Abstract
27597

Development of PRS-220, a potential best-in-class, inhaled CTGF/CCN2 inhibitor for the treatment of IPF

Vanessa Neiens, Marina Pavlidou, Gabriele Matschiner, Claudia Wurzenberger, Eva-Maria Hansbauer, Cornelia Wurzenberger, Stefan Grüner, Janet Peper-Gabriel, Thomas Jaquin, Antonio Konitsiotis, Josefine Morgenstern, Josef Prassler, Shane Olwill

Pieris Pharmaceuticals GmbH, Zeppelinstrasse 3, 85399 Hallbergmoos - Germany

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive, and ultimately fatal lung disease of unknown cause characterized by progressive scarring of the interstitial lung tissue. Median survival is two to five years from the time of diagnosis, with standard of care conferring only modest benefit for patients. In addition, adverse events often lead to the withdrawal of standard of care treatment. Therefore, there is a high medical need for novel, well-tolerated and effective IPF treatments.

To overcome the limitations of current IPF therapies, we developed PRS-220, a 20 kDa protein suitable for inhalation, based on the Anticalin® technology, targeting connective tissue growth factor (CTGF/CCN2). Pieris Pharmaceuticals' proprietary Anticalin platform comprises a novel class of biotherapeutics derived from lipocalins, low molecular weight proteins that are abundantly expressed in human tissues and body fluids including the lung. Like antibodies, Anticalin proteins are engineered to bind a variety of therapeutically relevant targets but, in contrast to antibodies, are smaller in size and composed of a single polypeptide chain. Most importantly, the biophysical properties of Anticalin proteins allow for inhaled delivery, making them ideal for local interventions in the lung and driving more targeted biology in lung diseases. CTGF, a protein localized in the extracellular matrix, is a driver of fibrotic tissue remodeling. Over-expression of this target is observed in lung tissues of patients suffering from IPF (Pan et al., Eur Resp J 2001 and Figure 2), and Phase 2 clinical data with a systemically-delivered antibody indicate that inhibition of CTGF reduces the decline in lung function among these patients (Richeldi et al., Lancet Respir Med 2019).

PRS-220 was selected from Pieris' proprietary Anticalin libraries using phage display technology to bind CTGF and was engineered to optimize high affinity and specificity. In addition, PRS-220 was designed with favorable biophysical properties giving this biologic the robustness and stability for large-scale manufacturing and nebulized inhaled administration. The local administration of PRS-220 directly into the lung offers the benefit of enhanced drug exposure and local target engagement while avoiding the systemic sink of CTGF and leading potentially to a more efficient CTGF inhibition than systemically administered antibodies. Compared with parenteral delivery, which is the most common route of administration for biologics, pulmonary delivery offers a non-invasive alternative which is of greater convenience for patients.

Here we provide a preclinical data set demonstrating the best-in-class potential of PRS-220. PRS-220 binds to the functionally active epitope of CTGF and shows more stable target engagement than the clinically validated anti-CTGF antibody pamrevlumab. PRS-220 has a favorable pharmacokinetic profile and lung biodistribution pattern upon lung delivery in mice. Nebulization of PRS-220 using a vibrating mesh nebulizer shows aerosol characteristics and molecular integrity suitable for effective lung deposition.

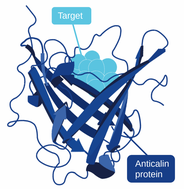


Figure 1. Schematic representation of an Anticalin protein comprising of four variable loops and a rigidly conserved beta-barrel backbone, which together form a pliable cup-like binding pocket.

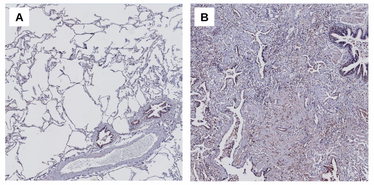
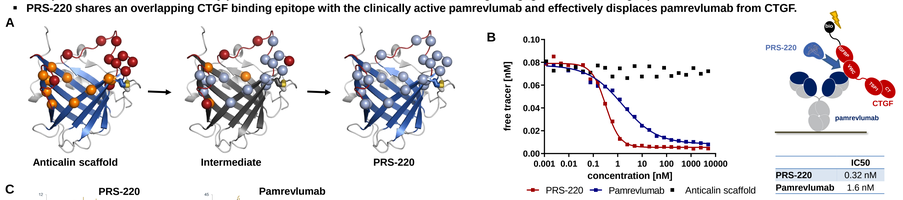


