The effects of lebrikizumab in patients with mild asthma following whole lung allergen challenge

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Summary

Background Interleukin 13 (IL13) is a T-helper type 2 (Th2) cytokine associated with inflammation and pathology in allergic diseases such as bronchial asthma. We have shown that treatment with lebrikizumab, an anti-IL13 monoclonal antibody, significantly improves prebronchodilator forced expiratory volume in 1 s (FEV_1) in a subset of subjects with uncontrolled asthma.

Objective To evaluate efficacy and safety of lebrikizumab in subjects with mild asthma who underwent bronchial allergen challenge.

Methods Twenty-nine subjects were randomized 1 : 1–5 mg/kg lebrikizumab ($n = 13$) or placebo ($n = 16$) administered subcutaneously every 4 weeks over 12 weeks, a total of four doses. Primary efficacy outcome was late asthmatic response (LAR) at Week 13, defined as area under the curve of FEV_1 measured 2–8 h following inhaled allergen challenge. Serum biomarkers were measured to verify IL13 pathway inhibition and identify patients with an increased response to lebrikizumab.

Results At Week 13, the LAR in lebrikizumab subjects was reduced by 48% compared with placebo subjects, although this was not statistically significant (95% confidence interval, −19%, 90%). Exploratory analysis indicated that lebrikizumab-treated subjects with elevated baseline levels of peripheral blood eosinophils, serum IgE, or periostin exhibited a greater reduction in LAR compared with subjects with lower baseline levels of these biomarkers. Lebrikizumab exerted systemic effects on markers of Th2 inflammation, reducing serum immunoglobulin E (IgE), chemokine ligands 13 and 17 by approximately 25% ($P < 0.01$). Lebrikizumab was well tolerated.

Conclusion and Clinical Relevance Lebrikizumab reduced the LAR in subjects with mild asthma. Clinical trial number NCT00781443.

Introduction

Interleukin 13 (IL13) is a multifunctional cytokine involved in T-helper type 2 (Th2) inflammation. It is produced by activated T cells and natural killer T cells and released from cytoplasmic granules upon degranulation of activated basophils, mast cells, and eosinophils. Preclinical models have demonstrated a dominant role for IL13 in the pathogenesis of allergic airway inflammation. Furthermore, IL13 mRNA and protein

levels are detectable at elevated levels in the airways of patients with asthma [1–3]. In subjects with mild asthma following local lung allergen challenge, active secretion of IL13 in the airway was shown during the late asthmatic phase [4]. Huang and colleagues (2008) also noted that there was a significant up-regulation of IL13 transcripts and IL13 protein following allergen challenge [5]. These studies provide significant evidence that IL13 is involved in the regulation of allergen-induced inflammatory response. As such, IL13 blockade is an attractive therapeutic approach for the treatment for bronchial asthma.

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Lebrikizumab is a novel humanized stabilized immunoglobulin G (IgG) four monoclonal antibody exhibiting high binding affinity to human IL13 [6]. Lebrikizumab was demonstrated to be effective in improving prebronchodilator forced expiratory volume in 1 s (FEV_1) in patients with uncontrolled moderate–severe asthma [7]. In this study, we evaluated the safety and efficacy of lebrikizumab in the context of a bronchial allergen challenge in subjects with mild asthma. Bronchial allergen challenge studies in patients with mild asthma conducted prior to the initiation of larger asthma trials [8] can be useful to test exploratory hypotheses and evaluate mechanism of action of therapeutics. The primary outcome measures of allergen challenge studies reflect the acute changes in airway function in response to a specific allergic stimulus and hence allow for more in depth mechanistic assessments of experimental asthma therapies that target components of allergic inflammation.

Pitrakinra [9], a non-signalling IL4 mutein that binds to IL4Rα, and IMA-638 [10], a humanized IgG1 monoclonal antibody to IL13 significantly reduced the late-phase allergen response in mild asthmatics. Furthermore, genetic analysis of the patients in this small study suggested that variants in the IL4Rα may have enriched for treatment response [11].

