# MULTI-OMIC APPROACHES TO INVESTIGATE THE ANTI-FIBROTIC EFFECTS OF BEXOTEGRAST ON HUMAN PCLS


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## ABSTRACT


### Background and Aims

Recent advances in transcriptomic methodologies at the single-cell and spatial level have enabled researchers to gain important insights into core pathological drivers in the fibrotic niche. Here we utilized single nuclei RNA-seq (snRNA-seq) and spatial analysis of precision-cut lung slices (PCLS) from IPF patients to evaluate the impact of bexotegrast, a dual inhibitor of TGF-β-activating integrins ($\alpha_V\beta_6$ and $\alpha_V\beta_1$), on ECM-related (matrisome) gene expression, and fibrogenic pathways.

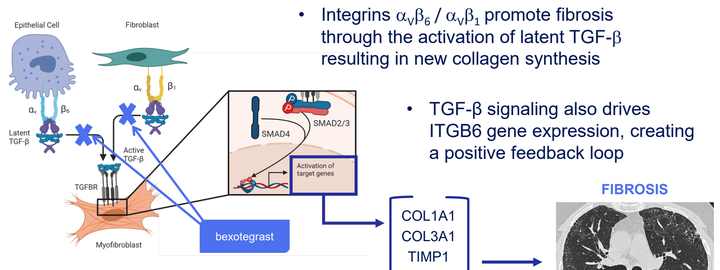
### Methods

PCLS prepared from fibrotic human lung explants were cultured for 7 days in the presence of bexotegrast, TGF-β receptor I kinase inhibitor (ALK5i: R-268712), or vehicle (DMSO). Single nuclei were isolated from PCLS and processed for snRNA-seq (10x Chromium Next GEM 3') or Xenium spatial transcriptomics. Matrisome gene expression and fibrotic pathway enrichment were performed on annotated cell subpopulations and spatial niches. Differentially expressed genes were defined as (|Log2FC| > 0.25, FDR < 0.05).

### Results

Differential gene expression analysis revealed a significant bexotegrast-mediated reduction of collagen family genes (e.g. *COL1A1* and *COL5A1*) across epithelial cell and fibroblast subpopulations, including aberrant basaloid and myofibroblast. Core matrisome analysis showed bexotegrast reduced expression of ECM glycoprotein and proteoglycan family genes, including *CTHRC1, THBS1, SPP1,* and *BGN*. Matrisome-related effects of bexotegrast were observed within spatially defined profibrotic niches. Bexotegrast also reduced expression of EMT pathway and senescence-associated signature genes in aberrant basaloid cells. Through comparative snRNA-seq analysis, bexotegrast showed distinct pharmacodynamic profiles compared to ALK5 inhibition.


## 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
- Bexotegrast, a dual inhibitor of integrins $\alpha_V\beta_6$ and $\alpha_V\beta_1$, is the subject of late-stage evaluation in the BEACON-IPF study

**Figure 1**. Schematic diagram summarizing the mechanism of action of bexotegrast in IPF

## PRECISION CUT LUNG SLICE (PCLS) PLATFORM

Fibrotic lung tissue explanted from patients with IPF at transplant is used to evaluate the effects of novel anti-fibrotic agents. snRNAseq approaches enable the analysis of drug effects on pathologic cell populations at the single cell level.

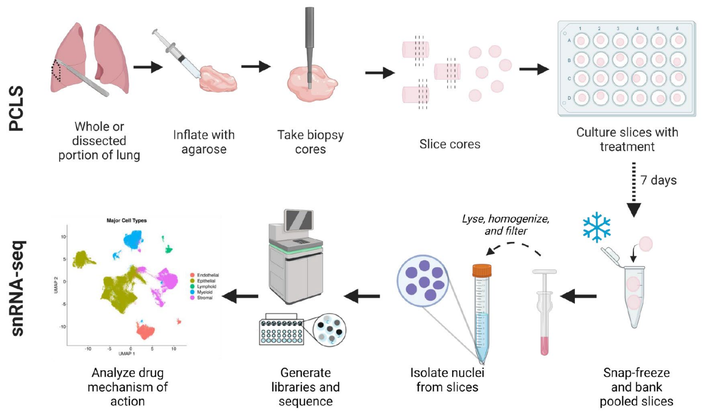



**Figure 2**. Schematic diagram summarizing the generation and culture of PCLS from fibrotic human lung tissue, followed by single nuclei isolation, 10x snRNA library generation, sequencing, and analysis to analyze drug mechanism of action.

