Biomarker Strategies in the Clinical Development of LTI-03 in IPF

IPF Summit 2024

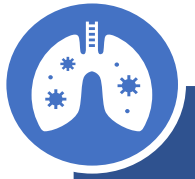
AUGUST 2024

Forward-Looking Statements

This presentation and various remarks we make during this presentation contain forward-looking statements of Aileron Therapeutics, Inc. ("Aileron", the "Company", "we", "our" or "us") within the meaning of the Private Securities Litigation Reform Act of 1995, including statements with respect to: the timing and expectation of the topline results from Cohort 1 of the Company's ongoing Phase 1b clinical trial of LTI-03; the sufficiency of the Company's cash resources; the status and plans for clinical trials, including the timing of data; future product development; and the potential commercial opportunity of LTI-03 and LTI-01. We use words such as "anticipate," "believe," "estimate," "expect," "hope," "intend," "may," "plan," "predict," "project," "target," "potential," "would," "can," "could," "should," "continue," and other words and terms of similar meaning to help identify forward-looking statements, although not all forward-looking statements contain these identifying words. Actual results may differ materially from those indicated by such forward-looking statements as a result of various important factors, including risks and uncertainties related to: changes in applicable laws or regulations; the possibility that the Company may be adversely affected by other economic, business, and/or competitive factors, including risks inherent in pharmaceutical research and development, such as: adverse results in the Company's drug discovery; preclinical and clinical development activities; the risk that the results of preclinical studies and early clinical trials may not be replicated in later clinical trials or that partial results of a trial such as the results from Cohort 1 of the Company's ongoing Phase 1b clinical trial of LTI-03 may not be indicative of the full results of the trial; the Company's ability to enroll patients in its clinical trials; and the risk that any of its clinical trials may not commence, continue or be completed on time, or at all; decisions made by the U.S. Food and Drug Administration and other regulatory authorities; investigational review boards at clinical trial sites and publication review bodies with respect to the Company's development candidates; competition; the sufficiency of the Company's cash resources to fund its planned activities for the periods anticipated and the Company's ability to manage unplanned cash requirements; and general economic and market conditions; as well as the risks and uncertainties discussed in the "Risk Factors" section of the Company's Annual Report on Form 10-K for the year ended December 31, 2023, and the Company's Quarterly Report on Form 10-Q for the quarter ended June 30, 2024, which are on file with the Securities and Exchange Commission, and in subsequent filings that the Company files with the Securities and Exchange Commission. These forward-looking statements should not be relied upon as representing the Company's view as of any date subsequent to the date of this presentation, and we expressly disclaim any obligation to update any forward-looking statements, whether as a result of new information, future events or otherwise, except as required by law.

This presentation contains estimates and other statistical data made by independent parties and by us relating to our clinical data, market size and other data about our industry. This data involves a number of assumptions and limitations, and you are cautioned not to give undue weight to such data and estimates. In addition, projections, assumptions and estimates of our future performance and the future performance of the markets in which we operate are necessarily subject to a high degree of uncertainty and risk.

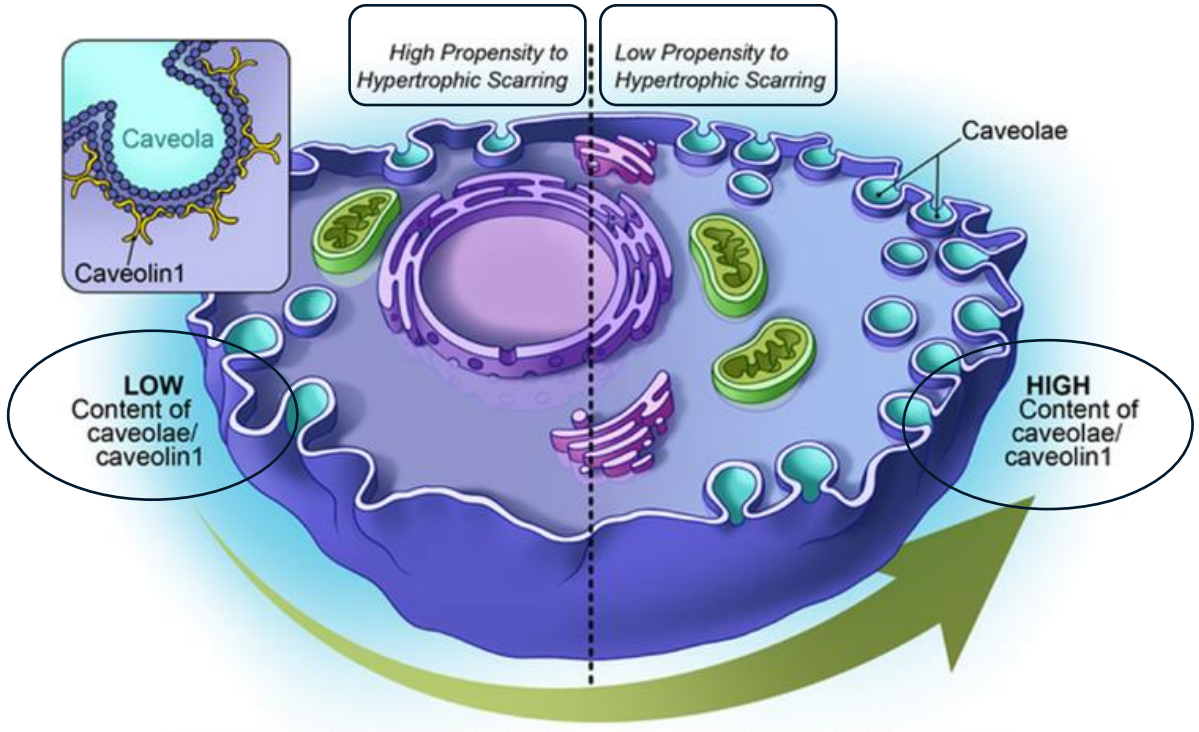
Clinical-stage Biotech with Pulmonary Pipeline



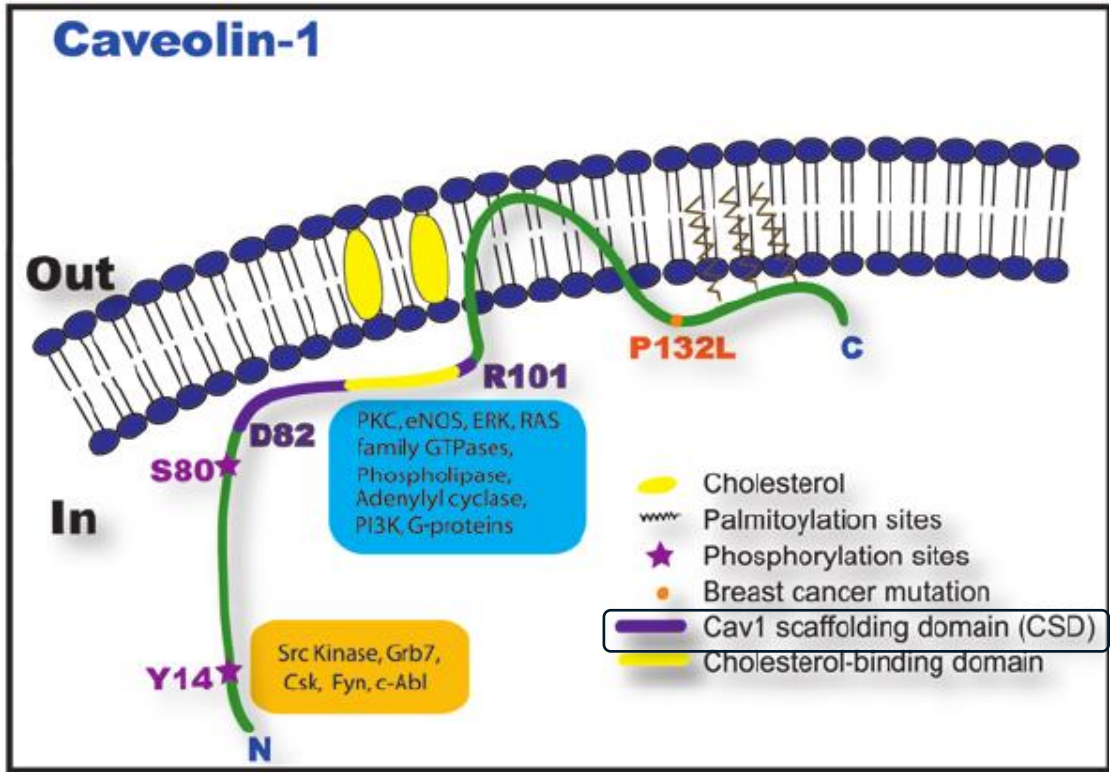
Therapies for Underserved Fibrosis and Pulmonary Conditions

LTI-03 ***Idiopathic Pulmonary Fibrosis***	***Phase 1b***	• Preclinical evidence supporting the ability to **protect healthy lung epithelial cells** and to **reduce pro-fibrotic signaling** • Demonstrated ability to increase sRAGE, a **prognostic biomarker** of IPF
LTI-01 ***Loculated Pleural Effusions***	***Phase 2b ready***	• **Potentially fatal disease** with no approved drugs • Completed Phase 1b and Phase 2 trials; similar mechanism as existing, off label therapeutic use
LTI-05 ***Cystic Fibrosis***	***PC***	• ENaC inhibitor intended for the **15-20% of CF pts.** who **do not respond to CFTR modulators** • **100% inhibition** and **localized** activity (safety profile) in preclinical studies

