The Discovery and Development of AZD1402/PRS-060 a Potent and Selective Blocker of the IL-4 Receptor alpha

G. Matschiner[±], S. Huang[+], S. Constant[+], B. Rattenstetter[±], H. Gille[#], A.M. Hohlbaum[§], B.H. Koller[**], D. Keeling[*] & M. Fitzgerald[±]

[±] Pieris Pharmaceuticals, 255 State St, 9th Floor, Boston, MA 02109, USA; [+] Epithelix Sàrl, Geneva, Switzerland; [*]Respiratory, Inflammation and Autoimmunity, IMED Biotech Unit, AstraZeneca, Gothenburg, Sweden; [#] Iason Consulting, Niederzier, Germany; [§]Ablynx, Ghent, Belgium; [*] Department of Genetics, UNC at Chapel Hill, USA

Introduction

In 5-10% of asthma patients control of disease is not achieved with standard of care therapies (inhaled corticosteroids in combination with long-acting beta-2 agonists). This inability to achieve asthma control significantly impacts patients' quality of life and healthcare costs. Type 2 (T2) cytokines, specifically interleukin (IL)-4, IL-5, and IL-13, play crucial roles in asthma pathogenesis[1,2]. Dupilumab, a fully human anti-IL-4 receptor alpha (IL-4Rα) monoclonal antibody given by subcutaneous injection, inhibits IL-4 and IL-13 signalling, key drivers of T2-mediated inflammation, and has been shown to reduce exacerbations and improve lung function in moderate to severe asthma subjects[3]. Pitrakinra, an inhaled IL-4 mutein that antagonises IL-4Rα, has also been shown to have beneficial effects in a subset of asthma patients[5]. PRS-060/AZD1402, is a human tear lipocalin derived Anticalin antagonist that has a high potency and selectivity for the human IL-4 receptor alpha. Selectivity has been established by testing against a range of cytokine receptors. PRS-060/AZD1402 is being developed as an inhaled treatment for moderate to severe asthma.

Methods

TF1 cells, known to express IL-4Rα[4], were used in a FACS assay measuring the signal transducer and activator of transcription 6 (STAT6) phosphorylation following IL-4 or IL-13 stimulation in the presence and absence of PRS-060/AZD1402. To demonstrate functional activity of PRS-060/AZD1402, a proliferation assay using hGM-CSF starved TF1 cells stimulated with a low dose of IL-4 (0.1 nM) or IL-13 (10 nM) was set up, using as readout the release of ATP by living cells.

In a human airway epithelium culture system (3D MucilAir™)[6], incubation with IL-13 (10 ng/ml, every 2 days for a total of 14 days) induced a goblet cell metaplasia as assessed by *in situ* Alcian blue staining.

As PRS-060/AZD1402 does not cross react with IL-4Rα from species commonly used for *in vivo* efficacy studies, a syntenic (humanised) mouse was generated by Dr. Beverly Koller at UNC-Chapel Hill and the mouse studies were performed in her laboratory. In this mouse the genes for IL-4Rα and IL-4/13 were replaced with the respective human orthologues. This mouse both responds to human IL-4 and IL-13 but also generates human IL-4 and IL-13 when the T2 cytokine pathway is activated.

A pharmacodynamic murine model for the evaluation of the potency and duration of action of PRS-060/AZD1402 was developed in this mouse. Human IL-13 (1µg) was given via the intra-tracheal (i.t.) route and the expression of Ccl11 (eotaxin-1) was quantified in lung tissue by qPCR 24-hour post challenge.

An ovalbumin (OVA) model of asthma was also developed in these mice. Mice were sensitised to OVA (20µg OVA in Alum i.p.) on day 0 and day 7 and were challenged with an aerosol of OVA on day 14. Animals were sacrificed 24 and 48hr later and the inflammatory response was assessed in the bronchoalveolar lavage fluid by performing total and differential cell counts. In separate animals, lungs were perfused with PBS/ heparin followed by 4% PFA in PBS. Lungs were then inflated with 4% PFA/ PBS at a constant head pressure of 20 cm H_20, and then maintained in the inflation solution until fixation. Three serial sagittal lung sections were then prepared from formalin fixed, paraffin blocked lung tissue and stained for haematoxylin and eosin, Periodic-acid Schiff and trichrome demonstrations, respectively.

