

Expert Opinion

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Pitrakinra for asthma

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Importance of the field: In asthma IL-4 and IL-13 have been demonstrated to play major pathogenic roles and therefore their blockade would potentially represent a plausible therapeutic approach.

Areas covered in this review: Pitrakinra is a dual IL-4/IL-13 inhibitor currently under development for asthma and the existing preclinical and clinical data are discussed.

What the reader will gain: Inhaled pitrakinra demonstrated a good anti-inflammatory potential and a good safety profile on a short-term basis but its place in asthma therapy is still to be found.

Take home message: Specific anticytokine therapies might in the near future reshape asthma therapy.

Keywords: asthma, efficacy, IL-13, IL-4, IL-4R α , pitrakinra, safety

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1. Introduction

Asthma is an inflammatory disease of the airways in which various allergens induce T_H2 lymphocyte proliferation and activation leading to complex inflammatory pathways resulting in activation of eosinophils, mast cells, IgE secretion and airways hyperreactivity (AHR) which are pathogenic hallmarks of this disease. Allergen exposure results in bronchoconstriction manifested clinically as wheezing, dyspnea and chest constriction sensation.

Bronchoconstriction occurs via complex mechanisms and has been evaluated clinically in inhaled allergen challenge models, which subsequently were validated for clinical development of asthma therapies. Allergen challenge with an inhaled allergen results in a bi-phasic bronchoconstriction response. The early asthmatic response (EAR), in which airway smooth muscle contraction is mainly the result of histamine and leukotrienes released from mast cells and lasts approximately 2 h with a peak between 10 and 30 min. In the late asthmatic response (LAR) bronchoconstriction is the result of T_H2-induced eosinophilic airways inflammation and occurs 3 – 8 h later, being able to continue for up to 24 h or even longer [1-3].

In LAR the inflammation is centred on eosinophils and on T_H2-related cytokines such as IL-4, IL-5 and IL-13 which interplay and produce airways hyperresponsiveness, IgE synthesis, airways remodelling and mucus hypersecretion, which are the main pathogenic features in asthma. IL-4 and IL-13 in particular are demonstrated to be potent pro inflammatory cytokines exerting their effects both independently and via a common receptor.

Asthma therapy is aimed at relieving the bronchospasm with rescue medication usually represented by a short-acting β 2-agonist and at reducing airways inflammation with inhaled corticosteroids or cysteinyl leukotriene blockers. However the airways inflammation cannot always be controlled with such therapies especially because such therapies are not able to inhibit airways inflammation evenly. Therefore in such situations further suppression of inflammation is necessary for example with anticytokine therapies, and IL-4/IL-13 blockade might be suitable for this purpose.

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Box 1. Drug summary.

Drug name	Pitrakinra
Phase	IIb
Indication	Asthma
Pharmacology description	IL-4 receptor antagonist
Route of administration	IL-13 receptor antagonist
Pivotal trial(s)	Nebulisation (inhalatory)
	Two Phase IIa trials (NCT00535028 and NCT00535031) evaluating the efficacy and safety of two formulations (subcutaneous and inhalatory) of pitrakinra and demonstrating the good anti-inflammatory efficacy and safety profile

This paper reviews the pathogenic role of IL-4 and IL-13, and discusses the preclinical and clinical data on pitrakinra, an IL-4 variant which blocks both IL-4 and IL-13 and which is a potential candidate for an asthma therapy (Box 1).

2. Overview of the market: anti-inflammatory therapies in asthma

In asthma currently airways inflammation is tackled with regular therapy represented by either inhaled corticosteroids, cysteinyl leukotriene antagonists (such as montelukast) or their combination: inhaled corticosteroids (e.g., mometasone, fluticasone, budesonide) represent the preferred approach in asthma, their potent and non-specific anti-inflammatory activity being demonstrated in both children and adults at various dosages, whereas cysteinyl leukotriene antagonists given orally are usually used when asthma is associated with allergic rhinitis [4]. Such therapies are usually able to reduce airways inflammation and this translates clinically as improvement of lung function and as reduction of respiratory symptoms and of asthma attacks. However, the lack of specific inhibitory effects on T_H2 -related cytokines limit sometimes their efficacy because the suppression of airways inflammation is suboptimal [5]. This can have important consequences for the disease outcome favouring, for example, airways remodeling which in itself limits subsequently the efficacy of these anti-inflammatory therapies. Therefore therapies able to inhibit specifically a cytokine-driven pathogenic pathway might represent efficacious alternative or additive therapies and the IL-4/IL-13 pathway is one such therapeutic target that is currently under evaluation.

