DUAL αVβ6/αVβ1 INTEGRIN INHIBITOR BEXOTEGRAST REDUCES FIBROGENESIS IN PATHOLOGICAL CELL POPULATIONS PRESENT IN THE FIBROTIC HUMAN LUNG

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ABSTRACT

Background

Single-cell RNA-sequencing analysis of fibrotic human lungs has recently identified novel pathological cell populations that drive disease progression; however, little is known about the impact of anti-fibrotic therapies on these cells. We investigated the effects of bexotegrast (PLN-74809), a dual inhibitor of TGF-β activating integrins $\alpha_v\beta_6$ and $\alpha_v\beta_1$ currently in development for the treatment of idiopathic pulmonary fibrosis (IPF), on pathological cell populations in fibrotic human lung tissue by single nuclei RNA-sequencing (snRNA-Seq) analysis of precision-cut lung slices (PCLS).

Methods

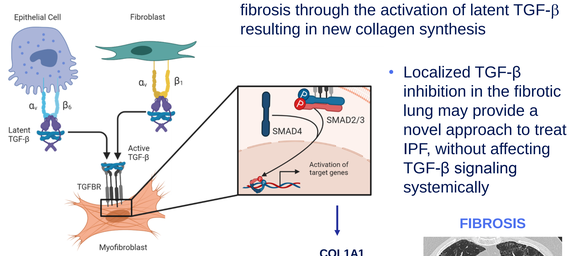
PCLS prepared from lung explants from patients with IPF were cultured for 7 days in the presence of bexotegrast or vehicle. Single nuclei were isolated from PCLS and processed using 10x Chromium 3' kits. Sequenced libraries were processed using CellRanger and analyzed using Seurat and BBrowserX.

Results

snRNA-Seq analysis of fibrotic PCLS identified several unique cell populations important to IPF pathology, including aberrant basaloid cells and high collagen-producing CTHRC1+ fibroblasts. Treatment with bexotegrast significantly reduced total fibroblast expression of TGF-β pathway and scar-associated genes (e.g. COL1A1, TIMP1) (FDR<0.05), while also lowering the number of CTHRC1+ fibroblasts. Bexotegrast treatment also significantly reduced the expression of epithelial-mesenchymal plasticity-related genes (e.g. COL1A2, VIM, FN1) in aberrant basaloid cells (FDR<0.05).snRNA-Seq analysis of fibrotic human PCLS treated with dual $\alpha_v\beta_6/\alpha_v\beta_1$ integrin inhibitor bexotegrast revealed clear reductions in pro-fibrotic gene expression within cell populations important to IPF pathology.

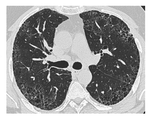
BACKGROUND

- Integrins $\alpha_v\beta_6$ and $\alpha_v\beta_1$ promote pulmonary fibrosis through the activation of latent TGF-β resulting in new collagen synthesis
- Localized TGF-β inhibition in the fibrotic lung may provide a novel approach to treat IPF, without affecting TGF-β signaling systemically
- Bexotegrast, a dual inhibitor of integrins $\alpha_v\beta_6$ and $\alpha_v\beta_1$, has recently initiated late-stage evaluation in the BEACON-IPF study



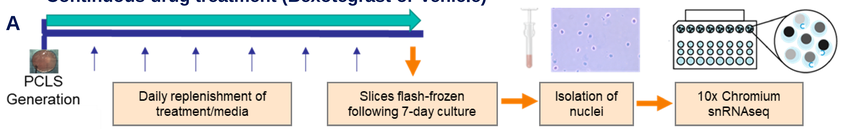
COL1A1
COL3A1
TIMP1
CCN2
ITGB6
...