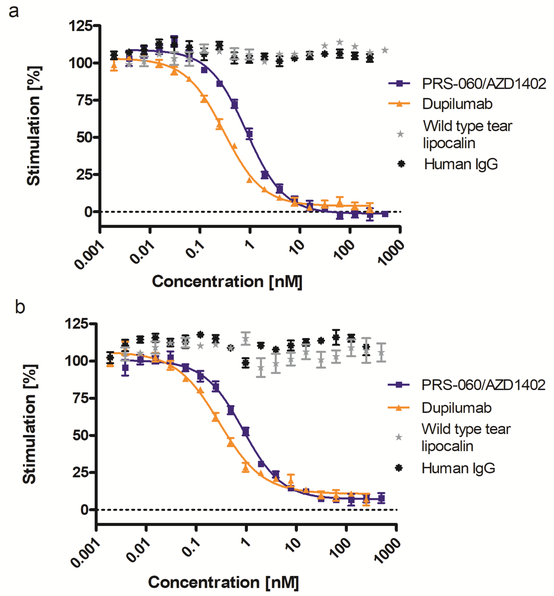
Results 1

Table 1. Dual inhibition of hIL-4 and hIL-13 induced STAT6 phosphorylation in TF1 cells by PRS-060/AZD1402

Test substance	IL-13 [10nM] induced TF-1 pSTAT6 IC_{50} (mean ± SD)	IL-4 [0.1nM] induced TF-1 pSTAT6 IC_{50} (mean ± SD)
PRS-060	0.097 nM ± 0.007	0.14 nM ± 0.04
IL-4 mutein (Pitrakinra)	9.1 nM ± 1.088	7.12 nM ± 0.06

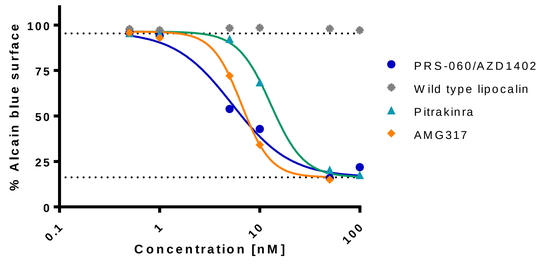
- In an *in vitro* assay using TF-1 cells, PRS-060/AZD1402 showed dual inhibition of IL-4R and IL-13R signalling by inhibiting both the IL-4 and IL-13 induced STAT6 phosphorylation with 50 - 90 fold greater potency compared to pitrakinra (table 1), respectively.

Figure 1. Inhibition of a) IL-4 and b) IL-13 induced TF1 cell proliferation by PRS-060/AZD1402



- In a functional cell-based assay, PRS-060/AZD1402 inhibited IL-4 and IL-13 induced proliferation of TF-1 cells with similar potency to dupilumab (figure 1).

Figure 2. Inhibition of IL-13-induced goblet cell metaplasia by PRS-060/AZD1402



Results 2

- In a human airway epithelium culture system (3D MucilAir™), PRS-060/AZD1402 effectively inhibits IL-13 induced goblet cell metaplasia with a similar potency to AMG317, an anti-IL-4 receptor alpha (IL-4Rα) monoclonal antibody, and a greater potency than pitrakinra (figure 2).

Figure 3. Effect of PRS-060/AZD1402 on IL-13-induced increases in eotaxin gene expression in murine PD model