Figure 2. CTGF immunohistochemistry of A) human control and B) IPF lung tissues (collaboration with Prof. Dr. Janette Burgess, University of Groningen, Netherlands)

PRS-220 demonstrates superior target binding properties to pamrevlumab

- PRS-220 was engineered to bind CTGF with high affinity (in the picomolar range).
- Compared to the anti-CTGF antibody pamrevlumab, PRS-220 retains a more stable target engagement over a longer period of time.
- PRS-220 shares an overlapping CTGF binding epitope with the clinically active pamrevlumab and effectively displaces pamrevlumab from CTGF.



	IC50
PRS-220	0.32 nM
Pamrevlumab	1.6 nM

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) Structural models comparing Lcn2 (Anticalin scaffold), an intermediate, and PRS-220 highlighting the evolution of residue variations towards high affinity binding to CTGF and favorable biophysical properties. Cartoon presentations are shown. The Anticalin scaffold represents the human Lcn2 structure (PDB ID: 1DFV). Yellow sticks: disulfide bond forming Cys residues. Red and orange spheres: residues in the loop and β-strand regions, respectively, that were mutated in Lcn2 to generate the intermediate. Blue spheres: Cα atoms of residues that remained fixed during subsequent engineering steps towards PRS-220. **B)** Competition of PRS-220 with pamrevlumab for binding to CTGF and competition of pamrevlumab with itself for binding to CTGF. Competition was assessed in an ELISA-based format using coated pamrevlumab and biotinylated CTGF as tracer (see schematic; CTGF domains: insulin-like growth factor binding protein (IGFBP), von Willebrand type C domain (vWC), thrombospondin type 1 repeat (TSP1), C-terminal domain (CT)). IC50 values of the competition are shown in the table. **C)** Surface Plasmon Resonance (SPR) binding experiment showing the binding of PRS-220 (left) and pamrevlumab (right) to CTGF. The 1:1 binding model was used to fit the data of PRS-220. The bivalent analyte model was used to fit the data of the antibody pamrevlumab. CTGF-Fc was immobilized on a CM5 chip and PRS-220 and pamrevlumab were used as analytes. Pamrevlumab was generated in-house from patent signatures.

PRS-220 binds to CTGF expressed on primary, activated lung fibroblasts

- TGF-β1 stimulation induces CTGF expression of primary normal human lung fibroblasts (NHLF).
- PRS-220 binds in a dose-dependent manner to CTGF endogenously expressed by TGF-β1 activated NHLF.

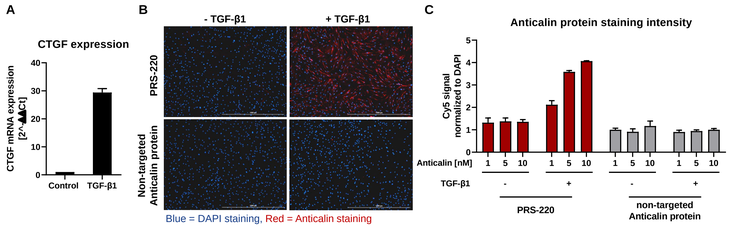


Figure 4. In vitro target binding of PRS-220. A) Increased CTGF mRNA expression upon 24 h TGF-β1 stimulation in primary NHLF. **B)** PRS-220 binds to CTGF expressing primary NHLF when activated by TGF-β1 stimulation. Cells (-/+ TGF-β1 stimulation) were treated with 1, 5 and 10 nM PRS-220 or non-targeted Anticalin protein as a control for 24 h followed by detection of Anticalin scaffold by immunofluorescence staining. **C)** Concentration dependent binding of PRS-220 in TGF-β1 activated NHLF as determined by quantification of fluorescence signal.

Mouse CTGF-directed analog of PRS-220 significantly reduces lung fibrosis in vivo

- An analog of PRS-220 with a higher affinity for mouse CTGF (KD = 0.039 nM) was used for in vivo efficacy studies in the mouse
- PRS-220 analog delivered to the lung led to a superior attenuation of fibrotic lung remodeling when compared to the systemically administered pamrevlumab.
- Intratracheal delivery of the PRS-220 analog significantly decreased Ashcroft score and reduced Col1a1 deposition in the lungs when compared to intratracheally administered vehicle control.
- Results from a pilot in vivo study support the functional activity of PRS-220.