An emerging body of evidence suggests that what is currently defined as asthma is pathophysiologically heterogeneous. In particular, Th2-driven inflammation as manifested by atopy and/or eosinophilic infiltration of the airways is evident in only a subset of asthma patients [12–17]. In a previous study with lebrikizumab, increased pretreatment serum periostin levels appeared to identify patients in which IL13 is a prominent driver of disease as a greater improvement in FEV_1 when compared to patients with lower pretreatment serum periostin levels [7]. Also, AMG-317, a humanized monoclonal anti-IL4Rα antibody, showed clinically significant activity only in a subset of patients with the most uncontrolled or symptomatic asthma [18]. Non-invasive biomarkers will be instrumental to further understand the heterogeneity of asthma and to assess and predict the potential effects of targeted therapies. Biomarkers associated with Th2 inflammation that are increased in the airways and blood of asthma patients include eosinophils, as well as serum immunoglobulin E (IgE), periostin [18, 19], chemokine ligands (CCL)13 [20], CCL17 [21] cartilage glycoprotein-39 (HC gp-39) (YKL-40) [22], and carcinoembryonic antigen-related cell adhesion molecule 5 (CEA) [23, 24].

Methods

Study design

This phase II, multi-centre, randomized, double-blind, parallel-group, placebo-controlled trial was designed to evaluate safety and efficacy of lebrikizumab compared with placebo in reducing the airway reaction to an inhaled aeroallergen solution in adult subjects with mild allergic asthma. The study included a 21-day screening period (Days −21 to −1) during which an allergen challenge was performed followed by a methacholine challenge 18–24 h later. Screening was followed by a 12-week treatment period (Weeks 0–12), during which subjects received 5 mg/kg lebrikizumab subcutaneously or placebo at study visits at Weeks 0, 4, 8, and 12; and a 16-week follow-up period (Weeks 13–28). At the Week 13 follow-up visit, subjects received a second allergen challenge followed by a methacholine challenge within 18–24 h (Fig. 1).

The protocol was approved by the ethics committee/institutional review board at each study site, and the study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. All subjects provided written informed consent prior to any study-specific procedure. The trial is registered at ClinicalTrials.gov (NCT00781443).
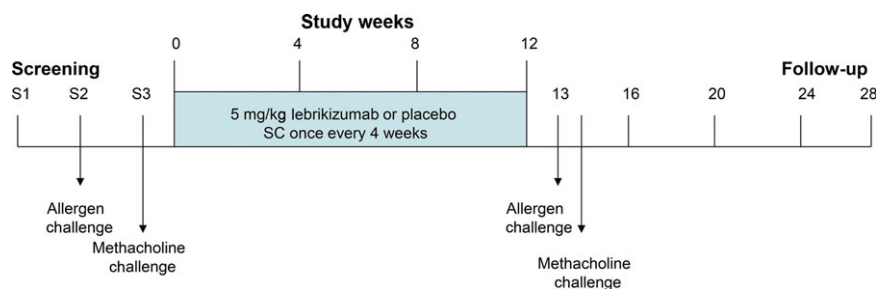

Fig. 1. Study design. The study included a 21-day screening period, a 12-week treatment period, and a 16-week follow-up period. At screening and at Week 13, subjects underwent an allergen challenge followed within 18–24 h by a methacholine challenge to determine PC_{20}, defined as the provocative concentration of methacholine that resulted in a > 20% reduction in FEV_1. Subjects were randomized 1 : 1 to lebrikizumab (5 mg/kg) or placebo, administered subcutaneously once every 4 weeks.

Screening and eligibility

Eligible subjects were 18–55 years of age, had allergic asthma diagnosed for $\geq$ 6 months, which was being treated with only intermittent short-acting inhaled β_2-adrenergic agonists, and had a normal chest X-ray $\leq$ 2 years prior to screening. At screening, eligible subjects had a positive skin test ($\geq$ 3 mm over negative control) for at least one of the following allergens: house dust mite, cat dander, ragweed, and had a FEV_1 of $\geq$ 70% of predicted, showed both an early asthmatic response (EAR) of $\geq$ 20% reduction in FEV_1 5–30 min following allergen challenge, and a late asthmatic response (LAR) of $\geq$ 15% reduction in FEV_1 2–8 h following allergen challenge, and had a $PC_{20} < 8$ mg/mL, defined as the provocative methacholine concentration that caused a > 20% fall in FEV_1 from the saline-alone value. Exclusion criteria are listed in the Supporting Information.