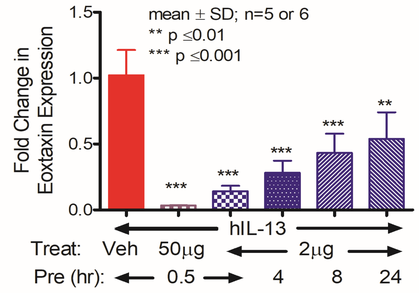
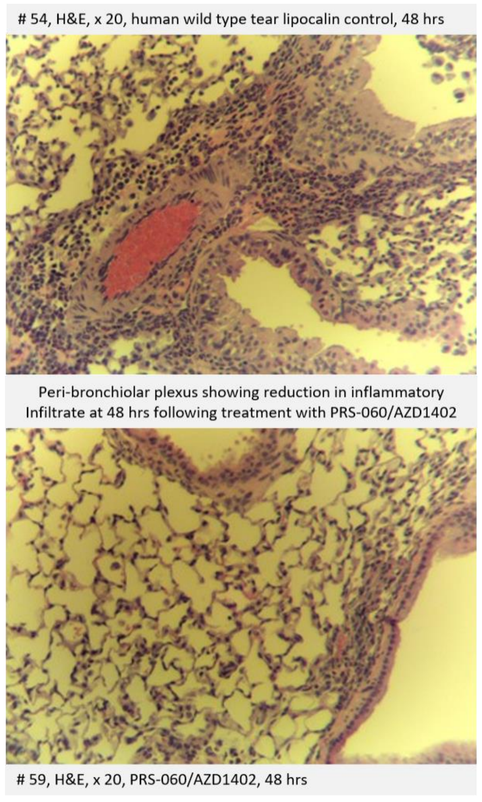
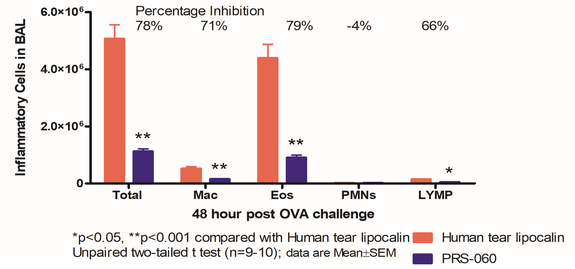


Figure 4. Effect of PRS-060/AZD1402 on OVA induced histopathological outcomes 48 hours post challenge in a murine asthma model



Results 3

Figure 5. Effect of PRS-060/AZD1402 on OVA induced inflammation 48 hours post challenge in a murine asthma model



- In an *in vivo* pharmacodynamic model developed in humanised mice, pre-treatment with PRS-060/AZD1402 given via the i.t. route, inhibited IL-13-induced eotaxin gene expression. A dose of 2µg/mouse inhibited this response over a 24-hour period (figure 3).
- In a murine asthma model developed in the humanised mouse, PRS-060/AZD1402 given i.t. (30µg/mouse) twice, 0.5 hours prior to and 24 hours post OVA challenge, inhibited antigen-induced pulmonary inflammation in the BAL 48 hours post challenge (figure 5). In this model, treatment with PRS-060/AZD1402 was associated with histologically significant reductions in bronchiolar and alveolar inflammation, eosinophil, neutrophil, macrophage infiltration, bronchiolar epithelial hyperplasia and fibroplasia (figure 4).

Summary

- PRS-060/AZD1402 is a potent and selective antagonist of the IL-4Rα that has a comparable profile to the monoclonal antibody to this receptor, dupilumab, and 50-90 fold greater potency than the inhaled IL-4 mutein, pitrakinra.
- PRS-060/AZD1402 effectively inhibits IL-13 induced goblet cell metaplasia *in vitro* supporting the concept that treatment will reduce mucus hypersecretion in moderate to severe asthma patients.
- In a murine PD model a single intra-tracheal dose of PRS-060/AZD1402 potently inhibited an IL-13 induced response and had a 24-hour duration of action.
- In a murine asthma model, intra-tracheal PRS-060/AZD1402 reduced pulmonary inflammation as assessed by an inhibition of BAL cell recruitment and via a reduction in antigen-induced histopathological changes.

Conclusions

The overall profile of PRS-060/AZD1402 supports its development as an inhaled therapy for moderate to severe asthma. Non-clinical safety studies suggest that this inhaled Anticalin therapeutic is safe and well tolerated and it is currently being evaluated clinically for safety, tolerability and efficacy in two Phase 1 studies (NCT03384290 and NCT03574805).

References

1. Voehringer et al. J Exp Med. 2006;203:1435-46.
2. Locksley Cell. 2010; 140:777-83.
3. Wenzel et al. Lancet. 2016; 388:31-44.
4. Lefort et al. FEBS Letters 1995; 366: 122-12.
5. Slager et al. JACI 2012; 130: 516-522.
6. http://www.epithelix.com/products/mucilair

Disclosure of Commercial Support and Relevant Financial Interests: Dr. Matschiner is an employee of Pieris Pharmaceuticals, Dr. Fitzgerald is a consultant for Pieris Pharmaceuticals, and Dr. Keeling is an employee of AstraZeneca; Professor Koller has provided scientific support and advice on the development of mouse models and the testing of novel Anticalin proteins.