THERAPEUTIC BIOMARKER DISCOVERY IN IDIOPATHIC PULMONARY FIBROSIS (IPF) THROUGH PROTEOMIC ANALYSIS OF PRECISION-CUT LUNG SLICE (PCLS) SUPERNATANTS

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RATIONALE

Idiopathic pulmonary fibrosis is a rare fibrotic disease that progressively destroys both lung structure and function and has a poor prognosis.^{1,2} Based on conservative estimates from Europe and North America, the estimate incidence is 3–9 cases per 100,000 per year.³ Identification of therapeutic biomarkers of idiopathic pulmonary fibrosis, which can better predict long-term therapeutic outcomes, is hampered by a lack of translational preclinical models. Precision-cut lung slices generated from explanted lung tissue from patients with idiopathic pulmonary fibrosis offer a physiologically relevant ex vivo model system to study fibrosis, preserving the diverse cellular composition and extracellular matrix architecture of fibrotic human lungs.^{1,4} Using a highly sensitive proteomics platform, we characterized the proteins secreted by fibrotic precisioncut lung slices in the presence or absence of a transforming growth factor-beta type I receptor kinase inhibitor (ALK5i), as proof-of-concept for biomarker identification in this model system.

PRECISION-CUT LUNG SLICE CULTURES PROVIDE A UNIQUE PLATFORM FOR THE DISCOVERY AND EVALUATION OF TREATMENT-SPECIFIC **TISSUE-SECRETED BIOMARKERS**

Background: Precision-cut lung slice culture methodology

- Precision-cut lung slices generated from idiopathic pulmonary fibrosis lung explant tissue can be cultured ex vivo for 7 days
- Replicate slices can be treated with low-molecular-weight inhibitors to evaluate treatment-mediated effects on protein or messenger ribonucleic acid expression

Figure 1. Schematic diagram showing the procedure to generate precision-cut lung slices



Experimental design

- Precision-cut lung slices, generated from explanted lung tissue from 5 patients with idiopathic pulmonary fibrosis at the time of transplant, were cultured to assess the impact of transforming growth factor-beta pathway inhibition on secreted proteins
- Precision-cut lung slices were cultured for 7 days with continuous treatment of either 0.1% dimethylsulfoxide as a vehicle or ALK5i (R-268712; 1 µM), a transforming growth factor-beta type I receptor kinase inhibitor. Medium was changed daily
- Hyper reaction monitoring deep proteomic profiling by liquid chromatographytandem mass spectrometry was performed on pooled conditioned media collected on the last day of culture (Day 7) from the precision-cut lung slices (n=6 pooled wells per donor/treatment)



SUMMARY OF PRECISION-CUT LUNG SLICE DONOR TISSUES Donor sample summary

Sample ID	Diagnosis	Previous patient treatment	Sex	Age
19-H-4	IPF	Nintedanib	М	58
19-H-6	IPF	Nintedanib	М	61
19-H-11	IPF (familial)	Pirfenidone	F	57
19-H-12	IPF	_	Μ	60
19-H-13	IPF	Nintedanib	М	62





ID. identification

99 PROTEINS WERE EXPRESSED AT SIGNIFICANTLY DIFFERENT LEVELS IN ALK5i-TREATED VS. VEHICLE-TREATED SAMPLES Quantification of proteins from precision-cut lung slice conditioned media samples by proteomic analysis

- media samples

References: 1. Decaris ML, et al. Respir Res 2021;22(1):265; 2. Maher TM, et al. Respir Res 2021;22(1):197; 3. Hutchinson JH, et al. Eur Respir J 2015;46(3):795-806; 4. Alsafadi HN, et al. Am J Physiol Lung Cell Mol Physiol 2017;312(6):L896-L902

Table 1. Characteristics of patients with idiopathic pulmonary fibrosis who donated lung tissue

F. female; ID, identification; IPF, idiopathic pulmonary fibrosis; M, male

Figure 3. Picrosirius red staining on adjacent fixed tissue from each donor







• In total, 3347 proteins represented by ~37,000 unique peptides were quantified by hyper reaction monitoring across all precision-cut lung slice conditioned