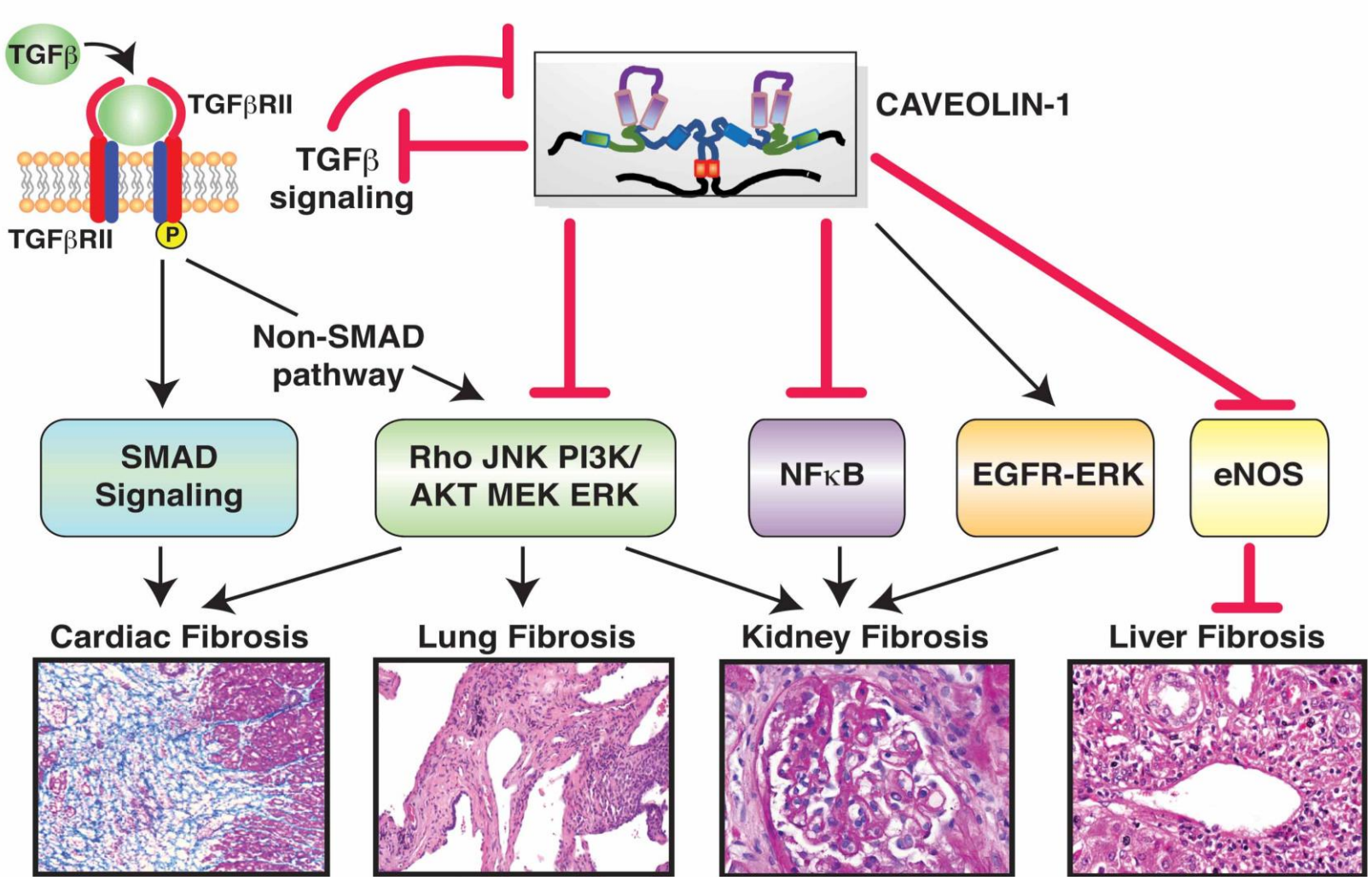
Caveolin-1: a Key Regulator in Fibrosis



Fibroblasts, epithelial cells, endothelial cells, myocytes, adipocytes, & immune cells.



Caveolin-1 Modulates Multiple Fibrosis-Related Pathways

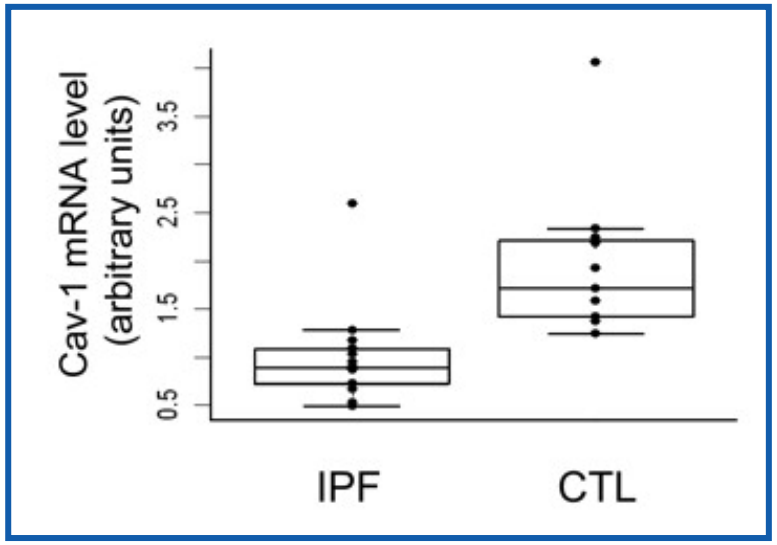


Adapted from Gvaramia et al, Matrix Biology, 2013

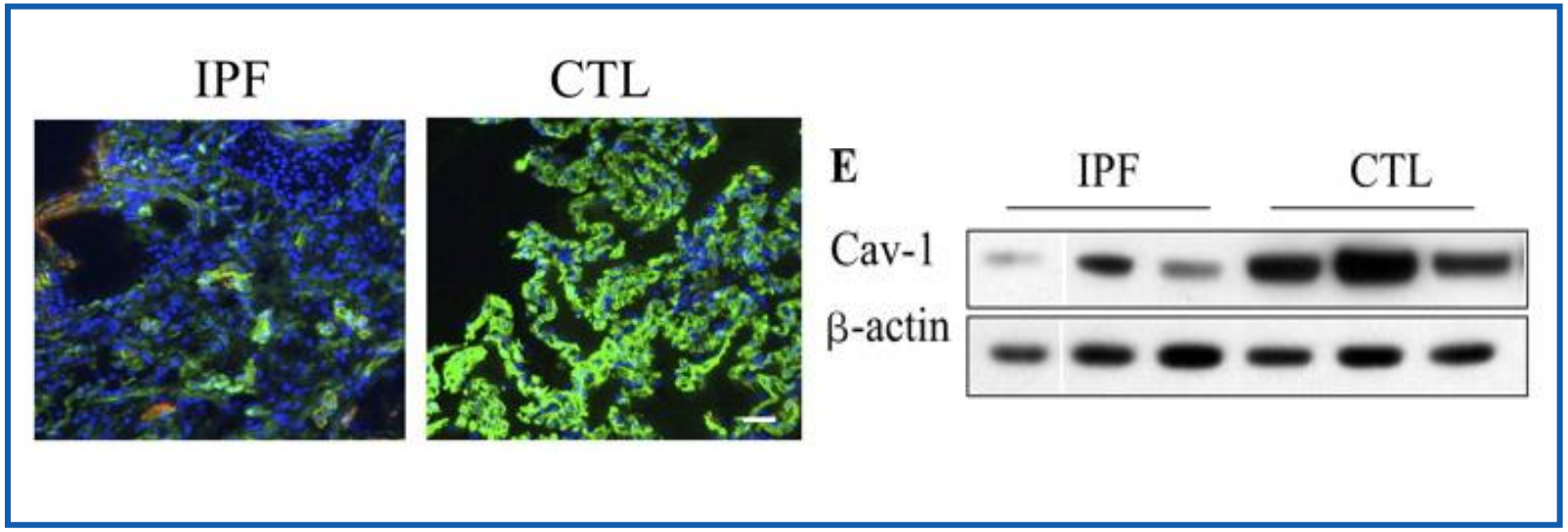
Caveolin-1 is Downregulated in IPF

Caveolin-1: a critical regulator of lung fibrosis in idiopathic pulmonary fibrosis

Xiao Mei Wang,[1] Yingze Zhang,[1] Hong Pyo Kim,[1] Zhihong Zhou,[1]
Carol A. Feghali-Bostwick,[1] Fang Liu,[1] Emeka Ifedigbo,[1] Xiaohui Xu,[2]
Tim D. Oury,[3] Naftali Kaminski,[1] and Augustine M.K. Choi[1]



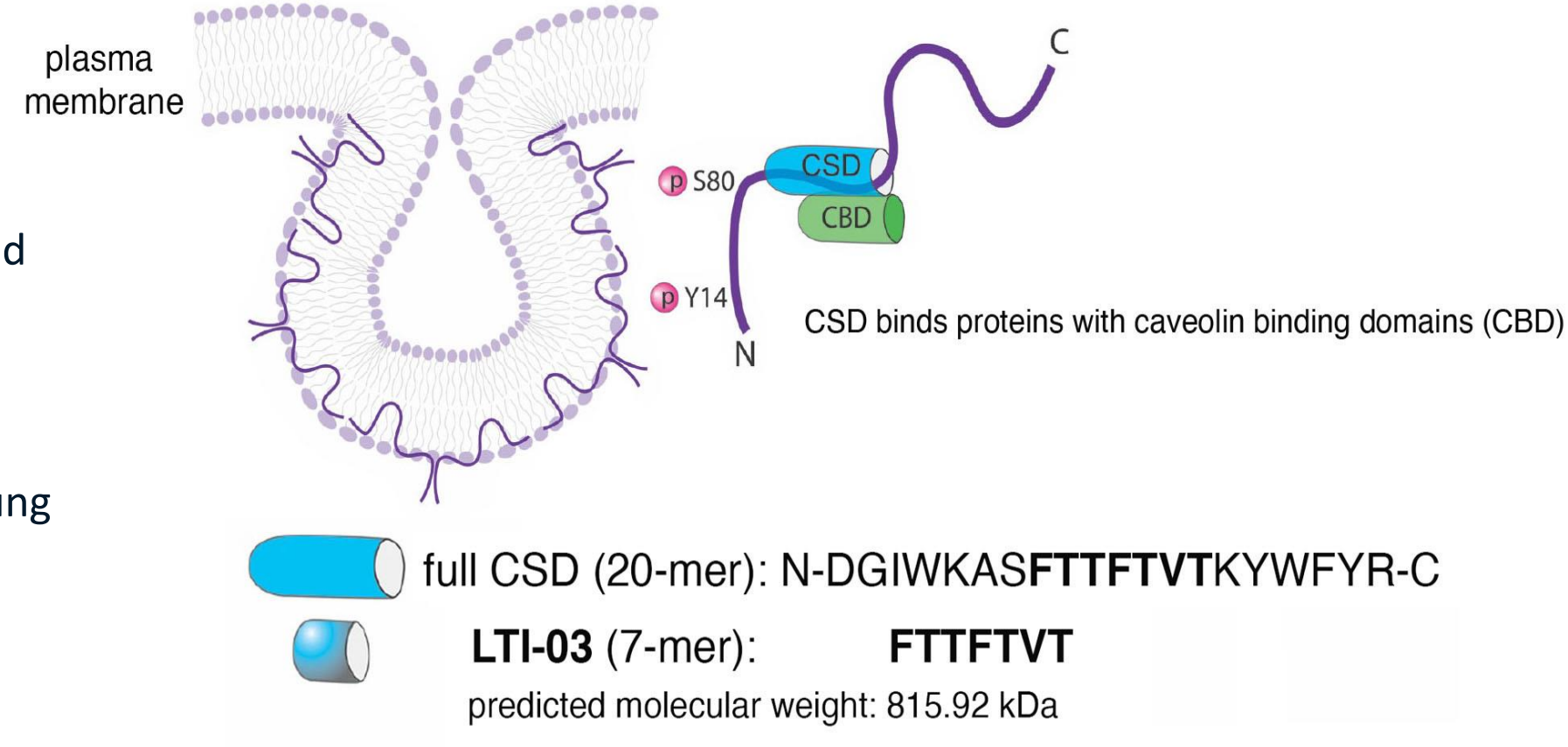
mRNA levels



Protein expression

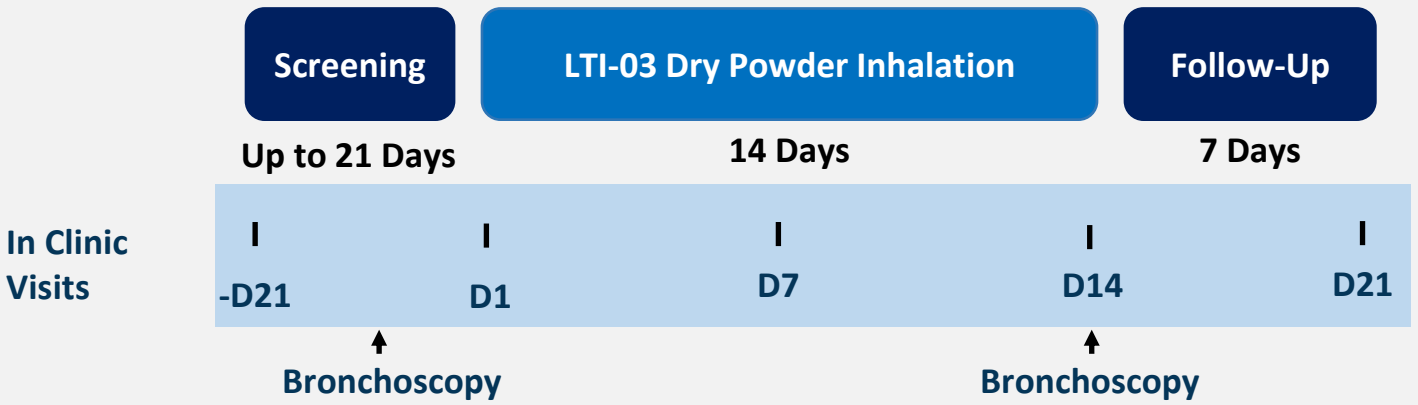
Simulation of Caveolin-1 Activity via CSD Peptide