FIBROSIS

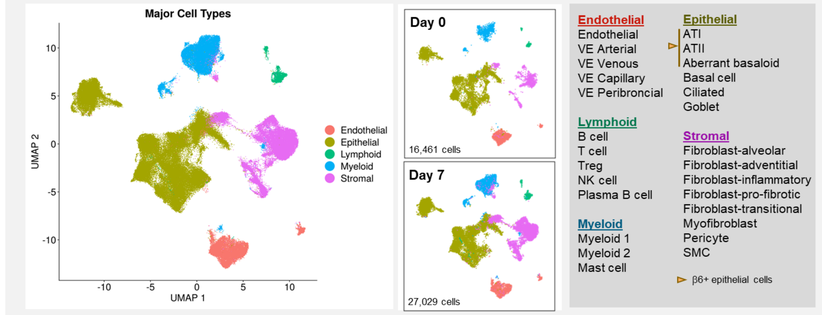


EXPERIMENTAL METHODS AND RESULTS

Continuous drug treatment (Bexotegrast or Vehicle)



B Marker Based Annotation Identifies Unique Cell Populations in PCLS



Marker-based Annotation (References: 1-3)

Figure 1. (A) Study design to evaluate the effects of bexotegrast treatment in PCLS prepared from fibrotic lung explants using snRNA-Seq. (B) UMAP representation of >130,000 cells identified in explant tissue and PCLS following 7 days of culture. Primary cell type categories are further broken down to cell subtype categories annotated using a marker-based approach informed by both single cell (1,2) and single nuclear (3) published fibrotic lung reference datasets.

Summary of snRNAseq Approach

- Explanted lung tissue from patients with IPF used to generate PCLS
- Bexotegrast- and Vehicle- (DMSO) treated PCLS compared following 7-day culture (n=4 IPF donor lungs)
- 132,657 cells successfully annotated from explant tissue and cultured PCLS (10x chromium snRNA-seq)
- A variety of unique cell populations known to play a role in the pathology of IPF were Identified, including cell populations known to express integrins $\alpha_v\beta_6$ and $\alpha_v\beta_1$

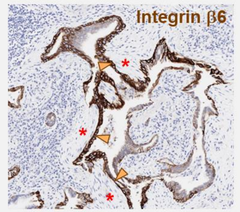
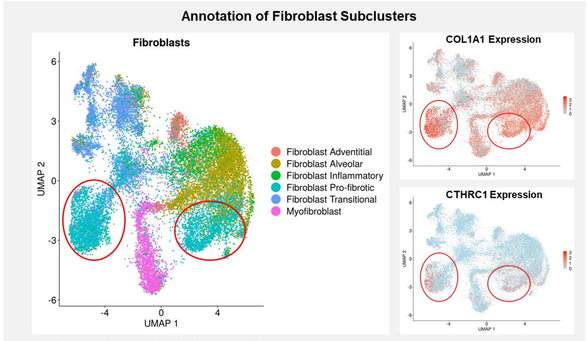


Figure 2. $\alpha_v\beta_6$ IHC staining of fibrotic human lung tissue showing colocalization of $\alpha_v\beta_6$+ cells and fibroblastic foci

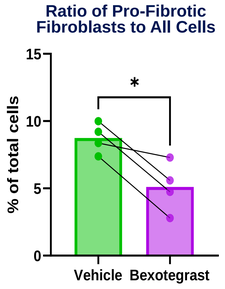
Bexotegrast Attenuates Pro-Fibrotic Stromal Cell Subpopulation in Precision-Cut Lung Slices Prepared from Fibrotic Human Lung Tissue



Marker-based Annotation (References: 1-3)

Figure 3. UMAP representation (left) of fibroblast subclusters annotated using a marker-based approach from published datasets (2,3). UMAPs (right) indicating populations with highest *COL1A1* and *CTHRC1* expression.

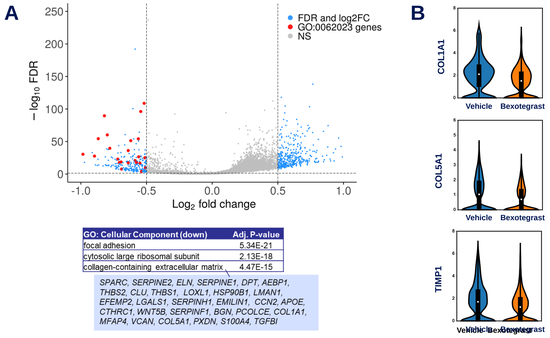
- Fibroblasts were subclustered into unique subpopulations using previously published markers[2,3] (Figure 3)
- A profibrotic fibroblast subpopulation consistent with that previously described[2] (*COL1A1*- and *CTHRC1*-high) was identified