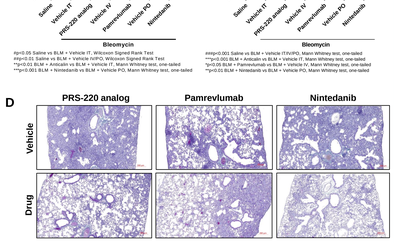
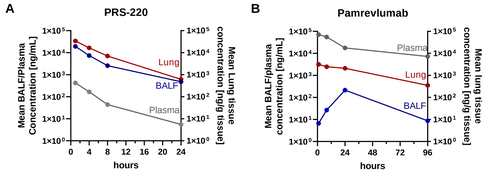


Figure 5. In vivo activity of mouse CTGF-directed analog of PRS-220. A) Scheme of the experimental study design. Bleomycin challenged mice were treated with an analog of PRS-220 (targeting a similar epitope on murine CTGF with higher affinity (KD = 0.039 nM)) by intratracheal delivery, the CTGF-targeting antibody pamrevlumab by intravenous infusion, nintedanib as a positive control via oral administration and vehicle controls administered via the respective routes of administration. **B)** Ashcroft score was determined from histopathological analyses of 10 individual tissue sections per subject. The mean % decline in Ashcroft score was calculated by normalization to the respective vehicle control group. **C)** Collagen1a1 (Col1a1) protein deposition was analyzed as the % of Col1a1 positive lung surface area determined by quantitative analysis of immunohistochemistry. The % decline of Col1a1 positive surface was calculated compared to the respective vehicle control-treated animals. **D)** Representative H&E staining of lungs from Bleomycin challenged mice treated with different drugs and respective vehicle controls. Data are shown as mean ±SEM, statistical analysis was performed as described in the figure (n = 6-15 per group). Pamrevlumab was generated in-house from patent sources.

PRS-220 achieves superior exposure in lung tissues

- Pharmacokinetic analysis of intratracheally delivered PRS-220 confirms significant exposure in the lung over 24 h supporting once daily pulmonary delivery.
- PRS-220 achieves high exposure in the lung while only ~ 1 % reaches the circulation.
- In comparison with PRS-220, pulmonary exposure of the systemically delivered pamrevlumab is significantly lower in BALF and lung tissue with only ~ 20 % reaching the lung tissue.



	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 6. Comparison of PRS-220 and pamrevlumab pharmacokinetic (PK) profile. A) PK analysis of PRS-220 in bronchoalveolar lavage fluid (BALF), lung tissue & plasma. PRS-220 (100 µg/mouse) was administered to the lungs of mice and exposure in different compartments were measured after 2, 4, 8 and 24 h by ELISA. **B)** PK profile of pamrevlumab in BALF, lung tissue and plasma. 100 µg antibody was administered to mice via intravenous infusion and exposure was measured after 1, 8, 24 and 96 h by ELISA. **C)** Comparison of lung and plasma exposure after PRS-220 and pamrevlumab dosing determined by NCA parameter analysis. Pamrevlumab was generated in-house from patent signatures.

PRS-220 lung biodistribution in fibrotic lung tissue

- PRS-220 reveals favorable lung tissue distribution upon intratracheal delivery in fibrotic lungs of bleomycin-challenged mice.
- Imaging suggests similar PK profile of PRS-220 when administered to fibrotic lungs of mice.
- PRS-220 is not only detected in the airways but also penetrates the fibrotic, interstitial lung tissue.

