

➤ Effect of an interleukin-4 variant on late phase asthmatic response to allergen challenge in asthmatic patients: results of two phase 2a studies

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Summary

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Background Increases in T helper (Th) 2 cytokine concentrations have been seen in atopic asthma, with interleukin 4 and interleukin 13 thought to have a role in the physiological response to allergen challenge. Our aim was to assess the therapeutic effect of pitrakinra, an interleukin-4 variant that targets allergic Th2 inflammation by potently inhibiting the binding of interleukin 4 and interleukin 13 to interleukin-4R α receptor complexes.

Methods In two independent randomised, double-blind, placebo-controlled, parallel group phase 2a clinical trials, patients with atopic asthma were treated with pitrakinra or placebo via two routes. In study 1, patients were randomly assigned to receive either 25 mg pitrakinra (n=12) or placebo (n=12) by subcutaneous injection once daily. In study 2, patients were randomly assigned to receive either 60 mg pitrakinra (n=16) or placebo (n=16) by nebulisation twice daily. Inhaled allergen challenge was done before and after 4 weeks of treatment. The primary endpoint for study 1 was maximum percentage decrease in forced expiratory volume in 1 s (FEV₁) over 4–10 h after allergen challenge, whereas that in study 2 was average percentage decrease in FEV₁ over 4–10 h after allergen challenge. All patients except those with baseline data only were included in our analyses. These trials are registered with ClinicalTrials.gov, numbers NCT00535028 and NCT00535031.

Findings No patients dropped out or were lost to follow-up in study 1; in study 2, two patients in the placebo group and one in the pitrakinra group dropped out or were lost to follow-up. These individuals had baseline data only, and were excluded from the analyses. In study 1, there was a 17·1% maximum percentage decrease in FEV₁ in the pitrakinra group; by contrast, the maximum decrease was 23·1% in the placebo group (difference 6%, 95% CI –4·37 to 16·32; p=0·243). In study 2, there was a 4·4% average percentage decrease in FEV₁ in the pitrakinra group; by contrast, the average percentage decrease was 15·9% in the placebo group (3·7 [95% CI 2·08–6·25] times lower in the pitrakinra group; p=0·0001). There were fewer asthma-related adverse events (p=0·069) and fewer adverse events requiring β -agonist rescue (p=0·031) after subcutaneous administration of pitrakinra than with placebo. There were too few asthma-related adverse events in study 2 to assess the effect of inhalation of pitrakinra on adverse events.

Interpretation Local treatment, targeted at inhibition of interleukins 4 and 13 in the lung, could substantially diminish the symptoms of asthma.

Introduction

The concept that upregulation of T helper (Th) 2 cytokines is critical for the allergic inflammation associated with asthma is nearly 20 years old.¹ However, confirmatory evidence that Th2 cytokines such as interleukin 4 or interleukin 13 have a critical role in the onset and development of clinical asthma has, until now, been lacking. Asthma has been reported to be characterised by infiltration of activated T lymphocytes and eosinophils into the bronchial mucosa.² These cells, along with resident mast cells, secrete soluble growth factors and inflammatory mediators (including interleukin 4 and interleukin 13) that could directly and indirectly modify the mucosal surface. In murine models, this Th2-type immune response leads to a bronchoconstrictive and inflammatory response to allergens which enhances non-specific bronchial hyper-responsiveness.^{3–7} However, recent clinical failure of drugs targeting the Th2 process (ie, anti-interleukin 5 and interleukin-4-specific antagonists) has lessened enthusiasm for this pathway's

singular importance. Despite these failures, concern has remained that targeting of interleukin 4, to the exclusion of interleukin 13, might have been too selective and that studies that inhibit both of these cytokines are needed.^{8,9}

One possible approach to inhibiting both interleukin 4 and interleukin 13 is through inhibition or antagonism of interleukin 4R α , the signalling component of the heterodimeric receptor complex for both interleukin 4 and interleukin 13.¹⁰ Interleukin 4R α forms a complex with the common γ receptor on T cells, where it specifically binds interleukin 4. Interleukin 4R α can also dimerise with interleukin 13R α 1 on other cell types to bind both interleukin 4 and interleukin 13.¹¹ We developed pitrakinra (Aerovant), a recombinant human interleukin-4 variant that competitively inhibits the interleukin-4R α receptor complex to interfere with the actions of both interleukin 4 and interleukin 13. This drug, administered either subcutaneously or via nebulisation, protected allergic cynomolgus monkeys from allergen-induced airways hyper-responsiveness and lung eosinophilia in

both prophylactic and therapeutic model settings.^{12,13} Subcutaneous administration in monkeys for 6 weeks or more also reduced the cutaneous wheal response and circulating concentrations of allergen-specific IgE.¹⁴ In human beings, pitrakinra decreased the eczema clinical score and circulating IgE concentrations, and normalised T-cell subsets in patients with severe atopic eczema after 4 weeks of subcutaneous administration.¹⁵ Our aim was to do two phase IIa trials of pitrakinra to investigate whether Th2 immunity was important in a clinical setting where asthmatics are experimentally challenged with aerosolised allergen.

Methods

Patients

For both studies, patients with atopic asthma (ages >18 years) were recruited to the Guy's Drug Research Unit, London, UK. Patients with asthma were included if they had a baseline forced expiratory volume in 1 s (FEV₁) of 70% or more of predicted, needed regular or as required use of β -agonists, and showed a late phase response ($\geq 15\%$ drop in FEV₁ between 4–10 h) to allergen challenge at screening. They must have been on a stable regimen of medications for asthma for 1 month or more, and could not have had systemic immunosuppressive therapy within 1 month of screening. Additionally, patients were screened for airways reactivity. In study 1, patients were required to have a PC₂₀ (provocative concentration that causes a 20% fall in FEV₁ from the saline alone value) to methacholine of less than 8 mg/mL and in study 2, participants were required to have a PC₂₀ to adenosine monophosphate of more than 3·125 mg/mL. Individuals were excluded if they had any medical condition that would preclude allergen challenge, had a greater than 10 pack-year smoking history, or had smoked in the 3 months before screening. Patients were also excluded if they had received any corticosteroid medications (systemic or inhaled) in the month before screening. Participants were to continue their non-steroidal concomitant treatments without change during the study. No participants used leukotriene-receptor antagonists while on study.

