Anti-fibrotic activity of Caveolin-1 scaffolding domain peptide LTI-03 in IPF precision cut lung slices

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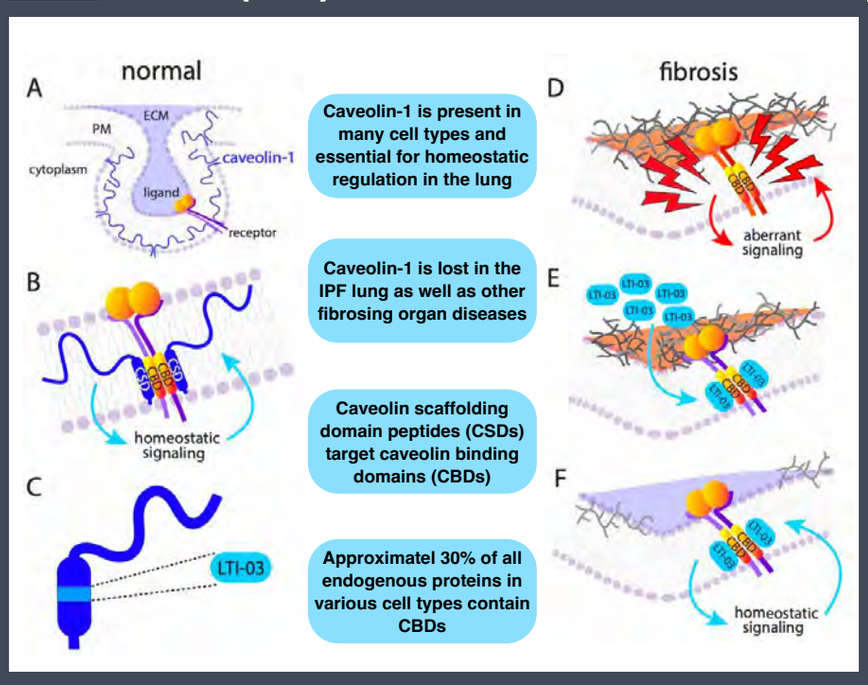
RATIONALE: *Cav-1 regulates homeostasis; down in IPF*

1 Caveolin-1 protein is lost in the fibrotic lung; LTI-03 is replacing the homeostatic regulator domain (CSD)

2 Cav-1 mRNA and protein is down in IPF

3 Proteins containing CBD domains are targets of LTI-03 and Nintedanib

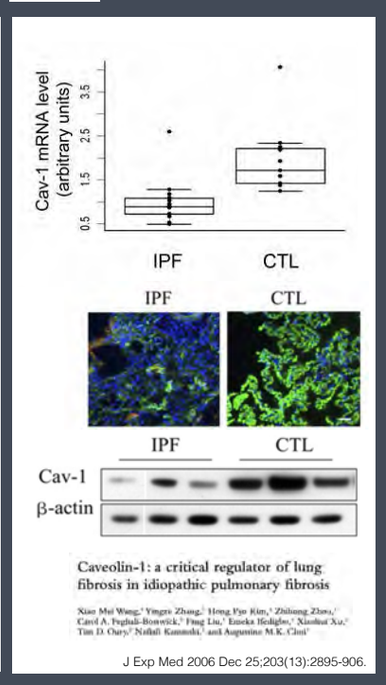


Caveolin-1 is present in many cell types and essential for homeostatic regulation in the lung

Caveolin-1 is lost in the IPF lung as well as other fibrosing organ diseases

Caveolin scaffolding domain peptides (CSDs) target caveolin binding domains (CBDs)

