Caveolin-1 derived peptide LTI-03 promotes epithelial cell survival and attenuates pulmonary fibrosis

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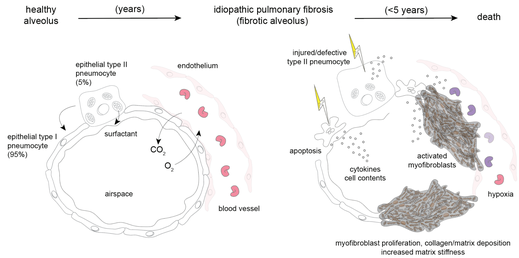
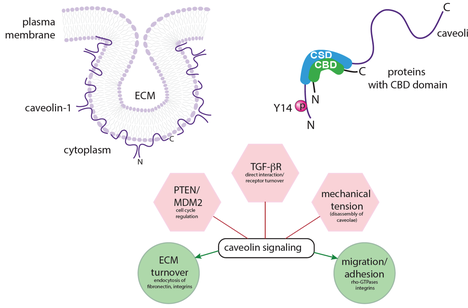
Rationale

1 Idiopathic Pulmonary Fibrosis (IPF) is a progressive lung disease involving many cell types



Idiopathic pulmonary fibrosis (IPF) is a lethal disease associated with progressive destruction of the lung parenchyma. Retrospective longitudinal studies suggest a median survival time from 2 to years from the time of diagnosis [1,2]. In the US, the annual incidence of IPF has been reported to be between 6.8 and 16.3 cases per 100,000 people, and it is considered a rare disease. The pathogenesis of IPF is characterized by alveolar epithelial cell (AEC) apoptosis, and the progressive accumulation of activated myofibroblasts which deposit excessive extracellular matrix (ECM). This results in progressive dyspnea and loss of lung function [3].

2 Endogenous caveolin-1 antagonizes fibrotic processes



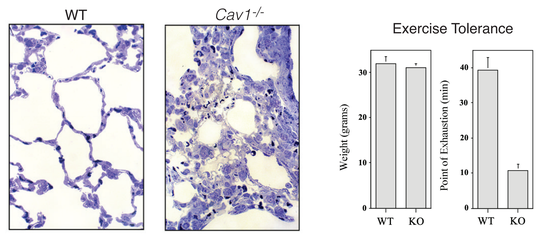
Caveolin-1 is part of a family (caveolin-1, -2 and -3) of cholesterol-binding membrane proteins that coat the intracellular surface of caveolae, small flask-shaped pits (50–100 nm in diameter) that form at the plasma membrane of most cells. It is essential for critical processes in tissue repair, such as migration, cell adhesion and ECM turnover due to its association with Rho GTPases and integrins. The caveolin scaffolding domain (CSD) can bind to any proteins with the caveolin binding domain (CBD) motif; ~30% of endogenous proteins [4]. Caveolin-1 antagonizes fibrotic processes by regulating membrane tension via rapid disassembly of caveolae, and antagonizes cell proliferation cell cycle regulation by facilitating PTEN phosphatase activity. It also inhibits TGFβ-1; figure adapted from [5]. LTI-03 peptide is derived from the CSD domain of Cav-1.

references (rationale):

1. Rudd RM, Prescott RJ, Chalmers JC, Johnston IDA. British Thoracic Society Study on cryptogenic fibrosing alveolitis: Response to treatment and survival. Thorax. 2007;62(1):62-66. doi:10.1136/thx.2005.045591
2. Mannino DM, Etzel RA, Gibson Parrish R. Pulmonary fibrosis deaths in the United States, 1979-1991: An analysis of multiple-cause mortality data. Am J Respir Crit Care Med. 1996;153(5):1548-1552. doi:10.1164/ajrccm.153.5.8630600
3. Sgalla G, Iovene B, Calvello M, Ori M, Varone F, Richeldi L. Idiopathic pulmonary fibrosis: Pathogenesis and management. Respir Res. 2018;19(1). doi:10.1186/s12931-018-0730-2
4. Byrne DP, Dart C, Rigden DJ. Evaluating Caveolin Interactions: Do Proteins Interact with the Caveolin Scaffolding Domain through a Widespread Aromatic Residue-Rich Motif? Haslam NJ, ed. PLoS One. 2012;7(9):e44879. doi:10.1371/journal.pone.0044879
5. Gvaramia D, Blaauboer ME, Hanemaaijer R, Everts V. Role of caveolin-1 in fibrotic diseases. Matrix Biol. 2013;32(6):307-315. doi:10.1016/j.matbio.2013.03.005

