Proteomic Analysis of Plasma and Tissue Samples for the Identification of Pharmacodynamic Biomarkers in IPF

M. Decaris[1], I. Pike[3], P. Wolters[2], M. Bremang[3], R. Gaster[1], P. Andre[1], S. Turner[1]
1 – Pliant Therapeutics, 700 Saginaw Drive, Suite 150, Redwood City, CA 94063, USA;
2 – University of California at San Francisco, San Francisco, USA;
3 – Proteome Sciences plc, Hamilton House, Mabledon Place, London, WC1H 9BB, UK

Rationale

There are no reliable biomarkers suitable for stratifying patients in clinical trials or monitoring treatment response in idiopathic pulmonary fibrosis (IPF). This lack of adequate tools contributes to large and lengthy clinical trials limiting the pace at which new therapies can be evaluated. Recent improvements in proteomics methodology and the development of tissue-enhanced fluid proteomics have increased the breadth and depth of plasma proteome coverage, providing a potential opportunity to identify novel pharmacodynamic biomarkers. We performed a proof of concept study to evaluate novel tissue and plasma proteomics approaches for the identification of novel biomarkers in IPF.

Methods

Longitudinal plasma samples from six individuals with IPF and individual samples from five healthy controls, along with lung tissue samples from IPF patients were analyzed with isobaric Tandem Mass Tags® (TMT®) on an Orbitrap® Fusion Tribrid® mass spectrometer. Plasma samples were depleted of the top ~70 abundant proteins using Seppro® IgY14 and Supermix® columns (Merck), trypsin digested, and analyzed with a TMTcalibrator™ approach to increase detection of lung-derived proteins[1]. **See accompanying abstract #13474 for full description of methods.**

Results

Proteomic analysis incorporating the tissue calibrator method roughly doubled the number of protein groups identified in plasma samples compared with protein depletion alone, from approximately ~4500 to 9000. Comparison of 5657 proteins quantified across all samples resulted in 887 proteins distinguishing IPF and healthy subjects (q < 0.05). Following rollup of IPF plasma samples by patient, we narrowed the list to 14 proteins and 334 individual peptides that were down-regulated, and 22 proteins and 311 peptides that were up-regulated in IPF vs. control plasma using the thresholds of fold change >2 and p-value <0.005. Significant proteins and peptides included a number of putative biomarkers of inflammation and fibrosis (e.g. CRP, WFDC2, ICAM1), as well as a variety of proteins known to play a role in pulmonary fibrotic disease including latent TGF-β binding protein 1 (LTBP1), pulmonary surfactant protein D (SFTPD), and mucin 5B (MUC5B). These peptides provide promising candidates for prospective MRM quantitation studies in larger patient groups. Future analyses using this methodology to study longitudinal correlations of plasma peptide abundance with disease progression and/or patient response to therapy may provide promising new prognostic and therapeutic biomarkers for IPF.

Patient Samples and Demographics

Patient #	Age	Sex	Date of ILD Dx	Plasma Collection	FVC range (%)
IPF #1	70	M	6/7/2013	7/13-9/14	71-62
IPF #2	82	M	4/19/2013	9/13-9/14	73-66
IPF #3	81	M	9/19/2013	3/14-8/14	55-51
IPF #4	71	F	4/8/2009	3/14-10/14	107-103
IPF #5	66	M	10/24/2013	4/14-6/15	56-51
IPF #6	71	M	2/21/2014	4/14-4/15	85-77
Healthy #1	56	M			
Healthy #2	66	M			
Healthy #3	68	F			
Healthy #4	74	F			
Healthy #5	72	F			

Longitudinal plasma samples collected from 6 IPF patients were analyzed alongside individual plasma samples from 5 age-matched controls

TMTcalibrator™ Methods

- Dual Protein Depletion and Tissue Calibrator[1] Method Used to Boost Detection of Lung-Related Proteins in Plasma