Both studies were done under a clinical trials authorisation from the UK Medicines and Healthcare products Regulatory Agency after review by the Guy's research ethics committee (London, UK). The studies were done according to the principles of good clinical practice and applicable laws and regulations and the Declaration of Helsinki. All participants gave written informed consent before any study-specific procedure.

Procedures

Both studies were double-blind, placebo-controlled, parallel group in design. Patients were screened and randomised within 1 month of dosing. Three screening visits consisted of assessment of medical history, allergen skin-prick test, lung function testing, determination of

lung function response to inhaled allergen, inhaled methacholine, or inhaled adenosine monophosphate, and basic safety screening. In study 1, participants were randomly assigned to receive pitrakinra or placebo subcutaneously once a day; whereas in study 2 participants were randomly assigned to receive pitrakinra or placebo via nebulisation twice a day. Allocation to treatment was according to a predetermined random order in block sizes of four. The randomisation list was generated with SAS version 8.2 by the Guy's Drug Research Unit. Participants were randomised to active treatment or placebo in a 1 to 1 ratio. Both pitrakinra and placebo were dispensed from the same nebuliser pots to maintain blinding. Randomised participants returned to the clinic once daily for 28 days to receive study medication. Final follow-up visits were held 5–10 days after the final dose for both studies (figure 1).

Pitrakinra is a recombinant form of the wild-type human interleukin 4 containing two functional mutations at positions 121 (arginine to aspartic acid) and 124 (tyrosine to aspartic acid). For these studies, the drug was manufactured by Bayer Ag (Wuppertal, Germany). The placebo drug product was sterile saline for injection or nebulisation. In study 1, 25 mg pitrakinra or placebo (1·5 mL) was injected subcutaneously once daily in the clinic. In study 2, 60 mg (nominal) of pitrakinra or matched volume placebo was nebulised twice daily with a Pari LC Plus nebuliser (Starnberg, Germany), with the morning dose given in the clinic and the evening dose self-administered at home. The nebuliser was expected to deliver about 10 mg pitrakinra to the lung, in view of its efficiency of delivery.

FEV₁ was assessed in accordance with American Thoracic Society (ATS)/European Respiratory Society (ERS) guidelines^{16,17} with a MasterScope spirometer

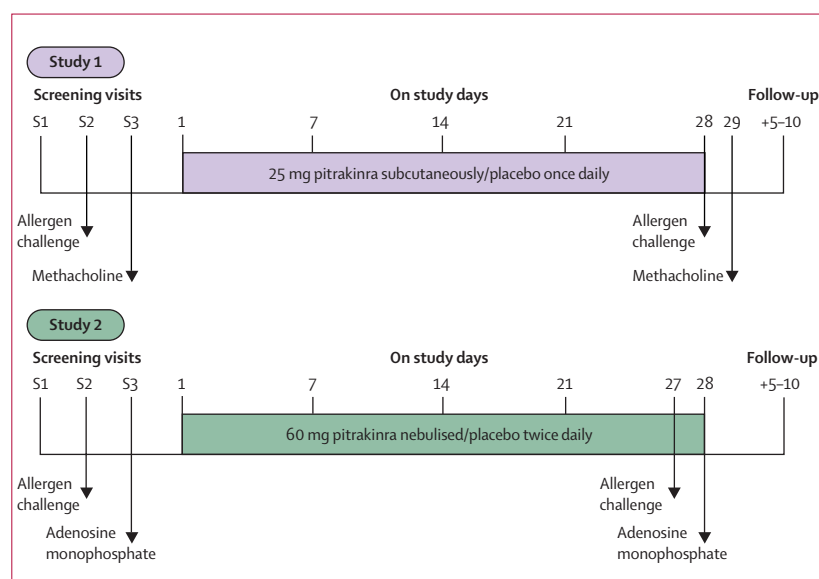


Figure 1: Treatment time line

(software version JLAB 4.61.0.1, Jaeger, Hoechst, Germany). Allergen inhalation challenge was done after determination of general sensitivity by standard skin-prick testing with cat, grass, and house dust mite allergens. All allergens were sourced from ALK-Abelló (Round Rock, TX, USA) and were nebulised with a Pari LC Plus nebuliser. In study 2, grass pollen skin-prick responders were excluded during hay fever season. Administration of allergens was done according to the five-breath dosimeter method by use of a calibrated Electro II inhalation dosimeter (Spira, Hämeenlinna, Finland), under the supervision of experienced respiratory clinical physiologists. The concentration of aerosolised allergen (starting at 250 standardised quality units per mL; SQU/mL) was doubled every 10 min until a decline in lung function of about 20% was achieved (maximum dose of 32 000 SQU/mL). This dose of allergen was then set as the target for the post-treatment challenge. However, for safety reasons, if a fall of more than 15% was achieved before reaching the target dose, the allergen challenge was terminated early. FEV₁ was measured after allergen inhalation challenge at 20 min, 30 min, 45 min, 1 h, and then every 30 min from 1 to 10 h. After the 10-h post-allergen pulmonary function test, the patient was given at least two puffs of β -agonist to relieve bronchoconstriction as part of standard operating procedure.

