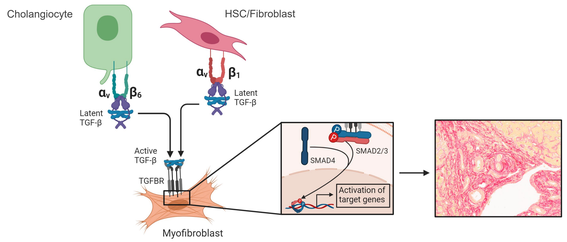
Dual $\alpha_V\beta_6/\alpha_V\beta_1$ integrin inhibitor bexotegrast attenuates profibrogenic gene expression across multiple pathologic cell types in human liver explant tissue with biliary fibrosis

Johanna Schaub, Steve Ho, Chris Her, Richard Ahn, Vikram Rao, Mahru An, Scott Turner, Martin Decaris | Pliant Therapeutics, Inc., South San Francisco, CA, USA

BACKGROUND

Transforming growth factor-beta (TGF-β) signaling is a key driver of liver fibrosis, 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. In primary sclerosing cholangitis (PSC), integrins expressed on injured cholangiocytes ($\alpha_V\beta_6$) and myofibroblasts ($\alpha_V\beta_1$) regulate TGF-β activity. Bexotegrast (PLN-74809), a dual inhibitor of integrins $\alpha_V\beta_6$ and $\alpha_V\beta_1$, is currently in clinical development for the treatment of PSC. To examine the effects of bexotegrast on the pathogenesis of PSC, we combined 10x single nuclei RNA sequencing (snRNA-seq) with the precision-cut liver slice (PCLivS) platform to characterize the response of unique cell populations in fibrotic PSC and primary biliary cholangitis (PBC) PCLivS to bexotegrast treatment.

Figure 1. Role of Integrins $\alpha_V\beta_6$ and $\alpha_V\beta_1$ in Biliary Fibrosis

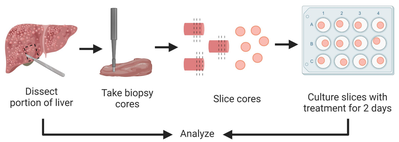


METHODS

Precision-cut Liver Slices

Liver explants were collected from patients with PSC (n = 3) and PBC (n = 1) at the time of transplant. PCLivS were generated and cultured for two days in the presence of bexotegrast or vehicle (DMSO). A TGF-β receptor I kinase inhibitor (ALK5i; R-268712) that blocks TGF-β signaling was also evaluated as a control.

Figure 2. Generation and Culture of PCLivS



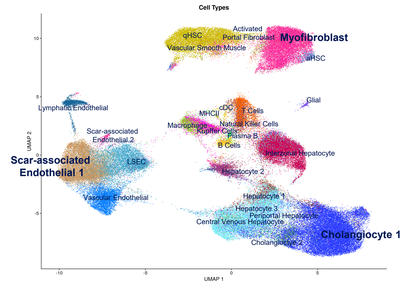
snRNA-seq and Analysis

Nuclei were isolated from two pooled slices per treatment using a combination of detergent-based lysis, mechanical disruption, and filtration. Nuclei were processed for single nuclear barcoding using 10x Chromium Next GEM 3' HT kits. Resulting libraries were sequenced, processed using CellRanger, and analyzed using Seurat. Custom annotation of cell types was performed using gene markers from published data sets[1,2]. Differential gene expression was determined using a non-parametric Wilcoxon rank sum test. Analysis focused on genes with $|\log2\text{ fold-change}| > 0.5$ and an FDR < 0.05. Pathway enrichment analysis was performed with Enrichr[3].

RESULTS

Identification of Cells Present in PCLivS

Figure 3. snRNA-seq Analysis Identified Major Hepatic Cell Populations



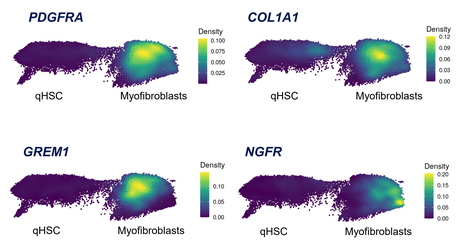
- **Cholangiocyte 1**
- Cholangiocyte 2
- Central Venous Hepatocyte
- Hepatocyte 1
- Hepatocyte 2
- Hepatocyte 3
- Interzonal Hepatocyte
- Periportal Hepatocyte
- LSEC
- Lymphatic Endothelial
- **Scar-associated Endothelial 1**
- Scar-associated Endothelial 2
- Vascular Endothelial
- Glial
- Activated Portal Fibroblast
- aHSC
- **Myofibroblast**
- qHSC
- Vascular Smooth Muscle
- B Cells
- cDC
- Natural Killer Cells
- Plasma B
- T Cells
- Kupffer Cells
- MHCII
- Macrophage

- Epithelial, endothelial, mesenchymal, myeloid, and lymphoid cells were identified in PCLivS
- Multiple mesenchymal, endothelial, and epithelial subpopulations were present including those previously identified to be relevant to the pathogenesis of liver fibrosis

Uniform manifold approximation and projection (UMAP) plots of filtered and normalized unique barcodes from 10x-sequenced single nuclei preparations of PCLivS samples from all donors and treatments. Cell type annotation for major cell type categories is indicated by color legend.