3. Pathogenic role of IL-4 and IL-13 in asthma

In asthma IL-4 has been demonstrated to be one of the classical T_H2 -related mediators of allergic inflammation, which along with IL-5 or IL-13 stimulates eosinophil migration, increases eosinophils survival at bronchial level, and

increases mucus hypersecretion [6]. IL-4 was also found to induce ϵ subtype B lymphocyte proliferation and consequent IgE synthesis [7].

Its involvement in airways inflammation was demonstrated in both asthma animal models and in subjects with atopic asthma. Sensitisation of wild type mice with aerosolised ovalbumin resulted in production of ovalbumin-specific IgE, airways hyperresponsiveness and increased eosinophil and lymphocyte counts in bronchoalveolar lavage (BAL), whereas in IL-4 knockout or in MHC Class II deficient (B6.Aa^{-/-}) mice this challenge did not have similar effects [8-10]. In subjects with allergies or atopic asthma IL-4 levels were found to be increased in serum and in BAL, dust mites challenge of PBMCs was associated with increased production of IL-4, whereas IL-4 nebulisation in mild asthma subjects augmented significantly the airways hyperresponsiveness and eosinophil count in sputum [7,11,12].

IL-4 exerts its pathogenic roles via IL-4 receptor, which is a heterodimeric structure composed of two subunits: a high-affinity alpha chain (IL-4R α) and the γ chain [7]. IL-4R α is a component of the complex IL-4R α /IL-13R $\alpha1$ (IL-4 type II receptor) which allow both IL-4 and IL-13 binding and a synergistic pro-inflammatory effect [13,14]. IL-4 also exert its pro-inflammatory effects independently from IL-13 by binding type I receptor IL-4R α / γ [7].

IL-13 is a cytokine that shares with IL-4 some similarities in amino-acid-related structural features and their various gene polymorphisms have been linked to atopy and asthma [15]. IL-13 is released by T_H2 lymphocytes, and by other cells involved in asthma pathogenesis such as mast cells or basophils, and along with IL-4 enhances airways hyperresponsiveness, mucus hypersecretion and airways remodeling [13,16,17]. Increased IL-13 expression was detected in mice and its levels were found to be increased in bronchial biopsies and BAL of asthma subjects after allergen challenge [18-20]. IL-13 can also bind to its specific IL-13R $\alpha2$ receptor but this results in inhibition of its proinflammatory activities [21,22].

Both IL-4 and IL-13 were found to induce airways hyperresponsiveness, mucus hyperproduction and airways remodeling [23-28].

IL-13 in particular was found to be directly involved in Ca^{2+} -mediated proliferation of the airways smooth muscle, and in the RhoA-induced airways hyperresponsiveness [29-31].

4. Chemistry of pitrakinra

Pitrakinra is a recombinant form of the wild-type human IL-4 containing two functional mutations at positions 121 (arginine to aspartic acid) and 124 (tyrosine to aspartic acid). This modified structure preserves the ability to bind to the IL4R $\alpha1$ receptor and prevents the IL-4 and IL-13 binding therefore interfering with the activation of the downstream signaling pathway.

5. Pharmacodynamics

Pharmacodynamics of pitrakinra were analysed with both subcutaneous and inhaled formulations *in vitro* and *in vivo* [32]. *In vitro* pitrakinra inhibited TF-1 cell line (erythroleukemia cell line) proliferation induced by human IL-4 with an IC₅₀ of 0.38 ± 0.25 nM and by human IL-13 with an IC₅₀ of 1.28 ± 1.59 nM. Pitrakinra had no independent agonistic activity. Pitrakinra was also demonstrated to inhibit human and monkey IL-4 induced proliferation in human and monkey T cells in a dose-dependent manner with an IC₅₀ value of 4.49 ± 1.17 (human IL-4) 3.18 ± 0.92 (monkey IL-4) in a human lymphocyte cell line (PBMC) and with an IC₅₀ of 3.69 ± 1.37 (human IL-4) and 2.10 ± 1.12 (monkey IL-4) respectively on monkey lymphocytes [32].

In vivo in the *Ascaris suum*-sensitive cynomolgous monkey, pitrakinra was given subcutaneously at dosages of 0.005, 0.025, 0.05 and 0.5 mg/kg twice per day or via inhaled route at dosages of 0.5, 1.3, and 100 mg twice per day). Compared with control treatment, the 0.05 mg/kg dosage was associated with a 3.8-fold reduction in AHR ($p = 0.0115$), whereas the 0.5 mg/kg dosage produced a 2.8-fold reduction in the AHR ($p = 0.0049$) [32]. Inhaled pitrakinra given at dosages of 100 mg twice per day and 3 mg trice per day respectively reduced significantly the AHR as compared with control therapy ($p = 0.002$ and 0.028 respectively) and both formulations were found to exert a similar maximal therapeutic effect consisting of a threefold reduction of AHR relative to control [32]. Airways eosinophilia was found to be reduced only by 0.025 mg subcutaneous twice per day ($p = 0.038$) and by 3 mg inhaled twice per day ($p = 0.005$).