Outcome measures

The primary efficacy outcome measure was LAR, defined as the area under the curve (AUC) of FEV_1 2–8 h following an allergen challenge at Week 13. Secondary efficacy outcome measures (all at Week 13) were EAR, defined as the AUC of FEV_1 0–2 h following allergen challenge, the maximal fall in FEV_1 that occurred 0–2 h following challenge, methacholine challenge PC_{20}, and change in methacholine challenge PC_{20} relative to the PC_{20} observed 24 h following allergen challenge at screening.

Safety outcome measures were the frequency and severity of treatment-emergent adverse events (AEs) and immunogenicity, measured by serum antitherapeutic antibodies (ATAs) in samples collected at Weeks 0, 12, and 28 (see Supporting Information for full methods and results).

Pharmacokinetics (PK) outcomes included trough serum concentration prior to dosing at Weeks 0, 4, 8, and 12; serum concentration at Weeks 1 and 13 (i.e. 7 days after the first and fourth doses); maximum observed serum concentration ($C_{max,obs}$); time of maximum observed serum concentration ($T_{max,obs}$); and terminal elimination half-life ($t_{1/2}$). Exploratory serum biomarkers were measured from samples taken during screening (prior to allergen challenge), at each treatment visit and at follow-up visits at Weeks 13, 16, 20, and 28. Details of assay methods are provided in the Supporting Information.

Randomization and blinding

Eligible subjects were randomized 1 : 1 to receive lebrikizumab or placebo, using an interactive voice response system. Randomization was stratified by study site. Subjects, investigating physicians, study site personnel, and the study sponsor and its agents were blinded to treatment assignment. The study sponsor was unblinded to treatment assignment until after verification of the data collected through Week 13 were verified. Subjects, investigating physicians, and study site personnel were unblinded at the end of the Week 28 follow-up visit.

Assessments

Spirometry. Spirometry was performed in accordance with the American Thoracic Society guidelines [25]. FEV_1 percentage predicted was computed using the Crapo formula [26].

Skin test. Allergen prick skin test was performed at screening by applying house dust mite, cat dander, and ragweed allergen extracts to the skin, scratching or pricking the skin to allow exposure, and evaluating the skin response. Details of methods are provided in the Supporting Information.

Allergen and methacholine challenges. All subjects were assessed as suitable for each allergen challenge including assessment of FEV_1 and history of symptoms or respiratory illness. Allergen challenges were administered at screening and at Week 13. A KoKo DigiDoser spirometer (nSpire Health, Inc., Longmont, CO, USA) was used to administer all challenge aerosols, according to the five-breath method [25, 27]. After baseline spirometry was performed, subjects were given doubling doses of the standardized extract of an allergen to which they had demonstrated a skin response during screening. The final allergen concentration used was the concentration that induced a $\geq$ 20% reduction in FEV_1 in the EAR during screening. Following the final allergen inhalation, FEV_1 measurements were taken every 10 min for the first 90 min and every hour from 2 to 8 h. The same concentrations of allergen used during the screening challenge were used for the Week 13 challenge, unless FEV_1 was reduced $\geq$ 20% at lower concentrations of allergen.

Methacholine challenges were performed within 18–24 h after allergen challenges at screening and Week 13. Subjects inhaled concentrations of methacholine aerosol ranging from 0.031 to 32.0 mg/mL until a fall in FEV_1 of $\geq$ 20% relative to saline control occurred. The highest two inhaled methacholine concentrations were used to estimate PC_{20} through linear interpolation. (For more information on the methacholine challenge procedure please see the Supporting Information).

Safety. AEs and serious AEs (SAEs) were monitored and reported in accordance with the International

Conference on Harmonisation (ICH)/WHO Good Clinical Practice requirements and coded according to MedDRA, version 8.0 (MeDRA, McLean, VA, USA). The severity of AEs was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE; NCI, Bethesda, MD, USA), version 3.0. Haematology, clinical chemistry, urinalysis, vital signs, and other protocol-specified tests that were deemed critical to safety evaluation were monitored. ATAs were measured from serum samples taken prior to dosing at Weeks 0 and 12 and at the Week 28 follow-up visit.