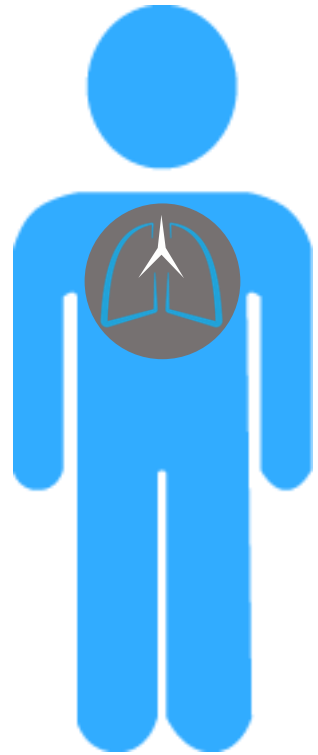
- LTI-03 is a seven amino acid peptide encompassing a portion of the Cav1 CSD
- LTI-03 is dosed direct-to-lung by dry powder inhaler



For a review on CSD/CBD binding domain list, see: Byrne et. al. PLOS One 2012

Phase 1b Clinical Trial Design (Status: In Process)

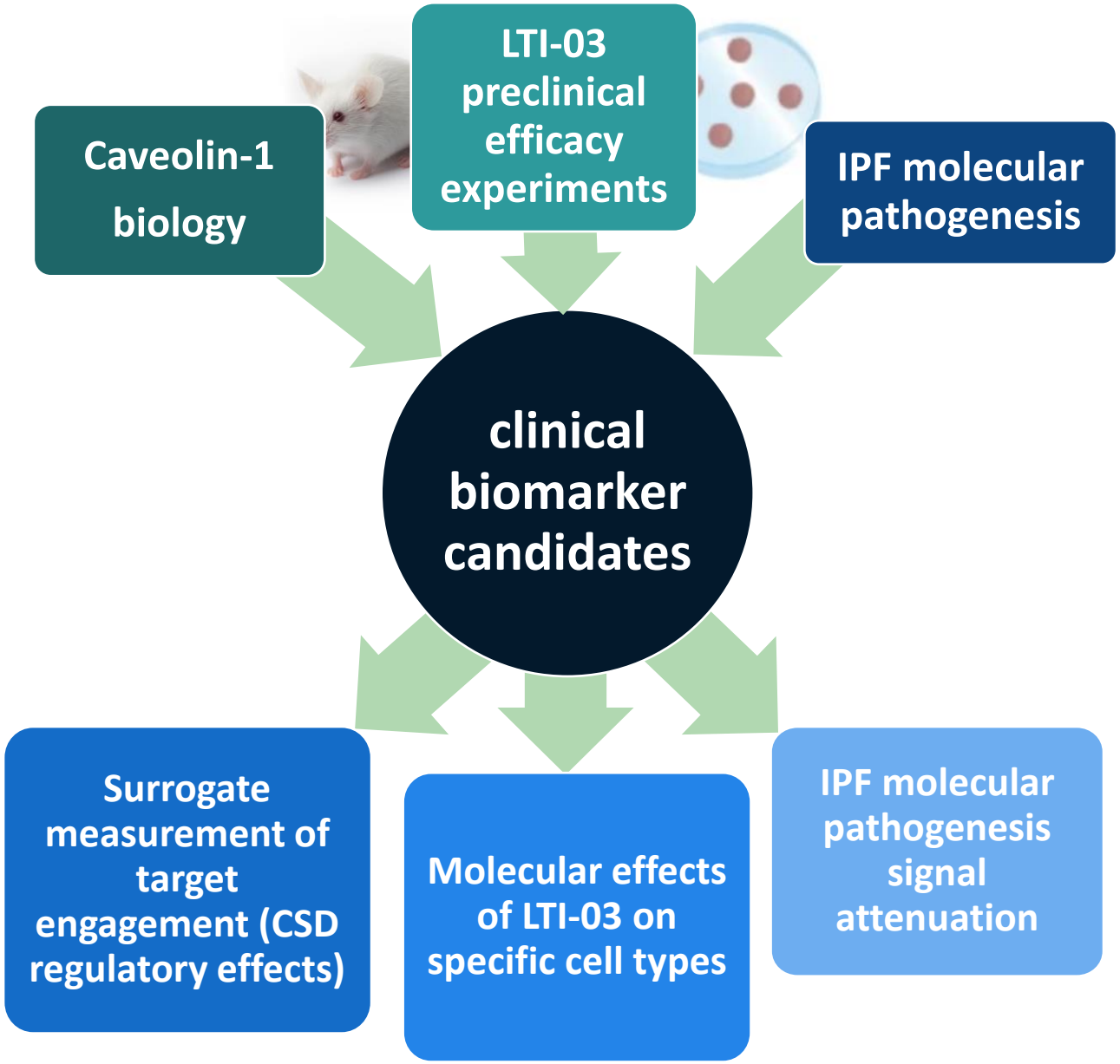




Study Design

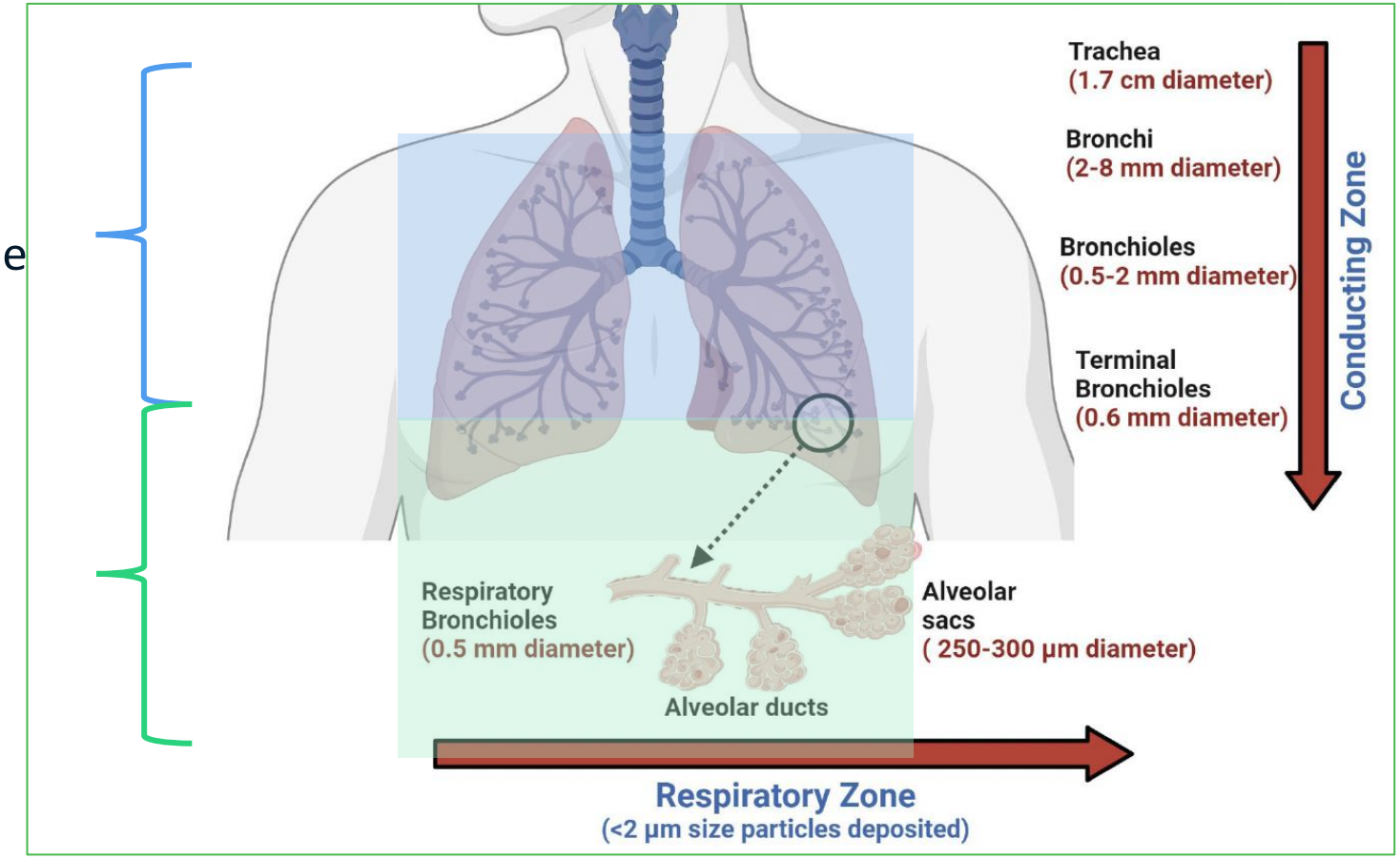
- IPF diagnosis ≤ 3 years; no previous antifibrotic therapy w/in 2 months of baseline
- 24 patients total (18 active, 6 placebo)
  - Low (2.5mg BID) and high (5mg BID) dose cohorts, sequential daily dosing for 14 days
- Bronchoscopy at screening and Day 14
- Primary endpoint: Safety/tolerability
- Key exploratory endpoint: Biomarkers (blood, BAL, brushings)

Movement of LTI-03 biomarkers indicates engagement of targets of various cellular origins implicated in IPF pathogenesis

