# SINGLE-CELL PROFILING DEMONSTRATES THE ANTIFIBROTIC EFFECTS OF BEXOTEGRAST ON PATHOLOGIC LUNG CELL POPULATIONS IN THE PRESENCE AND ABSENCE OF BACKGROUND THERAPY

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## RATIONALE & METHODS

Novel therapeutic strategies are needed for the treatment of idiopathic pulmonary fibrosis (IPF), as existing treatments have limited impact on life expectancy, disease progression, symptom burden, and are difficult to tolerate. Bexotegrast, a dual inhibitor of TGF-β-activating integrins $\alpha_V\beta_6$ and $\alpha_V\beta_1$, is undergoing evaluation for the treatment of IPF, where it is administered in the presence or absence of background therapy. Here, we utilized single nuclei RNAseq (snRNAseq) analysis of fibrotic human precision-cut lung slices (PCLS) to compare the pharmacodynamic effects of bexotegrast with an existing treatment for IPF.

PCLS prepared from IPF patient lung explants (n=5) were cultured for 7 days in the presence of bexotegrast (200nM), nintedanib (75nM), bexotegrast + nintedanib, or vehicle. Nuclei were isolated 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 (|Log2FC| > 0.25, FDR < 0.05) for each treatment relative to vehicle.

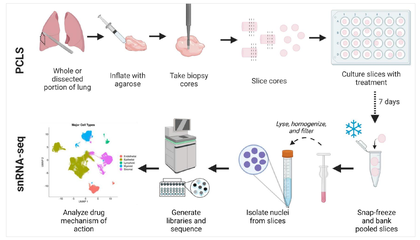
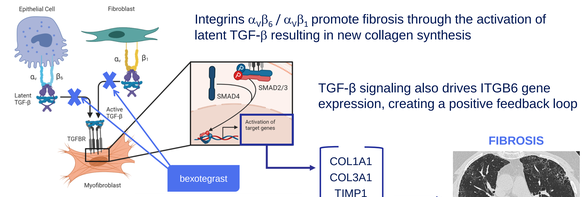



**Figure 1**. Generation, culture of PCLS, and snRNAseq analysis

## MECHANISM OF ACTION OF BEXOTEGRAST IN IPF

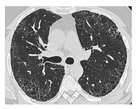



Integrins $\alpha_V\beta_6$ / $\alpha_V\beta_1$ promote fibrosis through the activation of latent TGF-β resulting in new collagen synthesis

TGF-β signaling also drives ITGB6 gene expression, creating a positive feedback loop

COL1A1
COL3A1
TIMP1
CCN2
ITGB6
…

FIBROSIS



Bexotegrast is an oral, once-daily dual inhibitor of integrins $\alpha_V\beta_6$ and $\alpha_V\beta_1$

## RESULTS

### Single nuclei RNAseq analysis of PCLS from fibrotic human lung explants enables analysis of $\alpha_V\beta_6$ and $\alpha_V\beta_1$ expressing sub-populations

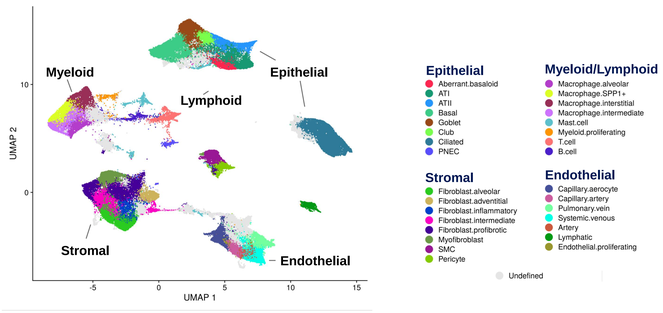
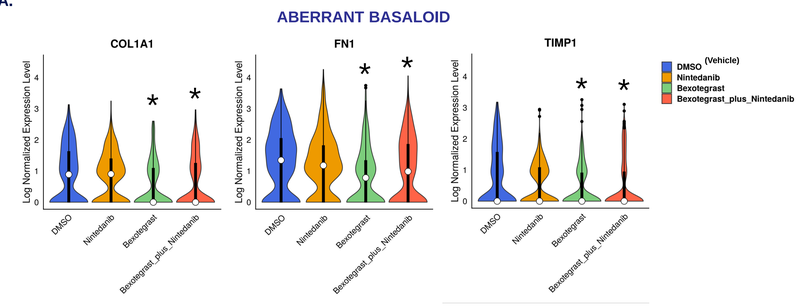



**Figure 2.** PCLS snRNAseq dataset summarized as a UMAP with annotated populations including aberrant basaloid, ATI, ATII, myofibroblasts, and profibrotic fibroblasts.

### Bexotegrast alone or in the presence of nintedanib significantly reduced profibrotic gene expression (e.g. *COL1A1, FN1, TIMP1*) in aberrant basaloid cells ($\alpha_V\beta_6$-expressing), while treatment with nintedanib alone did not.

A.




### Differential gene expression analysis of PCLS showed that bexotegrast, alone or in the presence of nintedanib, significantly reduced type 1 collagen (*COL1A1)* gene expression in fibroblasts ($\alpha_V\beta_1$-expressing), while treatment with nintedanib alone did not.

B.