Statistical analyses

This study was a small signal seeking proof-of-activity study and was not powered to demonstrate statistically significant differences between treatment groups. The study was designed to have a sample size of 24 patients, which was estimated to provide reasonable precision for estimating a clinically significant treatment effect on LAR when lebrikizumab treatment was compared with placebo. Assuming a relative difference of 50% in mean AUC LAR and subject variability consistent with similar studies, a 90% confidence interval (CI) on the population treatment effect is approximately $50 \pm 23\%$.

The treatment group comparability analysis, primary efficacy analysis, secondary efficacy analyses, and biomarker assessments were by treatment group based on the modified intent-to-treat population, consisting of randomized subjects who received at least one dose of study drug and had the postbaseline primary efficacy measurement. CIs for treatment effects and descriptive summary statistics for each treatment group overall were provided for the primary and secondary efficacy outcome measures. Biomarker measurements were expressed as absolute values or relative to Week 0 predose baseline values. The Wilcoxon rank sum test was performed on serum biomarkers analyses using SAS, version 9.2 (SAS, Cary, NC, USA), and a two-tailed $P < 0.05$ was considered statistically significant.

Safety outcomes, including AEs, SAEs, changes in clinical laboratory evaluations, and immunogenicity, were summarized for all randomized subjects who received at least one dose of study drug, grouped according to actual treatment received. All subjects who received lebrikizumab were included in PK analyses.

Results

Subject demographics and baseline characteristics

Twenty-nine randomized subjects received lebrikizumab ($n = 13$) or placebo ($n = 16$) at six U.S. study sites.

Table 1. Subjects demographics and baseline characteristics

	Placebo ($n = 16$)	Lebrikizumab ($n = 13$)
Age in years, mean (SD)	32 (11)	36 (11)
Male/female, n	9/7	6/7
Race white, n (%)	12 (75.0)	12 (92.3)
Percentage predicted FEV$_1$, mean (SD)	82.4 (8.9)	84.3 (13.6)
Baseline* serum biomarkers, mean (SD)		
IgE, IU/mL	239 (197)	309 (448)
Blood eosinophils, $10^3/\mu$L	0.264 (0.182)	0.258 (0.169)
CCL17, pg/mL	363 (197)	421 (230)
CCL13, pg/mL	187 (97)	195 (86)
CEA, ng/mL	1.22 (0.67)	1.09 (0.72)
YKL-40, ng/mL	35.9 (30.6)	29.6 (22.7)
Periostin, ng/mL	30.6 (7.6)	28.1 (7.7)

*Prior to dosing at Week 0.
CCL, chemokine ligand; CEA, carcinoembryonic antigen-related cell adhesion molecule 5; FEV$_1$, forced expiratory volume in 1 s; IgE, immunoglobulin E; IL13, interleukin 13; SD, standard deviation; YKL-40, cartilage glycoprotein-39 (HC gp-39).

Subject demographics and baseline characteristics were similar across treatment groups (Table 1).

Disposition and treatment

Twenty-eight (97%) subjects completed the study; each subject received a total of four doses of study drug over the 12-week treatment period. One subject who was randomized to the lebrikizumab treatment group decided to withdraw prior to study completion after receiving three doses of study drug (Fig. S1 in the Supporting Information).

Efficacy outcomes

Late asthmatic response 2–8 h following an allergen challenge at Week 13. At Week 13, after receiving four doses of study drug, the mean AUC of FEV$_1$ 2–8 h following allergen challenge of the lebrikizumab group was reduced by 48% compared with the placebo group, although this was not statistically significant (26.3 vs. 50.5; 95% CI, −19%, 90%) (Table 2; Fig. 2). Detailed analysis per investigation site are shown in Fig. S2 in the Supporting Information. Three patients received more allergen during the allergen challenge procedure at Week 13 than they received during the challenge at screening, two in the active group and one in the placebo group, and these events were considered protocol deviations. Twelve patients received less allergen during the Week 13 allergen challenge than they received during the screening challenge, five in the active group and seven in the placebo group in each case, as mandated