In study 1, 24 h after allergen challenge, doubling doses of nebulised methacholine (Provocholine, Methapharm Inc, Brantford, ON, Canada) were administered with a Pari LC Plus and Electro II inhalation dosimeter every 5 min starting with 0.03125 mg/mL and continuing to 32 mg/mL or until a 20% fall in FEV₁ was achieved. This approach was based on ATS guidelines.¹⁸ In study 2, adenosine monophosphate (Sigma Aldrich, St Louis, MO, USA) was nebulised initially at 3.125 mg/mL and increased to 400 mg/mL or until a 20% fall in FEV₁ was achieved.¹⁹ For each stimulus, the PC₂₀ was determined.

In study 2, fractional expiratory nitric oxide (F_ENO) was measured with a NIOX system (Aerocrine, Solna, Sweden) according to ATS F_ENO guidelines.²⁰ F_ENO was measured at the second screening and at day 27 (pre-allergen challenge), and at the third screening and day 28 (24 h post-allergen challenge). Total IgE was also measured in patients in study 2 from blood taken pre-dose on day 1 and day 28 by ImmunoCap1000 assay (Phadia, Uppsala, Sweden) at Laboratoire Marcel Merieux (Lyon, France).

Sputum induced with hypertonic saline was collected from participants in study 1 (but not study 2) on screening visit three and day 29 (24 h after allergen challenge and 1 h after methacholine challenge). Sputum cytopins were fixed within 2 h of collection and sent to Laboratory Marcel Merieux (Lyon, France) for cytology analysis. Eosinophils were reported as a proportion of non-squamous cells in the sample.

In addition to being done at screening, allergen skin-prick tests were done at day 28 in study 1. The

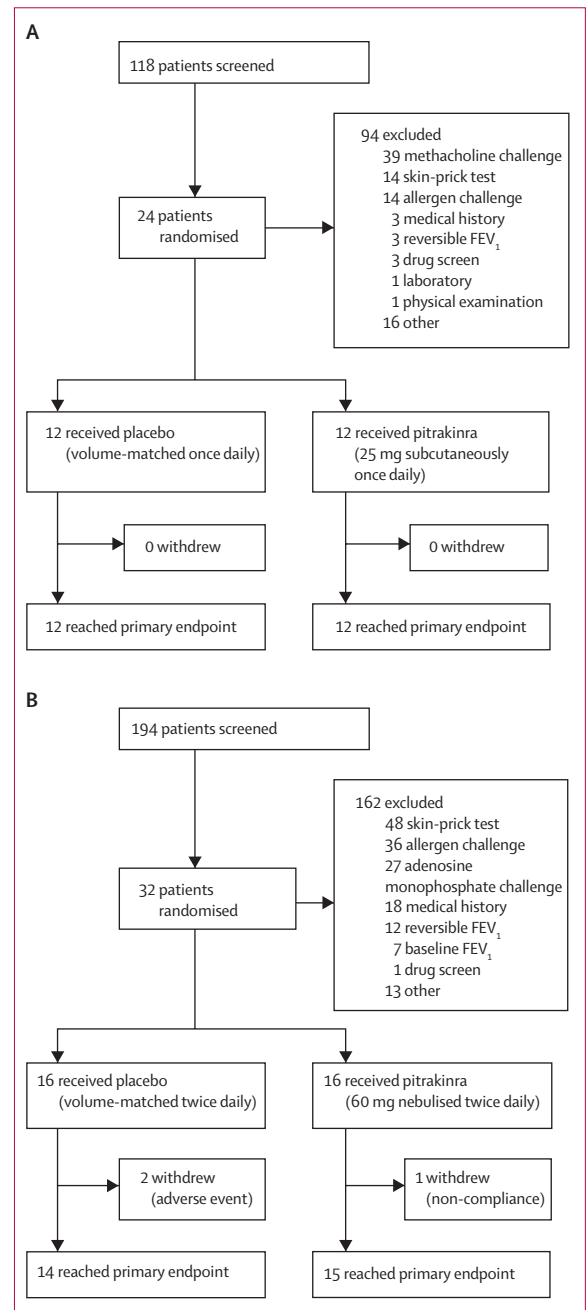


Figure 2: Trial profiles

cutaneous allergen response was determined at screening only in study 2, to guide the choice of allergen for respiratory challenge.

Clinical safety endpoints, including haematology, serum biochemistry, urinalysis, vital signs, and ECG, were assessed. Extra lung function measurements for safety were assessed in study 2 because this was the first time pitrakinra had been administered directly to the lung. Adverse events were reported in conventional format that reflect International Conference on

Harmonisation/WHO Good Clinical Practice requirements (coding with MedDRA version 8.0). IgG antibody levels against pitrakinra were also measured in blood samples taken pre-dose on day 1 and either 5–10 days after the last dose (study 1) or pre-dose on day 28 (study 2) and analysed by ELISA at Aerovance Inc. 96-well plates were coated with 1 µg/mL pitrakinra overnight, washed and blocked with casein (Pierce, Rockford, IL, USA). The samples were diluted (minimum dilution 1:20) and incubated on the plates. After washing, horseradish peroxidase-conjugated goat anti-human IgG Fc antibody (Jackson ImmunoResearch, West Grove, PA, USA) number 109-036-098, 1:10 000) and 3,3',5,5'-tetra-methylbenzidine (TMB) were used as the detection reagents. The neutralisation potential of samples that were positive for antibodies against pitrakinra was also assessed with an ELISA-based binding assay to detect the ability of such antibodies to block the binding of pitrakinra to interleukin 4Rα. Serum samples confirmed positive for IgG antibodies against pitrakinra were incubated with 5 µg/mL pitrakinra to allow formation of the antibody-analyte complex. Interleukin 4Rα (R&D Systems, Minneapolis, MN, USA) was immobilised on 96-well plates, then incubated with the antibody-analyte complex. Pitrakinra bound to interleukin 4Rα was detected with biotinylated rat antibody against the drug, followed by horseradish peroxidase-conjugated streptavidin and the TMB substrate. Cynomolgus monkey IgG against pitrakinra was used as a positive control in both assays. The assay formats and detection thresholds were designed in accordance with current guidelines.²¹

The primary endpoint of both studies was change in FEV₁ over 4–10 h post-allergen challenge (ie, during late asthmatic response). Study 1 defined the primary endpoint as the maximum percentage fall in FEV₁, while study 2 defined it as the average percentage fall in FEV₁. Secondary endpoints included F_ENO (study 2 only), PC₂₀, cutaneous allergen response (study 1 only), sputum eosinophils (study 1 only), and blood total IgE (study 2 only).

