study longer than 12 weeks in duration to establish.

We conclude that IL-13 has a role in causing allergen-induced airway responses in humans. Further study is required to determine whether anti-IL-13 monoclonal antibodies will be beneficial in chronic asthma.

Author Disclosure: G.M.G. was a consultant for Altair Therapeutics (up to \$1,000). She was on the Board or Advisory Board for Aim Therapeutics (\$1,001–\$5,000) and received grant support from Genentech, Schering Plough, and MedImmune (\$10,001–\$50,000). L.-P.B. was on the Board or Advisory Board for AstraZeneca (AZ), GlaxoSmithKline (GSK), and Novartis (\$1,001–\$5,000). He received lecture fees from AZ, GSK, Merck Frosst, and Novartis (\$1,001–\$5,000). He received grant support from Alexion, Wyeth, MedImmune, Cepion, Boehringer Ingelheim (BI), Altair, Asmacure Ltée, GSK, Genentech, Schering, AllerGen CIC (more than \$100,001), NIOSH (\$50,001–\$100,000), and CIHR (\$10,001–\$50,000). He was Research Chair of Knowledge Transfer for AZ and GSK (\$50,001–\$100,000). D.W.C. received lecture fees from AZ Canada, GSK Canada (up to \$1,000), and Pharmaxis (\$1,001–\$5,000). He received grant support from BI Canada, Genentech, Wyeth, Altair (more than \$100,001), and Genentech (\$50,001–\$100,000). J.M.F. received grant support from Wyeth (more than \$100,001). C.C. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. B.E.D. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. F.D. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. M.D. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. B.L.D. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. K.J.H. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. L.H. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. M.T.K. is a full-time employee for Pfizer (formerly Wyeth). K.J.K. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. T.X.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. R.M.W. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. N.Y. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. S.Z. is a full-time employee of Pfizer. D.R. was employed by Wyeth Research and he owned stock or options of Wyeth/Pfizer. P.M.O. was a consultant for AZ (\$10,001–\$50,000). He was on the Board or Advisory Board for Actelion (\$1,001–\$5,000), Asmacure, GSK, Merck, and Topigen (\$5,001–\$10,000). He received lecture fees from AZ (\$10,001–\$50,000), Chiesi (\$5,001–\$10,000), and Ono Pharma (\$1,001–\$5,000). He received grant support from Altair, AZ, Genentech, GSK, Wyeth, MedImmune, Merck, and Pfizer (more than \$100,001).