Table 2. Asthmatic responses to allergen challenge

	Screening			Week 13		
	Placebo ($n = 16$)	Lebrikizumab ($n = 13$)	(Lebrikizumab − placebo)/placebo	Placebo ($n = 16$)	Lebrikizumab ($n = 13$)	(Lebrikizumab − placebo)/placebo
Early asthmatic response						
Maximum percentage reduction in FEV_1	30.3	38.0	25.4	25.5	29.6	16.1
AUC FEV_1 (0–2 h post-challenge), percentage $FEV_1 \times$ hour	34.1	39.9	17.0	26.4	27.5	4.2
Late asthmatic response						
Maximum percentage reduction in FEV_1	24.8	30.8	24.2	16.4	13.8	−15.9
AUC FEV_1 (2–8 h post-challenge), percentage $FEV_1 \times$ hour	73.8	77.0	4.3	50.5	26.3	−47.9

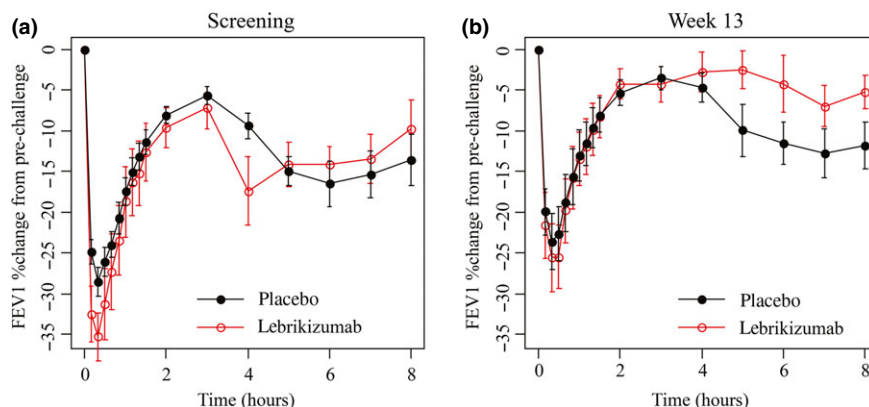AUC, area under the curve; FEV_1, forced expiratory volume in 1 s.

Fig. 2. Allergen-induced changes in FEV_1 following challenges at screening and Week 13. The allergen-induced percentage change in FEV_1 during screening (a) and at Week 13 (b) in placebo- and lebrikizumab-treated subjects. FEV_1 was collected every 10 min for the first 90 min and then every hour from 2 to 8 h following allergen challenge. Data points are means. Vertical bars are standard error of the mean.

by the protocol, the Principal Investigator terminated the allergen challenge procedures early.

Early asthmatic response 0–2 h following an allergen challenge at Week 13. Blocking the IL13 pathway did not affect the EAR substantively. At Week 13, both the mean AUC of FEV_1 0–2 h following allergen challenge and the maximum reduction in FEV_1 0–2 h following allergen challenge were similar between the lebrikizumab- and placebo-treated groups (AUC: 27.5 vs. 26.4; maximum percentage reduction: 29.6 vs. 25.5) (Table 2).

Methacholine challenge PC_{20}. Airway hyperresponsiveness was assessed by methacholine challenge doubling dose for 21 subjects who had methacholine challenges at both screening and Week 13. The arithmetic mean of the methacholine doubling dose in the lebrikizumab group was 0.33 doubling doses higher than that of the

placebo group mean (1.58 vs. 1.25, 95% CI, −0.64, 1.30), which is not considered as clinically meaningful inhibition.

