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

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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 Seppe® IgY14 and Supermix® columns (Merck), trypsin digested, and analyzed with a TMCalibrator™ approach to increase detection of lung-derived proteins. 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 pulmonary fibrosis.

Conclusions
- TMCalibrator™ 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

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

Table: Additional Proteins of Interest

Additional Peptides of Interest

- 379 of 645 significant peptides identified via tissue calibrator
- Additional Proteins of Interest
  - MUC5B peptides
  - LTBP1 peptides
  - TP peptides