

CHARACTERIZING THE ANTIFIBROTIC ACTIVITY OF BEXOTEGRASIT ON DISTINCT FIBROBLAST POPULATIONS IN PCLS FROM MULTIPLE ILD SUBTYPES

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BACKGROUND AND RATIONALE

Idiopathic pulmonary fibrosis (IPF) is the prototypical progressive fibrosing interstitial lung disease (ILD); however, progressive fibrosis can also be observed in other ILDs. ILD with radiological signs of fibrosis and progression over time is referred to as progressive pulmonary fibrosis (PPF) and includes a range of underlying diagnoses.¹

TGF- β signaling is a key driver of fibrotic disease, including pulmonary fibrosis. Integrins $\alpha_v\beta_6$ (epithelial cells) and $\alpha_v\beta_1$ (fibroblasts) promote pulmonary fibrosis through the activation of latent TGF- β , which leads to myofibroblast activation and new collagen synthesis (Figure 1).

Bexotegrast (PLN-74809) is an oral, once-daily, dual-selective inhibitor of integrins $\alpha_v\beta_6$ and $\alpha_v\beta_1$ undergoing evaluation for the treatment of IPF.² Preclinical evaluation of bexotegrast in precision-cut lung slices (PCLS) from IPF patient explants demonstrated decreased profibrogenic gene expression in specific pathologic cell populations.^{3,4}

Therefore, in this study, we used single-nuclei RNA sequencing (snRNA-seq) to evaluate the antifibrotic activity of bexotegrast in fibrotic PCLS generated from non-IPF ILD patient lung explants.

Figure 1. Bexotegrast reduces fibrosis by inhibiting integrin $\alpha_v\beta_6$ - and $\alpha_v\beta_1$ -mediated activation of latent TGF- β

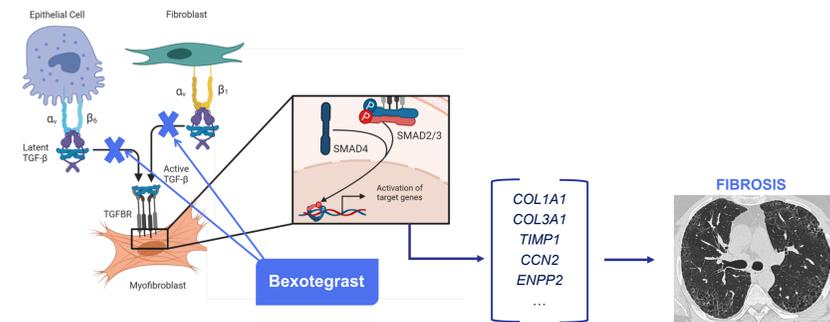
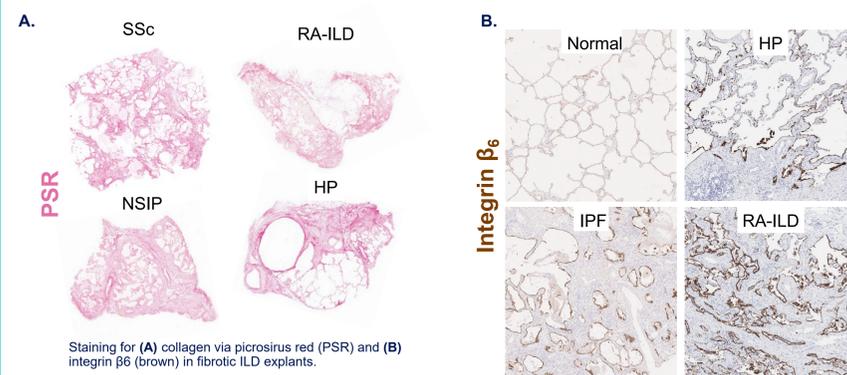