Safety outcomes

Eighteen subjects reported a total of 61 AEs (37 during the treatment period, 24 during the follow-up period) (Table 3). All reported AEs were NCI CTCAE Grade 1 or 2. The overall incidence of AEs was greater in placebo-treated subjects compared with lebrikizumab-treated subjects. Only one AE (decreased platelet count) was judged to be lebrikizumab related. This subject had a platelet count of 122×10^9/L at the end of the study (Week 28), which returned to 144×10^9/L in a week without treatment. Three placebo-treated subjects reported AEs (injection site reactions in two subjects and abdominal discomfort and diarrhoea in one subject) that were judged to be treatment related. In no case did

MedDRA preferred term	Placebo (*n* = 16)	Lebrikizumab (*n* = 13)
Any adverse event, *n* (%)	11 (68.8)	7 (53.8)
Upper respiratory tract infection	4 (25.0)	3 (23.1)
Nasopharyngitis	3 (18.8)	1 (7.7)
Muscle strain	0	2 (15.4)
Nausea	2 (12.5)	0
Injection site erythema	2 (12.5)	0
Injection site swelling	2 (12.5)	0
Viral upper respiratory tract infection	2 (12.5)	0
Arthropod sting	2 (12.5)	0
Headache	2 (12.5)	0

*Multiple occurrences of a specific adverse event for an individual subject were counted once. Includes treatment-emergent events that began on or after the first dose of study drug.

AEs lead to withdrawal of study drug or study discontinuation. No SAEs were reported. All subjects in the lebrikizumab-treated group tested negative for ATAs. No patterns of clinically significant change were observed in either treatment group for the haematology, clinical chemistry, and urinalysis parameter measures.

Pharmacokinetics

Lebrikizumab serum concentrations accumulated with multiple doses, as shown by an almost twofold increase in the predose trough concentrations between Week 4 (Day 28) and Week 12 (Day 84) (Supporting information Fig. S2). The mean predose serum concentration of lebrikizumab at treatment Weeks 4, 8, and 12 was 29.2 ± 4.93, 44.7 ± 8.96, and 54.5 ± 6.89 μg/mL, respectively. The mean $C_{max,obs}$ was 98.3 ± 13.8 μg/mL, which occurred at a $T_{max,obs}$ of 92.2 ± 1.55 days, approximately 7 days following the last dose. Based on non-compartmental analysis, the mean $t_{1/2}$ of lebrikizumab from serum was 27.4 ± 5.30 days, which suggests that lebrikizumab was approaching, but had not fully reached, steady state by the time the final dose was administered at Week 12. (Serum biomarker results are detailed in the Supporting Information.)

Predicting response to lebrikizumab based on circulating biomarkers

We explored whether baseline levels of serum IgE, periostin, CCL13, CCL17, CEA, YKL-40, and blood eosinophils were predictive of a treatment effect of IL13 blockade. Subjects in the placebo and lebrikizumab groups were divided into 'biomarker-high' and 'biomarker-low' groups based on the median level observed across all subjects. The median levels of serum IgE, CCL13, CCL17, CEA, periostin, YKL-40, and eosinophil

were 180 IU/mL, 167 pg/mL, 331 pg/mL, 1.0 ng/mL, 28 ng/mL, 22 ng/mL, and 200/μL, respectively. We also subdivided subjects in the placebo and lebrikizumab group based on a composite of serum IgE > 100 IU/mL and peripheral blood eosinophils > 140/μL, which have been shown to differentiate subjects with asthma that have increased Th2 inflammation in their airways (see Table S1 in the Supporting Information [17]). As this was a small study, the total number of subjects in active subgroups was low (5–8 active subjects/group) and statistical analyses were not performed on these exploratory endpoints. Nevertheless, increased baseline serum levels of IgE, eosinophils, composite of serum IgE > 100 IU/mL and peripheral blood eosinophils > 140/μL, and periostin appeared to identify asthma subjects who were more likely to respond to IL13 blockade. Compared with the 48% reduction in AUC of the LAR for all subjects in the study, a non-significant reduction of 73%, 57%, 63%, and 91% was observed in subjects with increased predose levels of those markers, respectively (Fig. 3). No increase in treatment effect was observed in subjects with elevated baseline levels of serum CCL13, CCL17, YKL-40, or CEA (data not shown).