• On average, there were 2605 proteins per sample; the number of proteins was relatively uniform across samples

Samples were protein-normalized; subsequent differential analysis was reflective of the relative protein amount across samples, not absolute protein amount

Differential analysis of protein abundance

- Differential protein abundance was calculated for ALK5i-treated vs. vehicle-treated samples
- In total, 99 proteins were expressed at significantly different levels in ALK5i-treated vs. vehicle-treated samples (p<0.05), with an average \log_2 fold change >1.5
- The volcano plot shows all proteins, plotted by log, fold change and p value for ALK5i-treated vs. vehicle-treated samples. The 99 differential proteins are highlighted in red, with the subset of genes identified by name



CRLF1, cytokine-like factor 1; CTSS, cathepsin S; DMSO, dimethylsulfoxide; ITGAL, integrin subunit alpha L MMP12, matrix metalloproteinase-12; NRCAM, neuronal cell adhesion molecule; SERPINE1, serpin family E member 1; SPON2, spondin 2; VSIG8, V-set and immunoglobulin domain containing 8

Table 2. Summary of top proteins in precision-cut lung slice conditioned media samples

 significantly downregulated by ALK5i treatment

Downegulated by terverVSIG9V-set and immunoglobulin domain-containing protein 8-2.8983.34E-02COL10A1Collagen type X alpha 1 chain-2.9174.44E-02TBP2Latent-transforming growth factor-beta-binding protein 2-2.1511.173E-02FAPD. Protyl endopeptidase FAP-1.9261.149E-02SPOCK1G. Testican-1-1.8463.37E-03SPORX2Angopoietin-like protein 4-1.4812.276E-02ANGPTL4Angiopoietin-like protein 4-1.4812.276E-02PLOD2Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2-1.4344.473E-02PLOD2Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2-1.4344.450E-02COL11A2Collagen type XI alpha 2 chain-1.1364.450E-02COL7A1Collagen type XI alpha 1 chain-1.13281.67E-03FRNS23I. Collagen type XI alpha 1 chain-1.13281.67E-03FRNS24Plasminogen activator inhibitor-1-1.3281.67E-03FRNS25I. G. Beta-2-glycoprotein 1-1.1253.65E-02OLFML28OLfactomedin-like protein 28-1.1063.96E-03DDX21Nucleolar RNA helicase 2-1.0264.45E-02DLAM41Laminin subunit alpha-1-0.9833.49E-02GAP43Nucreodulin-0.9774.64E-02GAP43Nucreodulin talpha-2-0.9651.61E-02GAP44Collagen type Y alpha 2 chain-0.9234.65E-02GAP43Collagen type Y alpha 2 chain-0.923<	Gene ID	Protein description	Average log ratio	Protein p value		
VSIG8V-set and immunoojobulin domain-containing protein 8-2.8983.34E-02COL10A1Collagen type X alpha 1 chain-2.19744.4E-02LTBP2Latent-transforming growth factor-beta-binding protein 2-2.1511.73E-02FAPProlyl endopeptidase FAP-1.9261.49E-02SPOCK1Testican-1-1.8463.34E-02SPON2Spondin-2-1.6463.37E-03ANGPTL4Angiopoietin-like protein 4-1.4812.76E-02ADAMTS7A disintegrin and metalloproteinase with thrombospondin motifs 7-1.4612.78E-02PLOD2Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2-1.4344.73E-02COL11A2Collagen