AZD1402/PRS-060, an inhaled Anticalin® IL-4Rα antagonist, potently inhibits IL-4 induced functional effects in human whole blood, which can be employed translationally in clinical studies.

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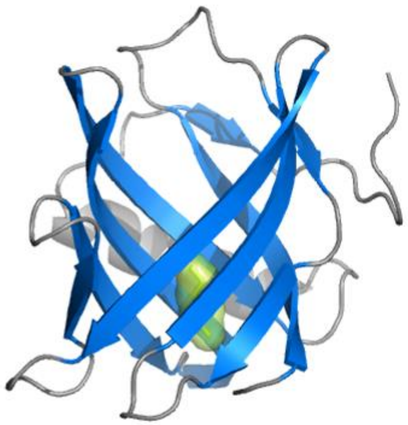
Abstract

Introduction: AZD1402 is an Anticalin® protein in clinical development that has the potential to offer an inhaled treatment for asthma patients suffering from T2-driven disease through selective blockade of IL-4Rα.
Aims and objective: To characterise the effect of AZD1402 on IL-4Rα signalling in human whole blood (WB) and establish a method to evaluate the functional impact of systemic exposure to AZD1402 following inhaled dosing.
Methods: WB from healthy subjects was stimulated with IL-4 in the presence or absence of AZD1402. Phosphorylation of signaling components and released soluble biomarkers were quantified using FACS and multiplex ELISA, respectively.
Results: Stimulation of human WB with IL-4 resulted in increased levels of phosphorylated STAT6 (pSTAT6) and in the release of eotaxin-3, TARC, and MDC. AZD1402, when added to WB samples (n=12), inhibited pSTAT6 in a concentration-dependent manner and with similar potency to the anti-IL-4Rα monoclonal antibody dupilumab (IC50 values1.3 and 0.8 nM, respectively). Inhibition of the release of the soluble cytokines eotaxin-3, TARC, and MDC by AZD1402, at equivalent potencies to dupilumab, was observed (IC50 values of 2.1 nM, 1.3 nM, and 2.0 nM, respectively). The low level of variation observed render this method suitable to detect the presence of systemic (pharmacologically active) levels of AZD1402 following inhaled dosing.
Conclusions: AZD1402, potently inhibits IL-4Rα signalling in human WB with IC50 values comparable to those of dupilumab. pSTAT6 responses in WB are used in the NCT03384290 Phase I trial to assess systemic exposure.

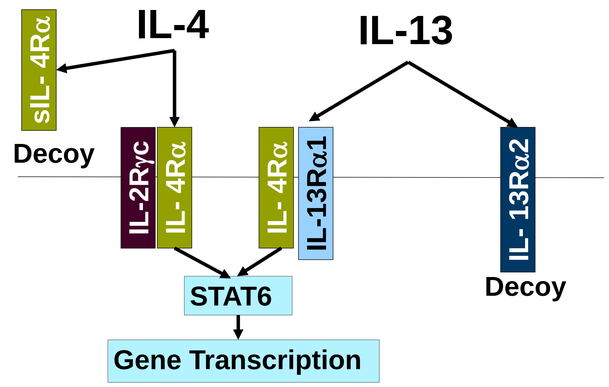


- AZD1402/PRS-060 is an Anticalin® protein engineered from human lipocalins developed by Pieris Pharmaceuticals. Its low molecular weight (16.9 kDa) makes it ideally suited for inhaled delivery.
- The antibody-like binding of Anticalin proteins allows inhibition of a wide range of important biological targets not tractable with small molecule drugs

Introduction

- AZD1402 is an Anticalin® antagonist of IL-4Rα, intended as inhaled treatment for moderate to severe asthma through selective receptor blockade in T2-driven disease.
- AZD1402 is currently in Phase 1 studies; a single ascending dose study in healthy volunteers and multiple ascending dose study in mild asthmatics.
- IL-4 signals via IL-4Rα and results in phosphorylation of STAT6, downstream gene transcription and cytokine release of mediators such as Eotaxin-3, TARC, and MDC (Fig. 1).
- Assessing AZD1402 functional effects in whole blood with robust assays allows us to determine systemic target engagement and potentially to help dissect local from systemic effects of the inhaled drug.