Discussion

This study demonstrates that lebrikizumab reduced the late response to bronchial allergen challenge in subjects with mild asthma, although the effect was not statistically significant. These data are in line with the
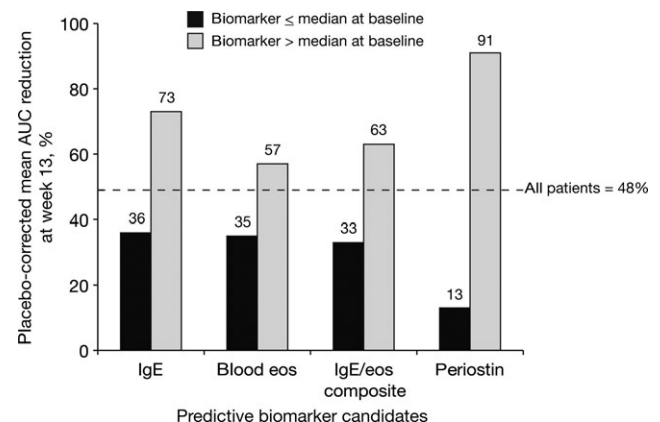

Fig. 3. Lebrikizumab appeared to have a greater but not statistically significant effect on allergen-induced late asthmatic response (LAR) in subjects with increased baseline levels of periostin, IgE, and/or peripheral blood eosinophils based on exploratory analysis. Black bars represent subjects with baseline levels of IgE, peripheral blood eosinophils, and periostin levels below the median of all subjects. Grey bars represent subjects with baseline levels of those biomarkers above the median of all subjects. Data are expressed as placebo-corrected mean LAR reduction at Week 13. The total number of subjects in active subgroups was low (5–8 active subjects/group), and statistical analyses were not performed on these exploratory endpoints.

hypothesis that IL13 blockade is an appropriate therapeutic approach for the treatment for asthma and plays a key role in the allergen-induced inflammatory response. Our data are similar to other monoclonal antibodies targeting the type 2 inflammation, such as the IMA-638, which inhibited the EAR and LAR in subjects with mild atopic asthma [10], and pitrakinra, which inhibited the LAR response in subjects with atopic asthma [9]. The anti-IgE, omalizumab, which was shown to suppress both the EAR and LAR in subjects with asthma [28, 29] while mepolizumab, an anti-IL5, did not inhibit the EAR or LAR following allergen challenge [30, 31]. Furthermore, while animal model data have shown that IL13 blockade inhibits AHR, this has not translated into humans with any of the biologics studied so far, indicating species differences between mice and men in the underlying mechanisms of airway hyperresponsiveness.

This study was a small proof-of-activity study to demonstrate the pharmacological activity of lebrikizumab and to explore the feasibility of IL13-related biomarkers as predictive biomarkers for response. Specifically, the results suggest that (i) IL13 contributes to allergen-induced airway obstruction in mild allergic asthma; (ii) asthma patients with increased levels of biomarkers correlating with Th2/eosinophilic airway inflammation (IgE, eosinophils, and periostin) appeared to have a larger treatment effect from lebrikizumab compared with subjects with lower levels of those biomarkers, and (iii) in addition to reducing the LAR, lebrikizumab significantly reduced the levels of systemic biomarkers (CCL13, CCL17, and IgE) that can be induced by IL13 (Fig. S3, Supporting Information). Although the effect of lebrikizumab on the LAR in the group as a whole was not statistically significant, the results of this study taken together [including the predictive and pharmacodynamic biomarkers (Figs. S4 and S5, Supporting Information)] highlight possible mechanisms of action of lebrikizumab. It is important to standardize the selection of subjects and to have procedures in place to try to minimize variability in the late-phase response; previous studies have adopted a number of measures to minimize variability in the late-phase response: (i) allergen challenge not performed within (at least) 4 weeks of a respiratory infection; (ii) avoidance of new sensitizing allergen exposure throughout the study; (iii) stability of FEV_1 prior to allergen challenge days (defined as FEV_1 to be not lower than 10% form baseline FEV_1 measured during the screening period; (iv) stability of AHR (PC_{20}) prior to allergen challenge days (defined as methacholine PC_{20} within one doubling dose lower than baseline PC_{20} measured during the screening period [32]). None of our subjects had a respiratory infection within 4 weeks of the screening allergen challenge, and exposure to new allergens was not specifically recorded but would have likely affected both treatment groups. We measured FEV_1 on four occasions during screening and at least once before allergen challenge and all subjects were within 10%. We did not measure methacholine prior to allergen challenge to minimize the number of visits and procedures, which is a limitation of our study. Finally, we recorded variations in the amount of allergen given at the post-treatment challenge compared with the individuals screening allergen challenge. These variations were unavoidable for the 12 cases where less allergen had to be delivered for safety reasons. Overall, the impact of these variations is likely to reduce the ability of the study to be precise in the conclusion about the magnitude of the treatment effect but given the relative balance of these variations across the treatment arms, it is unlikely to have had a major impact on the conclusions of the study. Thus, despite its limitations, this study provides supportive evidence to pursue additional studies of lebrikizumab as a potential novel therapeutic agent in asthma prior to embarking on large proof-of-concept studies.