The application of single cell transcriptomic techniques in PCLS enables assessment of drug effects on rare and disease-relevant cell sub-populations, assisting with drug MOA studies in IPF

## snRNA-SEQ ANALYSIS OF PCLS

Single nuclei RNAseq analysis was performed on PCLS from IPF lungs following culture with vehicle, bexotegrast, or ALK5 inhibitor

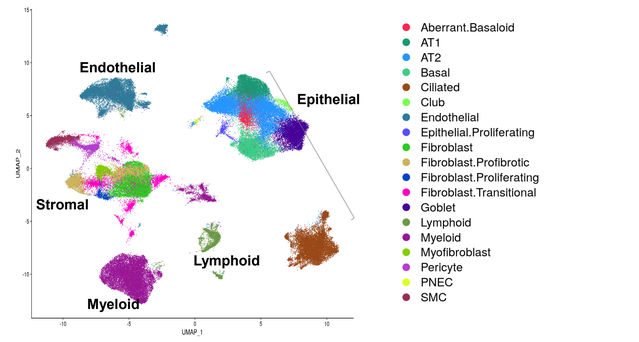



**Figure 3.** PCLS snRNAseq dataset summarized as a UMAP with annotated populations including aberrant basaloid, ATI, ATII, myofibroblasts, and profibrotic fibroblasts.

Collagen family members including *COL1A1* and *COL1A5* were downregulated in fibroblasts and aberrant basaloid cells in PCLS following treatment with bexotegrast. Top downregulated GO terms included focal adhesion and collagen containing extracellular matrix.

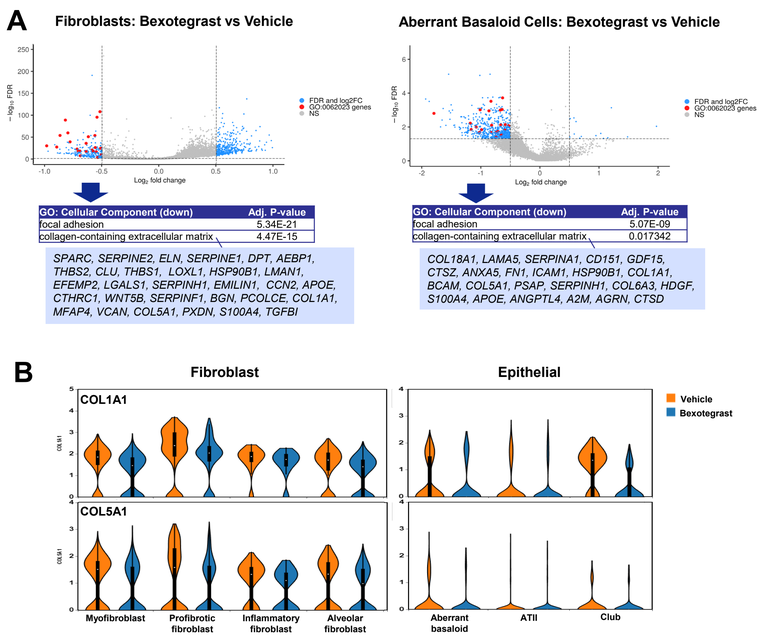



**Figure 4**. (A) Volcano plots of differential gene expression analysis comparing bexotegrast vs vehicle in fibroblasts and aberrant basaloid cells. Individual downregulated genes are highlighted in red and listed below. (B) Violin plots of COL1A1 and COL5A1 expression broken down by treatment in fibroblast and epithelial cell subtypes.

