Preclinical profile of PRS-220, a novel inhaled inhibitor of CTGF/CCN2, in clinical development for Idiopathic Pulmonary Fibrosis

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Rationale

Idiopathic pulmonary fibrosis (IPF) is a chronic and ultimately fatal lung disease characterized by progressive scarring of interstitial lung tissue. The unmet medical need for well-tolerated and effective therapies is very high due to the poor prognosis for patients and limited benefits conferred by current standard of care.

CTGF/CCN2, a matricellular protein highly abundant in IPF tissues (Pan et al., European Respiratory Journal 2001 and Figure 1) and a driver of fibrotic lung remodeling, has been validated as a novel intervention point for IPF treatment in a randomized clinical trial with the systemically delivered monoclonal antibody pamrevlumab (Richeldi et al., Lancet Respir Med 2019).

Delivery of a CTGF inhibitor directly to the lung via oral inhalation is expected to be advantageous compared to systemically administered antibodies for the following reasons:

- Increased pulmonary drug exposure associated with enhanced local target engagement
- Better local target saturation due to avoidance of systemic CTGF sink
- At-home administration provides greater convenience for patients

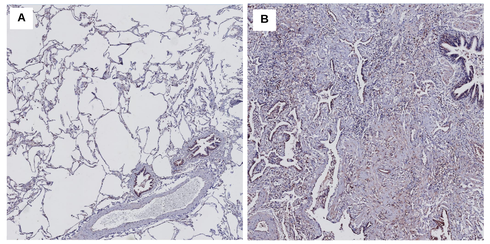
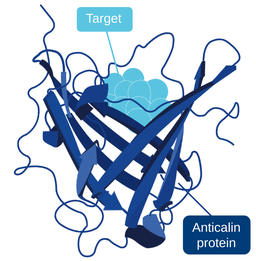


Figure 1. CTGF immunohistochemistry of A) human control and B) IPF lung tissues (collaboration with Prof. Dr. Janette Burgess, University of Groningen, Netherlands).

Anticalin® proteins are a novel class of biotherapeutics which, based on their biophysical properties and small size of approximately 20 kDa, are particularly well-suited for delivery via inhalation (Figure 2).

Here, we describe the preclinical profile of PRS-220, an Anticalin protein targeting CTGF, as a novel and promising inhaled therapy for IPF.

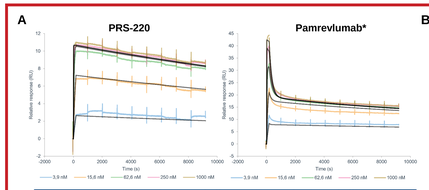


- **Human** – Scaffold derived from human lipocalins (extracellular binding proteins)
- **Specific** – High potency and selectivity for targets
- **Small** – Monomeric, monovalent, small size (~20 kDa vs ~150 kDa mAbs)
- **Stable** – High melting temperatures & insensitivity to mechanical stress
- **Formulable** – Nebulization & dry powder inhalation
- **Proprietary** – Broad IP position on platform and derived products
- **Validated** – Strong industrial partners and clinically tested

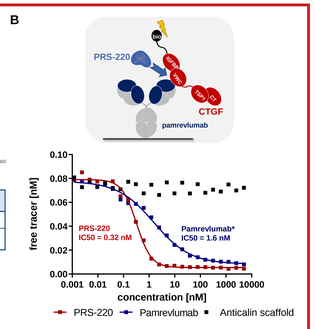
Favorable drug-like properties for lung delivery

Figure 2. Schematic representation of an Anticalin protein and characteristics supporting a favorable profile for lung delivery.

PRS-220 binds CTGF with higher affinity than pamrevlumab



Compound	Fit	kon [M-1*s-1]	koff [s-1]	KD [nM]	kon2 [RU-1*s-1]	koff2 [s-1]	KD2 [M]
PRS-220	1:1 binding	4.06E+05	2.78E-05	0.068			
Pamrevlumab*	Bivalent analyte	1.83E+05	2.74E-03	14.972	4.18E-04	4.17E-05	0.100



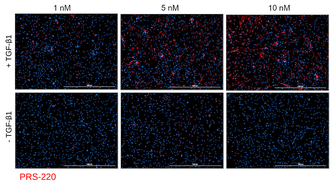
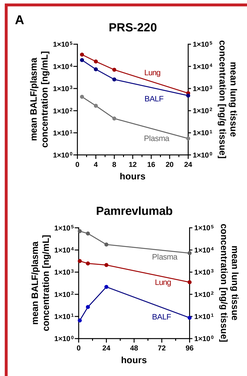


Figure 3. Binding properties of PRS-220 in comparison to pamrevlumab. A) Surface Plasmon Resonance (SPR) binding experiment showing the binding of PRS-220 (left) and pamrevlumab (right) to CTGF. B) Competition of PRS-220 with pamrevlumab for binding to CTGF and competition of pamrevlumab with itself for binding to CTGF by ECLA. C) Dose-dependent binding of PRS-220 to CTGF expressed by disease-relevant TGF-β1-activated primary normal human lung fibroblasts detected by immunofluorescence staining for the PRS-220 scaffold. *Pamrevlumab was generated in-house from patent-derived sequences for all investigations.