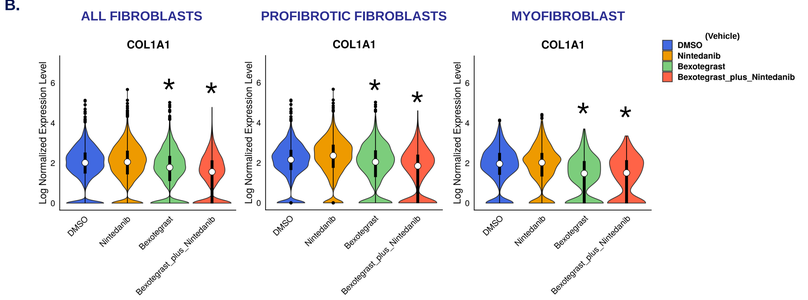



**Figure 3. (A)** Violin plots for aberrant basaloid cells showing gene expression for *COL1A1*, *FN1*, and *TIMP1* broken down by treatment group. **(B)** Violin plots for profibrotic fibroblasts, myofibroblasts, and all fibroblasts showing gene expression for *COL1A1* broken down by treatment group. Asterisks indicate significance relative to DMSO |log2FC| > 0.25, FDR < 0.05.

### Pathway enrichment analysis also showed that bexotegrast reduced genes associated with GO term collagen-containing extracellular matrix across multiple fibroblast subtypes, including *CTHRC1*$^+$-profibrotic fibroblasts.

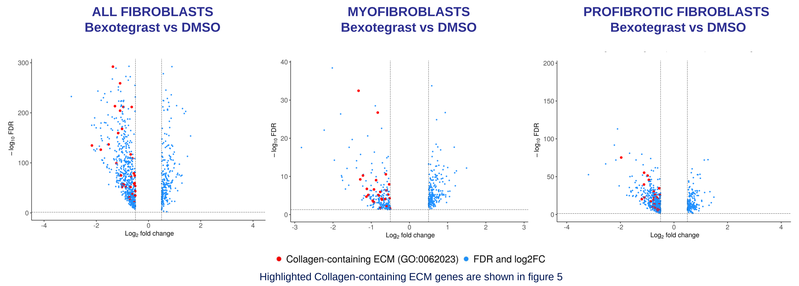



**Figure 4.** Volcano plots for Bexotegrast vs DMSO for all fibroblasts, myofibroblasts, and profibrotic fibroblasts. All differentially expressed genes shown by following criteria: |log2FC| > 0.5, FDR < 0.05. Downregulated genes for GO:Collagen-containing extracellular matrix (ECM) are highlighted in red.

### Treatment of PCLS with bexotegrast in the presence of nintedanib resulted in further downregulation of several ECM-related genes in profibrotic fibroblasts, including *COL1A1*.

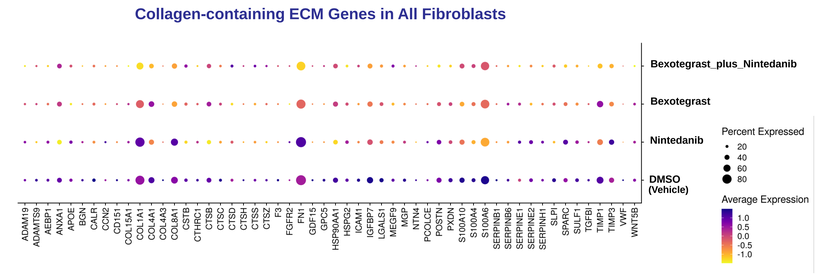



**Figure 5. (A)** Dot plot of all significant differentially expressed GO:collagen-containing ECM genes across all groups in all fibroblasts. Color and size of dots indicate average expression and percent of cells expressed respectively.

### Consistent with the cellular expression pattern of nintedanib drug targets *VEGFR1*, *VEGFR2*, and *PDGFRB*, greater numbers of DEGs were observed in smooth muscle cells, pericytes, artery, and capillary arterial cells in nintedanib-treated PCLS than bexotegrast-treated PCLS.

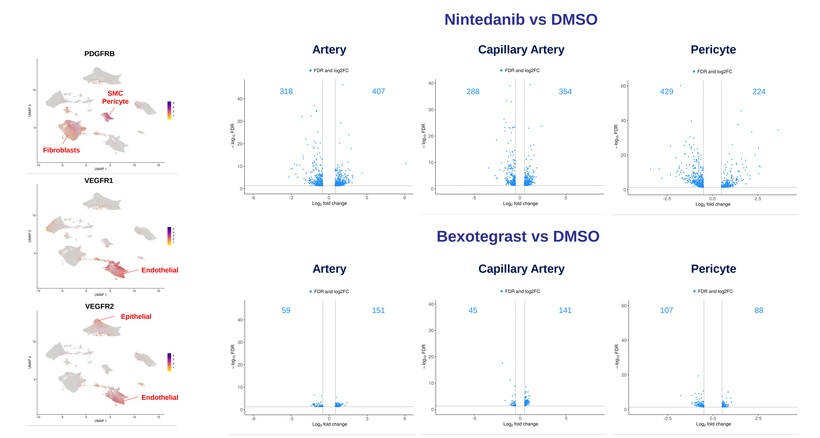



**Figure 6.** UMAP expression plots showing expression of *PDGFRB*, *VEGFR1*, and *VEGFR2*. Volcano plots summarize the differential effects of bexotegrast and nintedanib relative to vehicle in artery, capillary artery, and pericyte cell groups.

## CONCLUSIONS

- **Treatment of fibrotic human PCLS with bexotegrast or nintedanib showed distinct cell-specific pharmacodynamic profiles, with bexotegrast found to significantly reduce TGF-β-regulated gene expression (e.g. *COL1A1*) in $\alpha_V\beta_6$- and $\alpha_V\beta_1$-expressing pathologic lung cell populations such as profibrotic fibroblasts and aberrant basaloid cells.**
- **These findings demonstrate the potential for bexotegrast to provide antifibrotic benefit as a monotherapy or in combination with currently approved treatments for IPF.**

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**Disclosures:** MA, RA, SH, VR, HF, CH, ST, JY, JS, and MD are current or former employees and shareholders of Pliant Therapeutics