- Bexotegrast treatment reduced the percentage of pro-fibrotic fibroblasts in PCLS compared to vehicle (Figure 4)
- Effects were consistent across PCLS prepared from different IPF donor tissues (n=4)

Figure 4. Profibrotic fibroblast subpopulation plotted as percent of total cells per PCLS sample from each individual IPF donor. Each dot represents pooled PCLS replicates from an individual donor-treatment combination. Individual donor treatment pairs are indicated by solid lines. Statistical comparisons were performed as a paired t-test (* = p<0.05)

Bexotegrast Reduces Fibrogenic Gene Expression in Fibroblasts

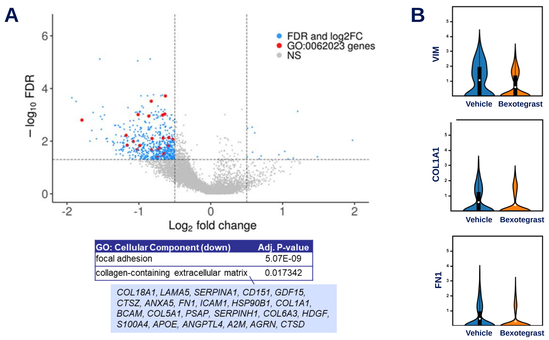


GO: Cellular Component (down)	Adj. P-value
focal adhesion	5.34E-21
cytosolic large ribosomal subunit	2.13E-18
collagen-containing extracellular matrix	4.47E-15

SPARC, SERPINE2, ELN, SERPINE1, DPT, AEBP1, THBS2, CLU, THBS1, LOXL1, HSP90B1, LMAN1, EFEMP2, LGALS1, SERPINH1, EMILIN1, CCN2, APOE, CTHRC1, WNT5B, SERPINF1, BGN, PCOLCE, COL1A1, MFAP4, VCAN, COL5A1, PXDN, S100A4, TGFBI

Figure 5. (A) Volcano plot of differentially expressed genes in fibroblasts from bexotegrast vs vehicle-treated PCLS. Thresholds for FDR<0.05 and LOG2FC>|0.5| are shown. Select gene ontology (GO):cellular component terms from pathway enrichment analysis of downregulated genes are shown, with collagen-containing extracellular matrix terms highlighted in the volcano plot. (B) Violin plots comparing *COL1A1, COL5A1, and TIMP1 expression* in fibroblasts from bexotegrast vs vehicle-treated PCLS.

Bexotegrast Reduces Fibrogenic Gene Expression in Aberrant Basaloid Cells



GO: Cellular Component (down)	Adj. P-value
focal adhesion	5.07E-09
collagen-containing extracellular matrix	0.017342

COL18A1, LAMA5, SERPINA1, CD151, GDF15, CTSZ, ANXA5, FN1, ICAM1, HSP90B1, COL1A1, BCAM, COL5A1, PSAP, SERPINH1, COL6A3, HDGF, S100A4, APOE, ANGPTL4, A2M, AGRN, CTSD

Figure 6. (A) Volcano plot of differentially expressed genes in aberrant basaloid cells from bexotegrast vs vehicle-treated PCLS. Thresholds for FDR<0.05 and LOG2FC>|0.5| are shown. Select gene ontology (GO):cellular component terms from pathway enrichment analysis of downregulated genes are shown, with collagen-containing extracellular matrix terms highlighted in the volcano plot. (B) Violin plots comparing *VIM, COL1A1, and FN1 expression* in aberrant basaloid cells from bexotegrast vs vehicle-treated PCLS.

CONCLUSION

- **Single nuclei RNASeq analysis of precision-cut lung slices generated from fibrotic patient tissue may be used to monitor the effects of anti-fibrotic drugs on specific cell populations**
- **Bexotegrast treatment reduced the expression of pro-fibrogenic genes and pathways in pro-fibrotic (CTHRC1-high) fibroblasts, as well as in aberrant basaloid cells known to express integrin $\alpha_v\beta_6$**

References: 1. Adams et al, Sci Adv. (2020); 2. Tsukui et al, Nature Comm. (2020); 3. de Rooij et al, Cardiovasc Res (2023)

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Disclosures: MA, SH, VR, BH, RA, JL, CH, ST, JS, and MD are employees and shareholders of Pliant Therapeutics