Approximatel 30% of all endogenous proteins in various cell types contain CBDs



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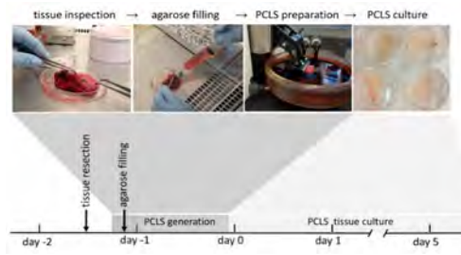
GENE	UNIPROT	CBD motif 1 ϕXϕXXXXϕ	CBD motif 2 ϕXXXXϕXXϕ	mixed domain ϕXϕXXXXϕXXϕ	Nintenadib targets
FGF2	P09038	0	1	0	
FGF5	P12034	0	1	0	
FGF8	P55075	0	1	0	
FGF9	P31371	0	1	0	
FGF11	Q92914	0	1	0	
FGF12	P61328	1	1	0	
FGF13	Q92913	1	1	0	
FGF14	Q92915	1	1	0	
FGF16	O43320	0	1	0	
FGF20	Q9NP95	0	4	0	
FGFR1	P11362	2	1	1	•
FGFR2	P21802	1	1	1	•
FGFR3	P22607	1	1	1	•
FGFR4	P22455	1	1	1	•
FZD1	Q9UP38	1	0	0	
FZD2	Q14332	1	0	0	
FZD4	Q9ULV1	1	2	1	
FZD5	O75084	1	0	0	
FZD6	Q9H461	3	0	0	
FZD7	O00144	1	2	1	
FZD10	Q9ULW2	0	1	0	
PGFRA	P16234	1	3	1	•
PGFRB	P09619	1	2	1	•
VGFR1	P17948	3	1	1	•
VGFR3	P35916	2	2	1	•

In silico identification of caveolin-1 binding domains in human proteome CBD motif 1 (ϕXϕXXXXϕ), CBD motif 2 (ϕXXXXϕXXϕ), and the CBD composite motif (ϕXϕXXXXϕXXϕ) were identified using the MOTIF Search tool (setting: nr-aa) available on GenomeNet (genome.jp). The symbol 'ϕ' represents either Tryptophan (Trp - W), Phenylalanine (Phe – F), or Tyrosine (Tyr – Y), while X is any other amino acid. These sequences were used as queries to identify protein sequences containing CBDs.

METHODS: *IPF PCLS*

4 PCLS were generated from end-stage IPF patients, cultured ex vivo, treated with LTI-03 every 12 hours up to 7 days

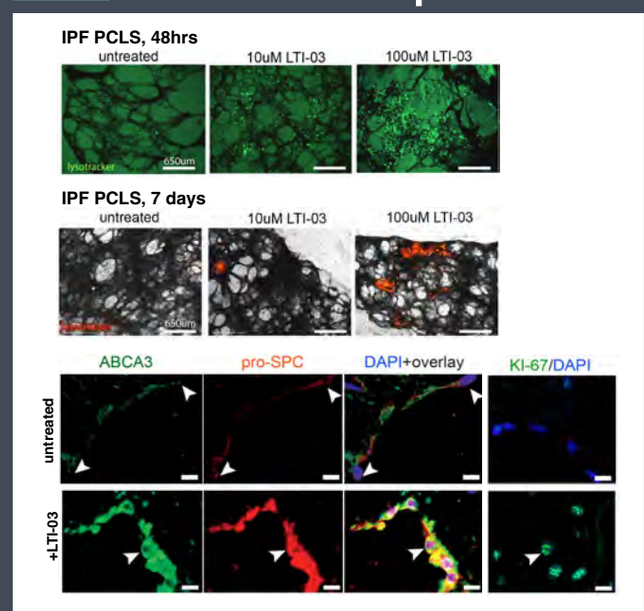
key patient data				applied models and assays					
age	sex	clinical diagnosis	pathological fibrosis pattern in explant tissue	PCLS	COL1A1 immuno-fluoresence (tissue)	Bio-Plex Human Inflammation Panel 1 (supernatant)	bulk RNA sequencing (tissue)	lysotracker (tissue)	solRAGE ELISA (supernatant)
53	M	IPF	UIP	•		•	•		
58	M	IPF	UIP	•		•	•		
60	M	IPF	UIP	•		•	•		
70	M	IPF	UIP	•		•	•		
70	M	IPF	UIP	•	•	•			
57	M	IPF	UIP	•	•	•			
66	M	IPF	UIP	•	•	•			
56	M	IPF	UIP	•	•	•			
67	M	IPF	UIP					•	•
51	M	IPF	UIP					•	•
50	F	IPF	UIP					•	•
67	M	IPF	UIP					•	•