type XI alpha 2 chain-1.3684.50E-02LOXL1Lysyl oxidase homolog 1-1.3281.67E-03PRSS23Serine protease 23-1.2403.61E-02APOHBeta-2.glycoprotein 1-1.1253.65E-02OLFML28Olfactomedin-like protein 28-1.0264.01E-02LAMA-1Laminin subunit alpha-1-0.9833.49E-02LAMA-1Laminin subunit alpha-1-0.9833.49E-02GAP43Neuromodulin-0.9774.64E-02MRC2Collagen type V alpha 2 chain-0.9553.28E-02ROK2Rho-associated protein kinase 2-0.9234.65E-02BGNBiglycan-0.9234.65E-02	Downregulated by treatment					
COL10A1Collagen type X alpha 1 chain-2.1974.444-02LTBP2Latent transforming growth factor-beta-binding protein 2-2.151-1.73E-02FAPProlyl endopeptidase FAP-1.926-1.49E-02SPOK1Collagen type X alpha 2 chain-1.846-3.37E-03SPON2Adisintegrin and metalloproteinase with thrombospondin motifs7-1.4612.76E-02ADAMT57Adisintegrin and metalloproteinase with thrombospondin motifs7-1.4512.78E-02PLOD2Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2-1.4344.73E-02COL11A2Collagen type XI alpha 2 chain-1.4162.20E-02COL1A1Collagen type VII alpha 1 chain-1.3684.50E-02LOXL1Collagen type VII alpha 1 chain-1.328-1.67E-03FRPN23Serine protease 23-1.4343.61E-02FRS23Serine protease 23-1.2403.61E-02OLFML28Olfactomedin-like protein 2B-1.026-3.65E-02OLFML28Olfactomedin-like protein 2B-1.026-3.49E-02DX21ANCeolar RNA helicase 2-1.026-4.64E-02LAMA1Laminin suburit alpha-1-0.965-3.49E-02GAP43Neuromodulin-0.9774.64E-02GNC42Collagen type V alpha 2 chain-0.955-3.28E-02GNC42Rho-associated protein Kinase 2-0.9234.65E-02GNC42Rho-associated protein Kinase 2-0.923-4.65E-02GNC42Biglycan-0.920-2.87E-02	VSIG8	V-set and immunoglobulin domain-containing protein 8	-2.898	3.34E-02		
LTBP2Latent-transforming growth factor-beta-binding protein 22.2.1511.7.3E-02FAPProlyl endopeptidase FAP1.9.261.4.9E-02SPOCK1Construction3.348E-02SPON2Spondin-21.6.463.37E-03ANGPTL4Angiopoletin-like protein 41.4.812.76E-02ADAMT57A disintegrin and metalloproteinase with thrombospondin motifs71.4.612.78E-02PLOD2Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 21.4.344.73E-02COL1142Collagen type XI alpha 2 chain1.4.162.11E-02COL1141Collagen type XI alpha 2 chain1.4.162.20E-02LOXL1Collagen type XI alpha 2 chain1.3.382.20E-02LOXL1Plasminogen activator inhibitor-11.3.281.67E-03PRSS23Serine protease 231.1.243.61E-02OLFML2BOlfactomedin-like protein 2B1.1023.65E-02DX21Nucleolar RNA helicase 21.0.264.01E-02LAMA-1Laminin suburit alpha-10.9833.49E-02GAP43Neuromodulin0.9774.64E-02MRC2Collagen type V alpha 2 chain0.9553.28E-02GNC42Rho-associated protein kinase 20.9234.65E-02BGNBiglycan0.9202.87E-02	COL10A1	Collagen type X alpha 1 chain	-2.197	4.44E-02		
