Pulmonary Delivery of PRS-400 Anti-Jagged-1 Anticalin® Proteins Reduce Inflammation-Driven Goblet Cell Metaplasia and Mucus Hypersecretion *in Vivo*

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Background

A pathogenic driver of many chronic lung diseases, including asthma, chronic obstructive pulmonary disease, cystic fibrosis (CF), primary ciliary dyskinesia and non-CF bronchiectasis, is obstruction caused by mucus hypersecretion and/or impaired mucociliary clearance.

Jagged-1, one of 5 human Notch receptor ligand members, is involved in cell fate specification. In the lung, Jagged-1, acting predominantly through Notch2, controls the balance of secretory club cells and ciliated cells. Local or systemic inhibition of the Jagged-1/Notch pathway *ex vivo* and *in vivo* redirects lineage specification towards ciliated cells and promotes loss of club cells, thus preventing their differentiation into mucus-secreting goblet cells which profoundly reduces mucus burden in the airways (Lafkas et al., Nature 2015). As the Notch pathway is active in multiple other organs, an inhaled intervention is especially suited to circumvent side effects previously described in clinical trials with systemically delivered molecules.

Anticalin® proteins derived from human lipocalins can be engineered to bind to their targets with high potency and selectivity. While their binding affinity is similar to that of antibodies, their small size and biophysical properties make Anticalin proteins uniquely suitable for lung delivery via inhalation, as demonstrated by the ongoing evaluation of Elarekibep (PRS-060) in a Phase 2 asthma study and inhaled PRS-220 in a Phase 1 study as a prelude to IPF patients.

Here, we characterize two PRS-400 Jagged-1 targeting Anticalin proteins (PRS-400 Parental/precursor molecule and PRS-400 Lead candidate) *ex vivo* and *in vivo* for the treatment of muco-obstructive lung diseases via inhalation.

Figure 1. Schematic representation of an Anticalin protein. Building block comprises four variable loops and a rigid, conserved beta-barrel, which together form a pliable cup-like binding pocket that provides target specificity and the required molecule stability to allow formulation for inhaled delivery.

The Anticalin scaffold's advantages at a glance:

- **Human** – Scaffold derived from human lipocalins (extracellular binding proteins)
- **Specific** – High potency and selectivity for targets
- **Small** – Monomeric, monovalent, small size (~18 kDa vs ~150 kDa mAbs)
- **Stable** – High melting temperatures & insensitivity to mechanical stress
- **Formulable** – Nebulization & dry powder inhalation
- **Proprietary** – Broad IP position on platform and derived products
- **Validated** – Strong industrial partners and clinically tested

Favorable drug-like properties for lung delivery

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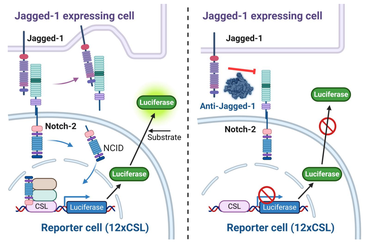
Results

PRS-400 Anticalin proteins inhibit Jagged-1-induced Notch2 activation *in vitro*



B

	PRS-400 Lead		PRS-400 Parental		Anti-Jagged-1 mAb (AMG430)
	Human	Murine	Human	Murine	Human
IC50[nM]	1.86	3.12	8.33	4.42	2.58
95% CI (profile likelihood) of IC50	1.07 to 3.29	2.25 to 4.31	4.63 to 15.16	2.78 to 7.10	1.38 to 4.85
R squared	0.90	0.97	0.90	0.86	0.91

Figure 2. A) Schematic overview of a Jagged-1/Notch2 reporter assay to assess *in vitro* potency of Anticalin proteins. Reporter cells lines expressing endogenous human or murine Notch2 were engineered to express luc2 luciferase under the control of a 12x repetitive CSL domain. Reporter cells were co-cultured for 24 h with overexpressing human or murine Jagged-1 cells resulting in a quantifiable luminescence signal upon Notch2 activation. The scheme was generated using BioRender. B) Table includes IC_{50}-values of PRS-400 Lead candidate, PRS-400 Parental clone (precursor of PRS-400 Lead candidate) and anti-Jagged-1 antibody (AMG430) that were retrieved from human and murine Jagged-1/Notch2 reporter assay experiments. Antibody AMG430 was generated in-house from patent-derived sequences.