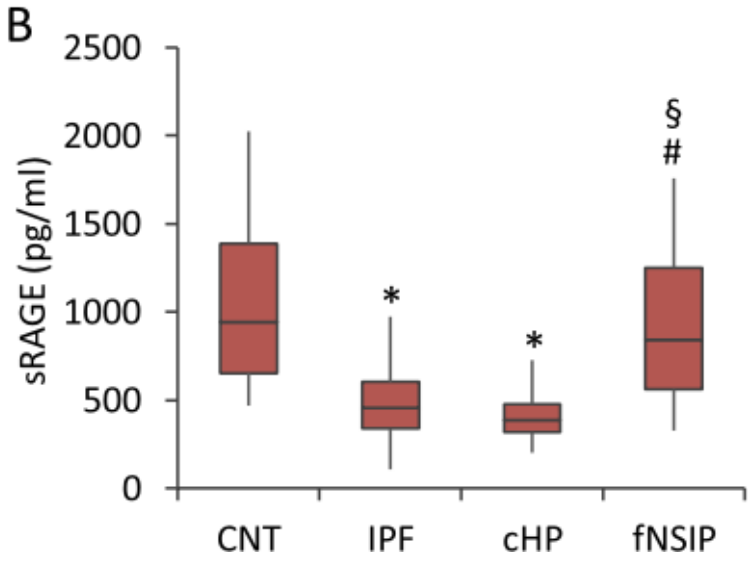


Cellular origin of LTI-03 biomarkers – lung specific

- Bronchoalveolar lavage fluid (conducting and terminal bronchioles)
    - BALF fluid components sample multiple lung regions
- Deep Bronchial Brushings (terminal bronchioles)
    - DBB samples contain deep lung components



Soluble RAGE (sRAGE) is Decreased in Fibrotic Lungs; decrease associated with rapid disease progression



Machahua *et al. Respiratory Research* (2016) 17:144
DOI 10.1186/s12931-016-0460-2

Respiratory Research

RESEARCH Open Access

Increased AGE-RAGE ratio in idiopathic pulmonary fibrosis

Carlos Machahua[1,2], Ana Montes-Worboys[1,2,3], Roger Llatjos[4], Ignacio Escobar[5], Jordi Dorca[1,2,3], Maria Molina-Molina[1,2,3*†] and Vanesa Vicens-Zygmunt[1,2†]

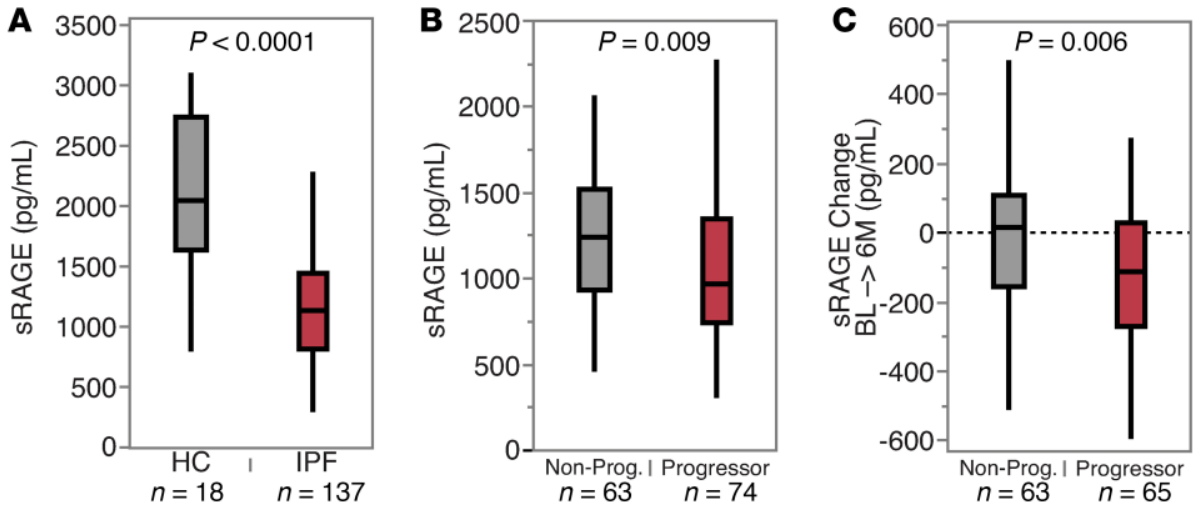


Figure 7. Decreased blood levels of RAGE were associated with more rapid disease progression in IPF patients. (A) Soluble RAGE levels in plasma from IPF patients and healthy controls. **(B)** Plasma sRAGE at baseline in IPF patients, dichotomized by disease progression (defined as loss of ≥10% predicted forced vital capacity [FVC] or death) from baseline to 1 year of follow-up. **(C)** Change in plasma sRAGE levels from baseline to 6 months in IPF progressors and nonprogressors. Statistical significance between the groups was determined by Wilcoxon rank sum test. BL, baseline.

Decrease in plasma sRAGE correlates with decline in %FVC in IPF and in other ILDs

Spearman correlation coefficients between plasma sRAGE and measures of disease severity

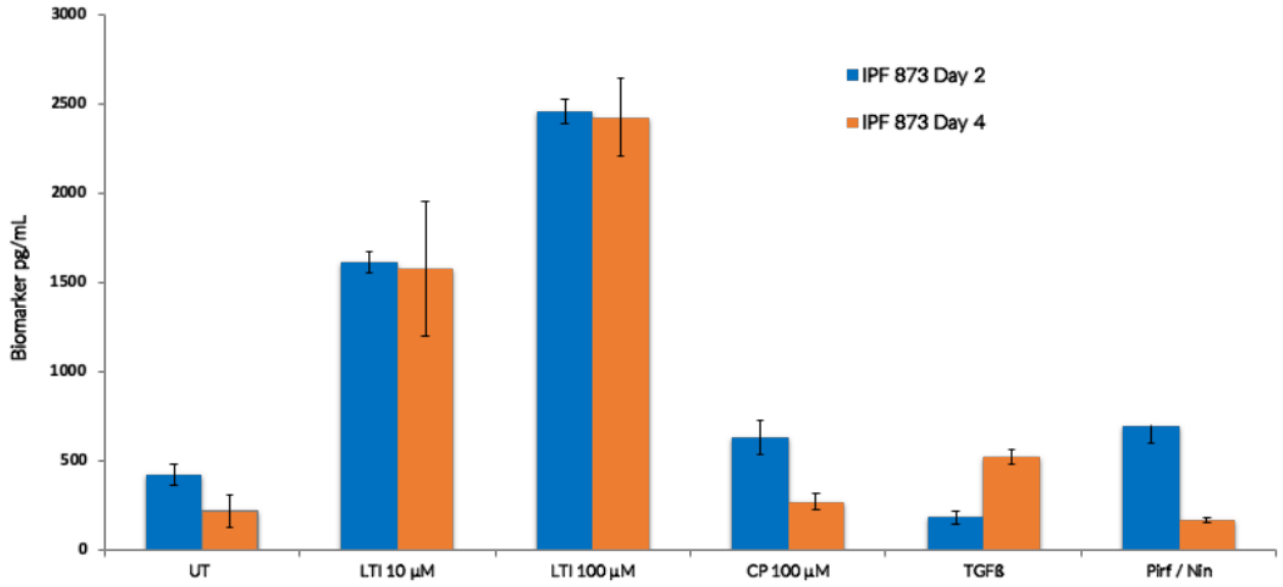
Variable	IPF		other ILDs	
	Spearman's rho	p-value	Spearman's rho	p-value
Age	0.22	0.02	0.11	0.13
BMI	-0.12	0.22	-0.16	0.04
FVC%	0.46	<0.001	0.27	<0.001
DLCO%	0.19	0.13	0.24	0.01
6MWD	0.36	0.02	0.17	0.09
GAP score	-0.23	0.07	-0.04	0.64

Spearman correlation coefficients between plasma sRAGE and measures of disease severity
Lederer, 2017 Ann Am Thorac Soc. 2017 May; 14(5): 628–635

Novel Prognostic Biomarker Data Supports LTI-03 Protection of Epithelial Cells

Biomarker Correlates with LTI-03 Impact in PCLS

Administration of LTI-03 in the PCLS system increased the soluble protein biomarker, sRAGE, while currently approved therapies had negligible effects on sRAGE levels



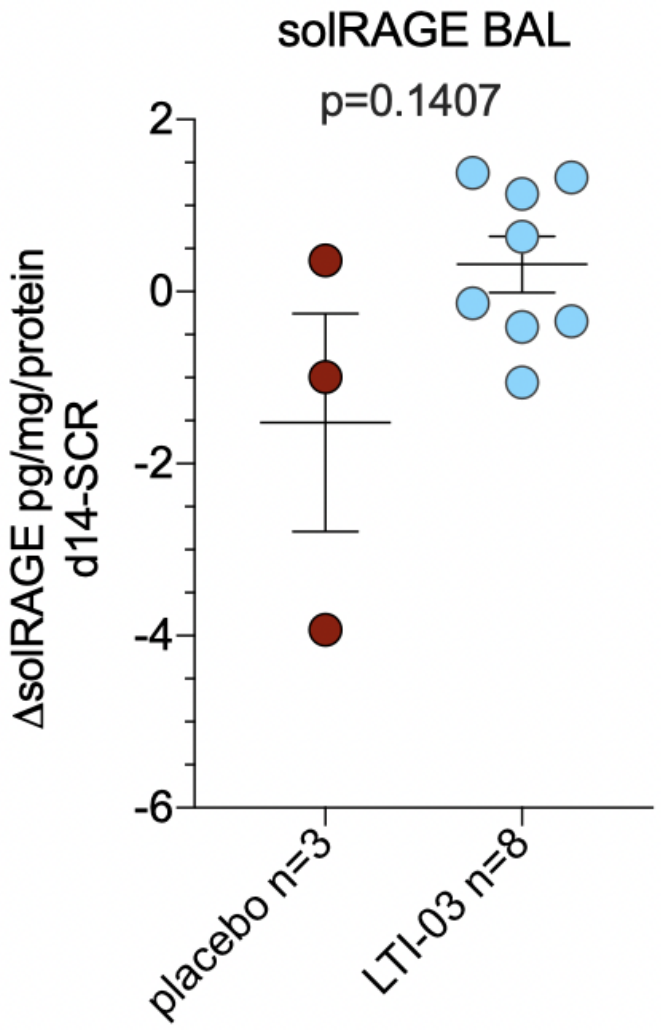
Low levels *of sRAGE at diagnosis predict poor survival in IPF*[1]

The increase in sRAGE *provides further evidence of increased AEC2 survival, leading to* ***greater AEC1 production*** *and thus* ***overall epithelial cell survival***

Ability to ***measure sRAGE in broncho-alveolar lavage fluid and blood*** *makes it a potentially* ***useful biomarker***

[1]Machahua, C., Montes-Worboys, A., Planas-Cerezales, L. et al. Serum AGE/RAGEs as potential biomarker in idiopathic pulmonary fibrosis. Respir Res 19, 215 (2018).

