Development of a Multi-biomarker Risk Score Based on Serum Proteins by the Prognostic Lung Fibrosis Consortium (PROLIFIC)

P. Schafer[1], J. Rassa[1], A. Seyda[1], S. Hersey[1], D. Eisinger[2], J. Melin[2], R. Bencher[2], M. Neighbors[3], M. Mouded[4], S. Turner[5], M. Decaris[5], Y. Bauer[6], C.L. Nickerson-Nutter[7], D. Ball[7], J. Larson[8], J. Swaney[9], S. R. Lim[10], A. C. MacKinnon[11], F. Marabita[12], J. Mefford[13], S. Staszak[13], A. Martin-Schwarze[14], Z. Zheng[14], P. Song[14], J. A. Lasky[15]

[1] Bristol Myers Squibb, Princeton, NJ, United States, [2] Rules-Based Medicine, A Q2 Solutions Company, Austin, TX, United States, [3] Genentech Inc., South San Francisco, CA, United States, [4] Novartis Biomedical Research Inc., Boston, MA, United States, [5] Pliant Therapeutics, South San Francisco, CA, United States, [6] Galapagos GmbH, Basel, Switzerland, [7] Three Lakes Foundation Trust, Northbrook, IL, United States, [8] Tvardi Therapeutics, Houston, TX, United States, [9] Lassen Therapeutics, San Diego, CA, United States, [10] Gilead Sciences, Foster City, CA, United States, [11]Galecto Biotech, Boston, MA, USA, [12] Chiesi Corporate R&D, Solna, Sweden, [13] Pulmonary Fibrosis Foundation, Chicago, IL, United States, [14] Biostatistics, University of Michigan, Ann Arbor, MI, United States, [15] Tulane University Health Science Center, New Orleans, LA, United States.

Introduction

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. (https://www.pulmonaryfibrosis.org/prolific).

Table 1. Markers selected for the PROLIFIC test

Category	Biomarker	Evidence of Prognostic or Pharmacodynamic Value (Ref)
Epithelial Damage	Cytokeratin 19 fragment (CYFRA 21-1)	Baseline CYFRA 21-1 was able to distinguish individuals at risk of 12-month disease progression (C-statistic 0.70 (95% CI 0.61 – 0.79), p < 0.0001) (Molyneaux 2022)
	Surfactant Protein-D (SP-D)	Significant improvement in the 1-year mortality prediction model when serum SP-A and SP-D (area under the receiving operator curve [AROC], 0.89) were added to the clinical predictors alone (AROC, 0.79; p = 0.03) (Kinder 2009)
	CA-19-9 (sialyl Lewis A)	Baseline of ≥22 U/mL was associated with a 3x increased risk of mortality (Maher 2017)
	CA-125 (MUC16)	Baseline of ≥12 U/mL was associated with a 3x increased risk of mortality (Maher 2017)
	KL-6 (MUC 1)	Serum baseline level >1000 U/mL is associated with worse prognosis (Yokoyama 2006) and >1300 U/mL with increased risk of acute exacerbation (Ohshimo 2014). KL-6 ≥ 1000 U/mL associated with disease progression (HR=2.761-2.845, p=0.040-0.045) (Chung 2022)
Fibrosis	Matrix Metalloproteinase 7 (MMP-7)	Higher levels (>3.5 ng/mL) lower transplant free survival (HR=2.3, p=0.016) (Richards 2012) Higher baseline (≥3.8 ng/mL) had higher risk of worsening (HR=2.2, p=0.001) (Bauer 2017)
	Tenascin C (TN-C)	Change from baseline Tenascin correlated with change from baseline FVC (van der Velden 2016)
	Periostin (POSTN)	Prognostic for FVC in the test cohort (Effect size=-3.6, p<0.001) and replication cohort (Effect size=-2.5, p=0.186) (Neighbors 2018)
Inflammation	CCL18 (PARC)	Prognostic for FVC in the test cohort (Effect size=-3.1, p=0.032) and replication cohort (Effect size=3.6, p=0.004) (Neighbors 2018)
	CXCL13 (BLC)	6-mo survival in the highest quartile of plasma CXCL13 was 65% versus 93% in the others (HR= 5.5, P = 0.0008) (Vuga 2014). >62.1 pg/mL shorter survival (DePianto 2015)
	sICAM-1	High level (>202.5 ng/ml) associated with lower transplant-free survival (Richards 2012)
Thrombosis	Plasminogen Activator Inhibitor 1 (PAI-1)	Stable IPF =45 ng/mL vs AEx =70 ng/mL (p=0.0004), predicts survival (p=0.14) (Collard 2010)

Methods

Assay Development

Twelve protein biomarkers were selected based on evidence for their prognostic and mechanistic value in IPF (Table 1), 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]).