Jagged-1-targeting Anticalin proteins reduce cytokine-induced goblet cell metaplasia and mucin expression in ALI cultures *ex vivo*

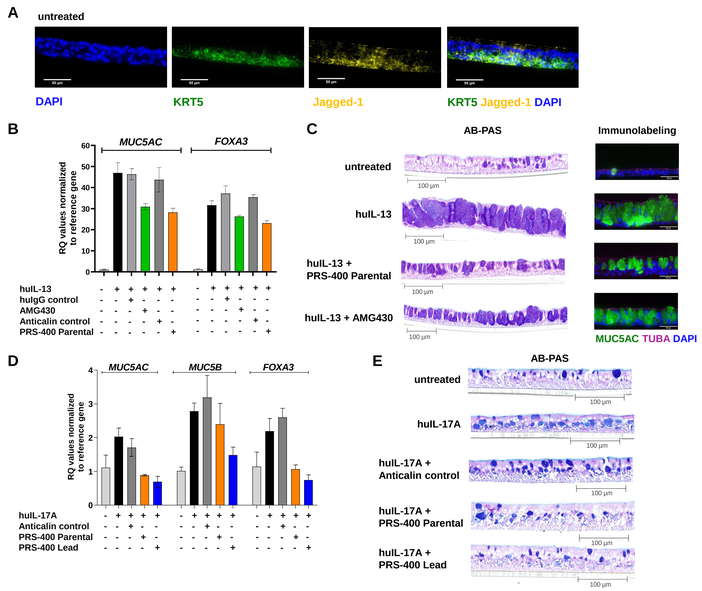


Figure 3. Air-liquid interface (ALI) cultured normal human bronchial epithelial cells, were air-lifted for 21 days prior to treatment to generate a pseudostratified lung epithelium. Goblet cell metaplasia (GCM) was induced by basolateral treatment with 1 ng/mL huIL-13 or 10 ng/mL huIL-17A every other day for three (B, D) or four (C, E) times in total. A) Cell type specific Jagged-1 expression was visualized by immunofluorescent co-immunolabeling in vertical ALI cell sections showing mainly expression in KRT5⁺ cells (basal cells). B, C) 20 nM of PRS-400 Parental or AMG430 were added simultaneously to huIL-13 every other day. Relative gene expression of mucin *MUC5AC* and secretory cell marker *FOXA3* was assessed by RT-qPCR (7 days post treatment), normalized to *HPRT* (B). Alcian blue Periodic acid–Schiff (AB-PAS) staining of mucus and MUC5AC and α-Tubulin (TUBA) specific immunolabeling in vertical ALI cell sections (10 days post treatment) is shown in (C). D, E) 100 nM of PRS-400 Parental or Lead candidate were added simultaneously to huIL-17A every other day. Relative gene expression of *MUC5AC*, *MUC5B* and *FOXA3* and mucus content was assessed by RT-qPCR (7 days post treatment), normalized to *HPRT* (D), and by AB-PAS staining in vertical ALI cell sections (10 days post treatment) (E).

Pulmonary delivery of PRS-400 Lead candidate dose-dependently prevents IL-13-induced goblet cell metaplasia and mucus hypersecretion *in vivo*