Pitrakinra when administered 'chronically' at 1 mg/kg three times a week as subcutaneous injections over a 12-week treatment period reduced significantly the acute cutaneous wheal and flare response to injected *A. suum* challenge ($p = 0.005$) as compared with placebo [32]. The maximum therapeutic effect was observed at week 10 (after 8 weeks of therapy) and consisted of a 68% inhibition of hypersensitivity reaction. However, afterwards its effect disappeared and the cutaneous wheal returned to its pretreatment levels and this was due to the blocking effect of an anti-IgG anti pitrakinra antibody produced over the treatment period [32].

The primary pharmacodynamic endpoint of each of the Phase IIa studies performed in asthma subjects was represented by allergen-induced decrease in FEV1 during late asthmatic response (LAR). Both formulations produced a significant therapeutic effect as compared with placebo, but this was reported to be uniform with inhaled pitrakinra and inconsistent with subcutaneous formulation. The therapeutic effect of the inhaled formulation was not found to correlate significantly with plasma exposure [33]. Nebulized pitrakinra was also found to reduce significantly the levels of FeNO after 4 weeks therapy as compared with placebo [33].

6. Pharmacokinetics

In the preclinical study performed in animal model of AHR the comparative analysis of pharmacokinetics of subcutaneous and inhaled formulations demonstrated that inhaled pitrakinra reduced AHR at plasma concentrations insufficient to demonstrate a similar effect if given subcutaneously. In the case of the inhaled formulation, a minor fraction of the inhaled compound was found to be absorbed rapidly, its subsequent plasma kinetics being found to be similar to those for subcutaneous injection [32].

Clinical pharmacokinetics on pitrakinra were analysed in 70 subjects with asthma or atopic eczema who received subcutaneous formulation and in 26 asthma subjects receiving inhaled (nebulised or dry powder) formulation in five Phase I, IIa or I/IIa studies [33]. The primary pharmacokinetics analysis following day 1 single dosing including pooled data from all 96 subjects and consisted of evaluation of concentration versus time profiles of the three formulations, the subcutaneous formulation being tested at dosages of 0.07, 0.7, 2, 7, 20 or 30 mg, the nebulized formulation at 60 mg, and dry powder inhaled formulation at 10 mg [33]. Irrespective of the formulation the absorption rate was rapid relative to the elimination, being more retarded with the inhaled formulation (about 17 – 18 min) as compared with the subcutaneous formulation (approximately 2 min), with the absorption half-life being 1 h with subcutaneous formulation and 0.5 – 0.6 h with inhaled formulations [33].

Systemic relative bioavailability was found to be larger with the dry powder formulation than with nebulised formulation and was found to increase with forced expiratory volume in 1 sec (FEV1) overall for these two inhaled formulations [33].

Systemic relative bioavailability of the subcutaneous formulation was found to be dose-dependent but this effect was of small amplitude: a 100-fold increase in pitrakinra dose (from 0.2 to 20 mg) resulted in a 55% increase in its relative systemic availability [33].

Like the single dose analysis, that of repeated subcutaneous dosing pharmacokinetics yielded a low plasma accumulation, the measured 1 h post-dose pitrakinra plasma levels following a 4 week course of twice daily 30 mg subcutaneous injection was found to be only 10.2% higher than that of the first dose.

Nebulised pitrakinra given as 60 mg twice daily for 4 weeks in subjects with mild to moderate asthma produced, in a subset of 12 patients who tested negative for anti-pitrakinra IgG antibodies after the last dose of the 4 week therapy course, a fourfold increase in systemic exposure without influencing the half-life, suggesting a time-dependence of systemic availability but not of clearance [33]. In the other three patients with anti-pitrakinra IgG the systemic exposure was found to be even larger, and this increase was attributed to the antibody effect itself, possibly due to increased neonatal Fc receptor-mediated absorption across the lung.