FAPProlyl endopeptidase FAP-1.9261.49E-02SPOCK1Testican-1-1.8463.34E-02SPON2Spondin-2-1.6463.37E-03ANGPTL4Angiopoletin-like protein 4-1.4812.76E-02ADAMTS7A disintegrin and metalloproteinase with thrombospondin motifs7-1.4612.78E-02PLOD2Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2-1.4344.473E-02COL1142Collagen type XI alpha 2 chain-1.1462.21E-02COL7A1Collagen type XI alpha 2 chain-1.3684.50E-02LOXL1Lysyl oxidase homolog 1-1.3281.67E-03SERPINE1Plasminogen activator inhibitor-11.3281.67E-03PRSS23Serine protease 23-1.1263.36E-02OLFML2BOlfactomedin-like protein 2B-1.1063.96E-03DDX21Nucleolar RNA helicase 2-1.0264.01E-02GAP43Neuromodulin-0.9774.64E-02MRC2Collagen type V alpha 2 chain-0.9653.28E-02GL5A2Collagen type V alpha 2 chain-0.9234.65E-02ROK2Rho-associated protein kinase 2-0.9234.65E-02	LTBP2	Latent-transforming growth factor-beta-binding protein 2	-2.151	1.73E-02		
SPOCK1Testican-1-1.8463.48E-02SPON2Spondin-2-1.6463.37E-03ANGPTL4Angiopoietin-like protein 4-1.4812.76E-02ADAMTS7A disintegrin and metalloproteinase with thrombospondin motifs 7-1.4612.78E-02PLOD2Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2-1.4344.73E-02COL1142Collagen type XI alpha 2 chain-1.4162.71E-02COL7A1Collagen type XI alpha 2 chain-1.4364.50E-02LOXL1Lysyl oxidase homolog 1-1.3684.50E-02SERPINE1Plasminogen activator inhibitor-11.3281.67E-03PRSS23Serine protease 23-1.1243.65E-02OLFML28Olfactomedin-like protein 2B-1.10264.01E-02DLX21Nucleolar RNA helicase 2-1.0264.01E-02GAP43Neuromodulin-0.9774.64E-02MRC2C.1ype mannose receptor 2-0.9653.28E-02GN24Scho-associated protein kinase 2-0.9234.65E-02BGNBiglycan-0.9234.65E-02	FAP	Prolyl endopeptidase FAP	-1.926	1.49E-02		
SPON2Spondin-2-1.646.3.37E-03ANGPTL4Angiopoietin-like protein 4-1.481.2.76E-02ADAMTS7A disintegrin and metalloproteinase with thrombospondin motifs 7-1.461.2.78E-02PLO02Procollagen-lysine, 2-oxoglutarets 5-dioxygenase 2.1.434.4.4.73E-02COL1142COllagen type XI alpha 2 chain.1.416.2.20E-02COL7A1COllagen type VII alpha 1 chain.1.416.2.20E-02LOXL1Lysyl oxidase homolog 1.1.328.2.20E-02SERPINE1Plasminogen activator inhibitor-1.1.328.3.61E-02SERPINE1Serine protease 23.1.125.3.65E-02OLFML28Olfactomedin-like protein 2B.1.106.3.96E-03DDX21Nucleolar RNA helicase 2.1.026.4.01E-02GAP43Nucleolar RNA helicase 2.1.026.4.64E-02MRC2C-type mannose receptor 2.0.995.3.63E-02GN24Collagen type V alpha 2 chain.0.9055.3.28E-02ROCK2Rho-associated protein kinase 2.0.9023.4.65E-02B6NBiglycan.0.921.2.87E-02	SPOCK1	Testican-1	-1.846	3.48E-02		
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PLOD2Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 21.4344.73E-02COL11A2Collagen type XI alpha 2 chain-1.4162.11E-02COL7A1Collagen type VII alpha 1 chain-1.3684.50E-02LOXL1Lysyl oxidase homolog 1-1.3432.20E-02SERPINE1Plasminogen activator inhibitor-1-1.3281.67E-03PRSS23Serine protease 23-1.12403.61E-02APOHBeta-2-glycoprotein 1-1.1253.65E-02OLFML28Olfactomedin-like protein 28-1.1063.96E-03DDX21Nucleolar RNA helicase 2-1.0264.01E-02GAP43Neuromodulin-0.9833.49E-02MRC2C-type mannose receptor 2-0.9651.61E-02CDL5A2Collagen type V alpha 2 chain-0.9553.28E-02ROCK2Rho-associated protein kinase 2-0.9202.87E-02BGNBiglycan-0.9202.87E-02	ADAMTS7	A disintegrin and metalloproteinase with thrombospondin motifs 7	-1.461	2.78E-02		
