PROTEIN SPECIFIC SIGNATURE OF OTR4132: DOSE EFFECTS Exploring the Antifibrotic Potential of the Heparan Sulfate Mimetic OTR4132 on Validated Human Precision-Cut Lung Slices (hPCLS) Model Mimicking Pulmonary Diseases

Mariia Pestriakova, Céline Moro, Thénot Jp, Ines Metatla, Kevin Rogers, Guerrera Ic, Jean-François Bernaudin, Xavier Brisbart, Florence Gazeau, Agnes Choppin, et al.

Exploring the Antifibrotic Potential of the Heparan Sulfate Mimetic OTR4132 on Validated Human Precision-Cut Lung Slices (hPCLS) Model Mimicking Pulmonary Diseases

Pestriakova M[1] , Moro C[2] , Thénot JP[2] , Metatla I[3] , Rogers K[3] , Guerrera IC[3] , Bernaudin J-F[4] , Brisbart X[5] , Gazeau F[1] , Choppin A[6] , Osman-Ponchet H[2] *, Chiappini F[6] *

1 NABI (Nanomédecine, Biologie Extracellulaire, Intégratome et Innovations en santé), CNRS UMR8175, INSERM U1334, Université Paris Cité, Paris, France, 2 Laboratoires PKDERM, R&D Department, Valbonne, France , 3 Proteomics platform Necker, Université Paris Cité-Structure Fédérative de Recherche Necker, INSERM US24/CNRS UAR3633, Paris, France , 4 Faculty of Medicine, Sorbonne Université, Paris; Hypoxia and Lung, INSERM U1272, Paris, Université Sorbonne Paris Nord, Bobigny France, 5 Cerba Path (Anatomo-cytopathology laboratory), Nice, France, 6 OTR3 (Organs Tissues Regeneration Reparation Replacement), Paris, France

BACKGROUND

Diffuse alveolar damage is a hallmark of acute lung injury and may progress to fibrosis. Extracellular matrix (ECM) and endothelial glycocalyx disruption, driven partly by heparan sulfate (HS) degradation, contributes to vascular dysfunction, edema and inflammation.

ReGeneraTing Agents (RGTA®) are synthetic HS mimetics designed to restore the glycan-based ECM scaffold after tissue injury.

Human precision-cut lung slices (hPCLS) preserve the native 3D lung architecture and major cell populations, providing a physiologically relevant system to assess therapeutic efficacy and safety. Here, a pro-fibrotic cocktail (PFC) is used to induce fibrotic remodeling, while nintedanib (Nin) serves as an anti-fibrotic reference treatment.

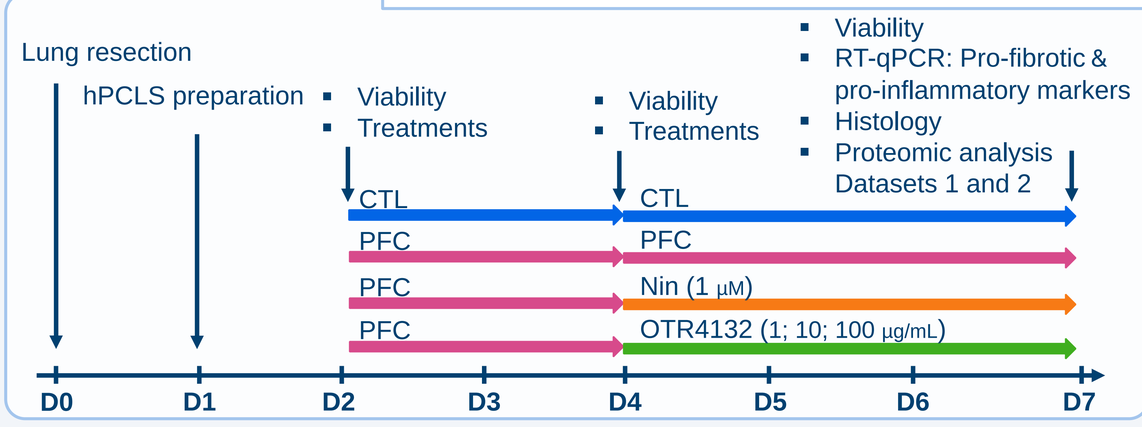
OBJECTIVES

1. **Specific protein signature (Sign)** showing a **consistent response** to PFC and Nin across **independent experiments**

