

RESEARCH

Open Access



Revisiting the INSPIRE trial: antibody profiling reveals high prevalence of occult autoimmunity

Marilyn K. Glassberg^{1*}, Cindy Burg², Simone Pereira-Simon³, Benjamin Trzaskoma², Lisa Harlow⁴, Stephanie Biedka⁵, Sharon Elliot^{1,3}, Yingze Zhang⁶, Hannah VanEvery⁷, Noreen Fertig⁷, Jonathan S. Minden^{5†} and Dana P. Ascherman^{7,8*†}

Abstract

Rationale In the INSPIRE trial, patients diagnosed with Idiopathic Pulmonary Fibrosis (IPF) failed to demonstrate improved survival after treatment with IFN-gamma-1 β . This outcome became the impetus to develop more personalized approaches to the diagnosis, classification, and management of pulmonary fibrosis.

Objective The present study was designed to assess autoantibody profiles in a randomly selected group of INSPIRE trial participants in order to better define IPF on a molecular diagnostic level and define subsets with potentially different underlying disease processes.

Methods We performed conventional, gel-based protein and RNA immunoprecipitation (IP) on 483 plasma specimens derived from patients enrolled in both the treatment and placebo arms of INSPIRE. Tandem immunoprecipitation and mass spectrometry proteomics (IP-to-MS) of selected specimens was used to confirm conventional IP interpretation and to identify unknown autoantigens.

Results Based on conventional IP approaches, approximately 30% of trial participants had evidence of autoimmune disease-specific autoantibodies and another ~ 10% had evidence of autoantibodies of unknown specificity. IP-to-MS revealed additional autoantigens, including Annexin 11.

Conclusions IP analyses demonstrated an unexpectedly high prevalence of autoantibodies potentially indicative of underlying connective tissue disease-associated ILD, underscoring the importance of classification schemes incorporating unbiased autoantibody profiling.

Keywords IPF (Idiopathic Pulmonary Fibrosis), Autoantibody, Immunoprecipitation

[†]Jonathan S. Minden and Dana P. Ascherman co-senior authors.

*Correspondence:

Marilyn K. Glassberg
Marilyn.Glassberg@lumc.edu
Dana P. Ascherman
DAscher@pitt.edu

¹Department of Medicine, Division of Pulmonary and Critical Care Medicine, Loyola University Stritch School of Medicine, South 1st Avenue, Maywood, IL 216060153, USA

²Roche Genentech, Inc., South San Francisco, CA 94080, USA

³Division of Pulmonary and Critical Care, University of Miami Miller School of Medicine, Miami, FL 33162, USA

⁴Division of Rheumatology, University of Miami Miller School of Medicine, Miami, FL 33162, USA

⁵Impact Proteomics, LLC., Pittsburgh, PA 15206, USA

⁶Division of Pulmonary, Critical Care, and Sleep Medicine, University of Pittsburgh, Pittsburgh, PA 15213, USA

⁷Division of Rheumatology and Clinical Immunology, University of Pittsburgh, Pittsburgh, PA 15213, USA

⁸Biomedical Tower South, 711, 3500 Terrace Street, Pittsburgh, PA 15213, USA



© The Author(s) 2026. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

Introduction

In 2009, publication of negative results from the INSPIRE (INternational study of Survival outcomes in idiopathic Pulmonary fibrosis (IPF) with InteRfEron gamma-1 β) phase 3 randomized, double-blind, placebo-controlled clinical trial encompassing 826 patients labeled as “IPF” [1] generated considerable disappointment, particularly given the limited treatment options for this relentlessly progressive disease. Participants in INSPIRE were 40–79 years of age, with IPF diagnosed according to clinical, radiological, and histological criteria that have changed with the publication of four consensus statements since that time [2]. The primary endpoint of INSPIRE was overall survival time, but the trial was terminated in early 2007 after a planned interim analysis showed no survival benefit from IFN gamma-1 β treatment. The authors of the paper summarizing the INSPIRE trial's unfavorable findings pointed out that those with quickly progressing illnesses might have been excluded by the eligibility requirements. They proposed that selection bias favoring enrollment of patients with more stable disease may have contributed to the reported lack of efficacy of IFN gamma-1 β , decreasing the chance of observing a therapeutic benefit.

Another key issue potentially contributing to the failure of this trial was the heterogeneity of the study population, as patients were not rigorously screened for abnormal serology (e.g., ANA positivity) or other clinical features of occult autoimmunity that might have skewed clinical outcomes. In fact, these considerations have prompted the search for novel biomarkers that can more accurately describe such patients and, eventually, create more individualized methods for their diagnosis and treatment [3].

The present study was therefore designed to revisit a randomly selected group of participants in the INSPIRE trial in order to define patient subsets with alternative disease processes that could potentially explain differing responses to therapy. In fact, as early as 2012 [4], the available literature highlighted subsets of patients previously diagnosed with IPF who manifested a more favorable clinical course and/or had subtle clinical features of autoimmune disease corresponding to the presence of disease-specific autoantibodies (e.g., myositis- or scleroderma-associated autoantibodies) [5] that were not assessed prior to enrollment in INSPIRE. Because the biologic behavior of interstitial lung disease and the response to immunomodulatory therapy may be quite different in the setting of underlying autoimmunity—even when associated with IPF-like histopathologic abnormalities of Usual Interstitial Pneumonia (UIP) [6, 7]—identification of these individuals is critical for appropriate placement in clinical trials and development of targeted therapeutic modalities.

Given the unique opportunity provided by the INSPIRE trial to assess the frequency of disease-specific autoantibodies in a large population of “idiopathic” ILD patients, we sought to serologically characterize these individuals based on the hypothesis that a subset of patients previously classified as IPF would possess autoimmune disease-specific antibodies targeting aminoacyl-tRNA synthetases (Jo-1, PL-7, PL-12, EJ, OJ, KS) or scleroderma-associated antigens (including Scl-70, Th/To, U3RNP, and Pm/Scl) indicative of an underlying autoimmune diathesis. We therefore performed a combination of protein and RNA immunoprecipitation on 483 plasma specimens derived from patients enrolled in both the treatment and placebo arms of this trial (Fig. 1A). Collectively, these studies demonstrated that a substantial percentage of patients (~40%) classified as IPF possessed serologic markers of occult autoimmunity (e.g., myositis- or scleroderma-associated antibodies)—many of which were confirmed by IP-to-MS, a quantitative, mass spectrometry-based method of autoantibody detection (Fig. 1B). These results demonstrate the power and versatility of newer mass spectrometry-based methodologies that can more accurately identify markers of occult myositis- and scleroderma-associated ILD as well as autoantibodies more uniquely linked to IPF.

Methods

Cohort

Based on availability, plasma samples collected from 483 consented patients enrolled in the INSPIRE trial were assessed in the current study. Baseline demographic characteristics included age, sex, and disease duration. Response to treatment (IFN-gamma-1 β) versus placebo was assessed through change in pulmonary function tests (absolute and percent predicted FVC), days to respiratory-related hospitalization, and survival. Banked samples from healthy subjects without interstitial lung disease and an independent cohort of IPF patients recruited through the University of Pittsburgh [GAP cohort, described in [8]] were used as controls for immunoprecipitation and other immunoassays. See online data supplement for additional details.

Protein immunoprecipitation

50 μ l of plasma were incubated overnight with Protein A sepharose beads, following which IgG-bound beads were co-incubated with ³⁵S-methionine labeled K562 extract* for 2 h according to established protocol [9]. Immunoprecipitated proteins were then eluted with SDS elution buffer and subjected to SDS-PAGE (Polyacrylamide Gel Electrophoresis) to delineate protein bands recognized by patient sera. Comparison to established reference sera allowed identification of specific autoantigen targets.