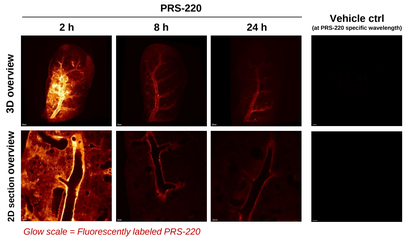
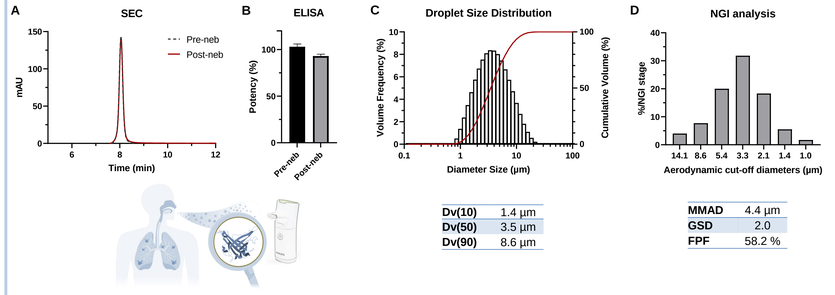


Figure 7. Lung biodistribution of PRS-220 upon intratracheal delivery to fibrotic lungs of mice. Alexa-647 labeled PRS-220 (100 µg/mouse) was administered to lungs of mice via the intratracheal route at day 21 after bleomycin challenge. The lung tissue distribution was analyzed 2, 8, and 24 h after PRS-220 administration by Light Sheet imaging of the left lung lobes (630 nm excitation channel). The figure shows representative 3D overview images (scale bars 500 µm) and magnified 2D sections from 3D scanned lungs (scale bars 150 µm). Lungs of saline treated mice imaged at the PRS-220 specific wavelength served as negative controls.

PRS-220 is suitable for pulmonary delivery using a nebulizer

- Favorable biophysical properties allow PRS-220 to retain stability and integrity upon nebulization.
- Aerosols generated using vibrating mesh technology show aerodynamic properties suitable for effective lung deposition.



Dv(10)	1.4 µm
Dv(50)	3.5 µm
Dv(90)	8.6 µm

MMAD	4.4 µm
GSD	2.0
FPF	58.2 %

Figure 7. In vitro nebulization performance of PRS-220 on one exemplary vibrating mesh device (Philips Innospire Go). A) Size exclusion chromatography (SEC) shows that the integrity of PRS-220 was preserved upon nebulization. PRS-220 elutes as a single peak from the SEC column (Sepax Zenix-C SEC-150) before and after nebulization **B)** ELISA-based potency measurement before and after nebulization shows that PRS-220 retains its potency upon nebulization **C)** Droplet size distribution measured using the nebulizer in conjunction with the Malvern Spraytec and inhalation cell. 10 % of the generated droplets are below 1.4 µm (Dv(10)), 50 % are below 3.5 µm (Dv(50)) and 90 % below 8.6 µm (Dv(90)) **D)** Aerodynamic Particle Size Distribution measured on a next generation cascade impactor (NGI) show a mass median aerodynamic diameter (MMAD) of 4.4 µm with geometric standard deviation (GSD) of 2 and a fine particle inhaled fraction (FPF) of 58.2 %.

Conclusions

- PRS-220 is an Anticalin-based biotherapeutic for the treatment of IPF designed for inhaled delivery via the nebulized route of administration.
- PRS-220 targets the functionally active epitope of CTGF with high affinity and shows more stable target engagement than the clinically active antibody pamrevlumab.
- An analog of PRS-220 targeting a similar epitope on the murine CTGF is functional in a preclinical model of lung fibrosis.
- PK and lung biodistribution behavior in mice support once daily inhaled dosing.
- PRS-220 is suitable for inhaled administration using a vibrating mesh nebulizer.

PRS-220's preclinical profile supports proceeding to clinical development with a planned start of Phase 1 studies in 2022. In addition to IPF, PRS-220 will be explored for the treatment of post-acute sequelae of SARS-CoV-2 infection (PASC) pulmonary fibrosis (PASC-PF), also known as post-COVID-19 syndrome pulmonary fibrosis.

ACKNOWLEDGEMENTS - We would like to especially thank the scientists and research assistants who performed these experiments or provided important pre-work to the presented experiments: Alexander Hahn, Mareike Maurer, Christina Grasmüller, Kristina Heinig, Nicolas Quilitz, Sarah Schmalbrock, Theresia Mosebach, David Goricanec, Patrick Zägel and Adam Cichy.

FUNDING - This work is partially funded by a grant from the Bavarian Ministry of Economic Affairs, Regional Development and Energy within the framework of the Bavarian Therapy Strategy to combat the COVID-19 pandemic ("BayTherapie2020").

Sponsored by
Bavarian Ministry of Economic Affairs, Regional Development and Energy