PRS-220 achieves superior exposure in the lung



	Lung exposure compared to plasma (%)	Plasma exposure compared to lung (%)
PRS-220	100*	0.9
Pamrevlumab	20.3	100*

* Exposure was set to 100% for compartment of administration.

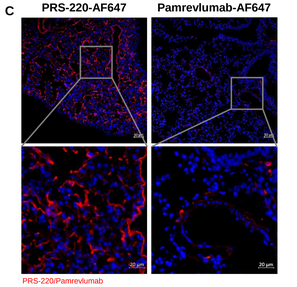
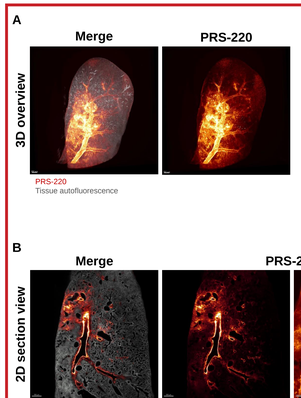


Figure 4. Comparison of PRS-220 and pamrevlumab pharmacokinetic (PK) profile in mice. A) PK analysis of PRS-220 delivered to the lung by oropharyngeal aspiration and pamrevlumab given intravenously (both 100 µg/mouse). Total drug levels were determined by ELISA. B) Comparison of lung and plasma exposure determined by non-compartmental analysis (NCA). C) Imaging of Alexa Fluor 647 labeled PRS-220 and pamrevlumab in the lung administered as described in A).

PRS-220 penetrates fibrotic tissue *in vivo*



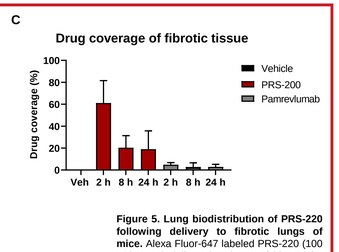
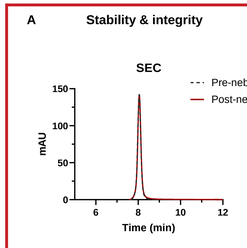
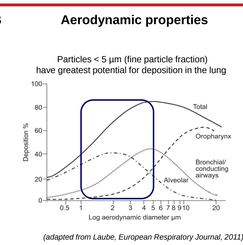


Figure 5. Lung biodistribution of PRS-220 following delivery to fibrotic lungs of mice. Alexa Fluor-647 labeled PRS-220 (100 µg/mouse) delivered to lungs of bleomycin-challenged mice (d21) by oropharyngeal aspiration was imaged by light sheet microscopy. A) 3D overview of PRS-220 in whole left lung 2 h after dosing (scale bars 500 µm). B) Magnified 2D sections from 3D scanned lung (scale bars 500 µm &150 µm) shown in A). C) Compound accumulation of PRS-220 delivered to the lung and pamrevlumab administered intravenously (both 100 µg/mouse) in fibrotic tissue determined by light sheet imaging & quantitative analysis (n = 2-5 per group). All images are representative for imaging of n=3-6 lungs. PRS-220 was imaged at 630 nm excitation channel and tissue autofluorescence at 560 nm.

PRS-220 is suitable for nebulized pulmonary delivery





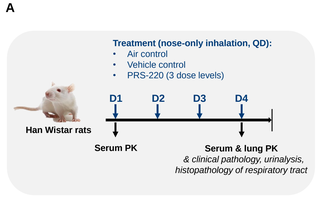
(adapted from Laube, European Respiratory Journal, 2011)

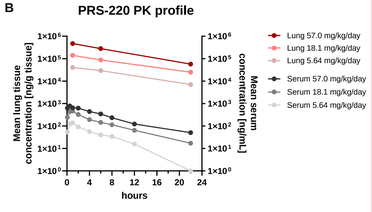
Figure 6. *In vitro* nebulization performance of PRS-220 with exemplary vibrating mesh devices. PRS-220 was nebulized by vibrating mesh nebulizers at a concentration of 50 mg/mL and with a fill volume of 1.2 mL for all measurements. A) Size exclusion chromatography (SEC) analyzing PRS-220 integrity pre and post nebulization. B) Assessment of PRS-220 aerosol delivery performance parameters by laser diffraction (LD), breathing simulator & Next Generation Cascade Impactor (NGI) analysis. The droplet size distribution varies depending on test set-up (temperature, humidity, etc.), explaining the slight difference between NGI and LD particle size measurements.