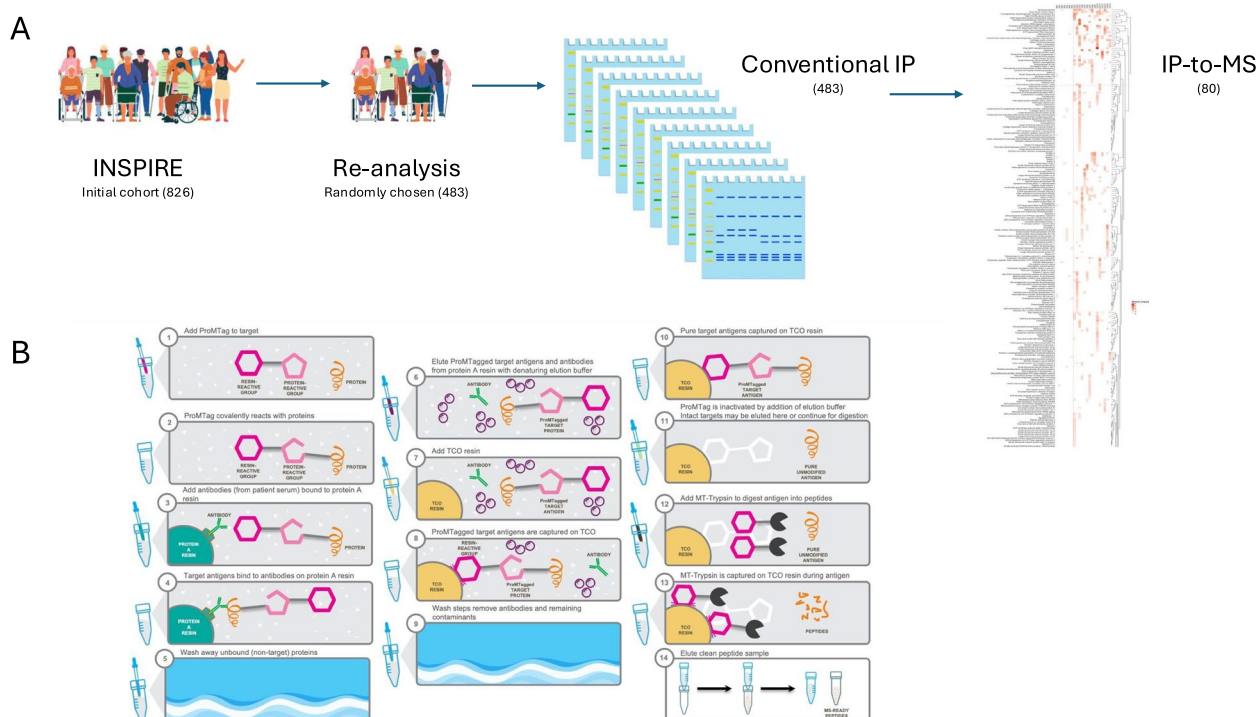


Fig. 1 Experimental design. **A** This schematic depicts the sample analysis progression from the original INSPIRE cohort to those assessed by conventional IP and then IP-to-MS, culminating in a heatmap to visualize the array of antigens recognized by autoantibodies derived from patient and healthy control samples. **B** This panel diagrams the IP-to-MS workflow that leads from ProMTagged whole-cell lysate proteins to purified peptides derived from antigens targeted by patient antibodies

*K562 extract is derived from an erythroleukemia cell line that is commonly used to detect ubiquitously expressed target antigens in immunoprecipitation assays based on favorable growth characteristics and high expression levels of autoantigens targeted in a variety of systemic autoimmune diseases.

RNA immunoprecipitation

IgG-bound beads generated as above and resuspended in NET-2 buffer (50 mM Tris/HCl, pH 7.4, 150 mM NaCl, 0.05% Igepal CA630) were incubated with K562 whole cell extract for 2 h at 4 °C. After washing with NET-2 buffer, the resulting complexes were resuspended in extraction buffer (NET-2, 0.25 M sodium acetate, 0.83% SDS, 1 μl glycogen) prior to phenol/chloroform/isoamyl alcohol (50:50:1) extraction. Subsequent ethanol precipitation of RNA and electrophoretic resolution on a 7 M urea 8% polyacrylamide gel permitted visualization through neutral silver staining. Comparison of electrophoretic mobility with controls of known specificity was used to characterize isolated RNA.

IP-to-MS

IP-to-MS analysis of plasma samples representing different patient and autoantibody subgroups was performed by Impact Proteomics, LLC [10]. IP-to-MS replaces

radiolabeled protein as a source of potential autoantigens with a reversible protein tag, called ProMTag (Fig. 1B). ProMTag has two key features: a reversible amine-reactive group and an irreversible beads capture functional group. Thus, whole-proteome K562 cell lysates were tagged with ProMTag where all proteins carry at least one ProMTag. This ProMTagged antigen pool was immunoprecipitated by patient antibodies bound to Protein A beads. After washing to remove non-target proteins, immunoprecipitated proteins were separated from immunoglobulins by binding to ProMTag-capture beads. The captured autoantigen target proteins were then subjected to in situ trypsin digestion; resulting peptides were then lyophilized and stored at -80 °C. Twenty percent of yielded peptides were separated by an Evosep One HPLC system and subjected to tandem mass spectrometric analyses by a timsTOF Pro 2 (Bruker) mass spectrometer (see Online Supplement for detailed MS methods).

CCP2 ELISA

To assess the presence of antibodies targeting citrullinated proteins, plasma samples (diluted 1:100 in diluent buffer) were separately assessed with a commercial IgG anti-CCP2 (cyclic citrullinated peptide 2) ELISA kit (Axis-Shield Diagnostics, United Kingdom) according to established protocol.

ANA testing

Selected plasma samples were assessed for anti-nuclear antibodies (ANA) using the Calbiotech (El Canon, CA) screening ELISA kit. This kit enables detection of antibodies against specific antigens such as RNP (U1RNP), Sm, Ro60/SS-A, SS-B, Scl-70, PCNA (Proliferating Cell Nuclear Antigen), dsDNA, histones, centromere B, and ribosomal P. Healthy control sera were used to establish an antibody index (OD450 of sample/calibrator cutoff) of 1.0 as the threshold for ANA positivity. All plasma samples were diluted 1:50 in diluent buffer.

Statistical considerations

Summary statistics included assessment of antibody frequency as well as concordance rates between conventional immunoprecipitation and IP-to-MS (that served as the gold standard). A concordance metric between the conventional IP gel interpretation and IP-to-MS analysis was established where agreement between methods was deemed “good” if one or more MS-identified protein had a band of similar apparent molecular mass on the corresponding IP protein gel. The degree of agreement was characterized as “fair” if there was no direct mass agreement, but the observed protein gel band could be explained by known breakdown protein products of the MS-identified protein. Samples with no demonstrable concordance between gel-based IP and IP-to-MS were ranked as “poor”.

Clinical, demographic, and functional characteristics of serologically-defined subgroups were compared using Fisher’s exact/Chi-square testing (depending on group sizes) for dichotomous variables. Continuous variables were compared using student’s t test for normally

distributed data and Mann Whitney U test for non-parametric data.

