BEXOTEGRAST TARGETS TGF-β INHIBITION TO SPECIFIC CELL TYPES IN THE FIBROTIC HUMAN LUNG

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RATIONALE & METHODS

TGF-β is a master regulator of fibrotic disease, however systemic inhibition of TGF-β signaling has limited utility as a therapeutic strategy due to the pleiotropic nature of TGF-β in regulating homeostatic cellular pathways. Targeting TGF-β inhibition strategies to the cell populations most involved in fibrogenesis is therefore desired. Bexotegrast, a dual inhibitor of TGF-β-activating integrins ($\alpha_v\beta6$ and $\alpha_v\beta1$) expressed by pathologic cell populations in fibrotic lungs, is currently in development for the treatment of idiopathic pulmonary fibrosis (IPF). Here we utilized single nuclei RNA-seq (snRNA-seq) analysis of precision-cut lung slices (PCLS) from IPF patients to test the hypothesis that bexotegrast inhibits TGF-β signaling in a restricted cell-specific manner.

PCLS prepared from fibrotic human lung explants were cultured for 7 days in the presence of bexotegrast (bexo; 200nM), TGF-β receptor I kinase inhibitor (ALK5i: R-268712; 1μM), or vehicle (DMSO). Single nuclei were isolated from n=6 slices per treatment per donor and processed for snRNA-seq using 10x Chromium Next GEM Single Cell 3' HT kits. Comparative differential gene expression and pathway enrichment analysis were performed on annotated cell subpopulations. Differentially expressed genes (DEGs) were defined as (|Log2FC| > 0.5, FDR < 0.05) for each treatment relative to vehicle in donor-matched samples.

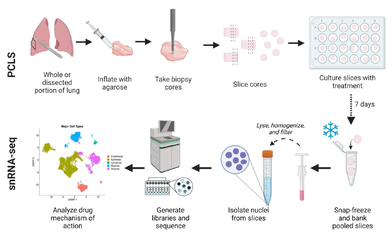
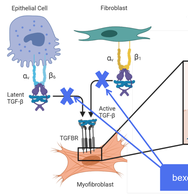


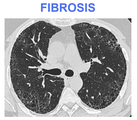
Figure 1. Generation, culture of PCLS, and snRNAseq analysis

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**
- **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**

COL1A1
COL3A1
TIMP1
CCN2
ITGB6
…



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**

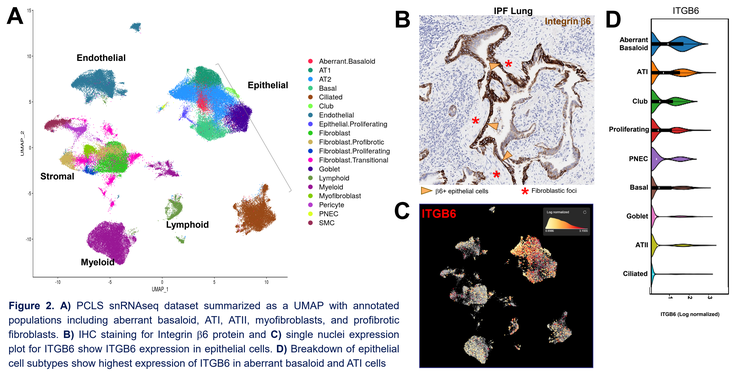


Figure 2. A) PCLS snRNAseq dataset summarized as a UMAP with annotated populations including aberrant basaloid, ATI, ATII, myofibroblasts, and profibrotic fibroblasts. **B)** IHC staining for Integrin β6 protein and **C)** single nuclei expression plot for ITGB6 show ITGB6 expression in epithelial cells. **D)** Breakdown of epithelial cell subtypes show highest expression of ITGB6 in aberrant basaloid and ATI cells

- **Differential gene expression analysis showed overlapping effects of bexotegrast and ALK5i in epithelial and mesenchymal cell subpopulations known to express $\alpha_v\beta_6$ or $\alpha_v\beta_1$, with attenuated effects of bexotegrast relative to ALK5i observed in other cell types**

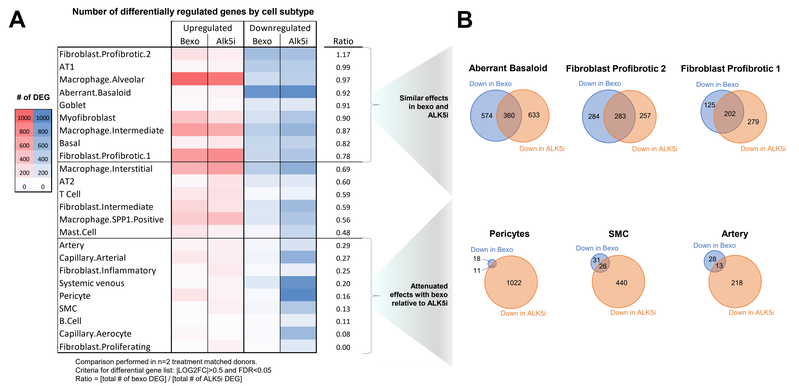


Figure 3. A) Number of differentially expressed genes per group comparison were compared across all cell types and summarized. The table is sorted by the bexo:ALK5i #DEG ratio. **B)** Cell populations with either overlapping effects or attenuated effects with bexo relative to ALK5i are further broken down by venn diagrams summarizing DEG for downregulated genes in bexotegrast or ALK5i (compared to vehicle).