PRS-220 aerosol delivery performance		
Laser diffraction	**Droplet size (VMD)**	3.0 µm
	FPF (LD)	89.3 %
NGI	**MMAD**	3.8 µm
	FPF (NGI)	74.5 %
Breathing simulator	**Delivered Dose**	57.7 %
	Nebulization time	02:47 min

VMD: Volumetric mean diameter
LD: Laser diffraction
FPF: Fine particle fraction
NGI: Next Generation Cascade Impactor
MMAD: Mass median aerodynamic diameter

Inhaled delivery of PRS-220 *in vivo* confirms favorable PK profile for lung delivery





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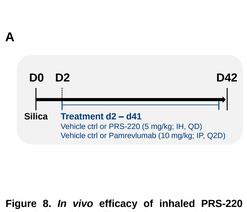
PRS-220 PK parameters in rat serum

	C_{max} [ng/mL]	T_{max} [h]	AUC_{0-24} [h*ng/mL]	$t_{1/2}$ [h]
5.65 mg/kg/day	140	3.00	776	4.26
18.1 mg/kg/day	465	3.00	2930	5.13
57 mg/kg/day	787	2.50	6050	6.57

Figure 7. PRS-220 pharmacokinetic profile upon inhaled administration to rats. A) Design of preclinical toxicology study in rats with daily (QD) delivery of PRS-220 at 3 different dose levels by nose-only inhalation for 4 days. B) PK profile of inhaled PRS-220 at day 4 including analysis of free drug levels in the lung and in serum measured by an electrochemiluminescence assay (ECLA). Figure legend indicates combined mean achieved delivered dose levels assessed at day 1 and day 4 of the study. C) Analysis of PRS-220 PK parameters in rat serum upon inhaled delivery on day 4.

Nebulized PRS-220 demonstrates superior anti-fibrotic effect compared to systemically delivered anti-CTGF antibody *in vivo*



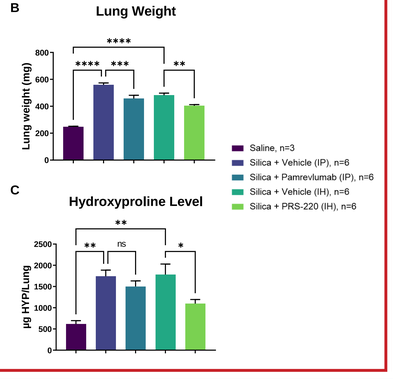


Figure 8. *In vivo* efficacy of inhaled PRS-220 in silica-induced lung fibrosis mouse model. A) Study design: PRS-220 (daily (QD) inhaled (IH) delivery, targeted dose 5 mg/kg), pamrevlumab (intraperitoneal (IP) delivery, 10 mg/kg every other day (Q2D)) or respective vehicle controls were administered to mice from day 2 to day 41 after silica challenge, and fibrotic remodeling of lung tissues was analyzed at day 42. B) Analysis of lung weights. C) Analysis of total collagen deposition in the lung by measuring lung hydroxyproline. Data shown as mean ± SEM, One-way ANOVA Šídák's multiple comparisons test (* p≤ 0.05, ** p≤ 0.01, *** p≤ 0.001, **** p≤ 0.0001).

Clinical development of PRS-220: Ongoing Phase 1 study in healthy subjects

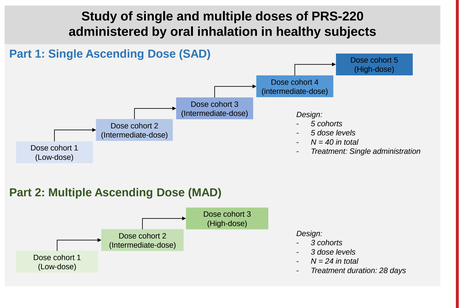


Figure 9. Design of PRS-220 Phase 1 study in healthy volunteers (NCT05473533). A Phase 1, randomized, blinded, placebo controlled, single and multiple ascending dose study to assess the safety, tolerability, pharmacokinetics, and immunogenicity of PRS-220 administered by oral inhalation in healthy subjects.

Conclusions

- Therapies for respiratory diseases such as IPF can be suboptimal due to off-target effects of small molecules and inefficiencies of a systemic route of administration for antibodies
- Inhaled administration of biologics may overcome these limitations, yet conventional biologics (e.g. mAbs) are not suitable for inhaled delivery
- Our proprietary Anticalin protein drug class combines the power of biologics with the efficiency of local lung delivery
- CTGF is an optimal intervention point for an inhaled biologic to treat IPF, achieving a high drug exposure for complete CTGF blockade in the lung that is limited with systemically delivered inhibitors, such as pamrevlumab
- PRS-220 possesses high potency and favorable drug-like properties for nebulized delivery, while demonstrating superior lung biodistribution and in vivo efficacy compared to systemically administered pamrevlumab
- A Ph1 study evaluating PRS-220 in healthy volunteers is ongoing as a prelude to initiating clinical development in IPF patients

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