LTI-03 Phase 1b – sRAGE (BAL)

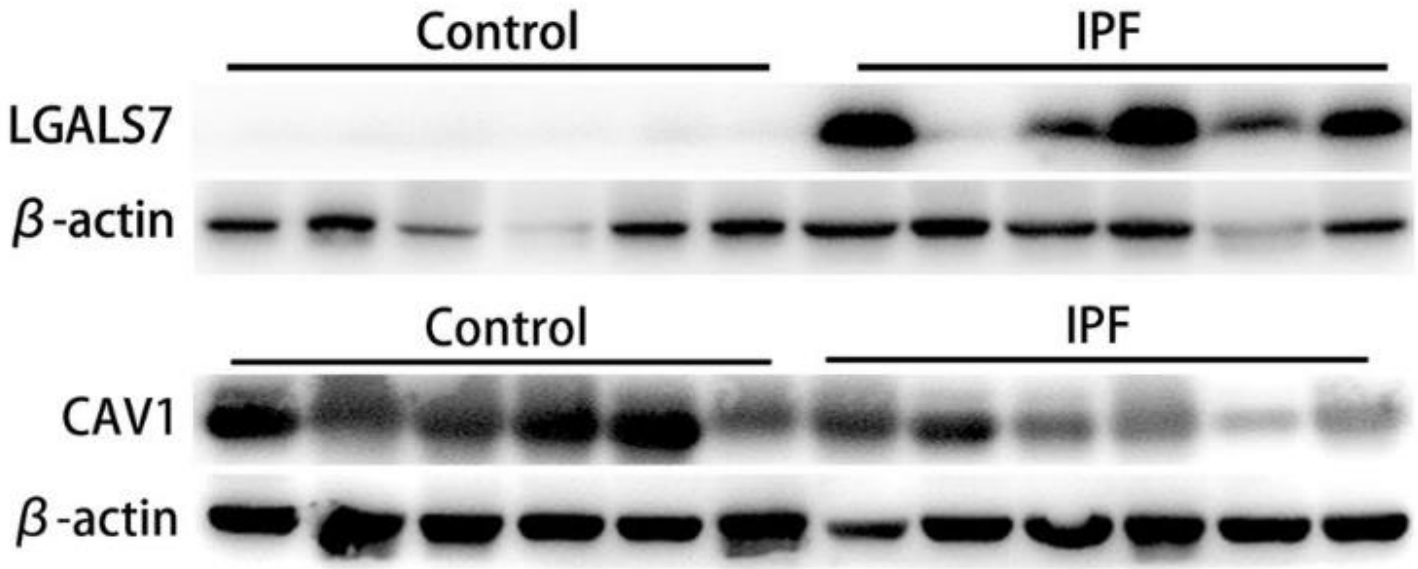


Galectin-7 is highly expressed in Caveolin-1 deficient bronchiolized areas in the IPF lung

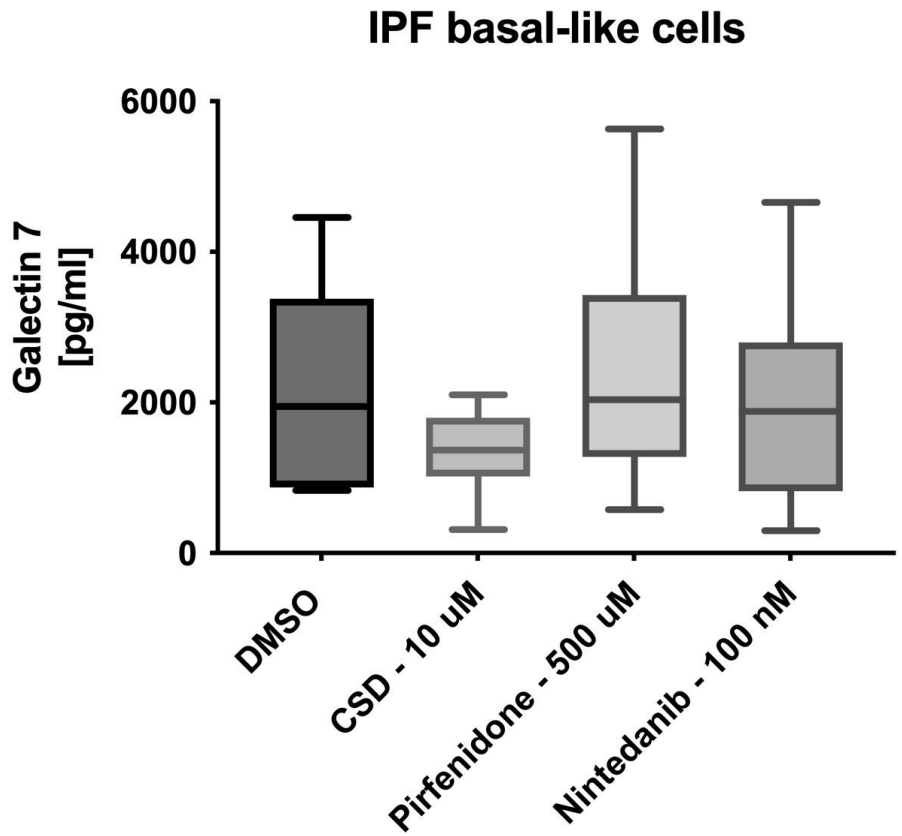
RESEARCH Open Access

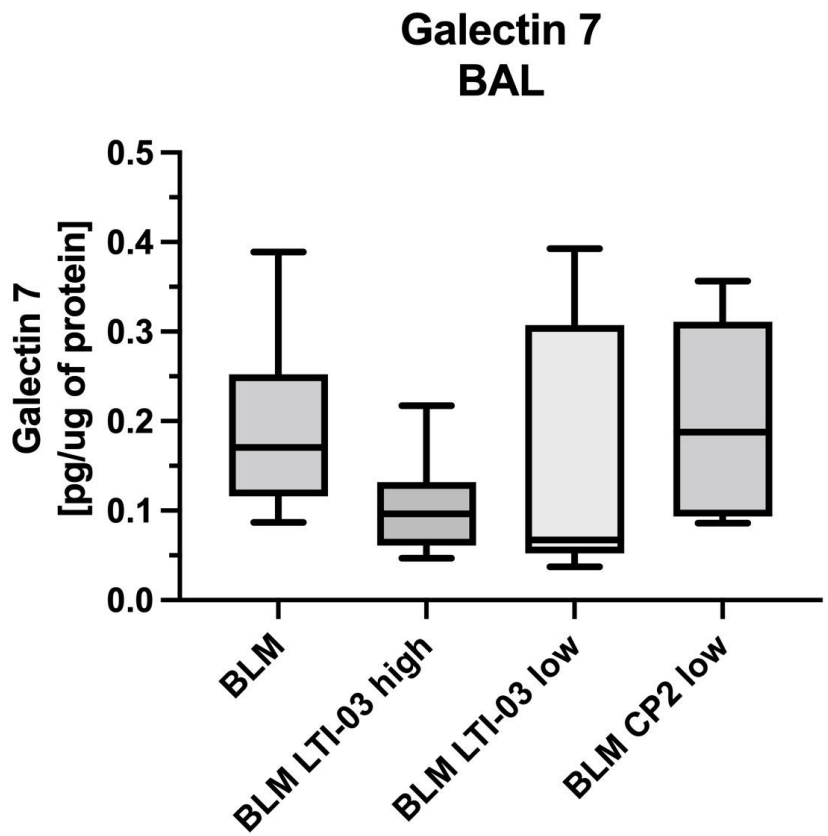
Quantitative proteomic characterization of lung tissue in idiopathic pulmonary fibrosis

Yaqiong Tian[1†], Hui Li[1†], Yujuan Gao[1†], Chuanmei Liu[2], Ting Qiu[3], Hongyan Wu[4], Mengshu Cao[1], Yingwei Zhang[1], Hui Ding[5], Jingyu Chen[6*] and Hourong Cai[1*]

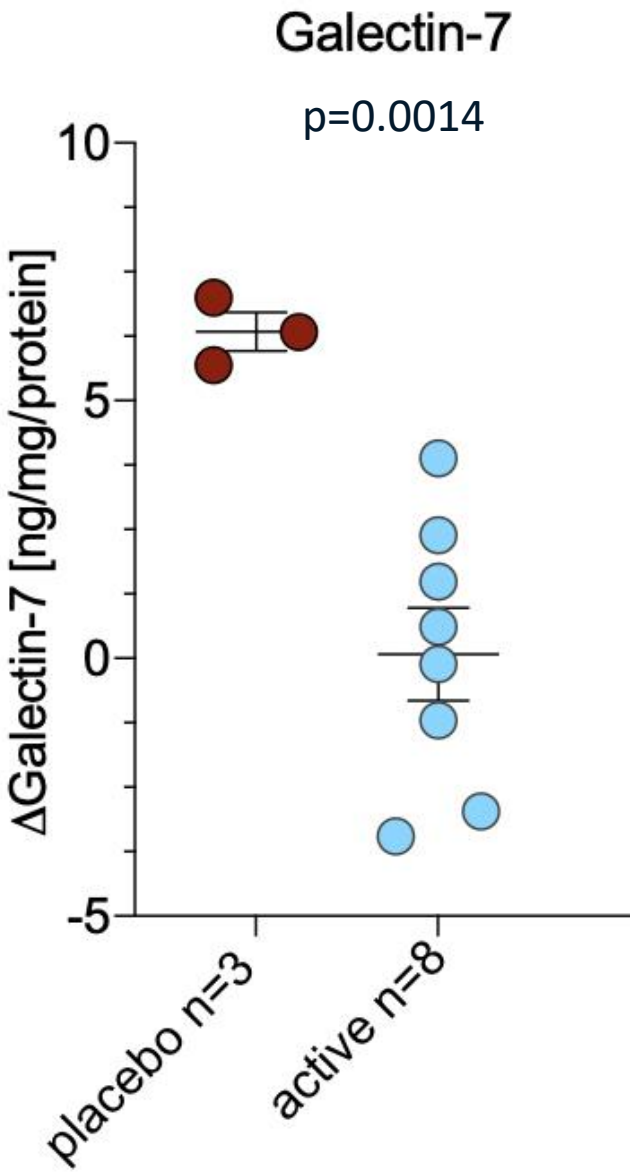


Galectin-7 was downregulated by LTI-03 in IPF basal like cell cultures and BAL collected from BLM-injured aged mice treated with LTI-03