Statistical analysis

The primary endpoint of each study was analysed independently by Aptuit (Edinburgh, Scotland). Additional analyses of safety endpoint data were done at Guy's Drug Research Unit and of efficacy endpoint data at Aerovance Inc.

Efficacy endpoints were interpreted with analysis of covariance (ANCOVA), with treatment group as the main variable of interest and a covariate for the baseline (screening) value. All patients, except those with baseline data only, were included in our analyses. The single exception was sputum eosinophils, for which the day 29 values were analysed non-parametrically (Kruskal-Wallis test) because of violation of residual normality assumptions not remedied by data transformation and poor within-patient correlation between baseline and

day 29 values for the few participants (four receiving study medication, seven receiving placebo) with paired (baseline and day 29) results.

To facilitate comparison between studies, FEV₁ in late asthmatic response was expressed in three ways for both studies: maximum percentage fall, average percentage fall, and area under the FEV₁-versus-time curve (AUC). The average percentage fall in FEV₁ was calculated by dividing the area under the curve of the percentage fall in FEV₁ by the duration of the response period. Maximum percentage fall in FEV₁ and AUC were analysed on the original scale. Estimated treatment arithmetic means and an estimate of treatment difference with 95% CI and associated two-sided p value were determined for maximum percentage fall in FEV₁ and AUC. Average percentage fall in FEV₁ was log transformed before analysis. After a back-transformation to the original scale, the estimated treatment geometric means and an estimate of treatment ratio with 95% CI and associated p value were determined for average percentage fall in FEV₁. Three of the participants who received pitrakinra and one of the placebo recipients in study 1, and two of those who received pitrakinra and none of the placebo recipients in study 2, had a negative average percentage fall in FEV₁ at the end of treatment. These negative values were truncated at zero to allow log transformation of the time-normalised areas. AUC and area under the curve of the percentage fall in FEV₁ were also calculated during the early asthmatic response period (defined as 0–2 h post-allergen).

F_ENO data were analysed on the original scale. PC₂₀, IgE, and cutaneous allergen response data were log-transformed before analysis. Blood eosinophil percentages were square-root transformed before analysis. Sputum eosinophil percentages were analysed non-parametrically on the original scale.

Adverse event data were analysed with a Pearson's χ^2 test, in which the number of days on which subjects had an asthma-related adverse event, or required a β -agonist, were assessed in the context of the number of days available for adverse event observations, or β -agonist treatment.

All data were analysed with SAS version 8.2 or Minitab release 14.13.

	Study 1		Study 2	
	Pitrakinra	Placebo	Pitrakinra	Placebo
Sex (male/female)	5/7	7/5	12/3	7/8
Ethnic origin (black/white/other)	2/10/0	2/9/1	0/12/3	1/13/1
Age (years)	31 (10)	30 (9)	25 (5)	29 (8)
PC ₂₀ * (mg/mL)	1.37 (1.51)	1.84(1.92)	16.6 (13.6)	34.5 (44.3)
FEV ₁ (L)	3.72 (1.01)	3.70(0.77)	4.09 (1.02)	3.52 (1.05)
FEV ₁ (percentage predicted)	102% (13)	100% (20)	99% (15)	96% (18)
Total IgE (kU/L)	315 (303)	459 (399)

Data are n or mean (SD). *Study 1 for methacholine; study 2 for adenosine monophosphate.

Table 1: Demographic and baseline characteristics of study participants

These trials are registered with ClinicalTrials.gov, numbers NCT00535028 and NCT00535031.

Role of the funding source

The study sponsor, the principal investigator, and the contracted research organisation had primary responsibility for the study design, conduct, data collection, and analysis. All authors had full access to the

data, and were responsible for the decision to submit for publication.

Results

The trial profiles are shown in figure 2. There were no study failures or withdrawals in study 1; by contrast, two patients in the placebo group of study 2 withdrew because of adverse events, one classified as serious (both were