7. Clinical efficacy

Two Phase IIa clinical double-blind placebo-controlled parallel-group studies evaluated the efficacy and safety as well as pharmacokinetics of two formulations of pitrakinra (subcutaneous, inhaled) treatment for 4 weeks in patients with atopic asthma and allergy to cats/grass/house dust mites demonstrated with skin prick tests [34]. Included were patients with known asthma who were stable non smokers in the last 3 months, not taking corticosteroids for asthma and having a baseline forced FEV1 of more than 70%, and demonstrated airways hypersensitivity expressed with a provocative concentration that causes a 20% fall in FEV1 from saline alone value (PC20), of less than 8 mg/ml metacholine in study 1 and of more than 3.125 mg/ml adenosine monophosphate. In study 1 patients were randomized to receive 25 mg pitrakinra or placebo once daily subcutaneously, whereas in study 2 60 mg inhaled pitrakinra or placebo were nebulised twice daily. During the initial screening (visit 2) the inhaled allergen challenges were performed in both studies and the inhaled allergen concentration causing a FEV1 fall of about 20% was also used for the post-treatment challenge (day 27). The primary efficacy endpoint of both studies was FEV1 change during late asthmatic response 4 – 10 h post-allergen challenge defined as maximum percentage fall in FEV1 in study 1 and as average percentage fall in FEV1, in study 2.

Secondary efficacy endpoints included blood eosinophil counts (studies 1 and 2) fractional expiratory NO (FENO) and blood total IgE in study 2, PC20, cutaneous allergen response and sputum eosinophils in study 1 [34].

Four weeks of pitrakinra therapy resulted in a decrease of allergen-induced FEV1 fall irrespective of the route of administration of the compound, but only inhaled formulation yielded a significant therapeutic effect as compared with placebo. Subcutaneous pitrakinra produced a threefold reduction in the late phase allergen-induced FEV1 fall ($p = 0.068$) whereas inhaled pitrakinra produced a 3.7-fold reduction in FEV1 fall ($p = 0.0001$). Inhaled pitrakinra unlike subcutaneous formulation also resulted in a significant reduction in the maximum percentage of FEV1 fall (placebo 27.6 versus 16.1% $p = 0.0009$).

Neither subcutaneous nor inhaled pitrakinra formulations significantly reduced airways hyperresponsiveness to metacholine with respective to AMP.

Pre-randomisation pre-allergen challenge FeNO was found to be significantly high (63.2 ± 39.2 p.p.b., normal range 0 – 25 p.p.b.) as well as after the initial (pre-randomisation) allergen challenge (111.7 ± 47.9 p.p.b., $p < 0.0001$ relative to screening visit 2). Inhaled pitrakinra reduced significantly pre-allergen FeNO after 4 weeks therapy (34.9 p.p.b. pitrakinra versus 63.1 p.p.b. in placebo, $p = 0.005$) [34].

Subcutaneous pitrakinra didn't reduce significantly the sizes of the skin prick tests or the sputum eosinophil counts and both formulation demonstrated a similar effect on blood eosinophil count. Inhaled pitrakinra didn't reduce blood IgE levels significantly ($p = 0.096$) [34].

8. Safety and tolerability

In the two Phase II studies clinical safety endpoints, included haematology, serum biochemistry, urinalysis, vital signs, ECG and lung function. 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) [34].

No significant changes in the vital signs, haematology, biochemistry, urinalysis, lung function or ECG tests safety endpoints were reported between treatment and placebo group in each study or overall in both studies. There were more general disorders reported with subcutaneous pitrakinra administration when compared with placebo, and these were mainly represented by injection-site reactions and/or discomfort: among the 672 injections administered, 39 injection-site-related adverse events were reported overall (35 in 8 out of 12 patients receiving pitrakinra and 4 in 2 out of 12 patients receiving placebo). In study 1 in pitrakinra group fewer asthma-related adverse events requiring β -agonist rescue were reported as compared with the placebo group (three events in three patients versus 11 events in five patients, $p = 0.031$). Similarly in study 2, two participants receiving pitrakinra as compared with five in the placebo group presented with such events. The incidence of asthma-related symptoms (wheezing, dyspnoea, and chest discomfort) was lower in patients receiving subcutaneous pitrakinra as compared with placebo (6 events in 4 patients versus 14 events in 7 patients $p = 0.069$). Anti-pitrakinra IgG antibodies were detected during follow up period (5 – 10 days after the last study treatment) in 3 of 10 participants receiving subcutaneous formulation (with titres of 1:40, 1:80 and 1:80) and in 3 of 15 participants after inhalation (with titres of 1:30, 1:60, and 1:480). However in all patients, irrespective of pitrakinra formulation received, these antibodies were demonstrated to be non-neutralising antibodies.

9. Regulatory affairs

Pitrakinra inhaled formulation is currently in Phase II of clinical development for asthma.