If validated, then:

2. **Anti-fibrotic** effect of **OTR4132:** functional signature

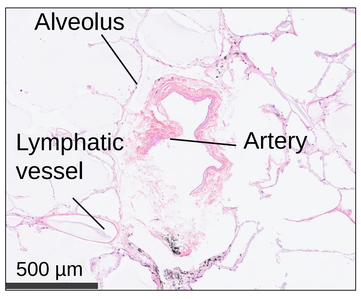
MATERIALS AND METHODS



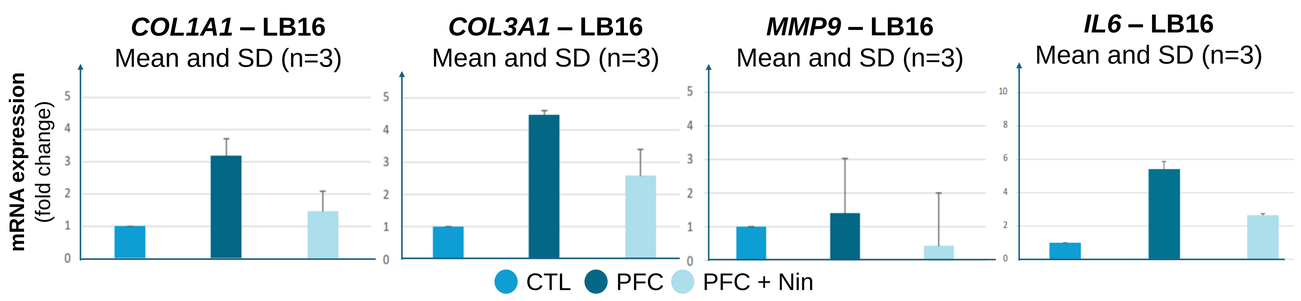
Dataset 1:
- 1 patient (LB10; N = 1)
  - CTL (n = 8)
  - PFC (n = 8)
  - PFC + Nin 1 µM (n = 8)

Dataset 2:
- 3 patients (LB17, LB18, LB19; N = 3)
  - CTL (n = 4)
  - PFC (n = 4)
  - PFC + Nin 1 µM (n = 4) (+1)
  - PFC + OTR4132 1 µg/mL (n = 4)
  - PFC + OTR4132 10 µg/mL (n = 4)
  - PFC + OTR4132 100 µg/mL (n = 4)

PRELIMINARY TESTS



hPCLS normal lung structure



Pro-fibrotic and pro-inflammatory marker mRNA expression increased after PFC and reduced after Nin.

WORKFLOW

1

Common proteins in two datasets

Dataset 1 → Missing value removal

Dataset 2 → Missing value removal

→ Batch effect correction (Limma: R function)

→ Venn Diagram and PCA: shared proteins

Sign-R: specific to PFC and reversed by Nin
CTL & PFC+Nin vs PFC

1 501 shared proteins	1 501 shared proteins

Random forest analysis
mtry=26;
ntree=122 880;
bootnumber = 500
	Random forest analysis
	mtry=57;
	ntree=122 880;
	bootnumber = 500

→ PCA: discriminative proteins

→ Venn Diagram: shared proteins

Sign-M: specific to PFC and modified by Nin
CTL vs PFC+Nin vs PFC

1 501 – Sign-R proteins	1 501 – Sign-R proteins

Random forest analysis
mtry=36;
ntree=122 880;
bootnumber = 500
	Random forest analysis
	mtry=55;
	ntree=122 880;
	bootnumber = 500

→ PCA: discriminative proteins

→ Venn Diagram: shared proteins

Model-Specific protein signature

Sign-R → Merge Sign-R and Sign-M ← Sign-M

2

OTR4132-specific signature
CTL vs PFC+Nin vs PFC vs OTR4132 1; 10; 100 µg/mL

Dataset 2
(After missing value removal and batch-effect correction)