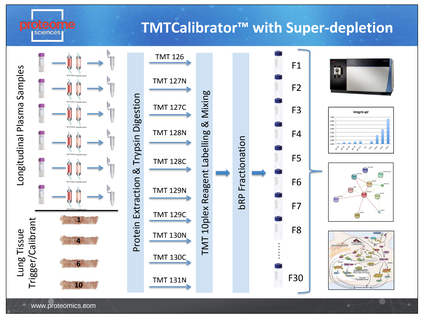


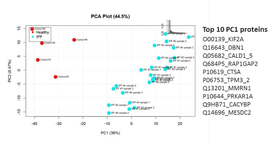
Table: Number of Peptides/Proteins Identified in each TMT Experiment

	TMTcalibrator™					TMT®MS2
10plex Number	TMT01	TMT02	TMT03	TMT04	TMT05	TMT06
PSMs [≥1 plasma channel]	104,217	164,242	124,941	178,938	172,052	118,725
PSMs [All 10 channels]	51,091	100,387	77,479	125,373	118,110	98,220
Peptides	57,890	76,698	66,954	84,543	81,053	40,938
ProteinGroups	8,903	10,640	9,783	11,027	10,909	4,617
Unique Gene Names	7,536	8,946	8,230	9,238	9,146	3,968

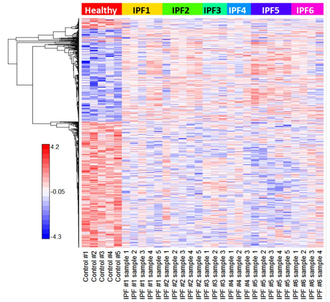
TMT01-05 analyzed plasma and tissue; TMT06 analyzed plasma only; PSM (peptide spectral matches)

- **Comparison of 5657 proteins quantified across all samples identified 887 proteins distinguishing IPF and Healthy subjects (q < 0.05)**

PCA Plot of Significant Proteins



Heatmap of Significant Proteins



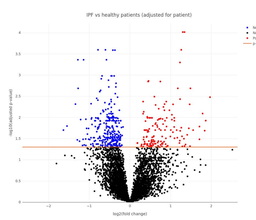
- Longitudinal correlation of plasma protein abundance with FVC/treatment did not result in strong statistical outcomes due to small sample size

1) Russell CL *et al*. Combined tissue and fluid proteomics with Tandem Mass Tags to identify low-abundance protein biomarkers of disease in peripheral body fluid: An Alzheimer's Disease case study. Rapid Commun Mass Spectrom 31(2):153-159 2017

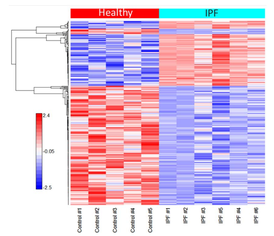
Plasma Protein Analysis

- Following rollup of IPF plasma samples by patient, 303 proteins were significantly different between IPF and Healthy Subjects (p <0.005)

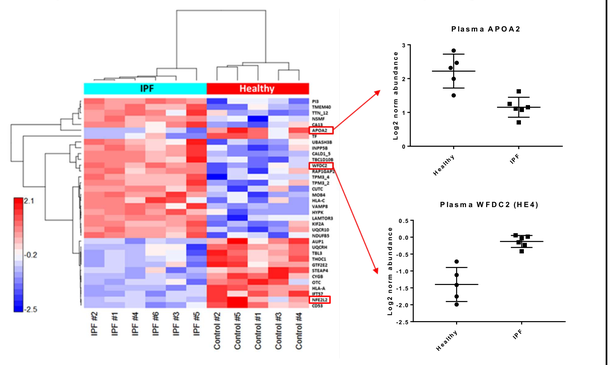
Volcano Plot of Protein Abundance



Heatmap of Significant Proteins



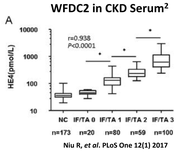
- 36 proteins were Identified with > 2-fold change and p-value <0.005 between IPF and Healthy Subjects



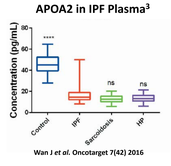
- Proteins distinguishing IPF from Healthy Subjects included some previously identified as markers of disease

Examples include: WFDC2: Serum levels correlate with fibrosis stage in CKD[2]; APOA2: Reduced levels in IPF plasma[3]; NFE2L2: Reduced levels in IPF Fibroblasts[4]

