CHARACTERIZING THE ANTIFIBROTIC ACTIVITY OF BEXOTEGRAST ON PATHOLOGIC CELL POPULATIONS IN MULTIPLE ILD SUBTYPES

Johanna Schaub[1], Mahru An[1], Richard Ahn[1], Jennifer Yuzon[1], Steve Ho[1], Vikram Rao[1], Chris Her[1], Selorm Tamakloe[1], Hanieh Farhadi[1], Edward Cantu[2], Jeremy Katzen[2], Paul Wolters[3], Martin Decaris[1]

[1]Pliant Therapeutics, Inc., South San Francisco, CA, USA; [2]University of Pennsylvania, Philadelphia, PA, USA [3]University of California, San Francisco, CA, USA

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 dual-selective small-molecule inhibitor of integrins $\alpha_V\beta_6$ and $\alpha_V\beta_1$. Preclinical evaluation of bexotegrast in precision-cut lung slices (PCLS) from IPF patient explants demonstrated decreased profibrogenic gene expression in specific pathologic cell populations, however clinical development of bexotegrast in IPF was ultimately discontinued due to an unfavorable risk-benefit profile.[2,3]

Here we used single-nuclei RNA sequencing (snRNA-seq) to evaluate the effects of bexotegrast in fibrotic PCLS generated from non-IPF ILD patient lung explants and characterize the role of integrins $\alpha_V\beta_6$ and $\alpha_V\beta_1$ in driving aberrant TGF-β signaling in non-IPF ILD.

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

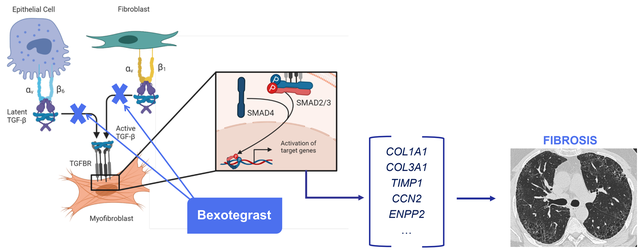
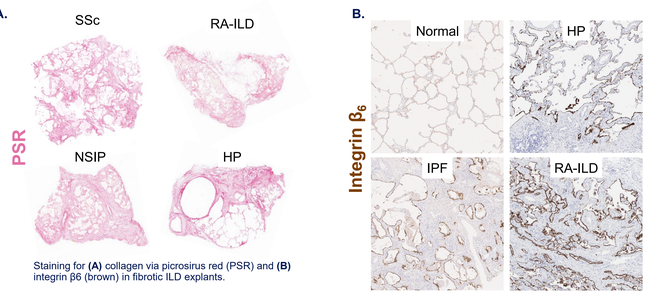


Figure 2. ILD explants have fibrosis and increased $\alpha_V\beta_6$ expression

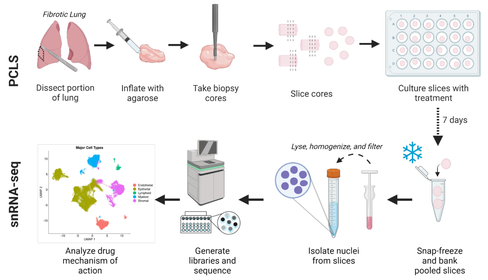


Staining for **(A)** collagen via picrosirus red (PSR) and **(B)** integrin β6 (brown) in fibrotic ILD explants.

STUDY DESIGN AND METHODS

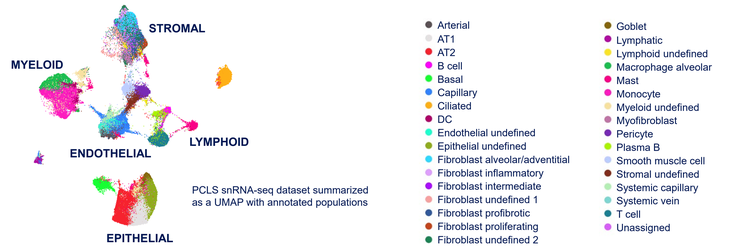
Precision-cut lung slices (PCLS) were generated from fibrotic lung explants (n = 2 RA-ILD, n = 1 HP) and cultured for 7 days in the presence of bexotegrast or vehicle. Nuclei were isolated from treated PCLS and processed for snRNA-seq (10x Chromium Next GEM 3'). Comparative differential gene expression and gene ontology (GO) pathway enrichment analyses were performed on annotated cell subpopulations. Differentially expressed genes (DEGs) were defined as ($|\text{Log2FC}| > 0.25$, 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