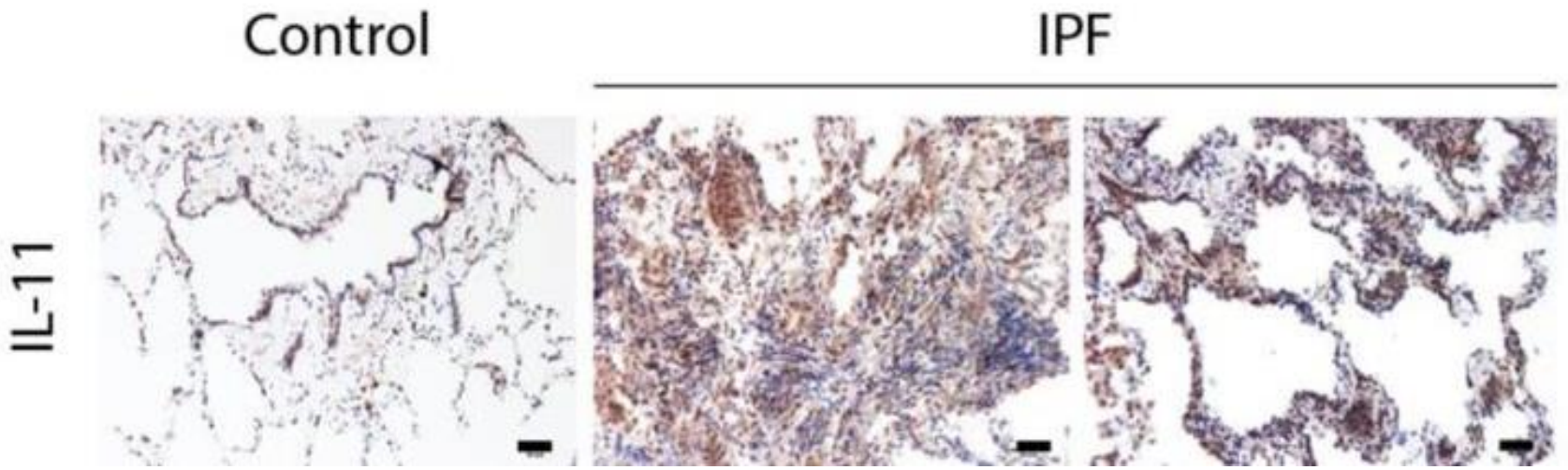


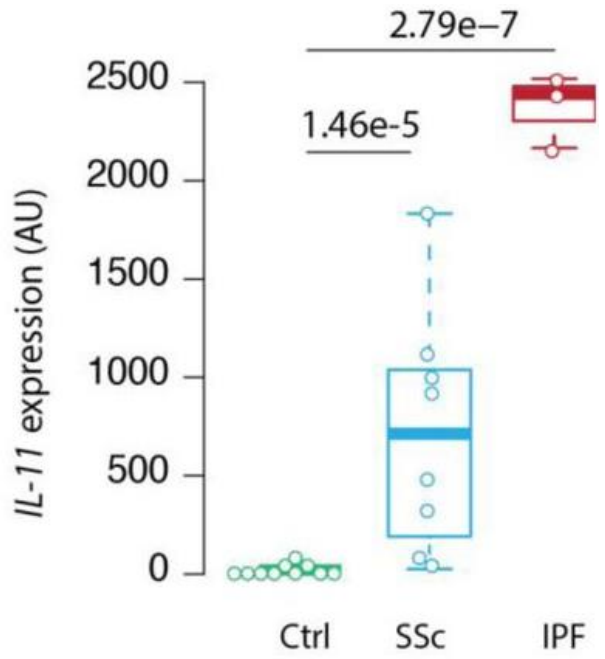


LTI-03 Phase 1b – Galectin-7 (GAL7)



IL-11 is highly upregulated in IPF tissue as well as in Cav-1ko mouse fibroblasts compared to WT fibroblasts





Cytokine/chemokine signaling		
Ccl2	Chemokine (C-C motif) ligand 2	−2.3
Ccl8	Chemokine (C-C motif) ligand 8	−2.6
Ccl11	Chemokine (C-C motif) ligand 11	−12.2
Cxcl7	Chemokine (C-X-C motif) ligand	2.7
Cxcl16	Chemokine (C-X-C motif) ligand	−3.0
Il11	Interleukin 11	2.4
Socs2	Suppressor of cytokine signaling	−2.2
Socs3	Suppressor of cytokine signaling	−2.0

IL-11 is an independent predictor of prognosis and acute exacerbation in IPF patients

Original Article

Interleukin-11 in idiopathic pulmonary fibrosis: predictive value of prognosis and acute exacerbation

Toru Arai[1]^, Masaki Hirose[1], Tomoko Kagawa[2], Kazuyoshi Hatsuda[1], Yoshikazu Inoue[1]^

Correlation between cytokine related parameters and other severity markers*

Parameters	mMRC score		%DLco	
	ρ	P value	ρ	P value
IL-11	−0.148	0.228	−0.153	0.221
PDGF	0.066	0.595	0.037	0.767
IL-11/%FVC	0.335	0.006	−0.518	<0.001
PDGF/%FVC	0.202	0099	−0.119	0.343

Highlight box

Key findings

- In this study, we measured the serum IL-11 level in patients with IPF and investigated its predictive significance for survival and AE occurrence. The serum IL-11/%FVC value was an independent predictor of prognosis and AE occurrence in patients with IPF, and the IL-11 level appeared to show pathophysiologic value in IPF.

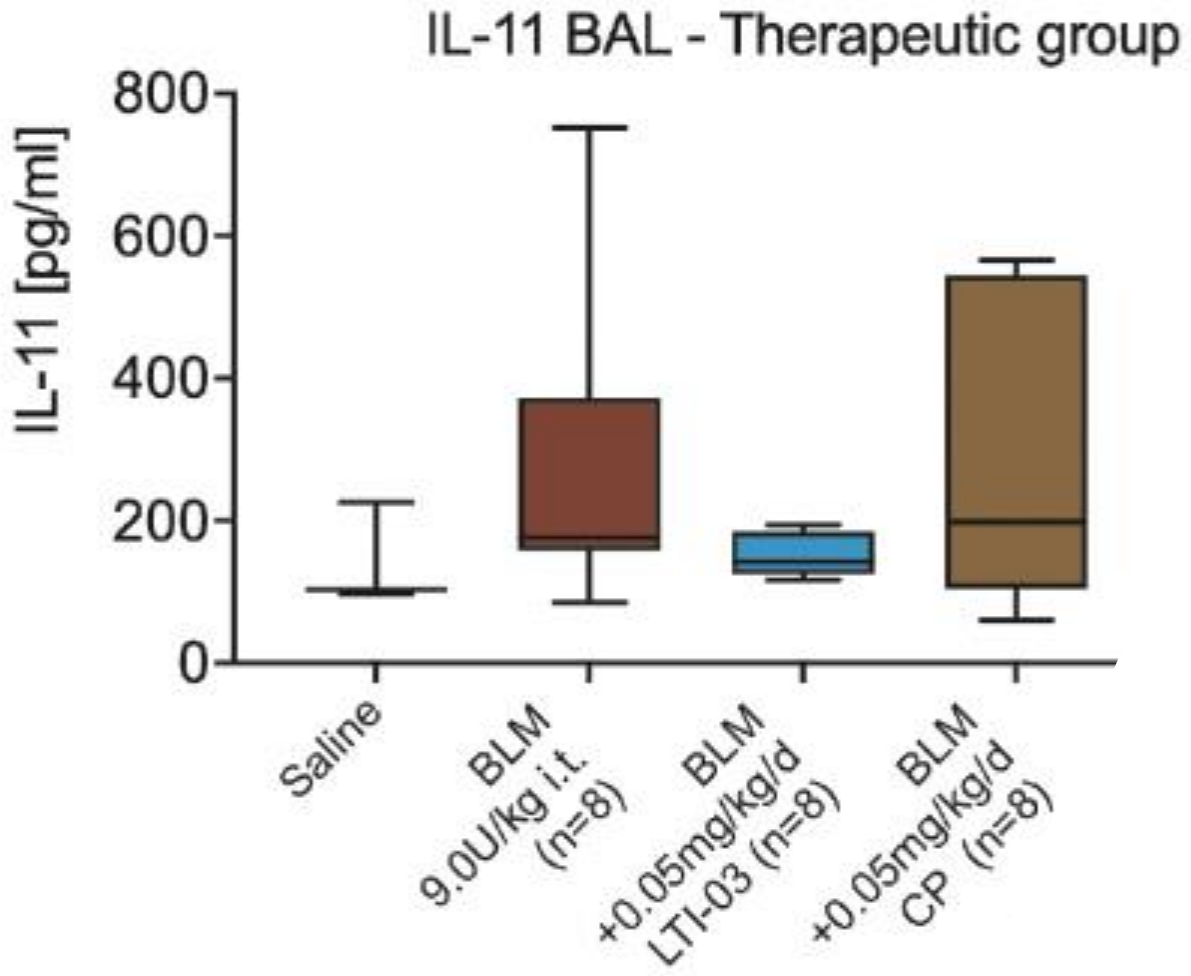
What is known and what is new?

- IL-11 is a member of the IL-6 family, and in vivo and in vitro studies have suggested that it has profibrotic effects in pulmonary fibrosis.
- This manuscript added significance of serum IL-11 levels to predict survival and AE occurrence of IPF.

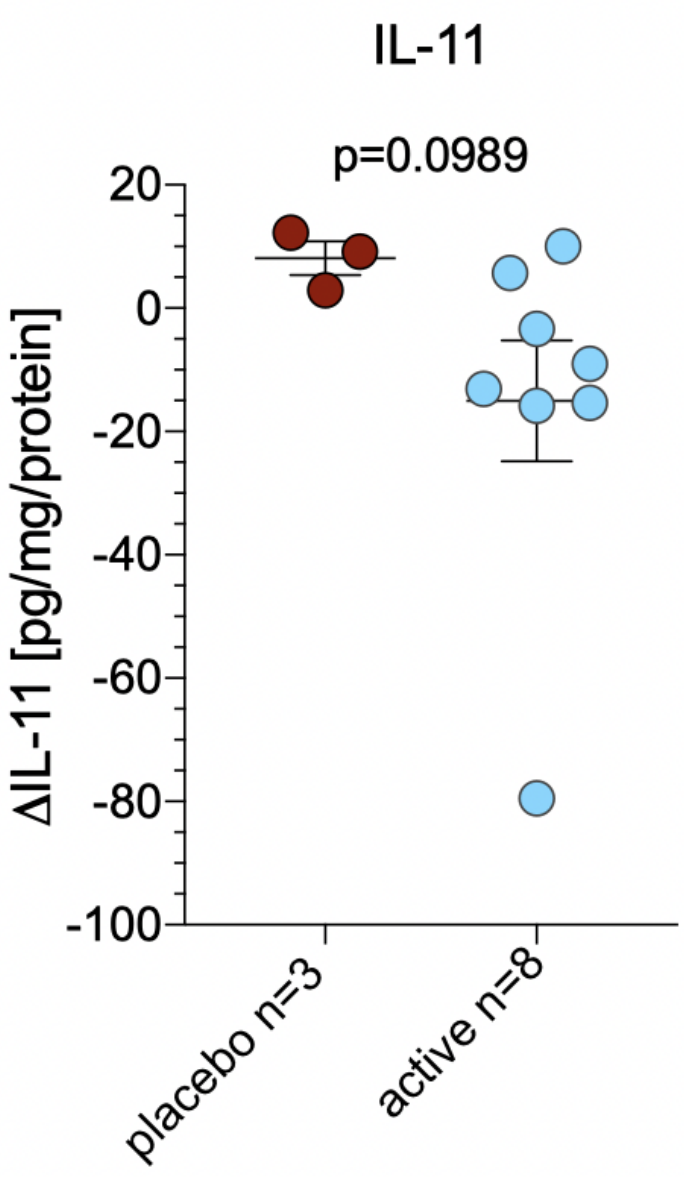
What is the implication, and what should change now?

- Serum IL-11 level appeared to show pathophysiologic roles in IPF. IL-11 might be a target molecule for treatment of IPF.