All 12 immunoassays were developed at Rules Based Medicine facility in Austin TX as either in single-plex or multiplex format, utilized the Luminex® xMAP® platform and consisted of antigen-specific antibodies optimized in a capture-sandwich format. The 12 assays were optimized into 3 multiplex panels and 2 singleplex panels. The assays were analytically validated for serum and EDTA plasma (MMP-7 for serum only) 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. All assays met pre-defined acceptance criteria.

Table 2. Patient characteristics of PFF Patient Registry serum samples

IPF patients Baseline data	Total N=657 Mean ± SD	
Age	70.69 ± 8.08 years	
Male	n=489 (74.4%)	
Asian	n=14 (2.1%)	
Black	n=10 (1.5%)	
White	n=617 (93.9%)	
Smoking history, Yes	n=427 (65.0%)	
Using anti-fibrotic meds	n=437 (66.5%)	
FVC	2.65 ± 0.78 liters	
FVC, % predicted	0.68 ± 0.17 %	
DLCO	11.71 ± 4.58 mL/min/mm Hg	
DLCO, % predicted	39.93 ± 14.17 %	
GAP score	5.67± 1.09	
Outcome data within 1 year		
Death	n=62 (9.4%),	time to death 0.52 ± 0.27 years
Lung transplant	n=37 (5.6%),	time to transplant 0.46 ± 0.25 years
≥10% absolute decline in % pred FVC	n=20 (3.0%)	
≥10% relative decline in % pred FVC	n=95 (14.5%)	

Pulmonary Fibrosis Foundation (PFF) Patient Registry

The analysis population included 657 IPF-diagnosed Registry patients who provided serum biosamples near the time of enrollment (baseline). Patients had FVC and DLCO measurements within 90 days of biosample collection. The analysis featured a longitudinal response and a time-to-event response. The longitudinal response was % predicted FVC (NHANES III; Hankinson et al., 1999) measured from 90 days before biosample collection through 455 days afterward. Subjects averaged 3.4 FVC measurements during this window. The time-to-event response was transplant-free survival within one year following biosample collection, censored for loss to follow-up. Demographic and clinical characteristics are shown in Table 2.

Statistical Analysis

Single biomarker statistical analyses were performed using a random coefficients longitudinal sub-model for the decline in % predicted Forced Vital Capacity (FVC) jointly with 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. Penalized logistic regression was used to select prognostic biomarkers using the LASSO method, and the resulting model with selected biomarkers was used to derive a multi-marker risk score.

Results

Single Marker Associations with FVC Decline

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 (Fig. 1, Table 3).

Figure 1. Single-marker analysis for annual change in % predicted FVC associated with a one standard deviation difference in log-scale baseline biomarker concentration

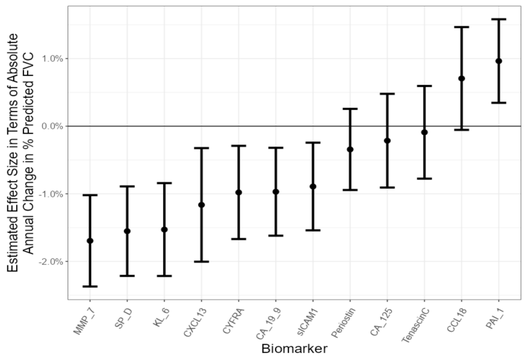


Table 3. Estimated longitudinal effects associated with a one standard deviation change in log-scale baseline biomarker concentration with standard errors and p-values.

Biomarker	Estimate	Std.Err	p-value
MMP-7	-1.70	0.344	< 0.001
SP-D	-1.55	0.338	< 0.001
KL-6	-1.53	0.351	< 0.001
PAI-1	0.96	0.315	0.002
CA-19-9	-0.97	0.332	0.003
CYFRA	-0.98	0.352	0.005
CXCL13	-1.16	0.429	0.007
sICAM1	-0.89	0.331	0.007
CCL18	0.71	0.388	0.069
Periostin	-0.34	0.306	0.26
CA-125	-0.21	0.354	0.54
TenascinC	-0.09	0.350	0.80

Single Marker Associations with Transplant-free Survival

Transplant-free survival was significantly associated with baseline SP-D, sICAM-1, TNC, and KL-6 (Fig. 2, Table 4).

Figure 2. Transplant-free survival hazard ratios associated with a one standard deviation difference in log-scale baseline biomarker concentration

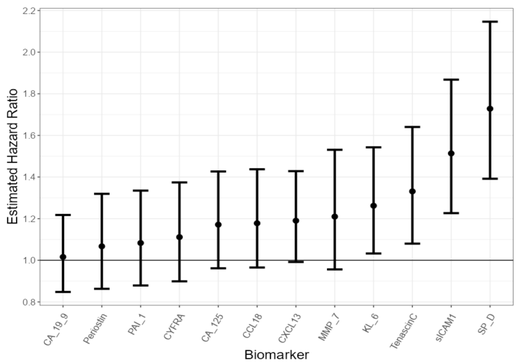


Table 4. Estimated hazard ratios associated with a one standard deviation difference in log-scale biomarker concentration with 95% confidence intervals and p-values.