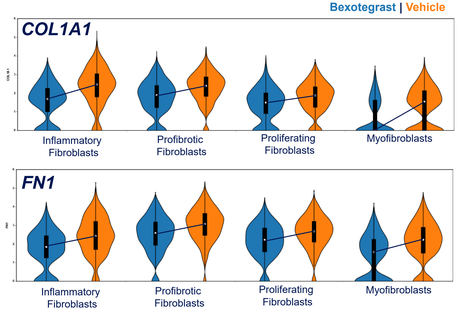
STROMAL CELLS

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

B.

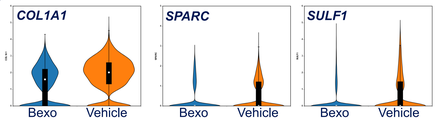


(A) Table of top 5 GO biological process terms for genes significantly downregulated by bexotegrast in the stromal cell population. **(B)** Violin plots for collagen, type 1, alpha 1 (*COL1A1*) and fibronectin (*FN1*) across 4 different fibroblast subpopulations broken down by treatment group.

FIBROBLAST MATRISOME

Figure 6. Bexotegrast significantly reduced matrisome components across fibroblast subtypes

A.



(A) Violin plots for expression of an example collagen (*COL1A1*), ECM glycoprotein (*SPARC*), and *ECM regulator (SULF1*) gene across all fibroblast subtypes combined.
Bexo = bexotegrast

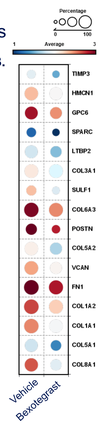
PROFIBROTIC FIBROBLASTS

Figure 7. 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

B.



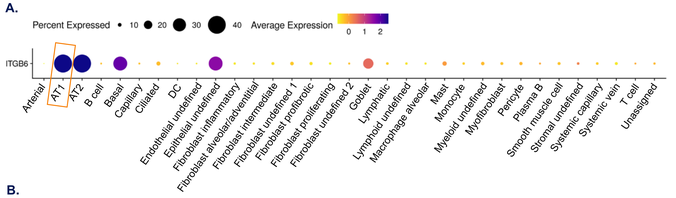
(A) Table of top 5 GO biological process terms for genes significantly downregulated by bexotegrast in profibrotic fibroblasts (*CTHRC1*^{Hi}*COL1A1*^{Hi}). **(B)** Dot plot of a subset of extracellular matrix-related genes significantly downregulated by bexotegrast in profibrotic fibroblasts.

- Fibrogenic and matrisome component genes were significantly reduced across multiple fibroblast subtypes
- In *CTHRC1*^{Hi}/*COL1A1*^{Hi} profibrotic fibroblasts, bexotegrast significantly reduce ECM-related gene expression
- Bexotegrast significantly reduced genes related to TGF-β signaling in AT1 cells (high expressors of *ITGB6*)

EPITHELIAL CELLS

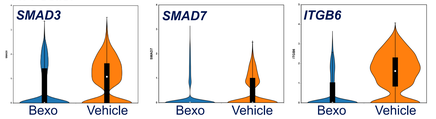
Figure 8. Bexotegrast reduced markers of TGF-β signaling in AT1 Cells

A.



B.

AT1 Cells:



(A) Dot plot of ITGB6 expression across all annotated cell types. **(B)** Violin plots for a subset of genes related to TGF-β signaling significantly reduced by bexotegrast in AT1 cells broken down by treatment group.
Bexo = bexotegrast

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, confirming the role of integrins $\alpha_V\beta_6$ and $\alpha_V\beta_1$ as drivers of non-IPF ILD pathology**

References: 1. Rajan et al. Eur Respir J (2023). 2. Decaris et al. Respir Res (2021). 3. An et al., ATS (2024)

Contact information: jschaub@pliantrx.com

Disclosures: JS, MA, RA, JY, SH, VR, CH, ST, HF, and MD are/were employees and shareholders of Pliant Therapeutics