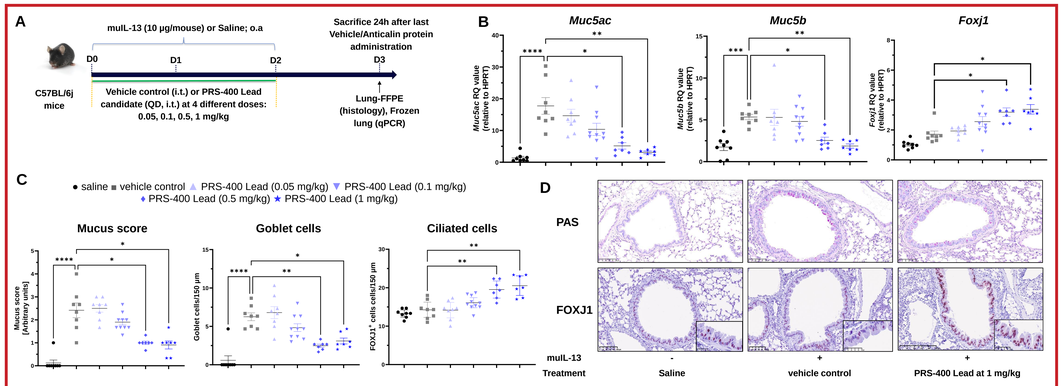


Figure 4. A) Experimental design. Goblet cell metaplasia was induced by daily oropharyngeal (o.a.) administration of muIL-13 for 3 days (D0-D2). Mice were treated by intratracheal (i.t.) instillation daily (D0-D2) with different doses (0.05, 0.1, 0.5 and 1 mg/kg) of PRS-400 Lead candidate. Lungs were harvested on D3 and processed either for gene expression, or formalin-fixed and paraffin-embedded (FFPE) for histological analysis. B) Relative gene expression of mucins *Muc5ac* and *Muc5b* and ciliated cell marker *Foxj1*. C) Histological evaluation of mucus production (mucus score), number of goblet and FOXJ1⁺ cells. D) Representative histological images of Periodic acid–Schiff (PAS)-stained and FOXJ1-immunolabeled airway sections. Statistics: non-parametric Kruskal-Wallis followed by Dunn's Multiple comparison test. * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001, **** p ≤ 0.0001.

Pulmonary delivery of PRS-400 Lead candidate reverts preexisting IL-13-induced goblet cell metaplasia and mucus hypersecretion *in vivo*

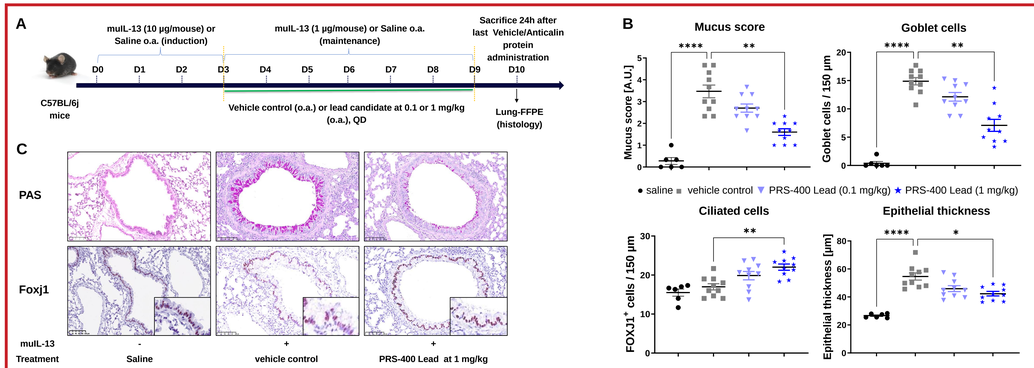


Figure 5. A) Experimental design. Goblet cell metaplasia was induced by daily oropharyngeal (o.a.) administration of muIL-13 for 10 days (D0-D9). Mice were treated by o.a. instillation daily from D3-D9 with different doses (0.1 and 1 mg/kg) of PRS-400 Lead candidate. Lungs were harvested on D10 and formalin-fixed and paraffin-embedded (FFPE) for histological analysis. B) Histological evaluation of mucus production (mucus score), number of goblet and FOXJ1⁺ ciliated cells, and epithelial thickness. C) Representative histological images of Periodic acid–Schiff (PAS)-stained and FOXJ1-immunolabeled airway sections. Statistics: non-parametric Kruskal-Wallis followed by Dunn's Multiple comparison test. * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001, **** p ≤ 0.0001.

Conclusions

- **In chronic lung diseases with mucus-driven pathology, Jagged-1/Notch signaling is an attractive therapeutic intervention point; however, systemic disruption of this pathway may cause undesired side effects.**
- **The inhalable Anticalin protein drug class, combining the power of biologics with the efficiency of lung delivery is able to reduce systemic target exposure to a minimum while being locally effective in the lung.**
- **PRS-400 Anticalin proteins potently inhibit Jagged-1-induced Notch2 activation *in vitro*, reduce mucin expression and goblet cell differentiation *ex vivo*, positively affect the mucus-ciliary axis locally *in vivo*, and potentially provide a therapeutic window that may be unavailable to systemically administered Jagged-1-targeting molecules.**