- western blot
- immunoflurescence
- bulk RNA sequencing
- lysotracker staining
- ELISA

A: *LTI-03 is anti-fibrotic and supports epithelium in end-stage IPF PCLS*

5 LTI-03 increased lysotracker staining as well as pro-SPC, ABCA3 and KI-67 positive cells



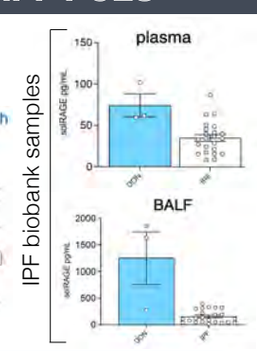
6 Decreased solRAGE in IPF plasma and BAL; increased post LTI-03 in IPF PCLS

Low levels of sRAGE predict poor survival in IPF

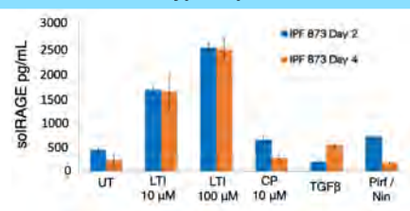
Decrease in plasma solRAGE correlates with decline in %FVC in IPF and in other ILDs

Spearman correlation coefficients between plasma sRAGE and measures of disease severity

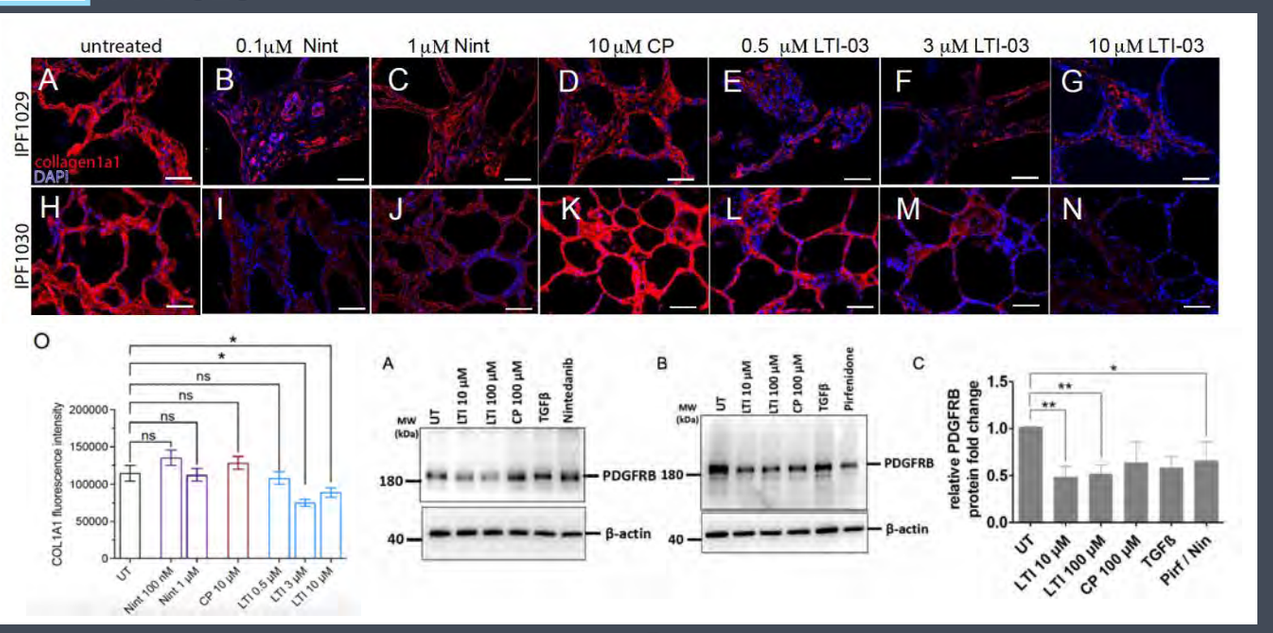
Variable	IPF Spearman's rho	IPF p-value	other ILDs Spearman's rho	other ILDs p-value
Age:	0.22	0.02	0.11	0.13
BMI	-0.12	0.22	-0.16	0.04
FVC%	0.46	<0.001	0.27	<0.001
DLCO%	0.19	0.13	0.24	0.01
6MWD	0.36	0.02	0.17	0.09
GAP score:	-0.23	0.07	-0.04	0.64