Results

Protein and RNA Immunoprecipitation

Plasma samples derived from 483 INSPIRE trial subjects, 15 patients with alternative interstitial lung diseases, and 12 healthy controls (without interstitial lung disease) were subjected to conventional protein immunoprecipitation (IP) using ^{35}S -methionine-labeled K562 cell lysate as potential target antigens according to the protocol outlined in the Methods section. Among the samples initially classified as IPE, 186 (38.5%) demonstrated clear protein bands of interest (Fig. 2 and Supplemental Fig. 1), while 119 other samples (28.6%) yielded equivocal bands. Four additional samples (out of 483) demonstrated anti-CCP2 antibodies by ELISA (variable titer), and the remaining 174 samples did not have evidence of autoantibodies by protein IP or CCP2 ELISA. RNA IP of 100 samples selected on the basis of protein IP patterns (potentially indicative of RNA-binding autoantigens) provided additional detail regarding autoantibody specificity (Fig. 3 and Supplemental Table 1). Based on these collective protein and RNA IP data, as well as the CCP2 ELISA results, we classified the INSPIRE trial samples into the following autoantibody-based subgroups (Table 1): Subgroup 1-A: antibodies targeting amino-acyl tRNA synthetases, topoisomerase I, TH/TO, Ro60, U3 RNP, or other snRNPs— $n=134$; Subgroup 1-B: antibodies targeting an unknown 56 kDa antigen— $n=27$; Subgroup 1-C: antibodies recognizing an undefined 31/35 kDa doublet— $n=25$; Subgroup 2: anti-CCP2— $n=4$; Subgroup 3: equivocal/other protein bands— $n=119$; and Subgroup 4: negative autoantibody screen— $n=174$. This classification

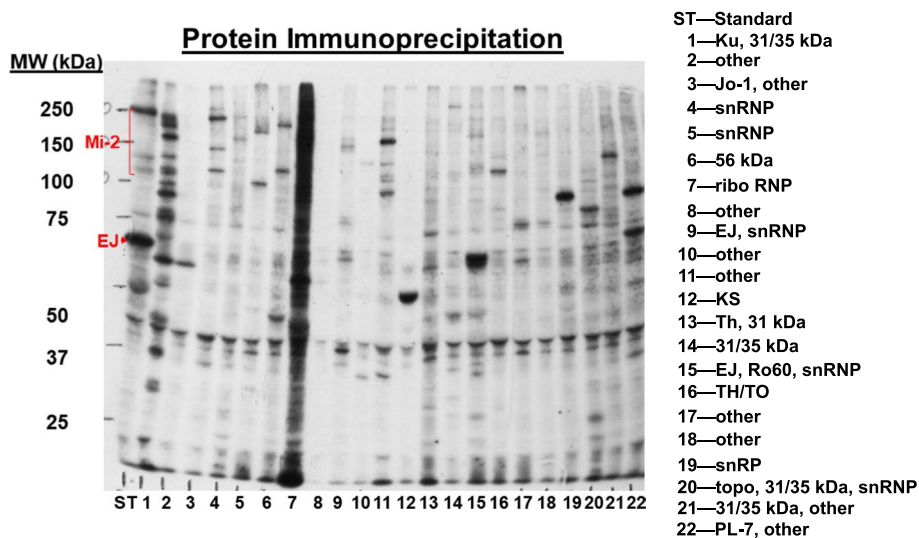


Fig. 2 Protein Immunoprecipitation. The depicted autoradiogram demonstrates K562-derived radiolabeled proteins immunoprecipitated by different sera; protein identities are listed on the right

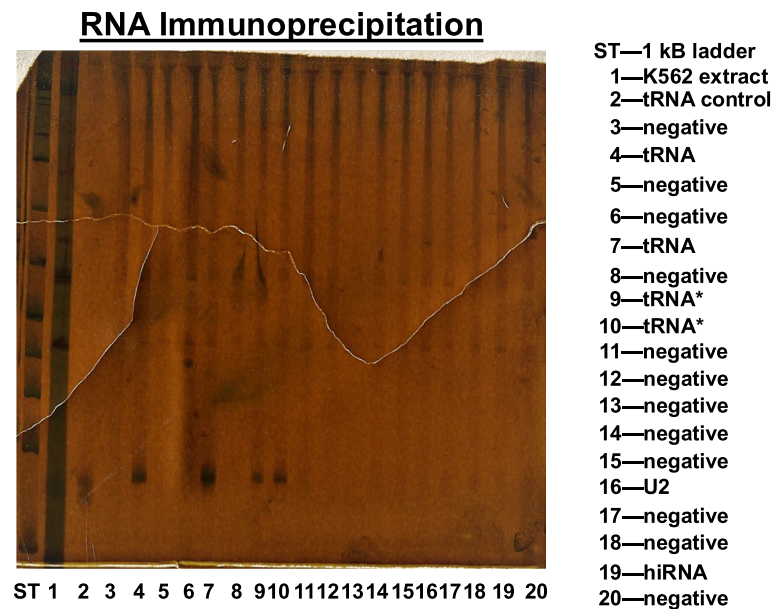


Fig. 3 RNA immunoprecipitation. The silver-stained gel shows different types of RNA complexes (listed at right) immunoprecipitated by individual sera; *duplicate samples

Table 1 Autoantibody classification of INSPIRE samples

Category	Number (%)	Description
1-A	134 (28)	tRNA synthetases, topoisomerase, TH/TO, snRNP
1-B	27 (6)	56 kDa protein
1-C	25 (5)	31/35 kDa bands
2	4 (1)	CCP-2
3	119 (25)	Other/undefined antigens
4	174 (36)	Negative

scheme encompasses patients with autoantibodies of interest ($n = 186$, Subgroups 1A-1C), those with bands of unknown identity ($n = 119$, Subgroup 3), and those without evidence of autoantibodies ($n = 174$, Subgroup 4).

Because the conventional, gel-based protein IP showed a diversity of novel protein bands that did not always correlate with known autoantigens—particularly in subgroups 1-B, 1-C, and 3—IP-to-MS was used to both confirm conventional IP autoantibody determinations and to identify novel autoantigens (as outlined in Fig. 1B). Based on availability of remaining plasma, IP-to-MS analysis was performed on 75 of the 483 INSPIRE trial samples previously assessed by gel-based IP: 68 samples yielding putative autoantigens by conventional IP and 7 samples that did not yield identifiable bands by conventional IP [Note that five samples were run as duplicates, bringing the total number of patient samples assessed by IP-to-MS experiments to 80]. In addition, 16 serum samples derived from healthy subjects without interstitial lung disease were assessed by IP-to-MS as negative controls.

A heatmap representing the \log_2 intensity score of detected proteins (based on spectral counts) was

generated to provide a full display of the IP-to-MS results, plotting patient sample ID horizontally versus MS-identified proteins vertically (Fig. 4A and Supplemental Table 2). In addition, a clustering algorithm was applied to sort proteins with similar detection patterns across patient samples. The top eleven protein rows appear to span the majority of patient samples and likely represent a set of common non-specific binding proteins. Conversely, the remaining protein rows demonstrate putative autoantigens that were immunoprecipitated by individual subjects, sometimes in patient-specific vertical clusters (G3, G75, and G76) not found in healthy control samples.

To better demonstrate specific immunoprecipitated proteins, individual rows of the heatmap were grouped together and magnified in panels B and C of Fig. 4. As shown in Fig. 4B and Table 2, IP-to-MS revealed numerous autoantibodies (in multiple patient samples) that target known autoantigens including tRNA synthetases (asparaginyl-, threonyl-, and tryptophanyl-tRNA synthetase), topoisomerase I, TH/TO, centromere V, and Ro60. These autoantigens are indicators of autoimmune diseases such as myositis, scleroderma, Sjogren's, and systemic lupus erythematosus. In addition, other known autoantigens were detected in single patient representatives, including X-ray repair cross-complementing proteins, exosome proteins, and RNase P subunits (Fig. 4A).