References

- de Vries JE. The role of IL-13 and its receptor in allergy and inflammatory responses. *J Allergy Clin Immunol* 1998;102:165–169.
- Chibana K, Ishii Y, Asakura T, Fukuda T. Up-regulation of cysteinyl leukotriene 1 receptor by IL-13 enables human lung fibroblasts to respond to leukotriene C₄ and produce eotaxin. *J Immunol* 2003;170:4290–4295.
- Laoukili J, Perret E, Willems T, Minty A, Parthoens E, Houcine O, Coste A, Jorissen M, Marano F, Caput D, et al. IL-13 alters mucociliary differentiation and ciliary beating of human respiratory epithelial cells. *J Clin Invest* 2001;108:1817–1824.
- Hashimoto S, Gon Y, Takeshita I, Maruoka S, Horie T. IL-4 and IL-13 induce myofibroblastic phenotype of human lung fibroblasts through c-Jun NH₂-terminal kinase dependent pathway. *J Allergy Clin Immunol* 2001;107:1001–1008.
- Grunstein MM, Hakonarson H, Leiter J, Chen M, Whelan R, Grunstein JS, Chuang S. 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.
- Finkelman FD, Hogan SP, Hershey GK, Rothenberg ME, Wills-Karp M. Importance of cytokines in murine allergic airway disease and human asthma. *J Immunol* 2010;184:1663–1674.
- Kasaian MT, Miller DK. IL-13 as a therapeutic target for respiratory disease. *Biochem Pharmacol* 2008;76:147–155.
- Huang SK, Xiao HQ, Kleine-Tebbe J, Paciotti G, Marsh DG, Lichtenstein LM, Liu MC. IL-13 expression at the sites of allergen challenge in patients with asthma. *J Immunol* 1995;155:2688–2694.
- Batra V, Musani AI, Hastie AT, Khurana S, Carpenter KA, Zangrilli JG, Peters SP. Bronchoalveolar lavage fluid concentrations of transforming growth factor (TGF)- β ₁, TGF- β ₂, interleukin (IL)-4 and IL-13 after segmental allergen challenge and their effects on α -smooth muscle actin and collagen III synthesis by primary human lung fibroblasts. *Clin Exp Allergy* 2004;34:437–444.
- Prieto J, Lensmar C, Roquet A, van der Ploeg I, Gigliotti D, Eklund A, Grunewald J. Increased interleukin-13 mRNA expression in bronchoalveolar lavage cells of atopic patients with mild asthma after repeated low-dose allergen provocations. *Respir Med* 2000;94:806–814.
- Kasaian MT, Tan XY, Jin M, Fitz L, Marquette K, Wood N, Cook TA, Lee J, Widom A, Agostinelli R, et al. Interleukin-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.
- Vugmeyster Y, Szklut P, Tchistiakova L, Abraham W, Kasaian M, Xu X. Preclinical pharmacokinetics, interspecies scaling, and tissue distribution of humanized monoclonal anti-IL-13 antibodies with different IL-13 neutralization mechanisms. *Int Immunopharmacol* 2008;8:477–483.
- Kasaian MT, Donaldson DD, Tchistiakova L, Marquette K, Tan XY, Ahmed A, Jacobson BA, Widom A, Cook TA, Xu X, et al. Efficacy of IL-13 neutralization in a sheep model of experimental asthma. *Am J Respir Cell Mol Biol* 2007;36:368–376.
- Bree A, Schlerman FJ, Wadanoli M, Tchistiakova L, Marquette K, Tan XY, Jacobson BA, Widom A, Cook TA, Wood N. IL-13 blockade reduces lung inflammation after *Ascaris suum* challenge in cynomolgus monkeys. *J Allergy Clin Immunol* 2007;119:1251–1257.
- Gauvreau GM, Boulet L-P, Fitzgerald JM, Durn BL, Zhou S, Burt D, Watson RM, Duong D, Killian KJ, Carlsten C, et al. The effects of IMA-638 on allergen induced airway responses in subjects with mild atopic asthma [abstract]. *Eur Respir J* 2008;32:827s.
- Cockcroft DW. Measure of airway responsiveness to inhaled histamine or methacholine; method of continuous aerosol generation and tidal breathing inhalation. In: Hargreave FE, Woolcock AJ, editors. Airway responsiveness: measurement and interpretation. Mississauga, ON, Canada: Astra Pharmaceuticals Canada Ltd; 1985. pp. 22–28.
- O'Byrne PM, Dolovich J, Hargreave FE. Late asthmatic responses. *Am Rev Respir Dis* 1987;136:740–751.
- Cockcroft DW, Murdock KY, Kirby J, Hargreave F. Prediction of airway responsiveness to allergen from skin sensitivity to allergen and airway responsiveness to histamine. *Am Rev Respir Dis* 1987;135:264–267.
- Pizzichini E, Pizzichini MM, Efthimiadis A, Evans S, Morris MM, Squillace D, Gleich GJ, Dolovich J, Hargreave FE. Indices of airway inflammation in induced sputum: reproducibility and validity of cell and fluid-phase measurements. *Am J Respir Crit Care Med* 1996;154:308–317.
- St Ledger K, Agee SJ, Kasaian MT, Forlow SB, Durn BL, Minyard J, Lu QA, Todd J, Vesterqvist O, Burczynski ME. Analytical validation of a highly sensitive microparticle-based immunoassay for the quantitation of IL-13 in human serum using the Erenna immunoassay system. *J Immunol Methods* 2009;350:161–170.
- Gauvreau GM, Watson RM, Rerecich TJ, Baswick E, Inman MD, O'Byrne PM. Repeatability of allergen-induced airway inflammation. *J Allergy Clin Immunol* 1999;104:66–71.
- Leigh R, Vethanayagam D, Yoshida M, Watson RM, Rerecich T, Inman MD, O'Byrne PM. Effects of montelukast and budesonide on airway responses and airway inflammation in asthma. *Am J Respir Crit Care Med* 2002;166:1212–1217.
- Arima K, Umeshita-Suyama R, Sakata Y, Akaiwa M, Mao XQ, Enomoto T, Dake Y, Shimazu S, Yamashita T, Sugawara N, et al. Upregulation of IL-13 concentration *in vivo* by the IL13 variant associated with bronchial asthma. *J Allergy Clin Immunol* 2002;109:980–987.
- Kawakami K, Taguchi J, Murata T, Puri RK. The interleukin-13 receptor α 2 chain: an essential component for binding and internalization but not for interleukin-13-induced signal transduction through the STAT6 pathway. *Blood* 2001;97:2673–2679.
- Feng N, Lugli SM, Schnyder B, Gauchat JF, Graber P, Schlagenhauf E, Schnarr B, Wiederkehr-Adam M, Duschl A, Heim MH, et al. The interleukin-4/interleukin-13 receptor of human synovial fibroblasts: overexpression of the nonsignaling interleukin-13 receptor α 2. *Lab Invest* 1998;78:591–602.
- Fichtner-Feigl S, Strober W, Kawakami K, Puri RK, Kitani A. IL-13 signaling through the IL-13 α 2 receptor is involved in induction of TGF- β ₁ production and fibrosis. *Nat Med* 2006;12:99–106.