Figure 1:
IL-4Rα is the common receptor subunit for signalling of IL-4 and IL-13



Aim and objectives

To characterise the effect of AZD1402 on IL-4Rα signalling in human whole blood and establish a method to evaluate the functional impact of systemic exposure to AZD1402 following inhaled dosing.

Methods

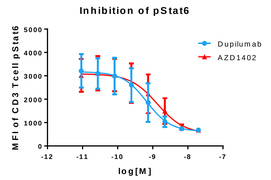
- Heparin treated whole blood was stimulated with 8 ng/ml IL-4 for 15 min with increasing concentrations of AZD1402 and pSTAT6 in the CD3+ T cell subpopulation was assessed.
- Heparin treated whole blood was stimulated with 8 ng/ml IL-4 for 24 h with increasing concentrations of AZD1402, followed by measurement of Eotaxin-3, TARC, and MDC using multiplex ELISA.

Results

***In vitro* addition of AZD1402 during IL-4 stimulation results in:**

1. Dose dependent inhibition of STAT6 phosphorylation with a similar potency to the IL-4Rα blocking monoclonal antibody, dupilumab (Fig. 2).
2. Dose dependent inhibition of the soluble biomarkers Eotaxin-3, TARC and MDC with similar potency to dupilumab (Fig. 2). These assays are being further developed using the TruCulture® System technology (HotScreen and Myriad RBM) and can easily be applied in a clinical setting (Fig. 3)

Results



Drug	IC_{50} [nM] pSTAT6	IC_{50} [nM] Eotaxin-3	IC_{50} [nM] TARC	IC_{50} [nM] MDC
AZD1402	1.3	2.1	1.3	2.0
Dupilumab	0.8	1.5	0.8	1.1

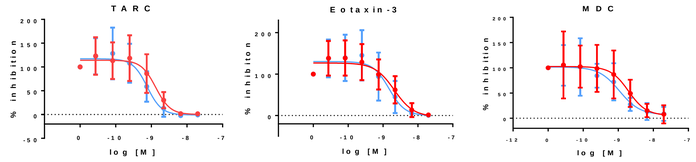


Figure 2: *In vitro* addition of AZD1402 reduces the levels of pSTAT6 and the levels of Eotaxin-3, TARC and MDC after IL-4 stimulation:
Inhibition of pSTAT6 (upper left), Eotaxin-3, TARC and MDC (lower panels) and table with the IC_{50} values of **AZD1402** (●) and **Dupilumab** (●) in the assays depicted (upper right).

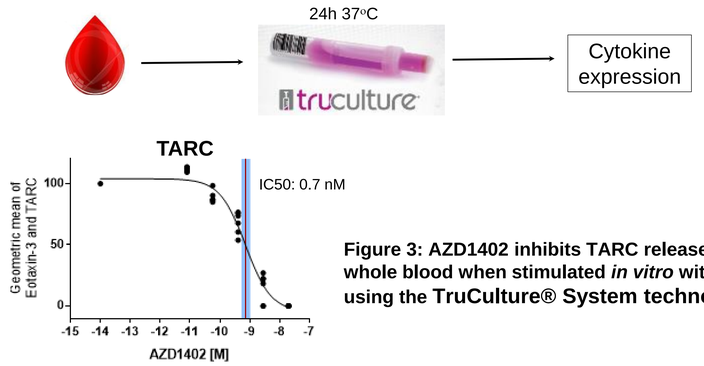


Figure 3: AZD1402 inhibits TARC release from whole blood when stimulated *in vitro* with IL-4 using the TruCulture® System technology.

Conclusions

- AZD1402 inhibits IL-4 signaling in whole blood as assessed by STAT6 phosphorylation as well as Eotaxin-3, TARC and MDC production induced by IL-4 stimulation. It had a similar potency to dupilumab in these functional assays.
- Measurement of *ex vivo* IL-4-stimulated pSTAT6 responses in whole blood as well as downstream cytokine release can be used to assess systemic target engagement following inhaled dosing of AZD1402. Furthermore, these assays will contribute to a more complete understanding of the site of action of this drug.

References
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2. IL-4 induces expression of TARC/CCL17 via two STAT6 binding sites; Wirnsberger G., Hebenstreit D., Posselt G., Horejs-Hoeck J., Duschl A.; *Eur J Immunol*; 2006.

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