Gel-based IP revealed previously uncharacterized 56 and 31/35 kDa proteins. IP-to-MS identified the commonly immunoprecipitated 56 kDa protein as Annexin A11 (confirmed by ELISA, data not shown) and suggested that the frequently observed 31/35 kDa bands could be prohibitin 1/prohibitin 2 (Fig. 4C). Other

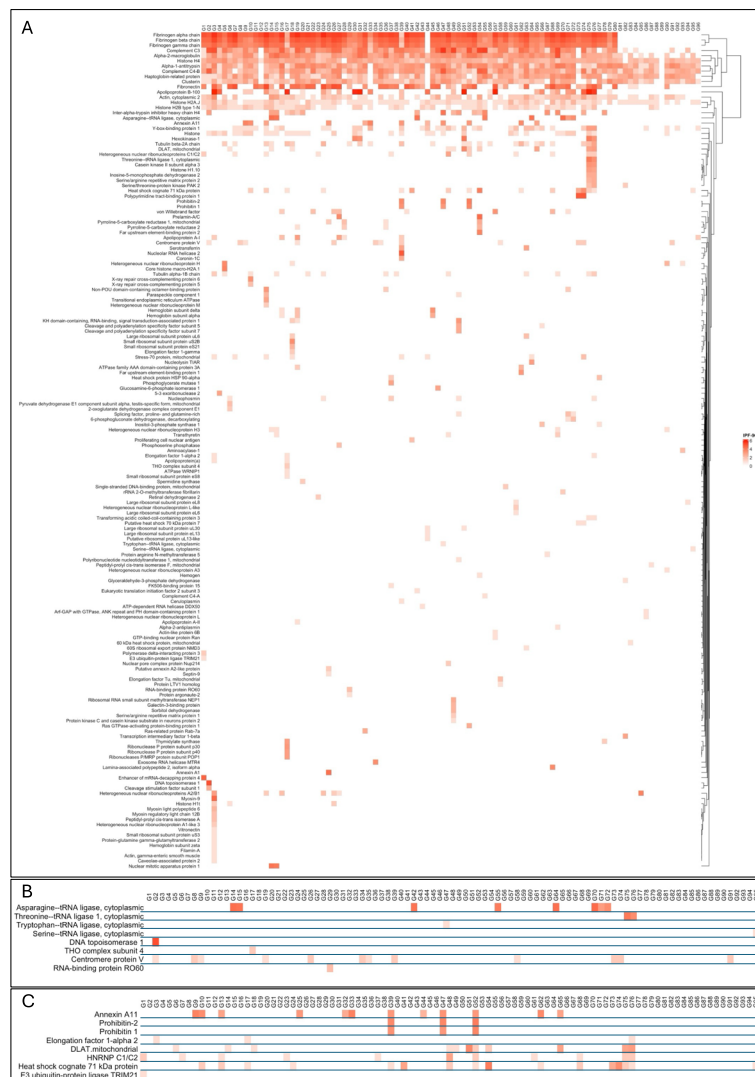


Fig. 4 IP-to-MS of 80 selected INSPIRE samples. **A** The full heatmap depicted in this panel demonstrates proteins identified by IP-to-MS in plasma specimens derived from INSPIRE participants ($n = 80$) and serum samples from healthy controls ($n = 16$). The intensity scale represents the \log_2 of spectral count data for all proteins identified. A very stringent cutoff was applied to only include proteins with a spectral count of at least two, where each spectral count represents a sequenced peptide associated with a unique protein and can be used as a proxy for relative protein abundance. The protein rows were sorted according to a pattern clustering algorithm. Note that fibrinogen only appears as a non-specific contaminant in the patient plasma samples, but not in the healthy control serum samples. **B** This expanded section of the heatmap highlights target antigens associated with myositis, scleroderma, and Lupus. **C** This portion of the heatmap shows target antigens not typically associated with ILD

potential autoantigens of interest detected in several samples, but not found in control samples, included elongation factor 1-alpha 2 ($n = 3$), CENPV ($n = 8$), DLAT, a component of the mitochondrial pyruvate dehydrogenase complex ($n = 9$), heterogenous ribonucleoproteins C1/C2 ($n = 10$), and heat shock protein 71 ($n = 15$) (Fig. 4C). As a complementary assessment of additional anti-nuclear antibodies, we performed ANA testing (using a commercial ELISA) on the subset of samples listed in Table 2. Of note, the results of ANA testing were highly discordant from the IP-to-MS analysis, as only 7 samples demonstrated low levels of anti-nuclear antibodies.

Because conventional, gel-based IP is widely considered to be the gold-standard for autoantigen classification, we compared the results from conventional IP versus IP-to-MS for the 80 INSPIRE samples analyzed by both methods (Tables 2 and 3). The results were compared on two levels: a) gel band apparent molecular mass versus MS-identified protein mass (Table 2) and b) expert interpretation of conventional IP versus MS protein identification (Table 3). Based on a concordance metric described in the "Methods" section, there was very good agreement between the gel-based protein bands and IP-to-MS assignments, where 75% (60/80) of the sample displayed good agreement (Table 2). Fifteen percent (12/80)

Table 2 IP-to-MS autoantibody profiling

Sam- ple ID	Observed Protein Gel Bands	Conventional IP-call	IP-to-MS 1st	IP-to-MS 2nd	IP-to-MS 3rd	IP-to-MS 4th	Agreement	AILD- known Ab	ANA posi- tive†
1	135,120	other	EDC4 (151)	HNRNPC (34)	POLDIP3 (46)	TRIM21 (54)	good	3	
2	100 smear,w63, w35, w31	prob topo, prob 35, prob 31	TOP1 (91)	ITIH4 (105)	CSTF1 (48)	CENPV (30)	good	1-A	Yes
3	63	poss KS	APOB (509)	MYH9 (226)	HNRN- PA2B1 (37)	MYL6 (17)	fair (1)	1-A	
4	100,110,74,w63	prob topo, poss KS, other	APOB (509)	XRN2 (109)			good	1-A	Yes
5	st50, st56	prob snRNP, prob 56	H2AFY (39)	HNRNPH1 (49)	TUBB2A (50)	TUBA1B (50)	good	1-A	
6(1)	mult hi bands, 110, 100,st80,78,st70, 61,st52,st46, st35,31mult hi bands, 110, 100,st80,78,st70, 61,st52,st46, st35,31mult hi bands, 110, 100,st80,78,st70, 61,st52,st46, st35,31mult hi bands, 110, 100,st80,78,st70, 61,st52,st46, st35,31mult hi bands, 110, 100,st80,78,st70, 61,st52,st46, st35,31mult hi bands, 110, 100,st80,78,st70, 61,st52,st46, st35,31	poss 35, poss 31, poss Ku, other	PDHA1 (43)	OGDH (116)	H1-2 (14)	DLAT (69)	good	1-A	
7	negative	poss Ro 60, other	TUBB2A (50)	HSPA8 (71)	HNRNPC (34)	YBX1 (36)	MS > gel	1-A	
8	90	other	CENPV (30)	YBX1 (36)			poor	3	
9	st 56, 260 st 56, 260 st 56, 260 st 56, 260 st 56, 260 st 56, 260	prob 56, other	ANXA11 (54)	APOB (509)	CENPV (30)	YBX1 (36)	good	1-B	
10	82, 70, st5682, 70, st5682, 70, st5682, 70, st5682, 70, st5682, 70, st56	prob 56, other	ANXA11 (54)	XRCC6 (70)	XRCC5 (83)	APOB (509)	good	1-B	
11	negative	negative	YBX1 (36)				MS > gel	4	
12	negative	negative	RNP A2/B1				good	4	Yes
13	63, st56, 7863, st56, 7863, st56, 7863, st56, 7863, st56, 7863, st56, 78	prob 56, poss KS, other	APOB (509)	NONO (55)	ANXA11 (54)	VCP (89)	good	1-A	
14	st 63	prob KS	NARS (63)	NUMA1 (238)	ITIH4 (105)	DLAT (69)	good	1-A	
15	st 63	prob KS	NARS (63)	NUMA1 (238)	ITIH4 (105)	APOB (509)	good	1-A	
16	w63, 56, 27	poss KS, poss snRNP, poss 56	HNRNPA2B1 (37)	APOA1 (31)	YBX1 (36)	VWF (309)	fair (1,2)	1-A	
17	mult hi bands, 63,60,56,45,37,31	poss KS, poss Ro 60, poss 56, poss 31, other	APOB (509)	RPP30 (29)	TYMS (36)	RPP40 (42)	good (1)	1-A	
18	125, 110, 63	poss KS, poss PL-12, other	RPSA (33)	ITIH4 (105)	RPS21 (9)	DLAT (69)	good	1-A	
19	63, 35,31	poss KS, prob 35, prob 31	APOA1 (31)	HBB (16)	HBA1 (16)	ITIH4 (105)	good	1-A	
20	130, 32	other	ITIH4 (105)	SRM (34)	APOB (509)		good (1)	3	
21	w160	other	TUBA1B (50)				poor	3	
22	70	other	VWF (309)				fair (2)	3	
23	40	other	ALDH1A2 (57)	HNRNPC (34)			fair	3	

