Development of a Multi-biomarker Assay for Serum Proteins by the Prognostic Lung Fibrosis Consortium (PROLIFIC)

P. H. Schafer[1], S. Hersey[1], J. Rassa[1], A. Seyda[1], D. Eisinger[2], J. Melin[2], R. Bencher[2], M. Neighbors[3], M. Mouded[4], A. Martin-Schwarze[5], S. Turner[6], M. Decaris[7], Y. Bauer[8], C. L. Nickerson-Nutter[9], D. Ball[10], J. Larson[11], J. Swaney[12], S. Lim[13], A. C. Mackinnon[14], F. Marabita[15], J. Mefford[16], S. Staszak[17], J. A. Lasky[18]; [1]Translational Medicine, Bristol Myers Squibb, Princeton, NJ, United States, [2]Rules-Based Medicine, A Q2 Solutions Company, Austin, TX, United States, [3]Genentech, South San Francisco, CA, United States, [4]Novartis Institute for Biomedical Research, Cambridge, MA, United States, [5]Biostatistics, University of Michigan, Ann Arbor, MI, United States, [6]Biology, South San Francisco, CA, United States, [7]Pliant Therapeutics, South San Francisco, CA, United States, [8]Galapagos NV, Basel, Switzerland, [9]Three Lakes Foundation, Georgetown, MA, United States, [10]Three Lakes Foundation, Northbrook, IL, United States, [11]Tvardi Therapeutics, Sugar Land, TX, United States, [12]Lassen Therapeutics, San Diego, CA, United States, [13]Biomarker Sciences, Gilead, Foster City, CA, United States, [14]Nine Bioquarter, Galecto Biotech, Edinburgh, United Kingdom, [15]Chiesi Corporate R&D, Solna, Sweden, [16]Corporate Relations, Pulmonary Fibrosis Foundation, Chicago, IL, United States, [17]Pulmonary Fibrosis Foundation, Chicago, IL, United States, [18]Tulane Univ Hlth Sci Ctr, New Orleans, LA, United States.

Corresponding author's email: peter.schafer@bms.com

Rationale: Multiple peer-reviewed publications have consistently reported a reoccurring set of blood-based protein biomarkers linked to idiopathic pulmonary fibrosis (IPF) disease progression. Despite the strength of the evidence, no harmonized and validated panel has been available to the scientific community for this context of use. To address this unmet need, the Prognostic Lung Fibrosis Consortium (PROLIFIC) was formed to develop well-qualified assays suitable for use as exploratory, prognostic or predictive biomarkers within the context of clinical trials. Methods: Twelve protein biomarkers were selected based on evidence for their prognostic and mechanistic value in IPF, including markers of epithelial damage (cytokeratin 19 fragment [CYFRA 21-1], surfactant protein D [SP-D], cancer antigen 125 [CA-125], cancer antigen 19-9 [CA-19-9], and Krebs von den Lungen 6 [KL-6]), fibrosis (matrix metalloproteinase 7 [MMP-7], tenascin C [TNC], and periostin [POSTN], inflammation (pulmonary and activation-regulated chemokine [PARC or CCL18], B lymphocyte chemoattractant [BLC or CXCL13], and soluble intercellular adhesion molecule 1 [sICAM-1], and thrombosis (plasminogen activator inhibitor 1 [PAI-1]). The 12 assays were optimized into 3 multiplex panels and 2 singleplex panels on the Luminex platform. The assays were analytically validated for serum and EDTA plasma (excludes MMP-7) under formal protocols with design controls and pre-defined acceptance criteria with respect to Limit of Detection, Sensitivity, Accuracy, Precision, Parallelism, Matrix Interference, Freeze/Thaw Stability, Short-term Analyte Stability, and Sample Reproducibility. The assays were used to measure biomarker levels in serum collected from IPF patients at the time of enrollment (baseline) into the Pulmonary Fibrosis Foundation Patient Registry (N=657). Statistical analyses were performed using a random coefficients longitudinal sub-model for the decline in % predicted Forced Vital Capacity (FVC) and a Cox proportional hazards sub-model for transplant free survival at one year, adjusting for sex, age, BMI, anti-fibrotic medication, % predicted FVC, and % predicted DLCO. Results: All assays met pre-defined acceptance criteria. The annual change in % predicted FVC was significantly

associated with baseline MMP-7, SP-D, KL-6, PAI-1, CA-19-9, CYFRA 21-1, BLC/CXCL13, and sICAM-1. Transplant-free survival was significantly associated with baseline SP-D, sICAM-1, TNC, and KL-6. In a joint model combining the outcome measures, SP-D had the best model fit, followed by KL-6, sICAM-1, MMP-7, TNC, CA-125, PAI-1, CYFRA 21-1, PARC/CCL18, and CA-19-9. Conclusions: All biomarkers except POSTN were associated with the decline in % predicted FVC and/or transplant-free survival. These results indicate the assay is well-qualified to measure these prognostic biomarkers within the context of IPF clinical trials.

This abstract is funded by: Bristol Myers Squibb and The PROLIFIC Consortium