LTI-03 attenuated IL-11 in BALF of aged mouse BLM model when administered preventatively or therapeutically



LTI-03 Phase 1b – IL-11

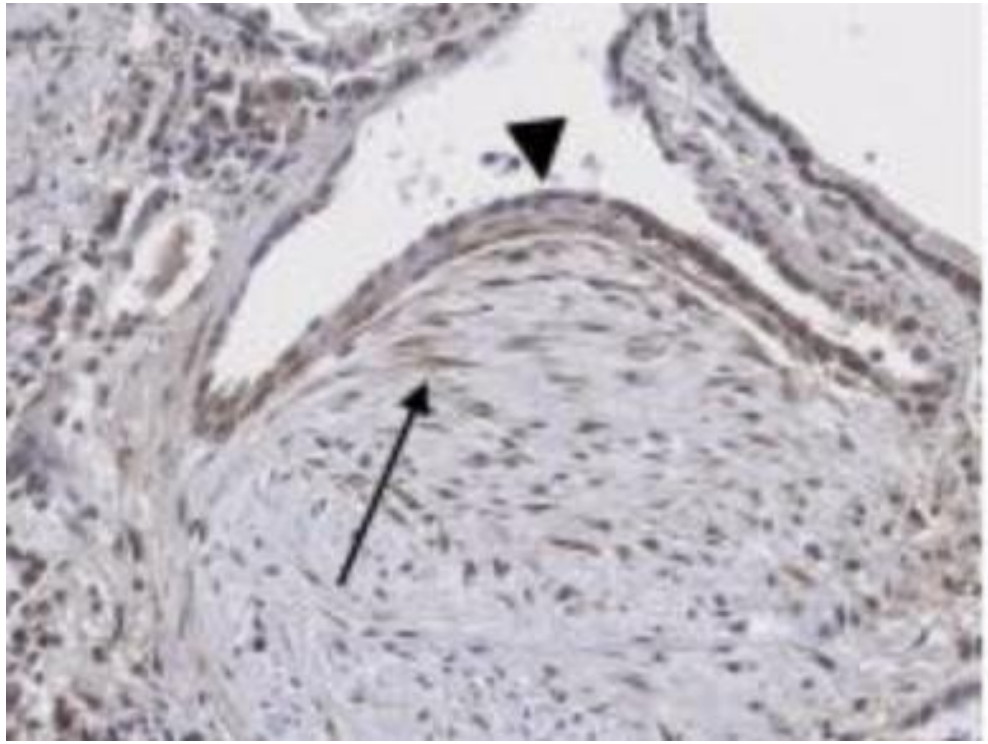


Thymic Stromal Lymphopoietin Protein (TSLP) is expressed in fibroblasts and basal like epithelium of IPF UIP lesions

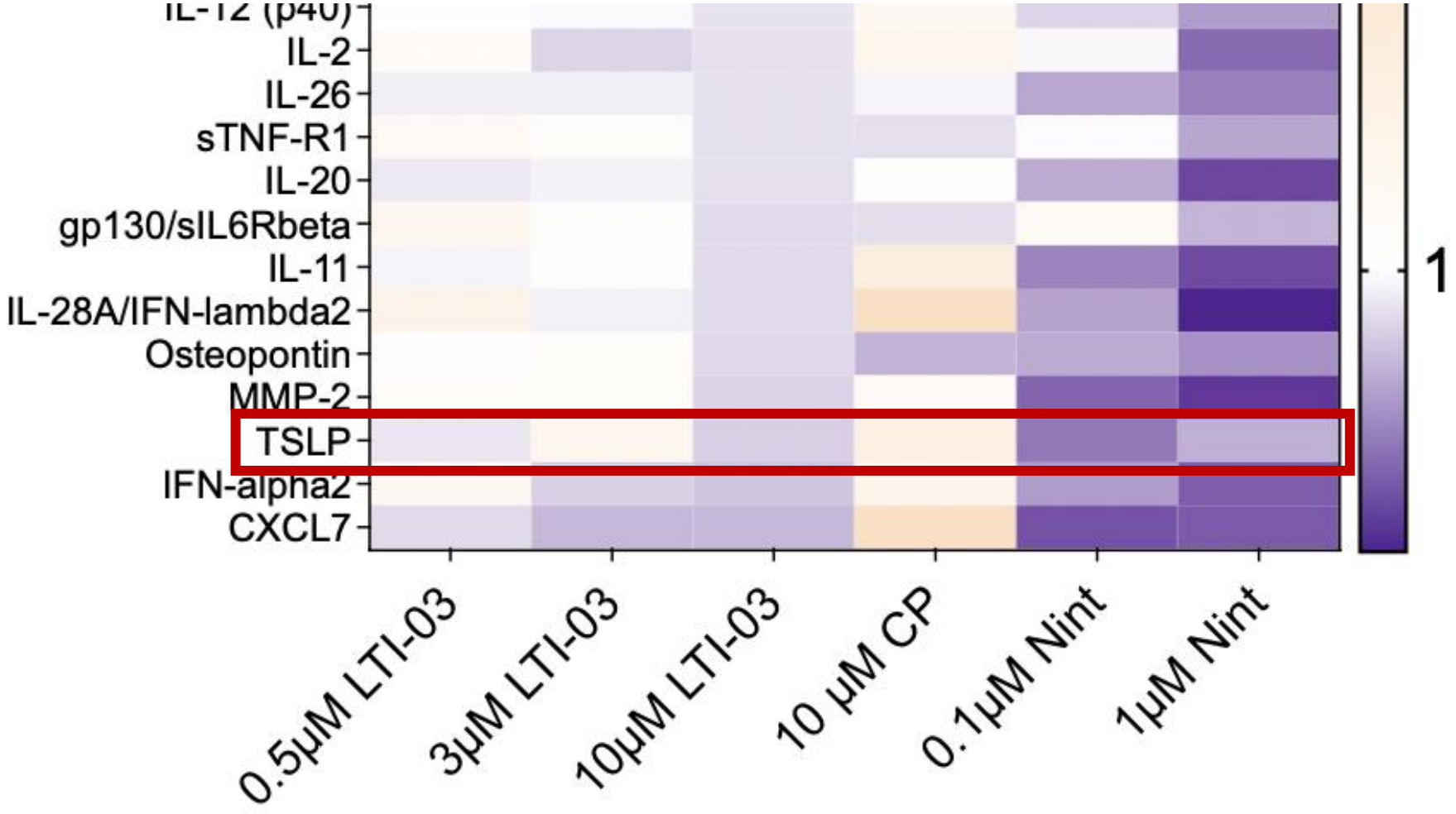
Evidence for a Functional Thymic Stromal Lymphopoietin Signaling Axis in Fibrotic Lung Disease

Arnab Datta,* Robert Alexander,* Michal G. Sulikowski,* Andrew G. Nicholson,† Toby M. Maher,† Chris J. Scotton,*,1 and Rachel C. Chambers*,1

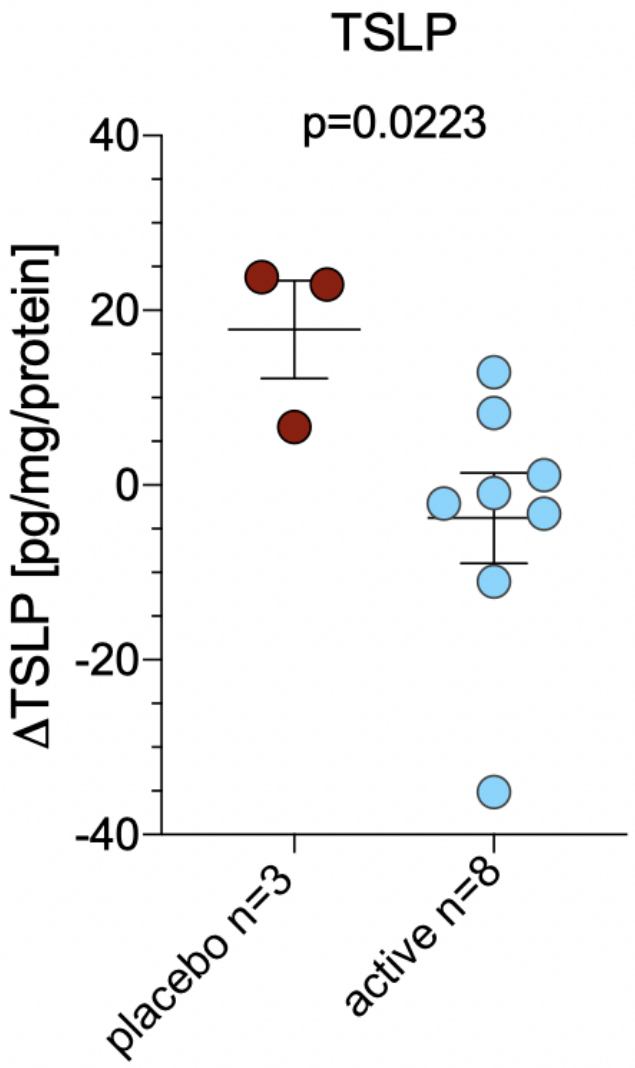
Thymic stromal lymphopoietin (TSLP) recently has emerged as a key cytokine in the development of type 2 immune responses. Although traditionally associated with allergic inflammation, type 2 responses are also recognized to contribute to the pathogenesis of tissue fibrosis. However, the role of TSLP in the development of non–allergen-driven diseases, characterized by profibrotic type 2 immune phenotypes and excessive fibroblast activation, remains underexplored. Fibroblasts represent the key effector cells responsible for extracellular matrix production but additionally play important immunoregulatory roles, including choreographing immune cell recruitment through chemokine regulation. The aim of this study was to examine whether TSLP may be involved in the pathogenesis of a proto-typical fibrotic disease, idiopathic pulmonary fibrosis (IPF). We combined the immunohistochemical analysis of human IPF biopsy material with signaling studies by using cultured primary human lung fibroblasts and report for the first time, to our knowledge, that TSLP and its receptor (TSLPR) are highly upregulated in IPF. We further show that lung fibroblasts represent both a novel cellular source and target of TSLP and that TSLP induces fibroblast CCL2 release (via STAT3) and subsequent monocyte chemotaxis. These studies extend our understanding of TSLP as a master regulator of type 2 immune responses beyond that of allergic inflammatory conditions and suggest a novel role for TSLP in the context of chronic fibrotic lung disease. ***The Journal of Immunology*, 2013, 191: 4867–4879.**



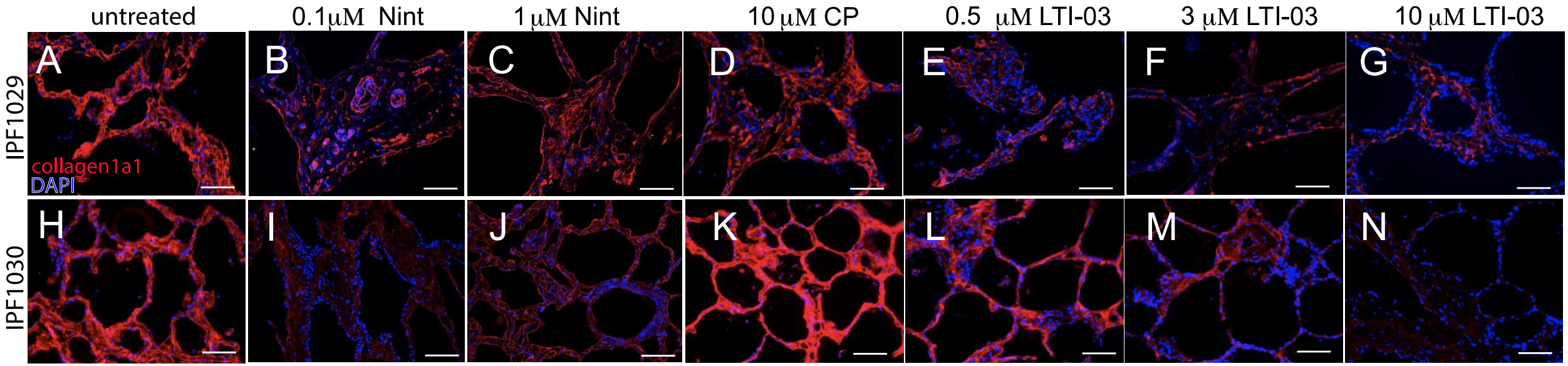
After 5 days, LTI-03 inhibits profibrotic and inflammatory mediator TSLP in the supernatant of IPF PCLS tissue