Table 2 (continued)

Sam- ple ID	Observed Protein Gel Bands	Conventional IP-call	IP-to-MS 1st	IP-to-MS 2nd	IP-to-MS 3rd	IP-to-MS 4th	Agreement	AILD- known Ab	ANA positiv et
24	48	other	APOB (509)	TUBB2A (50)	HNRN- PA2B1 (37)	HSPA8 (71)	good	3	
25	65, st56, st 35, st 3165, st56, st 35, st 3165, st56, st 35, st 3165, st56, st 35, st 3165, st56, st 35, st 31	prob 56, prob 35, prob 31, other	ANXA1 (39)	ANXA11 (54)	APOB (509)	PYCR1 (33)	good	1-B	
26	negative	poss snRNP	VWF (309)	HNRNPA2B1	CENPV (30)	H1-2 (14)	MS > gel	1-A	
27	w63, st 23	other	LMNA (74)	APOB (509)	VWF (309)	SERB (25)	good (1,2)	3	
28	70, st 35, 31	prob 35, prob 31, other	PYCR1 (33)	PYCR2 (34)	APOA1 (31)		good	1-C	Yes
29	Ro 60, 70,95	Ro 60, other	TROVE2 (61)	TUBB2A (50)	AGO2 (97)		good	1-A	
30(19)	st110, w63, w60, w56	poss snRNP	APOB (509)	HK1 (102)	YBX1 (36)		good	1-A	
31	st110, w63, w60, w56	poss snRNP	APOB (509)	HK1 (102)	YBX1 (36)	TUBB2A (50)	good	1-A	
32	st56	prob 56	ANXA11 (54)	RAB7A (23)			good	1-B	
33	st56, 38st56, 38st56, 38st56, 38	prob 56, poss Th, other	ANXA11 (54)	APOB (509)	YBX1 (36)	TUBB2A (50)	good	1-A	
34	st115, 110, w65, 60, 56, 28	poss snRNP, other	SKIV2L2 (118)	CENPV (30)			good	1-A	
35	135, 100, 70, 68, 35, w 31	poss topo, poss 35, poss 31, other	CENPV (30)				fair*	1-A	
36	220, 210, 160, 145, 110, 64, 35, 31	prob 35, prob 31, other	NONO (54)	PYCR1 (33)	PYCR2 (34)	TUBA1B (50)	good	1-C	
37	150, 125, st 84	other	PGAM1 (29)	NPM1 (33)	HS90B (83)	HNRNPC (34)	good	3	
38	negative	negative	TUBA1B (50)				good	4	
39	(63), st56, 37?, 31 (63), st56, 37?, 31 (63), st56, 37?, 31 (63), st56, 37?, 31	prob 56, poss 31, other; (poss KS)	DDX21 (87)	PHB2 (33)	PHB (30)	ANXA11 (54)	good	1-B	
40	63	KS	VWF (309)				good (2)	1-A	
41	175, 165, 88, st33	other	HSPA8 (71)	PCNA (29)			good	3	
42	st 63, 60, 50, 36st 63, 60, 50, 36st 63, 60, 50, 36st 63, 60, 50, 36	KS, poss Ro 60, other	NARS (63)	ITIH4 (105)	EIF2S3 (51)		good	1-A	
43	270, 175, 70, 68, 61, 58, 43, 27	other	YBX1 (36)	DDX50 (83)			good	3	
44	56, 6556, 6556, 6556, 65	prob 56	APOB (509)	ANXA11 (54)	YBX1 (36)	HSPA9 (74)	good	1-B	
45	200, 61, 36	prob snRNP, poss Th	HBD (16)	HBA1 (16)	TUBB2A (50)		fair	1-A	
46	130, 65, st31	prob 31, other	GNPDA1 (33)	YBX1 (36)			good	1-C	
47	60, 56, 31, 23, 7860, 56, 31, 23, 7860, 56, 31, 23, 78	KS, prob 56, poss snRNP, poss 31KS, prob 56, poss snRNP, poss 31KS, prob 56, poss snRNP, poss 31	ITIH4 (105)	PHB2 (33)	ANXA11 (54)	PHB (30)	good	1-A	
48	180, 115, 75, st 27	other	HNRNPC (34)	HSPA8 (71)	NUP214 (214)	DLAT (69)	good	3	
49	90, 80, 58, 38, 25	poss Th, poss snRNP, other	EMG1 (27)	LGALS3BP (65)	SORD (38)	DLAT (69)	fair	1-A	
50	hi bands, 82, 65, st 63, ~ 35	prob KS, poss 35, other	KHDRBS1 (48)	ITIH4 (105)	NUDT21 (26)	CPSF7 (52)	good	1-A	

Table 2 (continued)

Sam- ple ID	Observed Protein Gel Bands	Conventional IP-call	IP-to-MS 1st	IP-to-MS 2nd	IP-to-MS 3rd	IP-to-MS 4th	Agreement	AILD- known Ab	ANA posit- ivet
51	st110, 70st110, 70st110, 70	poss PL-12	APOB (509)	HK1 (102)	DLAT (69)	YBX1 (36)	good	1-A	
52	st56, 31st56, 31st56, 31	prob 56, poss 31	PHB2/PHB (33/30)	ANXA11 (54)	G3BP1 (52)	DLAT (69)	good	1-B	
53	65, 61, 56	poss 56, other	ITIH4 (105)	YBX1 (36)			fair (3)	1-B	
54	108, 82, 80, st63, 60, 35, 31, 42	poss 56, poss 35, poss 31, other	LMNA (74)	FUBP2 (73)	PYCR1 (33)	DLAT (69)	good	1-B	
55	70, st63, 60, 52, 4570, st63, 60, 52, 4570, st63, 60, 52, 45	prob KS, poss Ro 60, other	NARS (63)	ITIH4 (105)	C4A (40)		good	1-A	Yes
56	150, 120, 82, 72, 70, 43, 34, 32	other	TUBB2A (50)	APOB (509)			fair (1)	3	
57(3)	310, 300, 170, 150, st50, 43	poss Jo-1, other	APOB (509)	VWF (309)	ACTL6B (47)	RAN (24)	good (1,2)	1-A	
58	60, 56	prob 56, poss Ro 60	TUFM (50)	CENPV (30)	LTV1 (55)		good	1-A	
59	115, 76, 63, 52, 35	poss 35, poss snRNP, other	SSBP1 (17)				poor	1-A	
60	200, 140, 135, 105, w56, w35, w31	poss 35, poss 31	YBX1 (36)				good*	1-C	
61	65, 52	other	YBX1 (36)	HNRNPLL (60)	TUBB2A (50)	HNRNPC (34)	good	3	
62	w140, 125, st 56w140, 125, st 56w140, 125, st 56	prob 56, other	ANXA11 (54)	FUBP1 (68)	TUBB2A (50)	ATAD3A (71)	good	1-B	
63	175, 130, st63, 56?	poss KS, poss 56	TF (77)	YBX1 (36)	RPL9 (22)	TUBB2A (50)	good	1-A	
64	st 63, 43 st 63, 43	prob KS, other	NARS (63)	TIAL1 (42)	YBX1 (36)	HSPA9 (74)	good	1-A	
65	56, 7056, 70	prob 56, other	ANXA11 (54)	DLAT (69)	APOB (509)	YBX1 (36)	good	1-B	
66	> 300 smear, 88, 68, 61, 56, 28, 26	prob 56, other	APOB (509)	ISYNA1 (61)	YBX1 (36)	TUBB2A (50)	good (1)	1-B	
67	200, w63, 95	other	ITIH4 (105)	PRMT5 (73)			fair (3)	3	
68(14)	> 300, 250, (63), 56, 43, 35, 31	prob 35, prob 31, other	TMPO (75)	VWF (309)	APOB (509)	HSPA8 (71)	good (1,2)	1-C	Yes
69	negative	Ro 60	ITIH4 (105)	APOB (509)	TTR (16)	HNRH3 (37)	MS > gel	1-A	Yes
70(12)	st 63, 52 st 63, 52	KS, other	NARS (63)	FBL (49)			good	1-A	
71	st 63	KS	NARS (63)	APOB (509)	SFPQ (76)	NONO (54)	good	1-A	
72	st 63	KS	NARS (63)	APOB (509)	PGD (53)	SFPQ (76)	good	1-A	
73	KS, prob 56	poss KS, poss 56, other	PTBP1 (60)	HSPA8 (71)	CENPV (30)	HSPA6 (71)	good	1-A	
74	KS, prob 56	poss KS, poss 56, other	PTBP2 (57)	APOB (509)	HSPA8 (71)	CENPV (30)	good	1-A	
75(22)	st110, st 80 db, st 42st110, st 80 db, st 42	prob PL-7, other	TARS (83)	HK1 (102)	CSNK2A1 (45)	DLAT (69)	good	1-A	
76	st110, st 80 db, st 42st110, st 80 db, st 42	prob PL-7, other	TARS (83)	HK1 (102)	CSNK2A1 (45)		good	1-A	
77	w63, 37	other	HSP90AA1 (85)	ISYNA1 (61)			good	3	
78	33	poss U3	ITIH4 (105)	TYMS (36)			good	1-A	