10. Conclusions

Both IL-4 and IL-13 are recognized for their pathogenic role in airways inflammation and remodeling in asthma. They exert their effects mostly by binding the common IL4R α and this explains the high inflammation potential of these cytokine 'team' and the lower efficacy of non-specific anti-inflammatory therapies. Pitrakinra is a recombinant IL-4 variant, which by binding IL-4R α avoids the coupling of native IL-4 and IL-13 interleukins and the development of their downstream effects on eosinophils, IgE or mucus hypersecretion. Pitrakinra was evaluated in preclinical studies and was found to suppress IL-4-related antiproliferative effects

in vitro and to reduce airways inflammation and hyperreactivity in asthma animal models. Pivotal Phase IIa studies evaluating the short-term effects of pitrakinra in patients with asthma demonstrated that the inhaled formulation was superior to the subcutaneous formulation and this treatment reduced airways inflammation and improved lung function exhibiting also a good safety profile.

11. Expert opinion

In asthma the IL-4/IL-13 pathway has been examined as an appropriate therapeutic target given its prominent pathogenic role. Indeed this pathway can be inhibited nonspecifically with corticosteroids given both systemically and via the inhalatory route. However although inhaled corticosteroids are currently recognized as the therapeutic mainstay in asthma their long-term use, especially at higher doses, might be associated with safety problems such as osteoporosis or delayed growth. In such situations airways inflammation might be reduced with anti-inflammatory therapies able to suppress the downstream effects of cytokines such as IL-4, IL-5 or IL-13 and IL-4/IL-13 common pathway blockade in particular seems to be a plausible approach, given its prominent role in asthma-related eosinophilic inflammation.

Therefore pitrakinra which is a dual IL-4/IL-13 receptor antagonist demonstrating sustained anti-inflammatory effect in both animal models and asthma subjects seems to be a promising candidate as an asthma therapy.

Indeed the demonstration of significant anti-inflammatory activities and of a good safety profile are assets which, however, need to be reproduced in longer-duration studies in order to further support the use of pitrakinra as an anti-inflammatory therapy in asthma. Apart from demonstration of supportive efficacy and safety, pitrakinra also has the advantage of administration route, the topic (inhalatory) therapy allowing targeted delivery to the airways, dosage reduction and minimization of systemic adverse effects including immunogenicity.

The immunogenic potential of this therapy in particular should be further evaluated with long-term use because the demonstration of the lack of interference with therapeutic effect of the antibodies detected in clinical studies doesn't guarantee similar behaviour when pitrakinra is given on a long-term basis.

Other IL-4-blocking therapies are currently under evaluation for asthma [35]: humanized IL-4 monoclonal antibodies such as pascolizumab (SB 240683) demonstrated its

inhibitory potential in preclinical studies and is under clinical evaluation, anti IL-4 α antibodies (AMG317) were also tested in asthma, IL-4 receptor antagonism via antisense oligonucleotide therapy was also found to exert anti-inflammatory activities similar to those of dexamethasone and soluble recombinant human IL-4 receptor (altrakcept Nuvance, Immunex) was shown to improve lung function and reduce airways inflammation when given via the inhalatory route in asthma subjects.

Anti IL-13 therapies are also under development in asthma and monoclonal antibodies such as IMA-638 or CAT-354, or IL-13 antagonism with short interfering RNA (siRNA) or with soluble IL-13 α 2-IgGf fusion protein (sIL-13R) could also evolve as asthma-specific therapies [35].

Other specific therapies against cytokines such as IL-9 or IL-2 have been evaluated in asthma as well.

The place of such therapies in asthma should be established, taking into account their ability to block specifically certain allergy-related pathogenic pathways leaving others not tackled and potentially even more amplified to replace the missing ones. This may interfere significantly with their efficacy and may in fact explain the overall modest clinical efficacy of anti-cytokine therapies when given alone. A potential approach to address such an inconvenience would be a combination of a non-specific anti-inflammatory therapy (e.g., inhaled corticosteroids) and a specific anticytokine therapy. A synergistic effect is envisaged but must be demonstrated and this might result in a reshaping of anti-inflammatory maintenance therapy in asthma.

However not all asthma phenotypes are 'allergic' or even if they are the 'allergic load' is very low. In such situations other anti-inflammatory compounds able to reduce the airways inflammation adequately are needed.

To conclude, in atopic asthma inhibition of IL-4/IL-13 pathway with specific therapies is supported by the existing data, but the most appropriate compound(s) are still to be found. Pitrakinra might be one such therapy but further clinical studies are needed in order to better document its efficacy and its safety and in order to position it appropriately as an asthma therapy. Demonstration of inhibitory effects on airways remodeling would in particular represent a major advantage over corticosteroids and would further support development of pitrakinra as an asthma therapy.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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