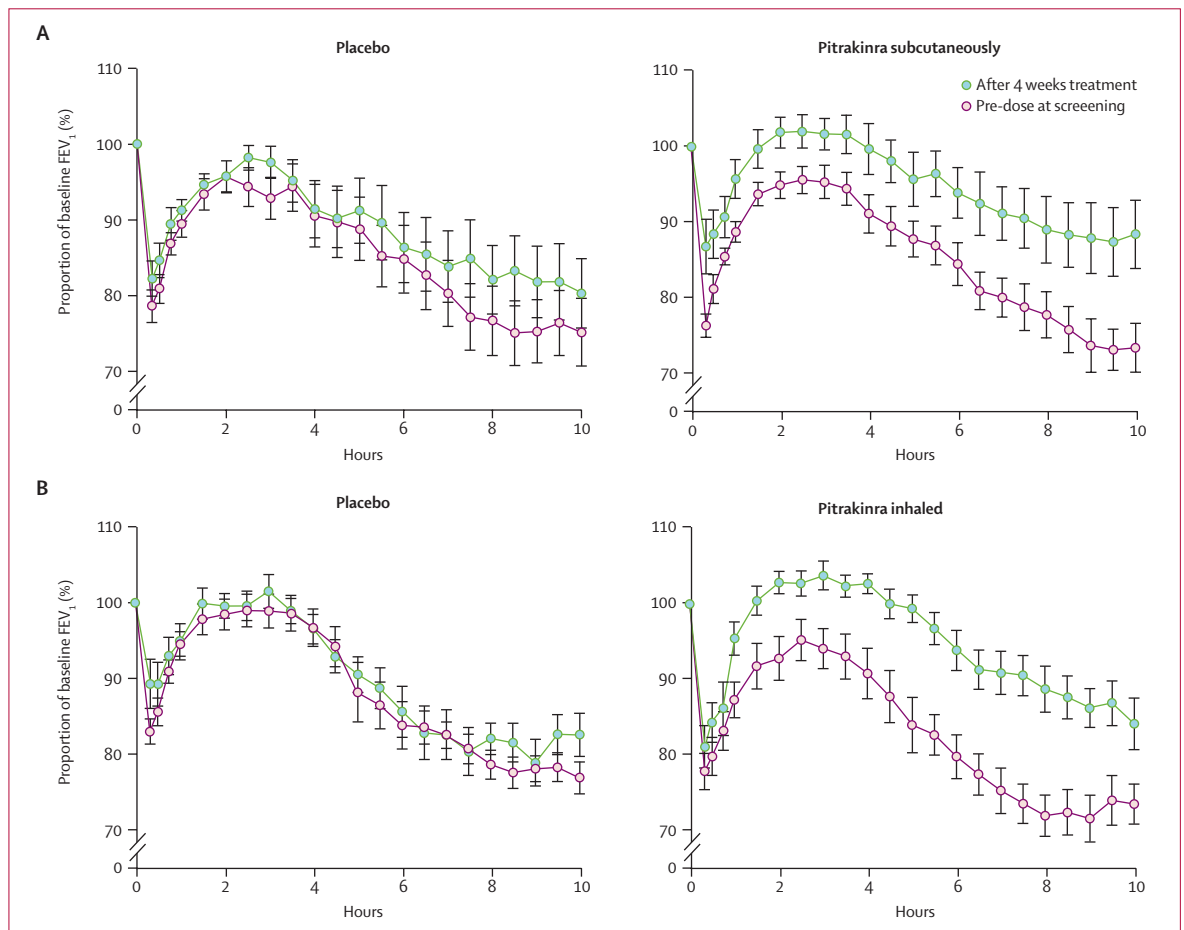


Figure 3: Average lung function after 4 weeks of treatment
Average lung function (FEV₁) response to allergen challenge shown for all patients in (A) study 1 and (B) study 2 by treatment group pre-dose at screening (red) and after 4 weeks of treatment (green). Error bars are SE.

	Placebo	Pitakinra	Comparison	Difference or ratio (95% CI)	p value
Study 1 (pitakinra group n=12, placebo group n=12)					
Average percentage fall in FEV ₁	9.4%	3.1%	Placebo/pitakinra	3.0 (0.91 to 10.12)	0.068
AUC (L·h)	17.6	19.2	Placebo-pitakinra	-1.6 (-3.25 to 0.03)	0.054
Maximum percentage fall in FEV ₁	23.1%	17.1%	Placebo-pitakinra	6.0% (-4.37 to 16.32)	0.243
Study 2 (pitakinra group n=15, placebo group n=14)					
Average percentage fall in FEV ₁	15.9%	4.4%	Placebo/pitakinra	3.7 (2.08 to 6.25)	0.0001
AUC (L·h)	18.4	21.6	Placebo-pitakinra	-3.1 (-4.63 to -1.65)	0.0002
Maximum percentage fall in FEV ₁	27.6%	16.1%	Placebo-pitakinra	11.5% (5.21 to 17.85)	0.0009

Treatment means (or geometric mean, for the analysis of average percentage fall in FEV₁ after log transformation) were determined from the ANCOVA model.

Table 2: Allergen-induced FEV₁ response during the late phase

asthma attacks precipitated by the allergen challenge protocol). These individuals provided no FEV₁ data after 1 h post allergen dose, and so did not contribute to the statistical analysis of the late asthmatic response or PC₂₀ data. Another patient in the pitrakinra group of study 2 withdrew because of poor clinic attendance and missed doses. This individual was also excluded from our analyses. Demographic and baseline data for the enrolled participants who completed the study are shown in table 1.

The decrease in FEV₁ in response to allergen challenge was attenuated after 4 weeks of treatment with pitrakinra, compared with placebo, irrespective of route of administration (figure 3 and table 2). After subcutaneous administration, pitrakinra conferred a 3.0 (95% CI 0.91–10.12, p=0.068) times reduction in the average allergen-induced percentage fall in FEV₁ from pre-challenge baseline during the late phase; by contrast, administration by inhalation conferred a 3.7 (2.1–6.3, p=0.0001) times reduction. The AUC, as well as the maximum percentage fall in late asthmatic response FEV₁ from pre-challenge baseline, were also improved after 4 weeks of subcutaneous or inhaled administration of pitrakinra (table 2), although treatment-related improvements were only significant after administration by inhalation. No significant difference between pitrakinra and placebo was seen on the early asthmatic response with either method of administration (table 3).