Table 2 (continued)

Sam- ple ID	Observed Protein Gel Bands	Conventional IP-call	IP-to-MS 1st	IP-to-MS 2nd	IP-to-MS 3rd	IP-to-MS 4th	Agreement	AILD- known Ab	ANA posit- ivet
79	58,56,52,45, 33	prob 56, poss snRNP, other	APOB (509)				fair (1)	1-A	
80	190, 76	other					poor	3	

good = reasonable mass match of 1 or 2 proteins

fair = approximate mass match, with possible protein breakdown products

poor = does not mass match

MS > gel = more MS protein identifications than observed gel bands

(x) the number in parenthesis indicate lane on gel in Fig. 2

†blank cells indicate negative ANA

* = more gel bands observed than identified by MS

(1) possible APOB breakdown products

(2) possible VWF breakdown products

(3) possible ITIH4 breakdown products

Blank cells indicate no proteins identified above threshold

Table 3 Concordance between IP-to-MS and conventional protein immunoprecipitation

IP call	# of calls	# MS of agreements	% MS agreement
prob 56	16	12	75
prob KS	11	8	73
prob TOP1	2	1	50
prob Ro60	2	1	50
prob snRNP	1	0	0
prob PL-7	1	1	100
poss 56	7	0	0
poss KS	11	0	0
poss TOP1	1	0	0
poss Ku	1	0	0
poss Ro60	5	0	0
poss snRNP	9	0	0
poss PL-12	2	0	0
poss Th	3	0	0
poss Jo-1	1	0	0
poss U3	1	0	0

of the samples were categorized as fair, as larger scale analysis will be required to determine if the observed gel bands are indeed breakdown products of the MS identified protein. Only 5% (4/80) of the comparisons were found to have poor agreement. Likewise, 5% (4/80) samples yielded more MS protein identifications than suggested by conventional IP banding patterns.

Conventional, gel-based IP interpretation relies on visual inspection of protein gel banding patterns relative to known standards. In this study, autoantigen identification was classified as “probable” or “possible” by an expert with more than 30 years of experience (NF). To assess concordance between these expert assessments of conventional IP versus the newer IP-to-MS profiling, we compared proteins identified by both methods (Table 3). Of the 33 probable calls, 23 (70%) agreed with MS data.

Table 4 Demographic and functional parameters of AILD versus IPF

	AILD ¹ (n = 134)	IPF ² (n = 174)
Sex (% Male)	66.9	70.8
Age (median, IQR)	67 (62–72)	68 (61–73)
Race (% Caucasian)	94	95.9
Smoking (% ever)	67.7	69
FVC% (median, IQR)	68.4 (61.7–81.1)	69.5 (62.0–80.1)
DLCO% (median, IQR)	45.4 (40.0–52.7)	46.3 (40.5–52.7)
6MWT (meters)	393 (321–468)	397 (343–459)
O ₂ (% requiring)	14.3	14

¹Antibody subgroup 1-A

²Antibody subgroup 4

In instances where the experts made possible assignments, none of the 41 possible calls agreed with the MS identifications. However, even in cases where mass spectrometry analysis did not confirm the interpretation of conventional IP, alternative autoantigens were routinely identified.

Clinical characteristics of autoimmune ILD and IPF subpopulations

To determine the clinical implications of this immunoprecipitation analysis, we divided the INSPIRE patient cohort by autoantibody profile (*as determined by conventional IP*) into the following *clinical* subsets: 1) Autoimmune ILD (AILD; antibody subgroup 1-A; *n* = 134) and 2) IPF patients without immunoprecipitation-based evidence of autoantibodies (antibody subgroup 4; *n* = 174). Samples with indeterminate profiles (antibody subgroup 3) and those associated with antigens of unknown significance (antibody subgroups 1-B and 1-C) were omitted from this analysis. Review of Table 4 indicates similar demographic characteristics and pulmonary function in individuals classified as AILD versus IPF by autoantibody

profile (antibody subgroup 1–A (AILD) versus subgroup 4 (IPF)). Specifically, there were no significant differences in sex distribution, race, or smoking history between AILD and IPF subjects. More importantly, there were no significant differences in baseline parameters of FVC percent predicted, DLCO percent predicted, or 6MWD between autoantibody subsets of AILD versus IPF. Correspondingly, the percentage of patients requiring supplemental oxygen was similar between AILD and IPF subgroups. Of note, comparison of these baseline pulmonary function parameters and demographic characteristics to those of the overall cohort [reported in (1)] did not reveal any significant differences indicative of selection bias. Similarly, comparative assessment of various outcome parameters (e.g., percent reduction in FVC over time, time to respiratory-related hospitalization, survival) in serologically-defined subgroups did not show any significant differences in response to IFN-gamma-1 β , paralleling results from the overall cohort (1).

Discussion

Among nearly 500 subjects enrolled in the INSPIRE trial based on a disease classification of IPF, 190 (39%) had evidence of probable/definite autoantibodies indicative of an underlying autoimmune process [186 with autoantibodies defined by IP (categories 1A–1C), 4 with positive CCP2 ELISA]. Mass-spectrometric analysis of selected immunoprecipitates substantiated these observations and, in some cases, identified novel autoantigens—demonstrating the utility of the IP-to-MS work flow. These collective findings exceeded our initial estimates of seroprevalence in this population and fully support our underlying hypothesis that a substantial portion of patients classified as IPF have an underlying autoimmune diathesis marked by autoantibodies targeting antigens previously associated with connective tissue diseases. In fact, while a number of the samples tested by conventional IP immunoprecipitated putative autoantigens of unknown identity (52/483, 11%; subgroups 1-B and 1-C), a sizeable percentage targeted defined antigens associated with the anti-synthetase syndrome, systemic sclerosis, or autoimmune overlap syndromes (134/483, 28%; subgroup 1-A).

While previous studies have, in fact, suggested the presence of occult anti-synthetase and other CTD-associated autoantibodies in patients with idiopathic interstitial pneumonia (IIP) [11–13], the rate of seroprevalence in the INSPIRE cohort of putative IPF patients was substantially higher than in these earlier analyses. Moreover, among more recent studies documenting the presence of autoantibodies in rigorously classified cohorts of IPF [14–18], none have consistently identified these CTD-associated autoantibodies—further demonstrating the specificity of autoantibodies detected in our study for classifiable, systemic autoimmune diseases.