## MATRISOME ANALYSIS IN PCLS

Sub-analysis of gene expression for core matrisome proteins was performed. DGE analysis highlights downregulation of collagens, ECM glycoprotein, and proteoglycans in profibrotic fibroblasts and aberrant basaloid cells

**A**

| Category | Family | Matrisome Database | Our Dataset (avg expression > 0) |
|---|---|---|---|
| Core matrisome | Collagens | 45 | 44 |
| | ECM Glycoproteins | 208 | 201 |
| | Proteoglycans | 37 | 35 |
| Total Matrisome | | | 280 |
| Non-matrisome | | | 28,949 |

DGE analysis of core matrisome genes

**B**

| | Collagen | | | ECM Glycoproteins | | | Proteoglycans | | |
|---|---|---|---|---|---|---|---|---|---|
| Cell Type | Total | Down | Up | Total | Down | Up | Total | Down | Up |
| **Aberrant Basaloid** | **19** | **9** | **0** | **51** | **6** | **0** | **4** | **1** | **0** |
| Basal | 13 | 3 | 0 | 51 | 9 | 2 | 5 | 1 | 0 |
| **Fibroblast Profibrotic** | **25** | **11** | **3** | **89** | **32** | **8** | **10** | **6** | **0** |
| Myofibroblast | 24 | 3 | 1 | 86 | 22 | 10 | 12 | 3 | 0 |
| Fibroblast Adventitial | 25 | 0 | 0 | 87 | 0 | 3 | 12 | 0 | 1 |

**C**

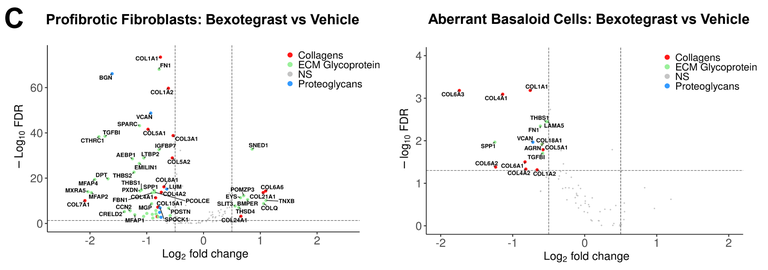
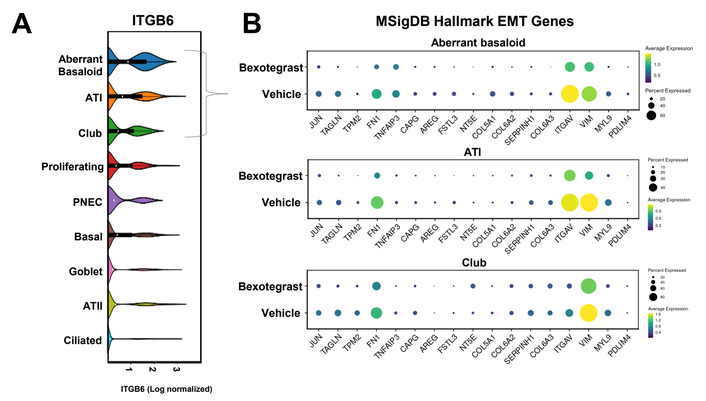



**Figure 5**. (A) Table summarizing matrisome family genes present in our dataset. DGE analysis of core matrisome categories summarized in table (B), showing number of down and upregulated genes by cell type and category. (C) Volcano plots showing significantly down and upregulated genes in profibrotic fibroblasts and aberrant basaloid cells.

## ANALYSIS OF ITGB6+ CELLS AND NICHES

In addition to matrisome effects, bexotegrast downregulated EMT pathway-related genes in *ITGB6* expressing cells




7 distinct spatial niches in PCLS were identified through Xenium spatial analysis. Analysis of top *ITGB6* expressing niches showed reduction of matrix proteins following bexotegrast treatment.

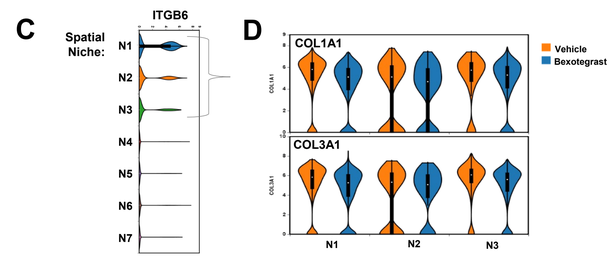



**Figure 6.** (A) Violin plots breaking down expression by epithelial cell type. (B) Significantly downregulated EMT pathway genes are shown in expression dot plots for aberrant basaloid, ATII, and club cells. (C) Violin plots breaking down *ITGB6* expression by spatial niches N1-N7 (D) Violin plots for *COL1A1* and *COL3A1* gene expression in niches N1, N2, and N3.