- **Similar numbers of differentially expressed genes observed in aberrant basaloid cells and profibrotic fibroblasts following treatment with bexotegrast and ALK5i treatment indicate integrin-driven TGF-β signaling in those cell populations**

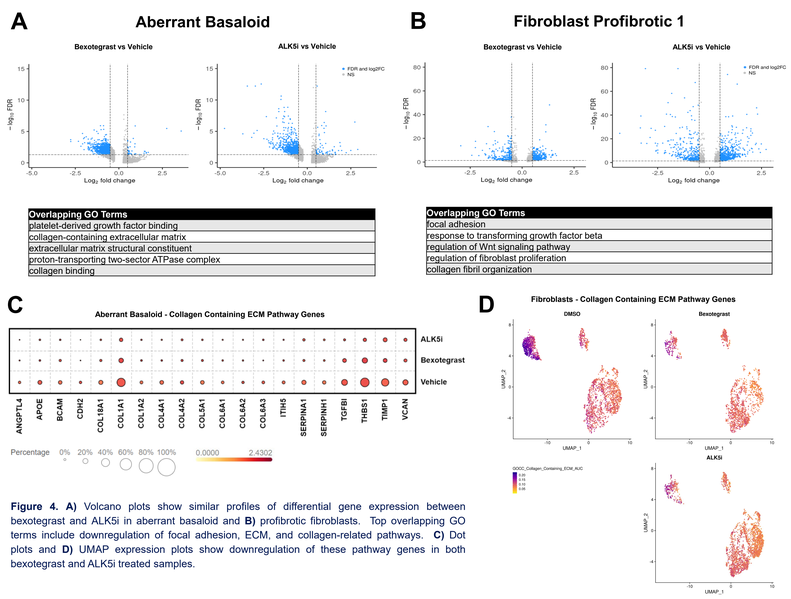


Figure 4. A) Volcano plots show similar profiles of differential gene expression between bexotegrast and ALK5i in aberrant basaloid and **B)** profibrotic fibroblasts. Top overlapping GO terms include downregulation of focal adhesion, ECM, and collagen-related pathways. **C)** Dot plots and **D)** UMAP expression plots show downregulation of these pathway genes in both bexotegrast and ALK5i treated samples.

- **Diminished effect of bexotegrast on pericyte, smooth muscle cell and vascular endothelial cell gene expression relative to ALK5i indicates non-integrin driven TGF-β signaling in those cell populations**

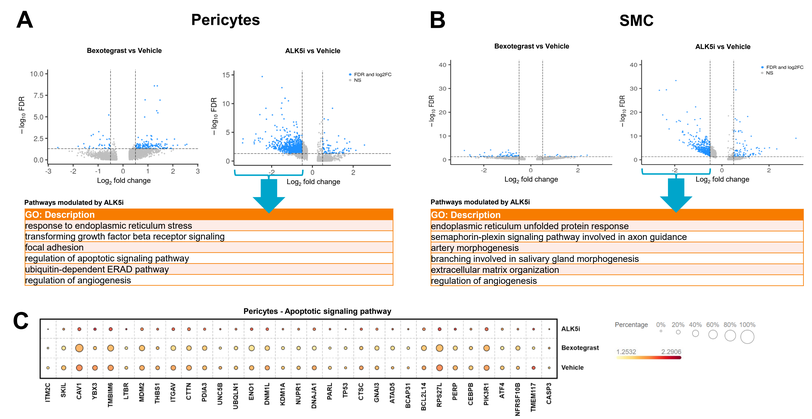


Figure 5. A) Volcano plots summarize the differential effects of bexotegrast and ALK5i relative to vehicle in pericytes and **B)** SMCs. The pathways modulated by ALK5i are shown. ALK5i treatment downregulates a number of pathways related to TGF-β signaling, focal adhesion, apoptosis, and angiogenesis. In contrast, bexotegrast did not modulate these pathways significantly. **C)** Dot plot shows differential effects of ALK5i and bexotegrast on apoptotic signaling pathway genes on pericytes.

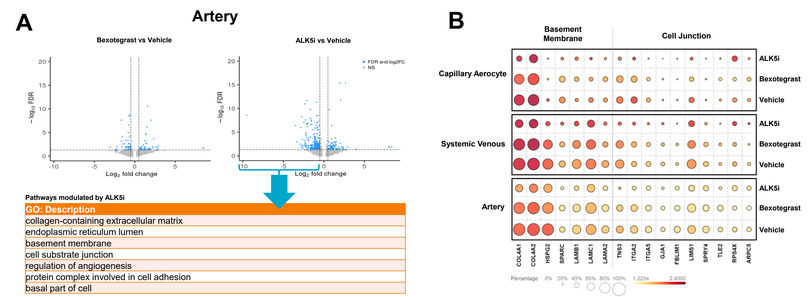


Figure 6. A) Volcano plots summarize the differential effects of bexotegrast and ALK5i relative to vehicle in arterial endothelial cells. Angiogenesis, basement membrane, and cell substrate junction are among the top pathways uniquely modulated by ALK5i and not by bexotegrast. **B)** Dot plot shows differential effects of ALK5i and bexotegrast on basement membrane and cell substrate junction pathway genes.

- **Effects of bexotegrast were found to be diminished relative to ALK5i in cells previously linked with TGF-β inhibition associated toxicities[1,2]**

CONCLUSIONS

- **Dual $\alpha_v\beta_6/\alpha_v\beta_1$ integrin inhibition with bexotegrast showed clear pharmacodynamic differences from ALK5 inhibition in fibrotic human PCLS, targeting reduction of TGF-β signaling pathways to fibrogenic cell populations.**
- **These findings provide valuable insight into the mechanism of action of bexotegrast and demonstrate the utility of this approach for distinguishing the cell-specific effects of anti-fibrotic therapies.**
- **Late-stage evaluation of bexotegrast is currently underway in the enrolling BEACON-IPF study (NCT06097260)**

References: 1. Sounni, N.E. et al. Dis Model Merch. 2010; 2. Mitra, M.S. et al. Toxicol Sci. 2020

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