Although some of the autoantibodies identified in this study do not have clearly defined associations with a specific autoimmune disease, they likely identify patients with an underlying autoimmune diathesis. Anti-Annexin 11 antibodies, for example, have previously been identified at low frequency (1–3%) in a mixed cohort of patients with IPF and idiopathic NSIP (Non-Specific Interstitial Pneumonia) [19]. These findings are consistent with our data (in which the prevalence of anti-56 kDa/Annexin 11 antibodies is approximately 6%, Table 1). Importantly, however, we have not detected anti-Annexin 11 antibodies in an independent cohort of well-annotated IPF patients in which individuals with occult features of CTD-ILD have been excluded (0/100 IPF sera positive for anti-Annexin 11 antibodies, data not shown)—suggesting that anti-Annexin 11 antibodies are not simply a marker of IPF. Collectively, these observations demonstrate the power of our IP-to-MS approach for autoantibody discovery, but also underscore the point that other, potentially novel, autoantibodies will need to be validated in independent cohorts of IPF and CTD-ILD to establish their relationship with disease phenotype.

What is equally striking about our results is that patients enrolled in INSPIRE had to have radiographic and/or biopsy evidence of UIP rather than NSIP, COP, or other histopathologic subtypes more likely to be associated with CTD-ILD or alternative inflammatory etiologies. Given the potential implications for clinical trials of anti-fibrotic and other emerging therapeutic agents, appropriate clinical and serological screening for underlying autoimmune disease is of paramount importance. In the INSPIRE trial, patients did not undergo routine laboratory testing for ANA, ANCA, CCP, or other commercially available autoantibodies. Although such screening would undoubtedly have identified some of the patients with autoimmune characteristics, it is important to point out that ANA testing alone would not detect a substantial portion of patients with myositis-associated antibodies targeting cytoplasmic antigens such as aminoacyl-tRNA synthetases—particularly when using ELISA-based approaches rather than indirect immunofluorescence (IIF). In fact, ELISA-based ANA testing of the 75 plasma specimens subjected to IP-to-MS analysis (Table 2) yielded only 7 low-moderate titer ANA-positive samples (including only 1/7 samples identified as synthetase-positive by IP-to-MS in this subset). Unfortunately, the limited availability of patient plasma precluded more comprehensive comparative assessment of ANAs in our study. Although future studies should employ ANA testing by IIF to fully capture the spectrum of potential autoantibodies, it is important to note that such testing is subject to interpretation and does not yield the identity of nuclear (or cytoplasmic) antigens.

A key question is how more detailed serological classification of patients with IIP/IPF might impact assessment of clinical outcomes and response to immunomodulatory therapies such as IFN-gamma-1 β . In fact, incorporation of serological profiles as a distinct domain in the previously developed clinical construct of Interstitial Pneumonia with Autoimmune Features (IPAF) [20] has yielded mixed results, with some studies demonstrating an intermediate phenotype between IPF and CTD-ILD and others showing no difference in clinical outcomes relative to IPF [21–26]. Within cohorts classified as IPF, a growing number of studies have revealed antibodies recognizing a range of cytoplasmic, cytoskeletal, and/or structural proteins [14–18]. Although it remains unclear whether these antibodies mark forme fruste manifestations of autoimmune disease, these studies do suggest that certain antibody specificities such as anti-THBS1 predict worse clinical outcome/reduced survival [18].

In our analysis of the INSPIRE trial, we were unable to detect statistically significant differences in clinical outcome parameters (such as reduction in percent predicted FVC, time to respiratory-related hospitalization, or survival) between patients reclassified as AILD (subgroup 1-A) and the autoantibody negative IPF subgroup following treatment with IFN-gamma-1 β . When interpreting these preliminary data, however, it is important to consider that our study was not designed to assess differences in clinical outcomes, in part because of limitations in sample availability that precluded serologic assessment of the entire study cohort. Furthermore, designation/classification as AILD based on IP-determined autoantibody profile alone was likely flawed, as shown by the IP-to-MS data in Table 2 indicating that a modest percentage of autoantibodies were incorrectly identified by conventional IP (that is reliant on molecular weight-based identification) and other potential autoantibodies were not detected by standard approaches. Equally important, combining different autoantibody subsets marking specific phenotypes (that could not be fully evaluated due to the lack of available data regarding extra-pulmonary clinical features of autoimmunity such as Raynaud's, esophageal dysfunction, arthritis, skin rashes, etc.) may have obscured altered biological responses to IFN-gamma-1 β in the INSPIRE trial. Given the apparent heterogeneity of the INSPIRE cohort, acquisition of data from the larger cohort of 826 patients enrolled in INSPIRE would likely be required to detect differences in specific subsets defined by autoantibodies and these additional extra-pulmonary clinical features.

As evidenced by the data presented in Table 2, IP-to-MS will serve as an invaluable tool that can overcome many of the technical limitations with conventional IP (use of radioactivity, reliance on banding patterns rather than mass spectrometry-based profiling) to help

address these issues and permit more precise classification of target autoantigens as well as associated disease phenotypes—a key consideration given the emerging paradigm that serologically-defined endotypes in IIP/IPF may predict biological behavior and clinical course [18]. It is important to point out, however, that even this more precise disease classification scheme may not have altered our analysis of outcomes in the INSPIRE trial, as there is no a priori reason to expect that IFN-gamma-1 β would be more effective in correctly designated AILD, particularly given the complex role of TH1/IFN γ -driven immune responses [which can indirectly promote fibrosis via M1 macrophage-derived cytokines such as TNF α and IL-1 β [27]] in different stages and subtypes of CTD-ILD.

Beyond these issues, another constraint on our findings is that the INSPIRE radiology database failed to use the same participant numbers for their initial and follow-up scans. More importantly, different databases were developed for serological markers that were not linked to the participant numbers in the imaging biobank. As a result, we could only compare our results to the overall imaging analysis conducted by the study's blinded radiologists, which noted that "definite fibrosis" was present in only 61% of the scans (301/491). We acknowledge that the inability to correlate identified biomarkers with their paired images hinders the application of our findings. The trial's impact from this omission should raise awareness of the critical importance of linked database entries in clinical studies. However, none of these limitations diminish the significance of our key observation that a substantial percentage of patients with clinical and radiologic features of IPF possess autoantibodies suggestive of underlying autoimmunity. With the advent of our novel, high throughput IP-to-MS approach that has shown excellent sensitivity/specificity compared to conventional ELISA-based methods and immunodiffusion in autoimmune disease patients [10], precise molecular characterization of autoantigen targets will become even more feasible—which will be crucial in defining subphenotypes of CTD-ILD as well as IPF.

Given the range of clinical data available from other completed clinical trials in IPF, retrospective identification/classification of autoimmune ILD should permit preliminary estimates of therapeutic efficacy for pirfenidone and other anti-fibrotic agents in this alternative disease subset [that will become better defined as we improve our understanding of clinical features associated with different autoantibodies (such as anti-annexin A11) identified in this cohort]. At the same time, more detailed serological profiling and exclusion of non-IPF patients from earlier data analyses may lead to revised assessments of therapeutic efficacy in IPF (or serologically-defined subsets of IPF)—underscoring the importance of correct disease classification. To address the

clinical response of subjects with non-IPF progressive pulmonary fibrosis (PPF), recent clinical trials encourage attention to the heterogeneous populations that develop fibrotic lung disease [28, 29]. By extension, the findings of the present retrospective study have clear implications for future clinical trials in which proper clinical and serological classification will be required to ensure appropriate patient selection/enrollment and allow more accurate assessment of therapeutic efficacy.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12931-025-03490-5>.

Supplementary Material 1.

Supplementary Material 2.

Supplementary Material 3.

Supplementary Material 4.

Authors' contributions

Data acquisition: MKG, CB, SP-S, JLS, LH, SB, SE, YZ, HV, JSM, DPA Data analysis/interpretation: BT, NF, HV, JSM, DPA Drafting manuscript: MKG, JSM, DPA Sources of support: Roche Genentech, Inc. (MKG, DPA).

Funding

Funding and statistical support were provided by Roche Genentech, Inc. Samples and trial data were collected by InterMune, Inc. prior to its acquisition by Roche Genentech, Inc.

Data availability

All research data generated during the preparation of this manuscript is provided in supplementary figures/tables.

Declarations

Ethics approval and consent to participate

The INSPIRE trial adhered to principles outlined in the Declaration of Helsinki, and all enrolled patients provided consent to participate as well as consent for publication of de-identified data encompassing clinical/demographic characteristics and associated biospecimens. Protocols and consent forms were approved by Institutional Review Boards or Ethics Committees representing individual institutions/clinical centers enrolling patients into the INSPIRE trial.