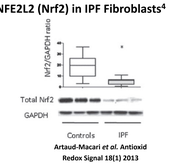
WFDC2 in CKD Serum[2]



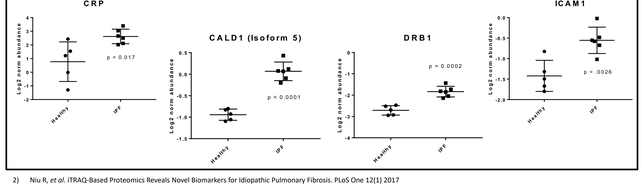
APOA2 in IPF Plasma[3]



NFE2L2 (Nrf2) in IPF Fibroblasts[4]



Additional Proteins of Interest

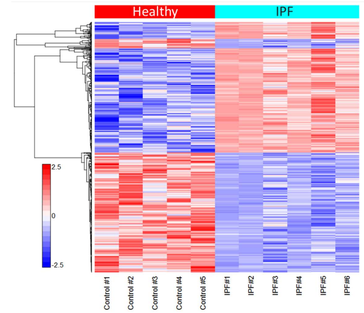


2) Niu R, *et al*. iTRAQ-Based Proteomics Reveals Novel Biomarkers for Idiopathic Pulmonary Fibrosis. PLoS One 12(1) 2017
3) Wan J *et al*. Elevated serum concentrations of HE4 as a novel biomarker of disease severity and renal fibrosis in kidney disease. Oncotarget 7(42) 2016
4) Artaud-Macari *et al*. Nuclear Factor Erythroid 2-Related Factor 2 Nuclear Translocation Induces Myofibroblastic Dedifferentiation in Idiopathic Pulmonary Fibrosis. Antioxid Redox Signal 18(1) 2013

Analysis of Individual Peptides

- 645 individual peptides distinguished IPF from healthy subjects (Fold Change > 2; p<0.005)
- 379 of 645 significant peptides identified via tissue calibrator approach

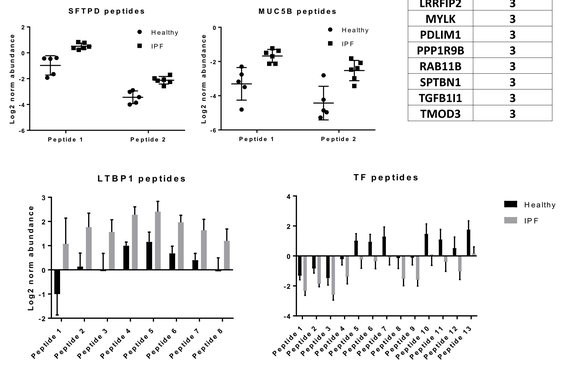
Heatmap of Significant Peptides



Proteins with ≥ 3 highly significant peptides

Gene Name	# sig peptides
FLNA	36
TF	13
CALD1	9
LTBP1	8
DYNC1H1	7
AHNAK	6
FLNB	6
APOA2	5
CA1	5
LRG1	4
MYH10	4
MYH11	4
NEXN	4
SPTAN1	4
VASP	4
CORO1C	3
CTNNA1	3
DCTN1	3
FYB	3
HSPG2	3
ICAM1	3
LRRFIP2	3
MYLK	3
PDLIM1	3
PPP1R9B	3
RAB11B	3
SPTBN1	3
TGFB1I1	3
TMOD3	3

Additional Peptides of Interest



Conclusions

- TMTcalibrator™ approach increased the number of quantifiable plasma proteins from ~4500 to 9000, and enhanced the detection of lung-derived proteins
- 36 proteins and 645 peptides were found to significantly differentiate IPF/healthy plasma (FC> 2; p<0.005), including known markers of inflammation and fibrosis
- Analysis of larger, well characterized IPF patient cohorts using this technique may help to identify novel circulating biomarkers for fibrotic disease

IP & MB are paid employees and hold stock and/or stock options in Proteome Sciences plc.
MD & ST are paid employees and hold stock and/or stock options in Pliant Therapeutics Inc.