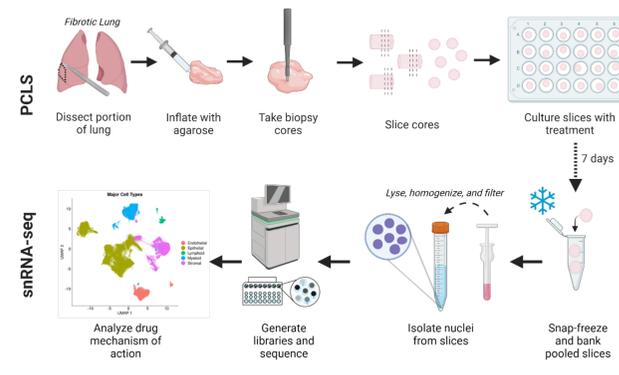
Figure 2. ILD explants have fibrosis and increased $\alpha_v\beta_6$ expression



STUDY DESIGN AND METHODS

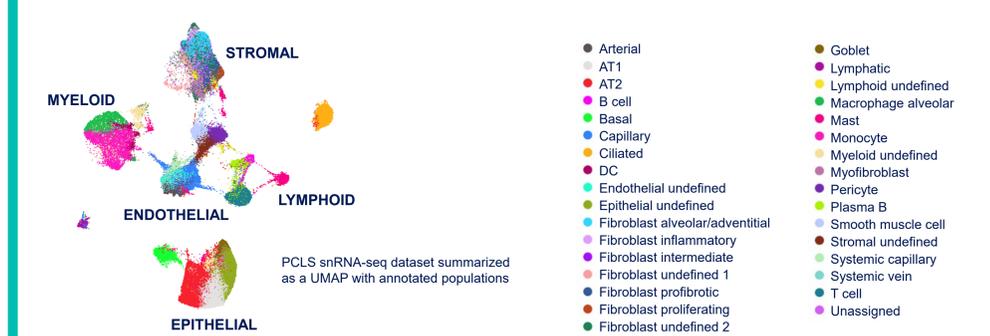
Precision-cut lung slices (PCLS) were generated from fibrotic lung explants (n = 2 RA-ILD, n = 1 HP) and cultured for 7 days in the presence of bexotegrast or vehicle. Nuclei were isolated from treated PCLS and processed for snRNA-seq (10x Chromium Next GEM 3'). Comparative differential gene expression and gene ontology (GO) pathway enrichment analyses were performed on annotated cell subpopulations. Differentially expressed genes (DEGs) were defined as ($|\text{Log}_2\text{FC}| > 0.25$, FDR < 0.05) relative to vehicle (Figure 3).

Figure 3. PCLS generation and snRNA-seq analysis



RESULTS

Figure 4. snRNA-seq of non-IPF fibrotic PCLS identified target cell populations

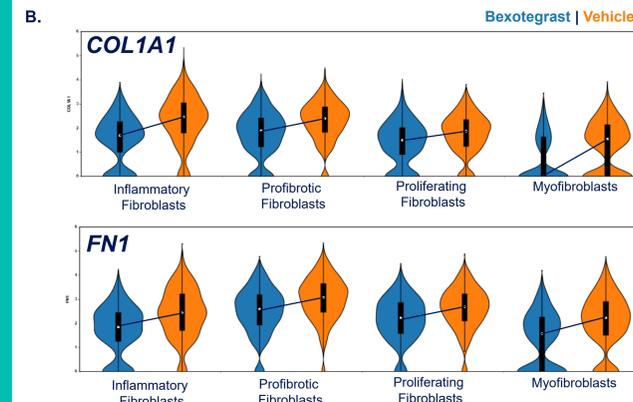


STROMAL CELLS

Figure 5. Bexotegrast significantly reduced fibrogenic gene expression in multiple fibroblast populations

A. Top Downregulated BP GO Terms in Stromal Cells

ID	Description	Adj. p value
GO:0030198	extracellular matrix organization	2E-23
GO:0043062	extracellular structure organization	2E-23
GO:0045229	external encapsulating structure organization	2E-23
GO:0030199	collagen fibril organization	5.5E-11
GO:0085029	extracellular matrix assembly	3.5E-08



(A) Table of top 5 GO biological process terms for genes significantly downregulated by bexotegrast in the stromal cell population. **(B)** Violin plots for collagen, type 1, alpha 1 (*COL1A1*) and fibronectin (*FN1*) across 4 different fibroblast subpopulations broken down by treatment group.