Competing interests

Both MKG and DPA received an independent investigator grant sponsored by Roche Genentech, Inc. However, neither author has any commercial interest from Roche Genentech, Inc., nor do these authors receive consulting fees. Both CB and BT are employees and shareholders at Roche Genentech, Inc., but have no ties to the INSPIRE clinical trial or the use of IFN-gamma-1beta as a therapeutic agent.

Received: 3 November 2025 / Accepted: 30 December 2025

Published online: 20 January 2026

References

- King TE Jr, Albera C, Bradford WZ, Costabel U, Hormel P, Lancaster L, et al. Effect of interferon gamma-1b on survival in patients with idiopathic pulmonary fibrosis (INSPIRE): a multicentre, randomised, placebo-controlled trial. *Lancet* (London, England). 2009;374(9685):222–8.
- Raghu G, Remy-Jardin M, Richeldi L, Thomson CC, Inoue Y, Johkoh T, et al. Idiopathic Pulmonary Fibrosis (an Update) and Progressive Pulmonary Fibrosis in Adults: An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline. *Am J Respir Crit Care Med*. 2022;205(9):e18–47.
- Spagnolo P, Maher TM. The future of clinical trials in idiopathic pulmonary fibrosis. *Curr Opin Pulm Med*. 2024;30(5):494–9.
- Luppi F, Spagnolo P, Cerri S, Richeldi L. The big clinical trials in idiopathic pulmonary fibrosis. *Curr Opin Pulm Med*. 2012;18(5):428–32.
- Cottin V. Significance of connective tissue diseases features in pulmonary fibrosis. *Eur Respir Rev*. 2013;22(129):273–80.
- Park JH, Kim DS, Park IN, Jang SJ, Kitaichi M, Nicholson AG, et al. Prognosis of fibrotic interstitial pneumonia: idiopathic versus collagen vascular disease-related subtypes. *Am J Respir Crit Care Med*. 2007;175(7):705–11.
- Strand MJ, Sprunger D, Cosgrove GP, Fernandez-Perez ER, Frankel SK, Huie TJ, et al. Pulmonary function and survival in idiopathic vs secondary usual interstitial pneumonia. *Chest*. 2014;146(3):775–85.
- Richards TJ, Kaminski N, Gibson KF. Plasma proteins for risk prediction in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med*. 2012;185(12):1329–30.
- Aggarwal R, Oddis CV, Goudeau D, Fertig N, Metes I, Stephens C, et al. Anti-transcription intermediary factor 1-gamma autoantibody ELISA development and validation. *Rheumatology* (Oxford). 2011;50(3):433–7.
- Biedka S, Yablonska S, Peng X, Alkam D, Hartoyo M, VanEvery H, et al. IP-to-MS: An Unbiased Workflow for Antigen Profiling. *J Proteome Res*. 2025;24(2):795–812.
- Tahara M, Sakamoto N, Satoh M, Ishimoto H, Yura H, Yamasaki K, et al. Clinical characteristics of idiopathic interstitial pneumonias with anti-Ro52/tripartite motif-containing 21 antibodies. *Sci Rep*. 2022;12(1):11122.
- Wakabayashi H, Iwasaki K, Murakami Y, Takashima K, Kaneko K, Matsuzawa Y. Prognostic value of myositis-specific antibodies in patients with idiopathic interstitial pneumonia. *BMC Pulm Med*. 2024;24(1):503.
- Watanabe K, Handa T, Tanizawa K, Hosono Y, Taguchi Y, Noma S, et al. Detection of antisynthetase syndrome in patients with idiopathic interstitial pneumonias. *Respir Med*. 2011;105(8):1238–47.
- Feghali-Bostwick CA, Wilkes DS. Autoimmunity in idiopathic pulmonary fibrosis: are circulating autoantibodies pathogenic or epiphenomena? *Am J Respir Crit Care Med*. 2011;183(6):692–3.
- Goobie GC, Ford-Sahibzada CA, Fritzier MJ, Johannson KA, Fell CD. Autoantibody status is not associated with change in lung function or survival in patients with idiopathic pulmonary fibrosis. *Respir Med*. 2019;153:85–90.
- Kirgou P, Sinis SJ, Dimeas IE, Papanikolaou IC, Tatsis K, Gogali A, et al. Clinical relevance of circulating autoantibodies in idiopathic pulmonary fibrosis; A NAt hard to break. *Front Med*. 2022;9:964722.
- Koether K, Besnard V, Sandig H, Carruthers A, Miranda E, Grootenboer-Mignot S, et al. Autoantibodies are associated with disease progression in idiopathic pulmonary fibrosis. *Eur Respir J*. 2023;61(5). <https://doi.org/10.1183/13993003.02381-2021>.
- Leuschner G, Semenova A, Mayr CH, Kapellos TS, Ansari M, Seeliger B, et al. Mass spectrometry-based autoimmunity profiling reveals predictive autoantigens in idiopathic pulmonary fibrosis. *iScience*. 2023;26(11):108345.
- Tansley SL, McMorrow F, Cotton CV, Adamali H, Barratt SL, Betteridge ZE, et al. Identification of connective tissue disease autoantibodies and a novel autoantibody anti-annexin A11 in patients with “idiopathic” interstitial lung disease. *Clin Immunol*. 2024;262:110201.
- Fischer A, Antoniou KM, Brown KK, Cadranet J, Corte TJ, du Bois RM, et al. An official European Respiratory Society/American Thoracic Society research statement: interstitial pneumonia with autoimmune features. *Eur Respir J*. 2015;46(4):976–87.
- Ahmad K, Barba T, Gamondes D, Ginoux M, Khouatra C, Spagnolo P, et al. Interstitial pneumonia with autoimmune features: Clinical, radiologic, and histological characteristics and outcome in a series of 57 patients. *Respir Med*. 2017;123:56–62.
- Fernandes L, Nasser M, Ahmad K, Cottin V. Interstitial Pneumonia with Autoimmune Features (IPAF). *Front Med*. 2019;6:209.
- Graney BA, Fischer A. Interstitial Pneumonia with Autoimmune Features. *Ann Am Thorac Soc*. 2019;16(5):525–33.
- Kim HC, Lee JH, Chae EJ, Song JS, Song JW. Long-term clinical course and outcome of interstitial pneumonia with autoimmune features. *Respirology*. 2020;25(6):636–43.
- Mackintosh JA, Wells AU, Cottin V, Nicholson AG, Renzoni EA. Interstitial pneumonia with autoimmune features: challenges and controversies. *Eur Respir Rev*. 2021;30(162). <https://doi.org/10.1183/16000617.0177-2021>.
- Sambataro G, Sambataro D, Torrisi SE, Vancheri A, Pavone M, Rosso R, et al. State of the art in interstitial pneumonia with autoimmune features: a systematic review on retrospective studies and suggestions for further

- advances. *Eur Respir Rev.* 2018;27(148). <https://doi.org/10.1183/16000617.0139-2017>.
27. Borthwick LA, Wynn TA, Fisher AJ. Cytokine mediated tissue fibrosis. *Biochim Biophys Acta.* 2013;1832(7):1049–60.
 28. Maher TM, Corte TJ, Fischer A, Kreuter M, Lederer DJ, Molina-Molina M, et al. Pirfenidone in patients with unclassifiable progressive fibrosing interstitial lung disease: a double-blind, randomised, placebo-controlled, phase 2 trial. *Lancet Respir Med.* 2020;8(2):147–57.
 29. Wells AU, Flaherty KR, Brown KK, Inoue Y, Devaraj A, Richeldi L, et al. Nintedanib in patients with progressive fibrosing interstitial lung

diseases-subgroup analyses by interstitial lung disease diagnosis in the INBUILD trial: a randomised, double-blind, placebo-controlled, parallel-group trial. *Lancet Respir Med.* 2020;8(5):453–60.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.