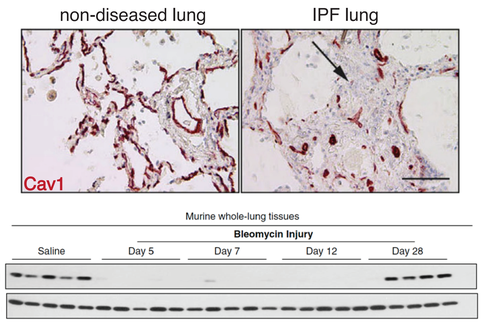
Background

3 Caveolin-1 mouse knockouts developed thickened lung septa and displayed reduced exercise tolerance due to lung defects



One-µm sections of lung parenchyma were cut and stained with toluidine blue. Cav1-deficient mice displayed significantly smaller, constricted, alveolar spaces with thickened alveolar septa and hypercellularity. In addition, an intolerance to exercise (failure to maintain buoyancy while swimming) was noted likely due to lung defects as weight was similar to wild type [6].

4 Caveolin-1 protein is decreased in IPF lungs and in lung homogenate of mice injured with bleomycin



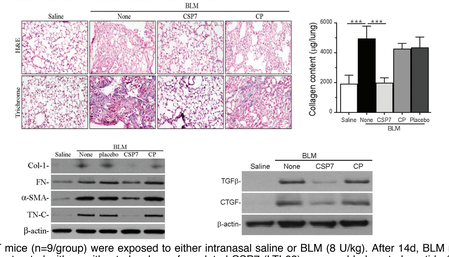
Caveolin-1 IHC revealed reduced Caveolin-1 protein staining in IPF lung sections. Caveolin-1 staining in non-IPF lung sections was comparatively increased in epithelial cells [7]. In bleomycin model of lung injury. Cav-1 expression was examined on d5 after injury (inflammation is relatively more active), as well as on d7 (fibrosis is developing), d12 (near peak fibrosis), and d28 (resolution). Cav-1 protein expression was markedly decreased in whole-lung lysates at d5, d7, and d12, with recovery of expression occurring on d28 after lung injury [8].

references (background):

6. Razani B, Engelman JA, Wang XB, et al. Caveolin-1 null mice are viable but show evidence of hyperproliferative and vascular abnormalities. J Biol Chem. 2001;276(41):38121-38138. doi:10.1074/jbc.M105408200
7. Nagaraja MR, Tiwari N, Shetty SK, et al. p53 Expression in Lung Fibroblasts Is Linked to Mitigation of Fibrotic Lung Remodeling. Am J Pathol. 2018;188(10):2207-2222. doi:10.1016/j.ajpath.2018.07.005
8. Sanders YY, Liu H, Scruggs AM, Duncan SR, Huang SK, Thannickal VJ. Epigenetic Regulation of Caveolin-1 Gene Expression in Lung Fibroblasts. Am J Respir Cell Mol Biol. 2017;56(1):50-61. doi:10.1165/rcmb.2016-0034OC

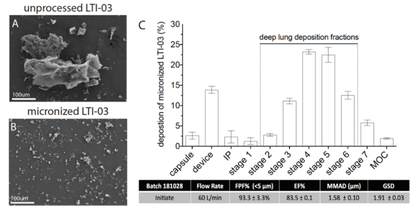
LTI-03 preclinical efficacy-mouse

5 Nebulization of LTI-03 (d14-d20) resulted in decreased lung fibrosis in mouse BLM-induced fibrosis



WT mice (n=9/group) were exposed to either intranasal saline or BLM (8 U/kg). After 14d, BLM mice were treated with or without placebo or formulated CSP7 (LTI-03) or scrambled contral peptide (CP). 5.8 mg were suspended in 30 ml of PBS containing lactose monohydrate stabilizer. Mice were exposed for 2h/d for 7d using a nose-only nebulization tower (CH technologies). H&E and Trichrome staining of perfused lung sections were performed. Whole lung homogenates were analyzed for total soluble collagen, immunoblotted for pro-fibrogenic proteins including tenascin-C, fibronectin, alpha-smooth muscle actin, TGF-β and CTGF against β-actin loading control. The experiments were repeated twice. *p<0.05, **p<0.01, ***p<0.001.

