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


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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, 5, and 13, play crucial roles in asthma pathogenesis1-5. Dupilumab, a fully human anti-IL-4 receptor alpha (IL-4Rα) monoclonal antibody given by subcutaneous injection, inhibits IL-4 and IL-13 signaling and produces T2-mediated inflammation, and has been shown to reduce exacerbations and improve lung function in moderate to severe asthma subjects5. Pitrakinra, an inhaled IL-4 mutein that antagonises IL-4Rα, has also been shown to have beneficial effects in a subset of asthma patients5. PRS-060/AZD1402, is a human 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, 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-M077-1 was performed 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®), incubation with IL-13 (30 ng/ml) per 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 (3µg) 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 (30µg 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 48h 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 H2O and then maintained in the inflation solution until fixation. Three 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.

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

<table>
<thead>
<tr>
<th>Test substance</th>
<th>IL-13 (10nM) induced TF-1 pSTAT6 IC50 (mean ± SD)</th>
<th>IL-13 (100nM) induced TF-1 pSTAT6 IC50 (mean ± SD)</th>
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<tr>
<td>PRS-060</td>
<td>0.10 ± 0.01</td>
<td>0.14 ± 0.04</td>
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<tr>
<td>IL-4-mutein (Pitrakinra)</td>
<td>1.00 ± 0.01</td>
<td>7.12 ± 0.06</td>
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In an in vitro assay using TF1 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

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

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 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

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

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

Table 2. Results 1

<table>
<thead>
<tr>
<th>Test substance</th>
<th>Mean ± SD</th>
<th>m5 or 6</th>
<th>** p &lt; 0.01</th>
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<tbody>
<tr>
<td>PRS-060/AZD1402</td>
<td>0.01 ± 0.01</td>
<td>0.01 ± 0.01</td>
<td>&lt; 0.001</td>
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</table>

Table 3. Results 2

Conclusion

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 NCT03179405).

References