Some participants received a lower dose of allergen on day 28 (study 1) or 27 (study 2) relative to the screening dose. The lower end-of-study allergen dose affected more placebo recipients than those who received pitrakinra, with similar changes in allergen dose across groups (study 1: four of 12 in the placebo group, mean change of 1.25 doubling doses, two of 12 in the pitrakinra group, mean change of 1.0 doubling dose; study 2: seven of 14 in the placebo group, mean change of 1.43 doubling doses, four of 15 in the pitrakinra group, mean change of 1.75 doubling doses). Despite the lower dose of allergen in some participants in the post-treatment challenge, the magnitude of the early response was not different from that at screening. Additionally, the effect of inhaled pitrakinra on late asthmatic response FEV₁ relative to placebo remained significant when the subset of participants with matching allergen dose at screening and study end (seven in placebo group, 11 in the pitrakinra group; study 2) was analysed (ANCOVA-adjusted ratio of average percentage fall in FEV₁ 3.3, 95% CI 1.4–7.6; p=0.009).

No significant difference between pitrakinra and placebo was seen on airway hyper-responsiveness to methacholine (study 1) or adenosine monophosphate (study 2). After subcutaneous administration, individuals receiving pitrakinra tolerated 0.84 doubling doses more methacholine before reaching a 20% fall in FEV₁ than did placebo recipients, although this increase was not significant: ANCOVA-adjusted geometric mean PC₂₀ was

	Placebo	Pitrakinra	Placebo/pitrakinra ratio (95% CI)	p value
Study 1 (pitrakinra group n=12, placebo group n=12)				
Average percentage fall in FEV ₁	6.5%	2.8%	2.3 (0.57–9.0)	0.56
Study 2 (pitrakinra group n=15, placebo group n=14)				
Average percentage fall in FEV ₁	4.2%	4.4%	0.97 (0.43–2.20)	0.94

Table 3: Allergen-induced FEV₁ response during the early phase

1.61 mg/mL in the pitrakinra group versus 0.90 mg/mL in the placebo group (pitrakinra/placebo ratio 1.79, 95% CI 0.67–4.83; p=0.234). Likewise, after inhaled administration, participants receiving pitrakinra tolerated 0.82 doubling doses more adenosine monophosphate before reaching a 20% fall in FEV₁ than did those in the placebo group; ANCOVA-adjusted geometric mean PC₂₀ was 34.6 mg/mL in the pitrakinra group versus 19.6 mg/mL in the placebo group (1.76, 0.84–3.70; p=0.128).

Baseline (pre-allergen, predose, screening 2) F_ENO was high (63.2 [SD 39.2] ppb, n=22, figure 4; normal range 0–25 ppb), as might be expected for the asthmatic population.²⁰ Resting (pre-allergen) F_ENO was significantly lower after 4 weeks of inhaled pitrakinra than with placebo (figure 4; ANCOVA adjusted mean 34.9 ppb with pitrakinra, 63.1 ppb with placebo; treatment difference 28.3 ppb, 95% CI 9.4–47.2; p=0.005).

24 h after allergen challenge (at screening 3), F_ENO was significantly increased in all patients pre-randomisation to 111.7 (SD 47.9) ppb (p<0.0001, relative to screening 2). A significant effect of allergen was also seen at study end across all participants (pre-allergen day 27 vs post-allergen day 28; p<0.0001; data not shown). There was a significant linear relationship between the change in post-allergen F_ENO (day 28–screening 3) and the change in resting F_ENO (day 27–screening 2; p<0.002, Pearson correlation coefficient 0.64, R² 0.41) in all participants in study 2.

At baseline in study 1, skin-prick area measurements ranged from 31.0 to 176.6 mm² (geometric mean 55.4 mm² [CV% 15%] in the pitrakinra group; 76.2 mm² [19%] in the placebo group). At screening in study 2, areas ranged from 21.7 to 108.5 mm² (geometric mean 63.1 mm² [15%] in the pitrakinra group; 56.3 mm² [19%] in the placebo group). At day 28 in study 1, there was no significant difference in the cutaneous allergen response between treatment groups (ANCOVA, 1.01, 95% CI 0.61–1.63; p=0.983).

At baseline, sputum eosinophils as a percentage of non-squamous cells ranged from 2 to 52%, with median percentages of 10% (n=9, placebo) and 11% (n=7, pitrakinra). There was no significant difference in sputum eosinophils at day 29 (p=0.965; data not shown), although this assessment was limited by the high proportion (54%) of participants without paired (baseline and day 29) data, due mainly to insufficient sputum volume.

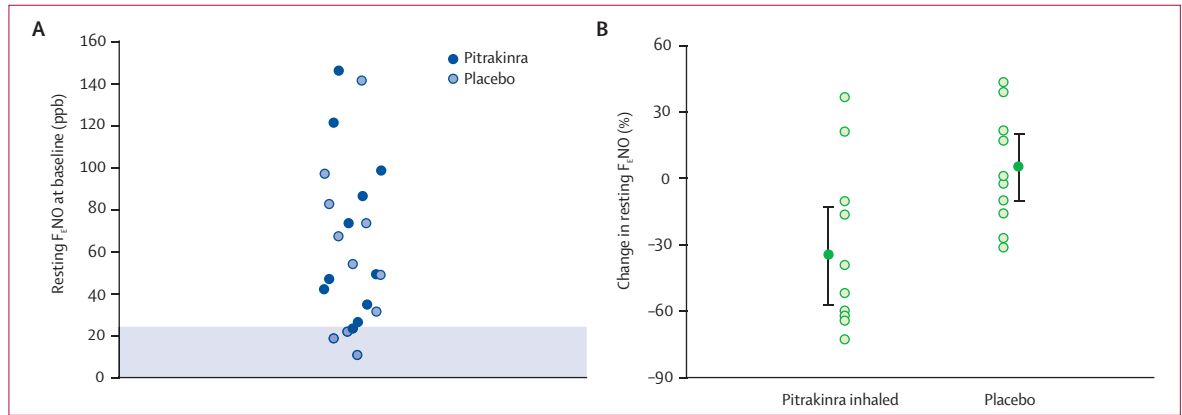


Figure 4: Pre-allergen baseline F_eNO at screening 2 (A) and after 4 weeks of treatment (B). Darker dots in (B) represent mean for each treatment group, with error bars representing 95% CI. Lighter dots are individual measurements.