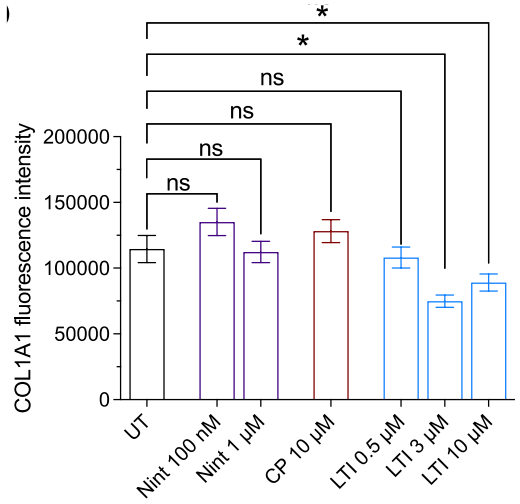


LTI-03 Phase 1b – TSLP



LTI-03 decreased fluorescence intensity for COL1A1staining in IPF PCLS tissue





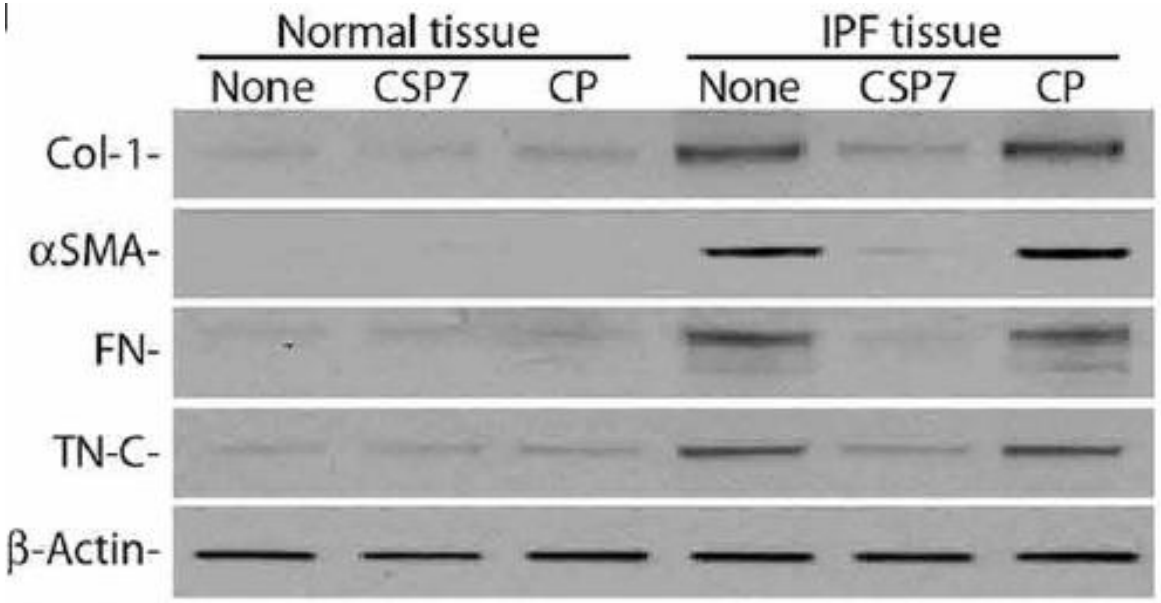
LTI-03 attenuated COL1A1 in tissue of mouse BLM model and in IPF lung tissue

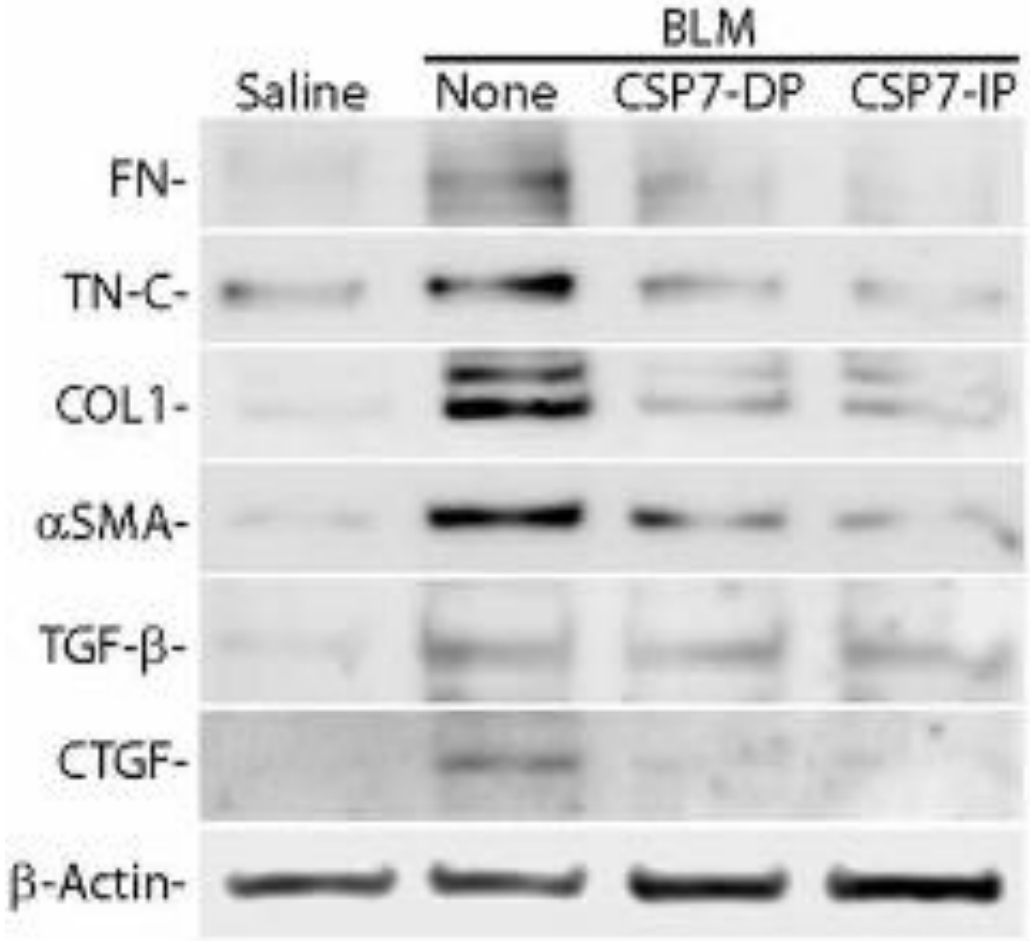
SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

PULMONARY FIBROSIS

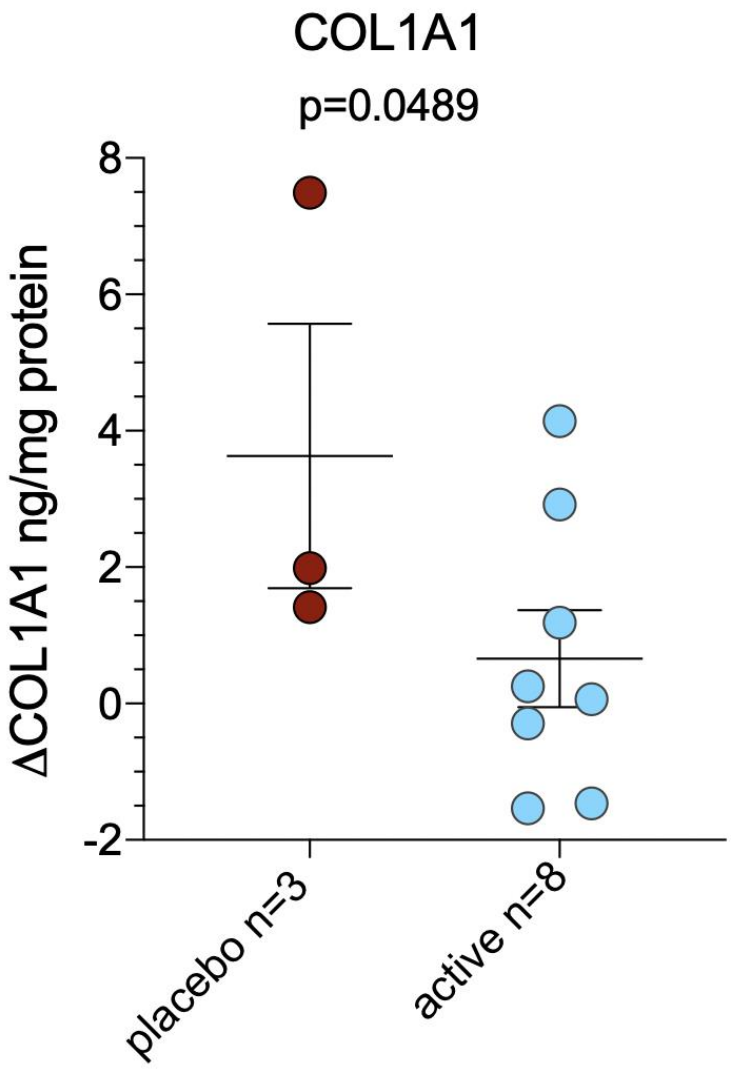
Caveolin-1–derived peptide limits development of pulmonary fibrosis

Amarnath Satheesh Marudamuthu[1]*, Yashodhar Prabhakar Bhandary[1]*, Liang Fan[1]*, Vijay Radhakrishnan[1], BreAnne MacKenzie[2], Esther Maier[3], Shwetha Kumari Shetty[1], M. R. Nagaraja[1], Venkadesaperumal Gopu[1], Nivedita Tiwari[1], Yajie Zhang[3], Alan B. Watts[3], Robert O. Williams III[3], Gerald J Criner[4], Sudhir Bolla[4], Nathaniel Marchetti[4], Steven Idell[1], Sreerama Shetty[1†]





LTI-03 Phase 1b – COL1A1



Biomarker Summary: LTI-03 Phase 1b Clinical Trial Cohort 1 (low dose; 2.5mg BID)

Biomarkers	Positive Trend	Statistically Significant (p<0.05)
Fibroblasts/myofibroblasts		
COL1A1	✓	✓
IL-11	✓	
Basal-like cells		
TSLP	✓	✓
GAL7	✓	✓
Alveolar epithelial health		
sRAGE	✓	

- Low dose **LTI-03 significantly reduced expression of 3 profibrotic proteins** of pathologic basal-like cells and/or fibroblast origin
- LTI-03 stimulated production of a factor indicative of **type I epithelial cell health (solRAGE)**.
- **LTI-03 did not induce inflammation in PBMCs**

NASDAQ: ALRN

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