COL11A2Collagen type XI alpha 2 chain-1.4162.11E-02COL7A1Collagen type VII alpha 1 chain-1.3684.50E-02LOXL1Lysyl oxidase homolog 1-1.3432.20E-02SERPINE1Plasminogen activator inhibitor-1-1.3281.67E-03PRSS23Serine protease 23-1.2403.61E-02APOHBeta-2-glycoprotein 1-1.1253.65E-02OLFML2BOlfactomedin-like protein 2B-1.1063.96E-03DDX21Nucleolar RNA helicase 2-1.0264.01E-02LAMA-1Laminin subunit alpha-1-0.9833.49E-02GAP43Neuromodulin-0.9774.64E-02MRC2Collagen type V alpha 2 chain-0.9653.28E-02COL5A2Collagen type V alpha 2 chain-0.9234.65E-02BGNBiglycan-0.9202.87E-02	PLOD2	Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2	-1.434	4.73E-02		
COL7A1Collagen type VII alpha 1 chain-1.3684.50E-02LOXL1Lysyl oxidase homolog 1-1.3432.20E-02SERPINE1Plasminogen activator inhibitor-1-1.3281.67E-03PRSS23Serine protease 23-1.2403.61E-02APOHBeta-2-glycoprotein 1-1.1253.65E-02OLFML2BOlfactomedin-like protein 2B-1.1063.96E-03DDX21Nucleolar RNA helicase 2-1.0264.01E-02LAMA-1Laminin subunit alpha-10.9833.49E-02GAP43Neuromodulin-0.9774.64E-02MRC2Collagen type V alpha 2 chain-0.9553.28E-02COL5A2Rho-associated protein kinase 2-0.9234.65E-02BGNBiglycan-0.9202.87E-02	COL11A2	Collagen type XI alpha 2 chain	-1.416	2.11E-02		
LOXL1Lysyl oxidase homolog 1-1.3432.20E-02SERPINE1Plasminogen activator inhibitor-1-1.3281.67E-03PRSS23Serine protease 23-1.2403.61E-02APOHBeta-2-glycoprotein 1-1.1253.65E-02OLFML28Olfactomedin-like protein 2B-1.1063.96E-03DDX21Nucleolar RNA helicase 2-1.0264.01E-02LAMA-1Laminin subunit alpha-1-0.9833.49E-02GAP43Neuromodulin-0.9774.64E-02MRC2Collagen type V alpha 2 chain-0.9553.28E-02ROCK2Rho-associated protein kinase 2-0.9234.65E-02BGNBiglycan-0.9202.87E-02	COL7A1	Collagen type VII alpha 1 chain	-1.368	4.50E-02		
SERPINE1Plasminogen activator inhibitor-1-1.3281.67E-03PRSS23Serine protease 23-1.2403.61E-02APOHBeta-2-glycoprotein 1-1.1253.65E-02OLFML2BOlfactomedin-like protein 2B-1.1063.96E-03DDX21Nucleolar RNA helicase 2-1.0264.01E-02LAMA-1Laminin subunit alpha-1-0.9833.49E-02GAP43Neuromodulin-0.9774.64E-02MRC2C-type mannose receptor 2-0.9653.28E-02COL5A2Collagen type V alpha 2 chain-0.9234.65E-02ROCK2Rho-associated protein kinase 2-0.9234.65E-02BGNBiglycan-0.9202.87E-02	LOXL1	Lysyl oxidase homolog 1	-1.343	2.20E-02		
PRSS23Serine protease 23-1.2403.61E-02APOHBeta-2-glycoprotein 1-1.1253.65E-02OLFML2BOlfactomedin-like protein 2B-1.1063.96E-03DDX21Nucleolar RNA helicase 2-1.0264.01E-02LAMA-1Laminin subunit alpha-1-0.9833.49E-02GAP43Neuromodulin-0.9774.64E-02MRC2C-type mannose receptor 2-0.9651.61E-02COL5A2Collagen type V alpha 2 chain-0.9234.65E-02ROCK2Rho-associated protein kinase 2-0.9202.87E-02	SERPINE1	Plasminogen activator inhibitor-1	-1.328	1.67E-03		