	Study 1, pitrakinra (n=12)		Study 1, placebo (n=12)		Study 2, pitrakinra (n=16)		Study 2, placebo (n=16)	
	Participants	Events	Participants	Events	Participants	Events	Participants	Events
Number of participants with an adverse event	12	73	11	41	12	45	15	44
Nervous system disorders*	4 (33%)	9	5 (45%)	5	6 (55%)	8	6 (42%)	7
General disorders and administration site conditions†	8 (67%)	40	4 (36%)	11	7 (63%)	7	3 (21%)	4
Skin disorders‡	3 (25%)	4	3 (27%)	4	1 (9%)	1	2 (14%)	2
Gastrointestinal disorders§	3 (25%)	4	1 (9%)	1	4 (36%)	7	4 (29%)	6
Respiratory, thoracic disorders¶	5 (42%)	8	7 (63%)	14	10 (90%)	15	11 (78%)	22
Ear, labyrinth	2 (17%)	2	0 (0%)	0	0	0	0	0
Musculoskeletal disorders**	2 (17%)	3	3 (27%)	4	3(27%)	3	2 (14%)	2
Infections††	1 (8%)	1	3 (27%)	3	0	0	0	0
Reproductive disorders‡‡	2 (17%)	2	0 (0%)	0	0	0	0	0

*For example, headaches, somnolence, dizziness. †For example, injection site discomfort, reaction, increased energy. ‡For example, rash, dry skin, pruritus. §For example, nausea, abdominal discomfort, diarrhoea. ¶For example, wheezing, chest discomfort, dyspnoea, nasal congestion. ||For example, ear ache. **For example, muscle soreness, back pain. ††For example, upper respiratory infection. ‡‡For example, menorrhagia, dysmenorrhagia.

Table 4: Treatment-emergent adverse events by system organ class (>15% of participants)

At baseline, blood eosinophils as a proportion of white blood cells were within the normal range of 0–6% for 18 of 24 (study 1) and for 25 of 30 (study 2) participants (data not shown). There was no significant difference in blood eosinophils between the two treatment groups in either study after 4 weeks of treatment (p=0.313, study 1; p=0.715, study 2; data not shown).

In study 2, baseline total IgE in peripheral blood was slightly above (geometric mean 277 kU/L [CV% 36%]) the normal range of less than 150 kU/L. There was no significant difference in blood IgE between the two treatment groups after 4 weeks of treatment (ANCOVA-adjusted geometric mean 312 kU/L in pitrakinra group vs 284 kU/L in placebo group; treatment ratio 0.91, 95% CI 0.81–1.02, p=0.096).

There were no significant changes in haematology, serum biochemistry, urinalysis, vital signs, or ECG tests over time after administration of pitrakinra by either route; there were also no significant differences in safety outcomes between those who received pitrakinra and

those who received placebo. Pitrakinra had no negative effects on pulmonary function. A summary of adverse events for both studies is shown in table 4.

There were more general disorders reported with subcutaneously administered pitrakinra than with placebo, mainly due to injection site reactions or discomfort. Of the 672 injections administered, there were 39 injection site-related adverse events reported across both groups (35 events reported by eight of 12 patients receiving pitrakinra and four events by two of 12 placebo recipients). Fewer asthma-related adverse events requiring β-agonist rescue occurred in the pitrakinra group than in the placebo group in study 1 (three events in three participants in the pitrakinra group compared with 11 events in five of the placebo recipients; p=0.031). Participants who received subcutaneous pitrakinra reported fewer adverse events related to asthma (wheezing, dyspnoea, and chest discomfort) than did those who received placebo (six events were reported by four participants in the

pitirakinra group vs 14 events in seven placebo recipients; $p=0.069$). There were fewer overall asthma-related adverse events requiring β -agonist rescue outside the allergen challenge protocol in study 2, with only two participants receiving pitirakinra and five of those in the placebo group affected.

IgG antibodies against pitirakinra were detected in follow-up blood samples from three of ten participants tested after subcutaneous administration (titres of 1:40, 1:80, and 1:80) and from three of 15 participants after inhalation (titres of 1:30, 1:60, and 1:480). All samples confirmed to contain IgG antibodies against pitirakinra were not able to block binding of the drug to interleukin 4R α —ie, they were non-neutralising antibodies.

Discussion

Our data show that, compared with placebo, decreases in FEV₁ after allergen challenge were significantly attenuated after 4 weeks of inhalation of pitirakinra, lending support to the hypothesis that dual inhibition of interleukin 4 and interleukin 13 can affect the course of the late asthmatic response after experimental allergen challenge. The frequency of spontaneous asthma attacks requiring rescue medication use was also diminished in the first study, suggesting improved control over asthma symptoms after treatment with pitirakinra.

Animal models and human studies suggest an important role of Th2 immune responses in atopic asthma,^{1,3-5} and interference with the Th2 immune pathway improves asthma symptoms in animal models.²²⁻²⁴ However, clinical data to support this hypothesis has been lacking. Experimental treatments that target interleukin 4 or interleukin 5 alone have not been especially effective, presumably because of redundancies with interleukin 13 in many allergic processes.⁸ Animal studies suggest that interleukin 4 is more important in the initiation of an immune response and interleukin 13 is more involved in the amplification or expansion of immune responses.^{5,8,25,26} Our data bolster support for the immunological mechanisms previously established in preclinical model systems that emphasise a cooperative or redundant role for interleukin 4 and interleukin 13 in allergic disease.