## PHARMACODYNAMIC CELL PROFILING

Comparative analysis of bexotegrast vs ALK5i treatment showed distinct pharmacodynamic profiles, with bexotegrast targeting anti-fibrotic activity to pathologic cell populations

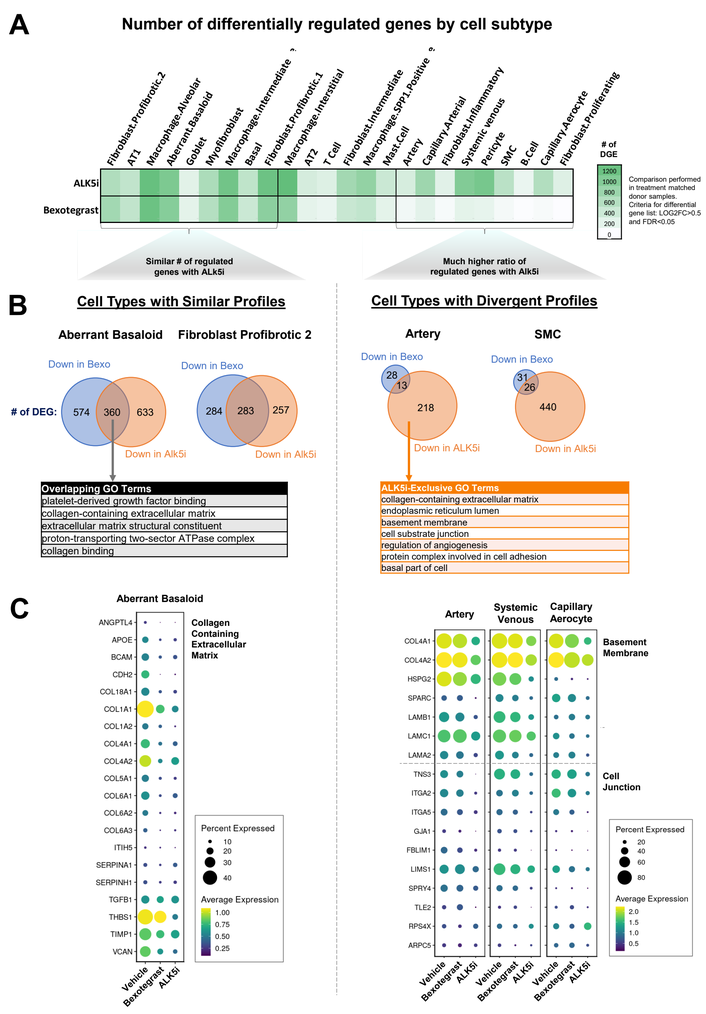



**Figure 7**. (A) Heatmap summarizing number of differentially regulated genes by cell subtype comparing bexotegrast and ALK5i to vehicle. (B) Venn diagrams summarizing number of downregulated DEG for example cell populations. Top unique GO terms are summarized. (C) Expression dot plots for significant genes from select GO terms.

## CONCLUSIONS

- **Treatment of fibrotic human PCLS with dual $\alpha_V\beta_6/\alpha_V\beta_1$ integrin inhibitor bexotegrast resulted in clear reductions in ECM-related gene expression within specific cell populations thought to be important to IPF pathology**
- **Bexotegrast showed clear pharmacodynamic differences from ALK5 inhibition in fibrotic human PCLS, targeting reduction of TGF-β signaling pathways to fibrogenic cell populations.**
- **Late-stage evaluation of bexotegrast is currently underway in the enrolling BEACON-IPF study (NCT06097260)**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MA, SH, VR, RA, JY, EB, CH, JS, and MD are employees and shareholders of Pliant Therapeutics BH, and ST are former employees of Pliant Therapeutics