→ Random forest analysis
mtry=235;
ntree=122 880;
bootnumber = 500

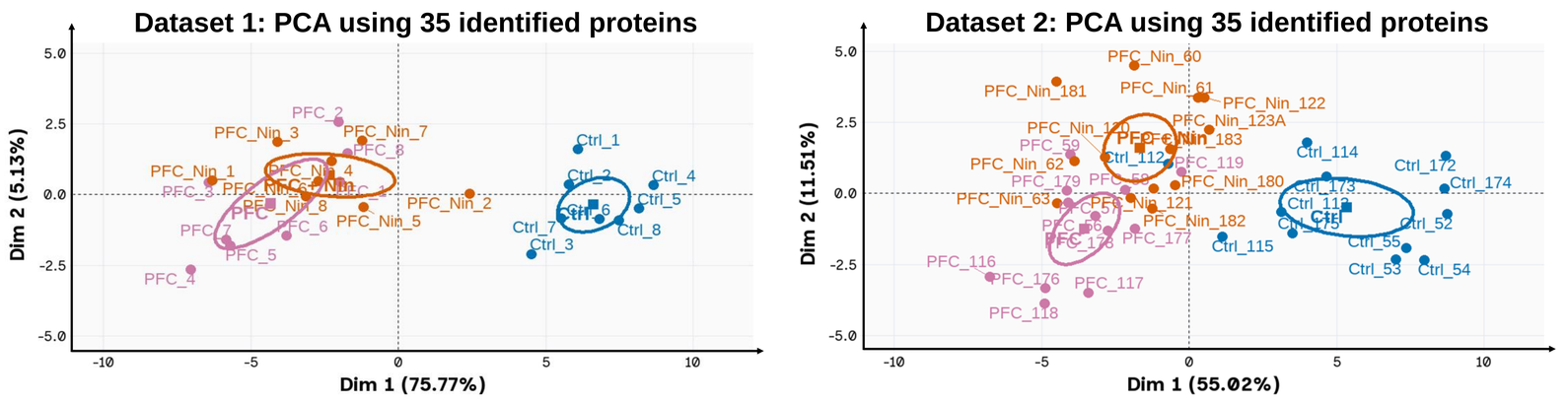
→ PCA : discriminative proteins

→ Functional analysis

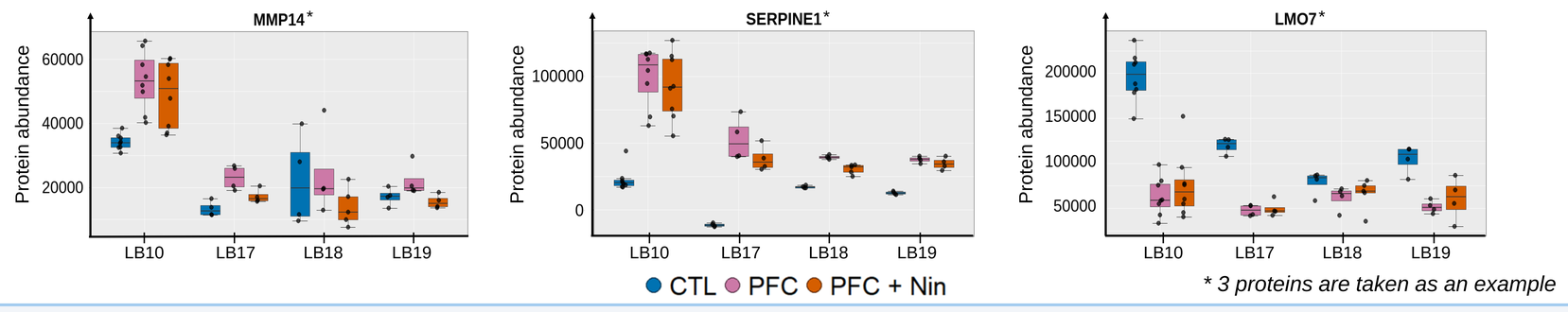
RESULTS

1

35 PROTEINS DISCRIMINATE CTL vs PFC vs PFC+NIN REGARDLESS OF THE PATIENT SAMPLE



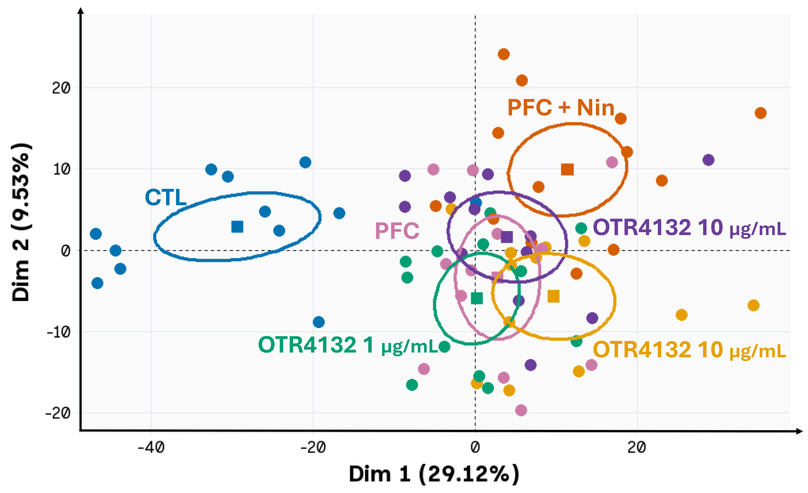
35 PROTEINS CONSISTENT WITH TREATMENT-INDUCED CHANGES REGARDLESS OF THE PATIENT SAMPLE



** 3 proteins are taken as an example*

2

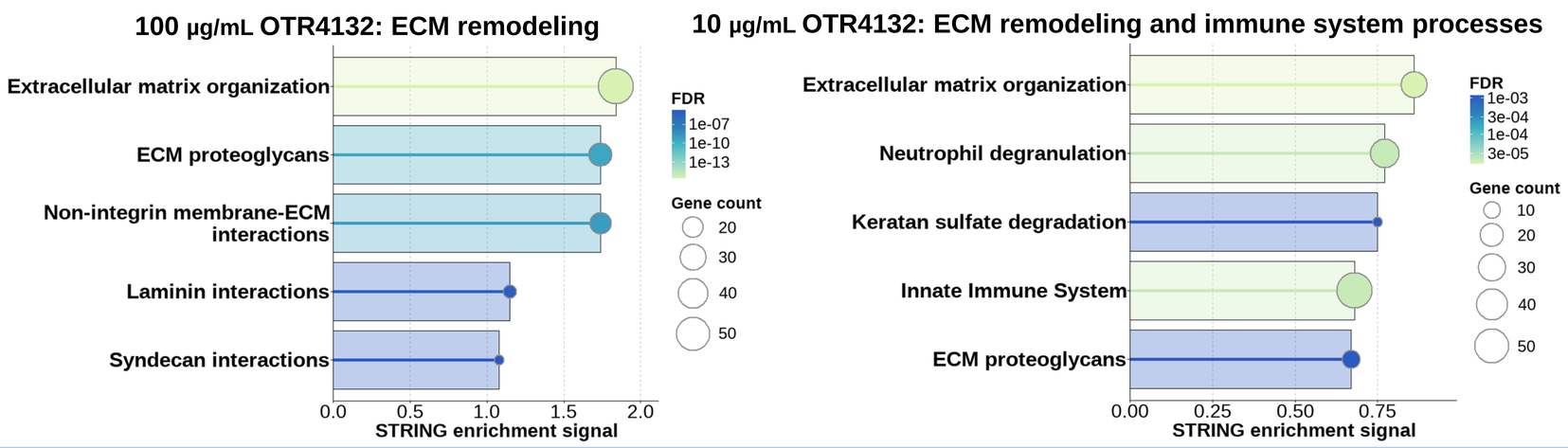
977 PROTEIN SPECIFIC SIGNATURE OF OTR4132: DOSE EFFECTS



PCA:
- **Dim1: 100 µg/mL** OTR4132 (787 proteins)
- **Dim2: 10 µg/mL** OTR4132 (457 proteins)

CTL
PFC
PFC + Nin
OTR4132 1 µg/mL
OTR4132 10 µg/mL
OTR4132 100 µg/mL

DOSE EFFECT OF OTR4132 ON ECM REMODELING AND IMMUNE SYSTEM PROCESSES



CONCLUSIONS

1. **35 SPECIFIC PROTEIN SIGNATURE**: **consistent response** to PFC and Nin across **independent experiments**, **validating** the model.
2. **977 PROTEIN SPECIFIC SIGNATURE OF OTR4132**: involved in **ECM remodeling** and **immune-related processes** consistent with an **anti-fibrotic effect.**

Acknowledgments:
This work has been co-founded by: The European Union and FEDER, Région Provence-Alpes-Côte d'Azur Program 2021-2027 (SUD004993) for PKDerm;
The Agence Nationale de la Recherche for OTR3 : ANR-22-CE18-0013 (MAT-PL);
The Domaine d'Intérêt Majeur (DIM) BioConverS-Région Île de France (PhD Grant) for Mariia Pestriakova.