A subsequent study evaluating the efficacy of lebrikizumab in subjects with uncontrolled asthma has shown that IL13 blockade improved lung function and this mainly driven by changes in a subset of patients with asthma with evidence of IL13-driven airway inflammation [7].

It is known that IL13 promotes eosinophilic inflammation in part by up-regulating expression of eosinophil-attracting CCR3-binding chemokines. In addition, IL13 acts on leucocytes and resident airway cells to induce the CCR4-binding chemokines, CCL17 and CCL22, which act on Th2 cells. Of these chemokines, peripheral blood levels of CCL13 [33] and CCL17 [34] are robustly increased in allergic asthma patients. IL13 can also mediate isotype switching of activated B cells to produce IgE, which along with eosinophils, is detectable at elevated levels in the peripheral blood of asthma patients with increased Th2 inflammation in their airways [17]. The reduction in serum CCL13, CCL17, and IgE observed after lebrikizumab dosing in this study increases our confidence that the reduction in the LAR is indeed mediated, at least in part, through inhibition of Th2 inflammation via IL13 blockade. Although CCL13 and CCL17 decreased after lebrikizumab treatment and subjects with the highest baseline chemokine levels had the largest reduction in serum chemokines, these subjects did not appear to have increased improvement in LAR response when compared with all subjects. This may reflect extrapulmonary sources of IL13-induced CCL13 and CCL17 production. As lebrikizumab was administered systemically and these biomarkers were assessed systemically, their tissue of origin cannot be determined in this study. The

pharmacodynamic effects of lebrikizumab on serum biomarkers of IL13 activity in mild asthmatics were similar to those observed in patients with moderate–severe uncontrolled asthma [7].

Exploratory analyses indicated that increased pre-treatment levels of serum periostin, IgE, and peripheral blood eosinophils identified subjects with increased treatment effects from lebrikizumab, indicating a more prominent role of IL13 and Th2 inflammation in the airways of these subjects. Of these predictive biomarkers of Th2 inflammation in patients with mild asthma periostin also enriched for clinical benefit from lebrikizumab in patients with uncontrolled moderate–severe asthma [7]. The ability of baseline IgE and eosinophils to significantly enhance for LAR benefit after lebrikizumab treatment in this bronchial allergen challenge study but not for prebronchodilator FEV_1 benefit in patients with moderate–severe asthma [7] may reflect the more direct link between IgE and the primary outcome measure, LAR upon allergen challenge, in this study and/or the variable effects of concomitant inhaled corticosteroid therapy on eosinophils in the study described by Corren et al. [7].

Taken together, the present study, using a bronchial aeroallergen challenge in subjects with mild allergic asthma and the study in patients with moderate–severe uncontrolled asthma [7] highlight the potential for therapeutic IL13 blockade in asthma. This study also illustrates how allergen challenge proof-of-activity studies can be used to explore mechanistic activity of novel therapeutics prior to exposing a large number of patients to experimental therapies.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Methods.
Figure S1. Study patient flowchart.
Figure S2. Late allergen response AUC FEV$_1$ at Week 13 for each subject plotted by site.
Figure S3. Serum concentration–time profile of lebrikizumab.
Figure S4. Serum levels of IgE, CCL13, and CCL17 and relationship between predose levels of serum IgE, CCL13, and CCL17 with magnitude of effect.
Figure S5. Serum levels of periostin, CEA, and YKL-40.