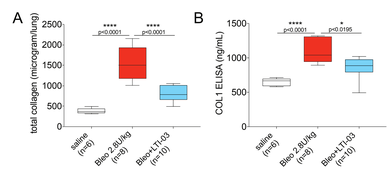
6 Air-Jet milling of cell-free, excipient free, synthesized LTI-03 produced particle profile optimal for deep lung delivery



Batch 181028	Flow Rate	FPF% (<5 µm)	EF%	MMAD (µm)	GSD
Initiate	60 L/min	83.3 ± 3.3%	83.5 ± 0.1	1.56 ± 0.10	1.91 ± 0.03

NGI results showing the deposition of micronized LTI-03 (CSP7). SEM of particle morphology; pre-air-jet milled (A) and micronized (post) air-jet milled (B). Air-jet milled (micronized) LTI-03 demonstrated excellent characteristics for inhalation; 95% of particles distributed in impactor stages 2 through 6, which represent aerodynamic size range appropriate for deep lung delivery. FPF: fine particle fraction, EF: emitted dose, GSD: geometric standard deviation, IP: induction port, MOC: micro-orifice collector.

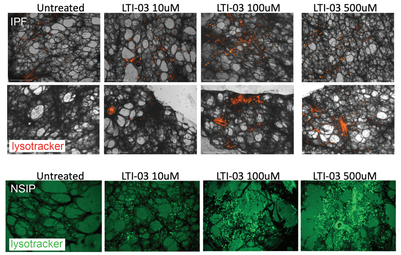
7 Dry powder LTI-03 (d14-d20) resulted in decreased lung collagen in mouse BLM-induced fibrosis



Female, C57Bl/6J mice, ages 8-10 weeks (Jackson labs #000664) were subjected to a single intratracheal installation of bleomycin (d0) via 26G plastic catheter inserted into the trachea; 40µl of bleomycin (Biotang, Cat# RB003) 2.8U/kg. Saline controls received 40µl of saline. On d14, *during the fibrotic phase of bleomycin injury*, animals were subjected to dry powder inhalation of LTI-03, 0.05 mg/kg/d (CH technologies) daily, for 7 consecutive days. The air jet-milled dry powder dosing of LTI-03 was based on the minimum efficacious dose of the previously used nebulization formulation. Collagen content of lung homogenate was assayed; Total Collagen Assay (Quickzyme); FilterMax F5, Molecular Devices, 580nm. ELISA for collagen 1a1 was performed on diluted lung homogenate (1:250) according to LS BioScience instructions;; Mouse COL1A1 / Collagen I Alpha 1 ELISA Kit (Sandwich ELISA) LS-F11155. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

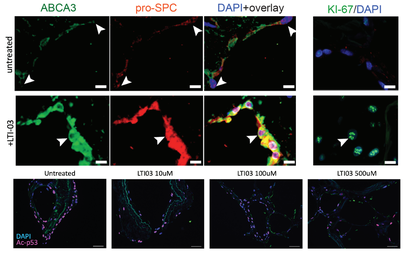
LTI-03 preclinical efficacy-IPF PCLS

8 Dose dependent increase in lysotracker staining of NSIP and IPF prision cut lung slices (PCLS)



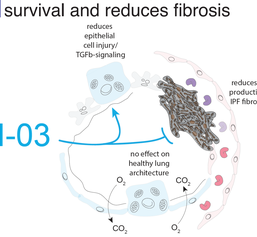
Precision cut lung slices (PCLS) of donor and NSIP and IPF tissues were cultured ex vivo and treated with LTI-03 for up to7 days. In brief, lysotracker staining, which stains acidic compartments in live cells and selectively accumulates in the lamellar bodies of lung AEC2 cells was performed on PCLS generated from patients with non-specific interstitial pneumonia (NSIP) and end-stage IPF. LTI-03 was suspended in DMEM/5%FBS and PCLS slices (n=5 replicates/treatment group) were treated with 10, 100 or 500uM of LTI-03. Dose dependent increases in AEC2 cell viability was observed with lysotracker staining.

9 Increased expression of AEC2 cell markers pro-SPC and ABCA3; as well as KI-67, decreased Ac-p53 on IPF PCLS



d7 IPF PCLS' were fixed in 4% PFA for immunofluorescence. Increased lysotracker staining correlated with increased intensity for AEC2markers pro-surfactant protein C (SpC) and ATP binding cassette subfamily A member 3 (ABC3A)and KI-67, a marker for proliferation which also co-localized with these markers. Finally, the % of acetylated-p53 cells were decreased in 100 and 500uM treated groups by d7 according to immunofluorescence staining (Ac-p53 ab62376 1:1500).

10 LTI-03 promotes epithelial cell survival and reduces fibrosis



11 Contact Lung Therapeutics, Inc for more information!

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