APOHBeta-2-glycoprotein 1-1.1253.65E-02OLFML2BOlfactomedin-like protein 2B-1.1063.96E-03DDX21Nucleolar RNA helicase 2-1.0264.01E-02LAMA-1Laminin subunit alpha-1-0.9833.49E-02GAP43Neuromodulin-0.9774.64E-02MRC2C-type mannose receptor 2-0.9651.61E-02COL5A2Collagen type V alpha 2 chain-0.9553.28E-02ROCK2Rho-associated protein kinase 2-0.9234.65E-02BGNBiglycan-0.9202.87E-02	PRSS23	Serine protease 23	-1.240	3.61E-02		
OLFML2BOlfactomedin-like protein 2B-1.1063.96E-03DDX21Nucleolar RNA helicase 2-1.0264.01E-02LAMA-1Laminin subunit alpha-1-0.9833.49E-02GAP43Neuromodulin-0.9774.64E-02MRC2C-type mannose receptor 2-0.9651.61E-02COL5A2Collagen type V alpha 2 chain-0.9553.28E-02ROCK2Rho-associated protein kinase 2-0.9234.65E-02BGNBiglycan-0.9202.87E-02	АРОН	Beta-2-glycoprotein 1	-1.125	3.65E-02		
DDX21Nucleolar RNA helicase 2-1.0264.01E-02LAMA-1Laminin subunit alpha-1-0.9833.49E-02GAP43Neuromodulin-0.9774.64E-02MRC2C-type mannose receptor 2-0.9651.61E-02COL5A2COllagen type V alpha 2 chain-0.9553.28E-02ROCK2Rho-associated protein kinase 2-0.9234.65E-02BGNBiglycan-0.9202.87E-02	OLFML2B	Olfactomedin-like protein 2B	-1.106	3.96E-03		
LAMA-1Laminin subunit alpha-1-0.9833.49E-02GAP43Neuromodulin-0.9774.64E-02MRC2C-type mannose receptor 2-0.9651.61E-02COL5A2Collagen type V alpha 2 chain-0.9553.28E-02ROCK2Rho-associated protein kinase 2-0.9234.65E-02BGNBiglycan-0.9202.87E-02	DDX21	Nucleolar RNA helicase 2	-1.026	4.01E-02		
GAP43Neuromodulin-0.9774.64E-02MRC2C-type mannose receptor 2-0.9651.61E-02COL5A2Collagen type V alpha 2 chain-0.9553.28E-02ROCK2Rho-associated protein kinase 2-0.9234.65E-02BGNBiglycan-0.9202.87E-02	LAMA-1	Laminin subunit alpha-1	-0.983	3.49E-02		
MRC2C-type mannose receptor 2-0.9651.61E-02COL5A2Collagen type V alpha 2 chain-0.9553.28E-02ROCK2Rho-associated protein kinase 2-0.9234.65E-02BGNBiglycan-0.9202.87E-02	GAP43	Neuromodulin	-0.977	4.64E-02		
COL5A2Collagen type V alpha 2 chain-0.9553.28E-02ROCK2Rho-associated protein kinase 2-0.9234.65E-02BGNBiglycan-0.9202.87E-02	MRC2	C-type mannose receptor 2	-0.965	1.61E-02		
ROCK2Rho-associated protein kinase 2-0.9234.65E-02BGNBiglycan-0.9202.87E-02	COL5A2	Collagen type V alpha 2 chain	-0.955	3.28E-02		
BGN Biglycan -0.920 2.87E-02	ROCK2	Rho-associated protein kinase 2	-0.923	4.65E-02		
	BGN	Biglycan	-0.920	2.87E-02		
CRLF1Cytokine receptor-like factor 1-0.9088.86E-04	CRLF1	Cytokine receptor-like factor 1	-0.908	8.86E-04		
PXDN Peroxidasin homolog -0.867 3.21E-02	PXDN	Peroxidasin homolog	-0.867	3.21E-02		
ANTXR1 Anthrax toxin receptor 1 -0.833 3.22E-02	ANTXR1	Anthrax toxin receptor 1	-0.833	3.22E-02		
PLXDC2 Plexin domain-containing protein 2 -0.819 3.63E-02	PLXDC2	Plexin domain-containing protein 2	-0.819	3.63E-02		
POSTN Periostin -0.815 4.41E-02	POSTN	Periostin	-0.815	4.41E-02		
GSTM3 Glutathione S-transferase Mu 3 -0.739 2.02E-02	GSTM3	Glutathione S-transferase Mu 3	-0.739	2.02E-02		