In addition to improvements in the late asthmatic response, the resting inflammatory status of the lungs (as measured by F_eNO) was significantly attenuated after inhalation of pitirakinra for 4 weeks, compared with placebo,²⁷ in line with observations that interleukin 4 and other pro-inflammatory mediators induce nitric oxide synthase (iNOS) through STAT1 and STAT6 in epithelial cells.²⁸⁻³¹ There have been some studies to suggest that nitric oxide could have a beneficial effect in asthma patients by enhancing bronchodilation.³² However, our data do not seem to support this hypothesis, since the fall in F_eNO was accompanied by, and even marginally correlated with, improved lung function after allergen challenge. The observation that resting F_eNO was

significantly decreased by pitirakinra, but post-challenge F_eNO was not, is interesting and could be caused by differences in pathways contributing to the increased concentrations of nitric oxide. Basal F_eNO could be more dependent on up-regulation of iNOS by interleukin 4 and interleukin 13, whereas the increase in F_eNO after allergen challenge could involve additional pathways in epithelial cells and perhaps macrophages that are not affected by the inhibition of interleukin 4R α . One could postulate that pitirakinra down-regulates baseline Th2 inflammation in the asthmatic lung while not interfering with the lung's natural defences in the face of large amounts of foreign allergen.

Although F_eNO is believed to give some indication of the degree of eosinophilic inflammation in the airways, the relationship is not completely reliable. These preliminary studies did not include any additional measures of inflammation, such as sputum or biopsy studies. Because numerous animal and in-vitro studies support the role of interleukin 4R α in mucus production from airway epithelial cells, it is certainly possible that some of the effect of pitirakinra is on epithelial cell mucus production.³³ Subsequent studies will be needed to determine the broader effect of interleukin 4R α inhibition on eosinophils, mast cells, mucus and goblet cell hyperplasia, and even smooth muscle hypertrophy or hyperplasia.³⁴ However, as a number of genetic studies have suggested that polymorphisms in the interleukin 4/interleukin 13/interleukin 4R α pathways contribute to both lower lung function and severe exacerbations in asthma, a potential for a long-term effect of this approach on disease outcomes clearly exists.^{35,36}

Pitirakinra did not have any effect on the early response to allergen challenge, nor did we anticipate that it would have such an effect. The early response is largely believed to be driven by a mast cell response secondary to cross-linking of IgE receptors by allergen. We have no reason to believe that a 1-month treatment would be sufficient to limit the activation of the mast cells. Inhibition of the early response would require a reduction in the numbers of mast cells present in the airways or in the degree of binding of IgE to the mast cell. Neither of these is likely to occur within the first month of therapy, but might be expected to occur after more prolonged therapy. More and longer studies will be required to determine whether inhibition of interleukin 4R α has any effect on mast cells.

Treatment with pitirakinra did not significantly affect airway hyper-responsiveness to either methacholine or adenosine monophosphate measured 24 h after allergen challenge. However, the difference between active treatment and placebo was close to one doubling dose, which suggests that an improvement might have been seen with greater numbers of participants. Furthermore, variability of the measurement was high, so the lack of statistical significance could be due to a type 2 error

rather than implying that airway hyper-responsiveness was not affected by pitrakinra. It is not uncommon for the sample sizes used in these types of studies to be associated with non-significant changes, partly because of the intrinsic variability of the test. Whereas some laboratories have reported very consistent effects of allergen to increase airway hyper-responsiveness, others have not. In fact, in study 1, an increase in airway hyper-responsiveness was seen in only 13 of the 24 participants. When an increase in airway hyper-responsiveness is not seen after allergen challenge, it is much less likely that the compound of interest will have a significant effect. Additionally, the drug was administered for only 4 weeks. Even inhaled corticosteroids have a modest effect on airway hyper-responsiveness after 4 weeks.³⁷ Our own animal models and those from other laboratories support an effect of interleukin 4 and interleukin 13 on airway hyper-responsiveness.^{12,13} However, larger studies will be needed to determine whether significant improvements in this endpoint will be seen in human beings.

Pitrakinra was associated with few adverse events in the three phase 1 (unpublished data) and three phase 2 clinical studies to date, whether administered by subcutaneous injection (up to 30 mg, for up to 13 weeks) or by inhalation (up to 60 mg nebulised, for up to 4 weeks) in participants with atopic asthma or atopic eczema (133 participants in total). The most common adverse event that we saw after subcutaneous administration was injection site-related discomfort, a common event with most injectable drugs. One should note that the events were not associated with the development of antibodies, nor were they associated with any discernible pattern (ie, they were not more common at the end of the 4 weeks of exposure). There were also fewer spontaneous asthma attacks requiring rescue medication in participants who received pitrakinra subcutaneously than in those in the placebo group. There were also fewer respiratory-related adverse events that required rescue medications in the pitrakinra group than in the placebo group in study 2, although there were too few events to determine significance.

The effects of pitrakinra on late phase asthmatic response are promising when compared with similar studies with other successful anti-inflammatory asthma therapies, including anti-IgE,³⁸ leukotriene antagonists,³⁹ and inhaled corticosteroids.⁴⁰ No current class of asthma drugs has failed in an allergen challenge model only to succeed in a real world setting. Whether the effect is due to inhibition of interleukin 13 alone, or both interleukin 13 and interleukin 4, is not yet known. Future studies of this drug, as well as molecules that specifically inhibit interleukin 13, in asthmatic individuals of all levels of severity over longer periods of time are clearly warranted.

Contributors

DW was the principal investigator. All authors contributed to discussions of protocol design, data analysis, and interpretation of the results of the study.

Conflict of interest statement

RF, EBG, and ML are all employed by Aerovance Inc. DW is employed by Quintiles Ltd, who were contracted by Aerovance to do the clinical study. SW is a consultant for Aerovance Inc and has been paid about US\$1500 in honoraria in the past 3 years for participation in advisory panel discussions.

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