27. O'Toole M, Legault H, Ramsey R, Wynn TA, Kasaian MT. A novel and sensitive ELISA reveals that the soluble form of IL-13R- α 2 is not expressed in plasma of healthy or asthmatic subjects. *Clin Exp Allergy* 2008;38:594–601.
28. Chen W, Sivaprasad U, Tabata Y, Gibson AM, Stier MT, Finkelman FD, Hershey GK. IL-13R α 2 membrane and soluble isoforms differ in humans and mice. *J Immunol* 2009;183:7870–7876.
29. O'Byrne PM, Gauvreau GM, Brannan JD. Provoked models of asthma: what have we learnt? *Clin Exp Allergy* 2009;39:181–192.
30. 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.
31. Shore SA. Direct effects of Th2 cytokines on airway smooth muscle. *Curr Opin Pharmacol* 2004;4:235–240.
32. Eum SY, Maghni K, Tolloczko B, Eidelman DH, Martin JG. IL-13 may mediate allergen-induced hyperresponsiveness independently of IL-5 or eotaxin by effects on airway smooth muscle. *Am J Physiol Lung Cell Mol Physiol* 2005;288:L576–L584.
33. Elias JA, Lee CG, Zheng T, Shim Y, Zhu Z. Interleukin-13 and leukotrienes: an intersection of pathogenetic schema. *Am J Respir Cell Mol Biol* 2003;28:401–404.
34. Kaur D, Hollins F, Woodman L, Yang W, Monk P, May R, Bradding P, Brightling CE. Mast cells express IL-13R α 1: IL-13 promotes human lung mast cell proliferation and Fc ϵ RI expression. *Allergy* 2006;61:1047–1053.
35. Study evaluating the effect of IMA-638 in subjects with persistent asthma: ClinicalTrials.gov Identifier: NCT 00425061.
36. Corren J, Busse W, Meltzer EO, Mansfield L, Bensch G, Fahrenholz J, Wenzel SE, Chon Y, Dunn M, Weng HH, *et al.* A randomized, controlled, phase 2 study of AMG 317, an IL-4R α antagonist, in patients with asthma. *Am J Respir Crit Care Med* 2010.
37. Nair P, Pizzichini MM, Kjarsgaard M, Inman MD, Efthimiadis A, Pizzichini E, Hargreave FE, O'Byrne PM. Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. *N Engl J Med* 2009;360:985–993.
38. Haldar P, Brightling CE, Hargadon B, Gupta S, Monteiro W, Sousa A, Marshall RP, Bradding P, Green RH, Wardlaw AJ, *et al.* Mepolizumab and exacerbations of refractory eosinophilic asthma. *N Engl J Med* 2009; 360:973–984.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.