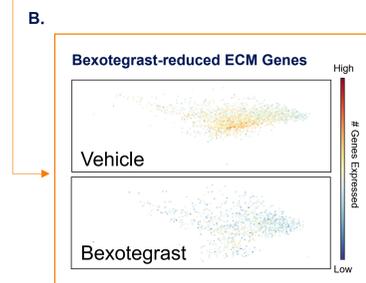
- Fibrogenic genes (e.g. *COL1A1* and *FN1*) were significantly reduced across multiple fibroblast subtypes
- In *CTHRC1^{hi}/COL1A1^{hi}* profibrotic fibroblasts, bexotegrast significantly reduce genes related to extracellular matrix
- ITGB6* is expressed most highly in AT1, AT2, and basal cells
- In AT1 cells, bexotegrast significantly reduced genes related to TGF- β signaling

PROFIBROTIC FIBROBLASTS

Figure 6. Bexotegrast significantly reduced fibrogenic gene expression in profibrotic fibroblasts

A. Top Downregulated BP GO Terms in Profibrotic Fibroblasts

ID	Description	Adj. p value
GO:0030198	extracellular matrix organization	1.1E-13
GO:0043062	extracellular structure organization	1.1E-13
GO:0045229	external encapsulating structure organization	1.1E-13
GO:0030199	collagen fibril organization	8.94E-07
GO:0030111	regulation of Wnt signaling pathway	0.000113

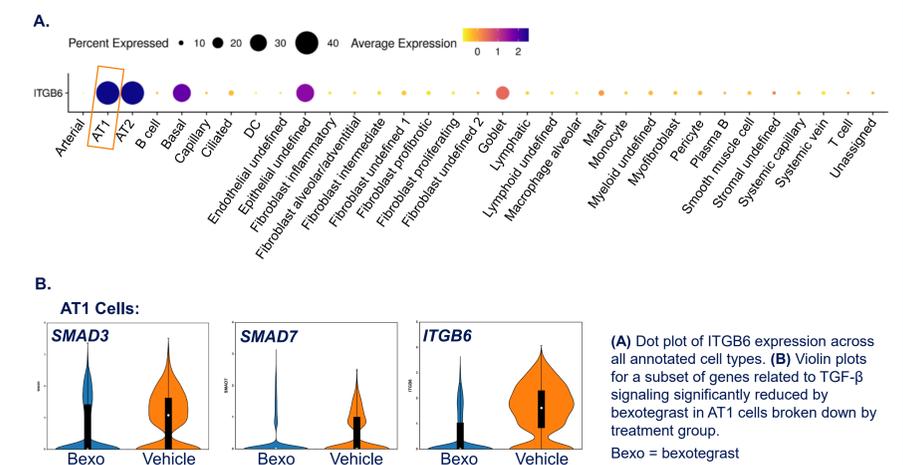


(A) Table of top 5 GO biological process terms for genes significantly downregulated by bexotegrast in profibrotic fibroblasts (*CTHRC1^{hi}/COL1A1^{hi}*). **(B)** Density map overlaid on the profibrotic fibroblast UMAP showing expression of the extracellular matrix organization (GO:030198) genes downregulated by bexotegrast. **(C)** Dot plot of a subset of extracellular matrix-related genes significantly downregulated by bexotegrast in profibrotic fibroblasts.

- ITGB6* is expressed most highly in AT1, AT2, and basal cells
- In AT1 cells, bexotegrast significantly reduced genes related to TGF- β signaling

EPITHELIAL CELLS

Figure 7. Bexotegrast reduced markers of TGF- β signaling in AT1 Cells



CONCLUSIONS

- snRNA-seq of PCLS can be used to evaluate the effects of novel therapeutics on specific cell populations within fibrosing ILD explants
- Integrin $\alpha_v\beta_6$ expression is increased in lungs of patients with multiple fibrotic ILD subtypes
- Bexotegrast, a dual $\alpha_v\beta_6/\alpha_v\beta_1$ inhibitor, reduced expression of genes related to TGF- β signaling and fibrogenesis in AT1 cells and multiple fibroblast subpopulations
- These data are consistent with our observations in IPF explants and support further investigation of the antifibrotic activity of bexotegrast in PPF