- FAP, fibroblast activation protein; ID, identification; RNA, ribonucleic acid

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 Table 3. Summary of top proteins in precision-cut lung slice conditioned media samples
 significantly upregulated by ALK5i treatment

Gene ID	Protein description	Average log ratio
	Upregulated by treatm	ent
MMP12	Macrophage metalloelastase	2.647
TNFSF13B	Tumor necrosis factor ligand superfamily member 13B	1.998
ITGAL	Integrin alpha-L	1.841
NUDT9	ADP-ribose pyrophosphatase, mitochondrial	1.685
PTX3	Pentraxin-related protein	1.630
SECTM1	Secreted and transmembrane protein 1	1.618
AMY1A	Alpha-amylase 1	1.613
LIPA	Lysosomal acid lipase/cholesteryl ester hydrolase	1.464
CD48	CD48 antigen	1.304
IFITM3	Interferon-induced transmembrane protein 3	1.302
VPS36	Vacuolar protein-sorting-associated protein 36	1.297
SLC34A2	Sodium-dependent phosphate transport protein 2B	1.260
C1QA	Complement C1q subcomponent subunit A	1.259
C1QC	Complement C1q subcomponent subunit C	1.243
PCSK9	Proprotein convertase subtilisin/kexin type 9	1.242
LRRN4	Leucine-rich repeat neuronal protein 4	1.217
HLA-A	HLA class I histocompatibility antigen, A alpha chain	1.192
GBP2	Guanylate-binding protein 2	1.180
TGFB2	Transforming growth factor-beta-2 proprotein	1.168
IST1	IST1 homolog	1.127
RAB27B	Ras-related protein Rab-27B	1.118
SMPDL3A	Acid sphingomyelinase-like phosphodiesterase 3a	1.116
NRCAM	Neuronal cell adhesion molecule	1.099
ITGB2	Integrin beta-2	1.094
HLA-C	HLA class I histocompatibility antigen, C alpha chain	1.087
FERMT3	Fermitin family homolog 3	1.086
PTPN6	Tyrosine-protein phosphatase non-receptor type 6	1.085
A2M	Alpha-2-macroglobulin	1.080
SARG	Specifically androgen-regulated gene protein	1.079
CD47	Leukocyte surface antigen CD47	1.066

ADP, adenosine diphosphate; CD, cluster of differentiation; HLA, human leukocyte antigen; ID, identification

DOWNREGULATION OF COLLAGEN SUBTYPES IN CONDITIONED MEDIA SAMPLES OF ALK5i-TREATED PRECISION-CUT LUNG SLICES

- Differential abundance of collagen subtypes are indicated on a heat map of all proteins, sorted by log, fold change in ALKi5-treated vs. vehicle-treated precision-cut lung slices
- Upon treatment, the majority of detectable collagen subtypes demonstrated a trend for relative downregulation

Figure 5. Heatmap of collagen subtypes sorted by log, fold change in ALK5i-treated vs. vehicle-treated precision-cut lung slices



Collagen type XVII alpha 1 chain Collagen type XVI alpha 1 chain Collagen type XVIII alpha 1 chain Collagen type XIV alpha 1 chain Collagen type IV alpha 5 chain Collagen type VI alpha 3 chain Collagen type II alpha 1 chain Collagen type III alpha 1 chain Collagen type VI alpha 2 chain Collagen type VI alpha 6 chain Collagen type I alpha 1 chain Collagen type IV alpha 3 chain Collagen type I alpha 2 chain Collagen type IV alpha 1 chain Collagen type VI alpha 1 chain Collagen type IV alpha 2 chain Collagen type XV alpha 1 chain Collagen type XXI alpha 1 chain Collagen type IV alpha 4 chain Collagen type XII alpha 1 chain Collagen type V alpha 2 chain Collagen type V alpha 1 chain Collagen type VIII alpha 1 chain Collagen type V alpha 3 chain Collagen type VII alpha 1 chain Collagen type XI alpha 2 chain Collagen type X alpha 1 chain