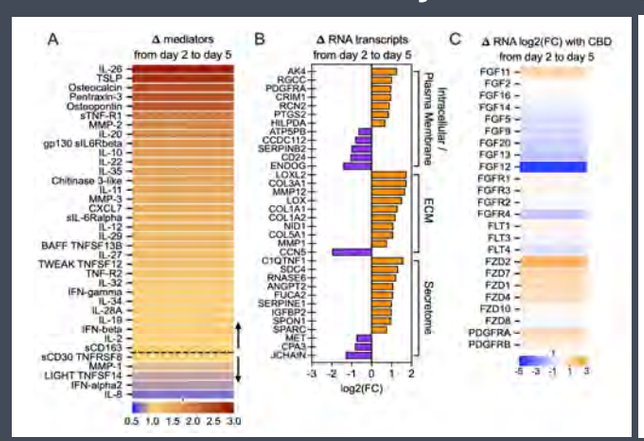
In IPF PCLS, LTI-03 stimulated production of sRAGE, a factor indicative of type I epithelial cell health



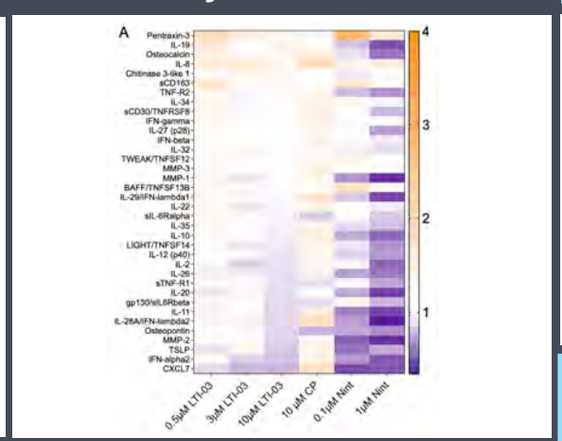
7 LTI-03 decreased COL1A1 and PDGFRB expression in end-stage IPF PCLS



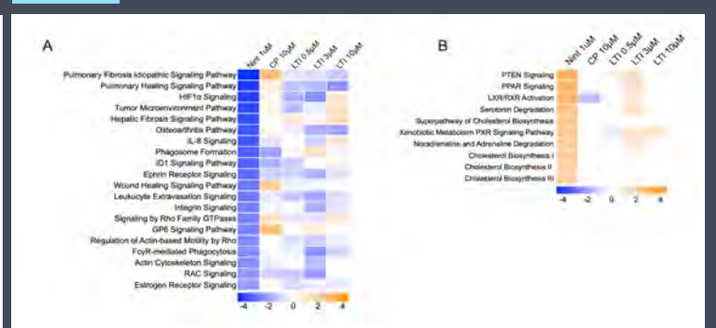
8 Progressive fibrotic activity was observed in IPF PCLS cultures over 5 days



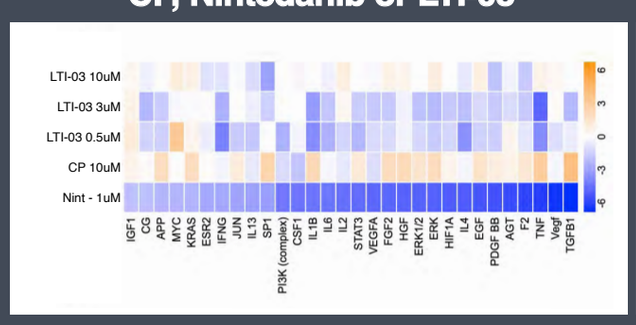
9 LTI-03 inhibits profibrotic and inflammatory factors +5 days of treatment.