Biomarker	Hazard Ratio	95% CI	p-value
SP-D	1.73	(1.39, 2.15)	< 0.001
sICAM1	1.51	(1.23, 1.87)	< 0.001
TenascinC	1.33	(1.08, 1.64)	0.007
KL-6	1.26	(1.03, 1.54)	0.023
CXCL13	1.19	(0.99, 1.43)	0.061
CCL18	1.18	(0.97, 1.44)	0.11
MMP-7	1.21	(0.96, 1.53)	0.11
CA-125	1.17	(0.96, 1.43)	0.12
CYFRA	1.11	(0.9, 1.37)	0.33
PAI-1	1.08	(0.88, 1.33)	0.45
Periostin	1.07	(0.86, 1.32)	0.55
CA-19-9	1.02	(0.85, 1.22)	0.86

Multi-Marker Model for Joint Outcome of FVC decline and Transplant-free Survival

Logistic regression using the LASSO method identified the most important biomarkers for prediction of clinical outcomes. Four biomarkers (MMP-7, TNC, SP-D, and CXCL13) were identified to be associated with the composite binary outcome of death, lung transplant, or ≥ 10% relative decline of % predicted FVC. The coefficients for these important proteomic features were estimated while adjusting for sex, age, BMI, anti-fibrotic medication, % predicted FVC and % predicted DLCO at baseline. The resulting multi-marker risk score (SP-D*0.33150 + CXCL13*0.29234 + TNC*0.14501 + MMP-7*0.13118) predicts the disease progression binary outcome with an AUROC= 0.796 (Figure 3).

Using this 4-marker prediction model, the quartiles of the individual risk scores show clear separation for the composite event rate, with a similar separation patterns among males and females, with or without antifibrotic medication (Figure 4).

Figure 3. LASSO logistic regression and solution paths of biomarker coefficients for disease progression model (death, lung transplant, or ≥10% decline in % predicted FVC at one year).

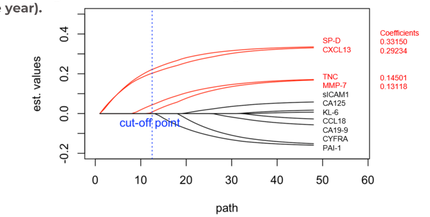
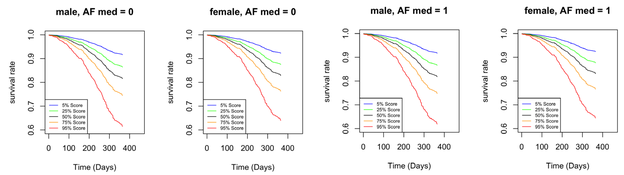


Figure 4. Probability of disease progression for multi-marker model in males vs. females, without or with antifibrotic medication.



Conclusions

A multi-marker algorithm confirmed the important serum proteins consistent with findings from single-marker analyses and yielded a subject-level prognostic biomarker-based score to predict both FVC decline and transplant-free survival in IPF.

References

1. Molyneaux PL et al. 2022 Am J Respir Crit Care Med. 15;205(12):1440-1448.
2. Kinder BW et al. 2009 Chest. 135(6):1557-1563.
3. Maher TM et al., 2017 Lancet Respir Med. 5(12):946-955.
4. Yokoyama A et al. 2006 Respirology 11(2):164-8
5. Chung C et al. Sci Rep. 2022 May 20;12(1):8564.
6. Richards TJ et al. 2012 Am J Respir Crit Care Med. 1;185(1):67-76.
7. Bauer Y et al. 2017 ERJ Open Res. 22;3(1). pii: 00074-2016.
8. van der Velden JL et al. 2016, Clin Transl Med. 5(1):36.
9. Neighbors M et al. 2018 Lancet Respir Med. 6(8):615-626.
10. Vuga LJ et al. Am J Respir Crit Care Med. 2014 Apr 15;189(8):966-74.
11. DePianto DJ et al. 2015 Thorax 70(1):48-56.
12. Collard HR et al. 2010 Am J Physiol Lung Cell Mol Physiol 299(1):L3-7.
13. Hankinson JL et al. 1999. Am J Respir Crit Care Med 159(1):179-87.

Disclosures

This work was funded by the members of the PROLIFIC consortium. All authors were employees of the institutions listed in the affiliations at the time the work was conducted.