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Protein p value
3.40E-02
3.67E-02
8.18E-03
1.64E-02
9.11E-03
2.05E-02
3.76E-02
4.85E-02
2.54E-03
3.90E-02
9.03E-03
1.10E-02
3.46E-02
2.65E-02
3.41E-02
4.29E-02
3.52E-02
4.88E-02
4.15E-02
4.96E-02
1.87E-02
2.85E-03
1.20E-03
2.37E-02
1.01E-02
3.22E-02
2.76E-02
2.96E-03
3.75E-02
2.75E-02

MODULATION OF CLINICAL BIOMARKERS AFTER ALK5i TREATMENT OF PRECISION-CUT LUNG SLICES FROM IDIOPATHIC PULMONARY FIBROSIS LUNG EXPLANTS

- Multiple idiopathic pulmonary fibrosis clinical biomarkers were quantified using this approach in precision-cut lung slices conditioned media samples
- Plasminogen activator inhibitor-1 was significantly (p=0.0079) reduced in the ALK5i-treated vs. vehicle-treated samples, while periostin showed a strong trend toward downregulation (not significant, Mann–Whitney t-test)

Figure 6. Quantification of idiopathic pulmonary fibrosis biomarkers in precision-cut lung slices conditioned media samples



CA, cancer antigen; CCL1-18, CC-chemokine ligand 18; COMP, cartilage oligomeric matrix protein; DMSO, dimethylsulfoxide; KL-6, Krebs von den Lungen-6; MMP7, matrix metalloproteinase-7; MUC1, mucin 1; MUC16, mucin 16; ns, not significant; PAI-1, p lasminogen activator inhibitor-1; POSTN, periostin; UPAR, urokinase-type plasminogen activator receptor

STABLE ISOTOPE LABELING EXPERIMENTS CONFIRMED THAT SECRETION OF **NEWLY SYNTHESIZED PROTEINS OCCURRED DURING CULTURE**

- To evaluate the presence of protein turnover in precision-cut lung slice cultures, lung slices were cultured in the presence of heavy water, and conditioned media samples were collected on Day 7 for tandem mass spectrometric analysis of mass isotopomer abundances of trypsin-generated peptides
- A substantial fraction of heavy water-labeled peptides were quantified for several matrix proteins
- Peptides measured in conditioned media samples from 7-day cultured precision-cut lung slices contained stable isotopes, indicating that they were newly synthesized during ex vivo culture

 Table 4. Percentage of heavy water-labeled proteins synthesized during precision-cut lung
 slice culture

Select D ₂ O-labeled proteins	Protein synthesized during PCLS culture (D ₂ O labeled), %	Unlabeled control, %		
Collagen type I alpha 1 chain	30	6		
Fibronectin	55	0		
Collagen type III alpha 1 chain	17	5		
Lumican	30	3		
Collagen type I alpha 2 chain	32	6		
Metalloproteinase inhibitor 1	49	0		
Collagen type VI alpha 1 chain	64	4		
D ₂ O, heavy water; PCLS, precision-cut lung slice				

CONCLUSIONS

- Proteomic profiling of conditioned media samples from ex vivo cultured fibrotic lung tissue was performed to identify putative biomarker candidates for idiopathic pulmonary fibrosis
- We identified 99 proteins present at significantly different levels between precision-cut lung slices treated with transforming growth factor-beta type I receptor kinase inhibitor (ALK5i) vs. vehicle
- This study demonstrates the utility of the precision-cut lung slice culture methodology, combined with deep proteomic profiling, to identify idiopathic pulmonary fibrosis biomarkers modulated by drug treatment of lung tissue from patients with idiopathic pulmonary fibrosis

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