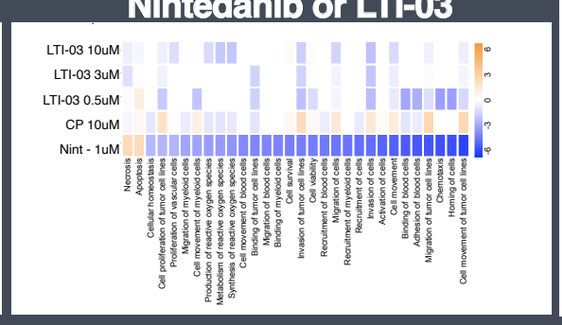
10 Canonical pathways modulated by Nintedanib or LTI-03 treatment of cultured IPF PCLS.



11 Expression of upstream regulators of fibrotic signaling in IPF PCLS treated with CP, Nintedanib or LTI-03



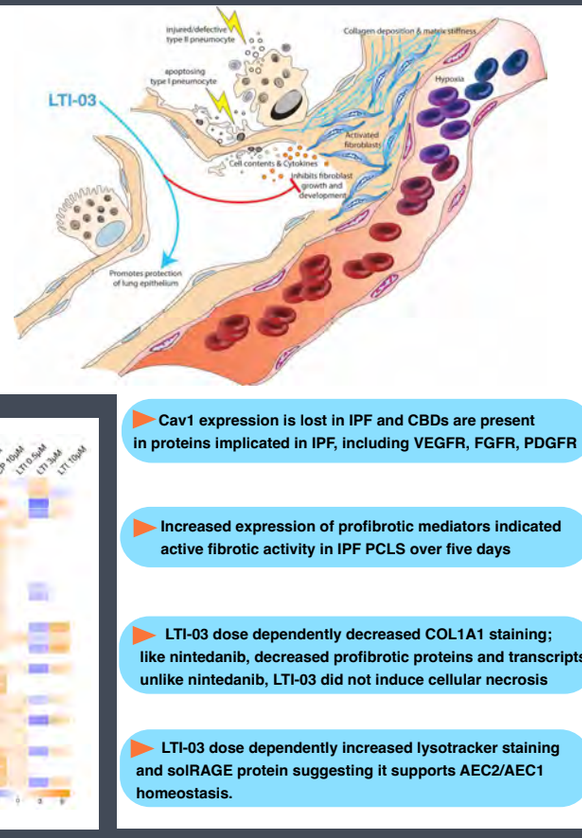
12 Disease pathways related to fibrotic signaling in IPF PCLS treated with CP, Nintedanib or LTI-03



13 LTI-03 inhibits profibrotic and inflammatory mediators in IPF PCLS following 2 days of treatment

SUMMARY

14 LTI-03 is demonstrates anti-fibrotic activity in end-stage IPF PCLS ex vivo cultures



- Cav1 expression is lost in IPF and CBDs are present in proteins implicated in IPF, including VEGFR, FGFR, PDGFR
- Increased expression of profibrotic mediators indicated active fibrotic activity in IPF PCLS over five days
- LTI-03 dose dependently decreased COL1A1 staining; like nintedanib, decreased profibrotic proteins and transcripts; unlike nintedanib, LTI-03 did not induce cellular necrosis
- LTI-03 dose dependently increased lysotracker staining and solRAGE protein suggesting it supports AEC2/AEC1 homeostasis.