Evaluation of Differentially Expressed Genes in Individual Cell Populations

Figure 4. snRNA-seq Analysis Identified *PDGFRA*⁺ Myofibroblast Population

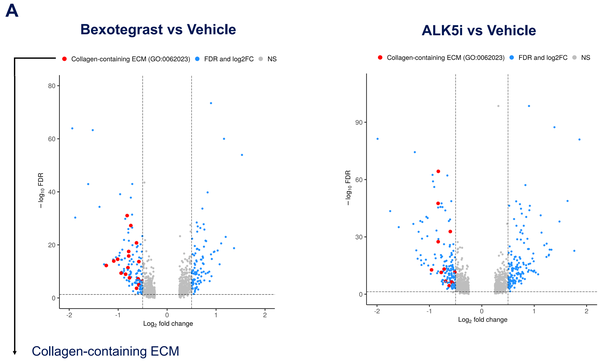


- Myofibroblasts had high expression of type I collagen (*COL1A1*) and expressed markers of activated portal fibroblasts (*GREM1*⁺) and HSCs (*NGFR*⁺)
- Differential gene expression analysis of bexotegrast-treated or ALK5i-treated versus vehicle-treated PCLivS showed downregulation of genes related to extracellular matrix and collagen fibril organization
- Bexotegrast significantly reduced myofibroblast expression of *COL1A1* to a similar degree as ALK5i

UMAPs density plots indicating *PDGFRA*, *COL1A1*, *GREM1*, and *NGFR* expression

Figure 5. Bexotegrast Treatment Significantly Decreased Profibrogenic Pathways in Myofibroblasts

A

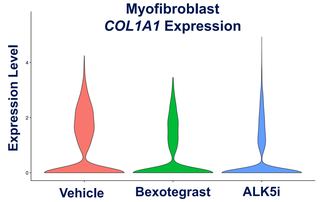


TGFB1, COL16A1, BGN, FBLN1, LTBP2, AEBP1, SULF1, LOXL1, COL1A1, CCDC80, CXCL12, SRPX2, ADAMTS3, COL5A1, COL7A1, MGP, ANXA6, COL8A1, IGFBP7, CTSC

B

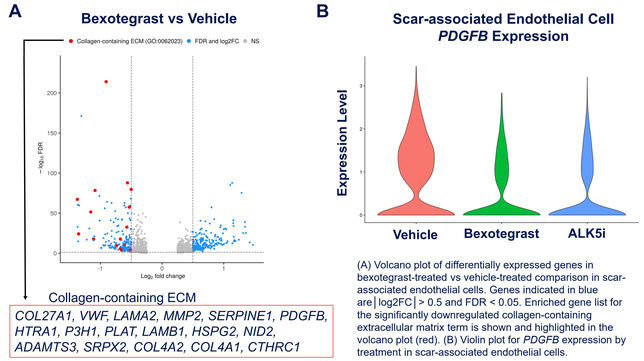
GO: Biological Process	Adj. P-Value
extracellular matrix organization (GO:0030198)	1.28E-05
extracellular structure organization (GO:0043062)	1.19E-03
external encapsulating structure organization (GO:0045229)	1.19E-03
collagen fibril organization (GO:0030199)	1.19E-03

C



(A) Volcano plots of differentially expressed genes in bexotegrast-treated or ALK5i-treated vs vehicle-treated comparison in myofibroblasts. Genes indicated in blue are $|\log2FC| > 0.5$ and FDR < 0.05. Enriched genes for the significantly downregulated collagen-containing extracellular matrix term are highlighted in the volcano plots (red) and listed for the bexotegrast-treated comparison. (B) Select GO:biological process terms from pathway enrichment analysis of downregulated genes for the bexotegrast-treated comparison ($|\log2FC| > 0.5$, FDR < 0.05). (C) Violin plot for *COL1A1* expression by treatment in myofibroblasts.

Figure 6. Bexotegrast Treatment Significantly Reduced Profibrogenic Gene Expression in Scar-associated Endothelial Cells



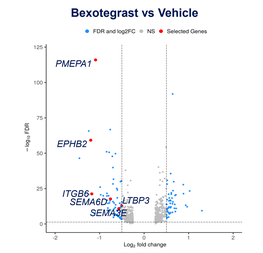
COL27A1, VWF, LAMA2, MMP2, SERPINE1, PDGFB, HTRA1, P3H1, PLAT, LAMB1, HSPG2, NID2, ADAMTS3, SRPX2, COL4A2, COL4A1, CTHRC1

(A) Volcano plot of differentially expressed genes in bexotegrast-treated vs vehicle-treated comparison in scar-associated endothelial cells. Genes indicated in blue are $|\log2FC| > 0.5$ and FDR < 0.05. Enriched gene list for the significantly downregulated collagen-containing extracellular matrix term is shown and highlighted in the volcano plot (red). (B) Violin plot for *PDGFB* expression by treatment in scar-associated endothelial cells.

- Scar-associated endothelial cells have previously been shown to be enriched in fibrotic human livers and present in the fibrotic niche[1]
- Differential gene expression analysis of bexotegrast-treated versus vehicle-treated PCLivS showed downregulation of genes related to extracellular matrix
- Bexotegrast significantly reduced expression of *PDGFB*, suggesting a disruption of profibrogenic signaling from endothelial cells to myofibroblasts

Figure 7. Bexotegrast Treatment Reduced TGF-β-related Gene Expression in Cholangiocytes

- Differential gene expression analysis of bexotegrast-treated versus vehicle-treated PCLivS showed downregulation of genes related to TGF-β signaling and liver fibrosis



Volcano plot of differentially expressed genes in bexotegrast-treated vs vehicle-treated comparison in cholangiocytes. Genes indicated in blue are $|\log2FC| > 0.5$ and FDR < 0.05. Selected genes are highlighted in the volcano plot (red).

CONCLUSIONS

- **Bexotegrast treatment resulted in clear reductions in profibrogenic gene expression across multiple pathologic cell populations in PCLivS from liver explants with biliary fibrosis**
- **The anti-fibrotic effect from bexotegrast was similar to ALK5i demonstrating the importance the $\alpha_V\beta_6/\alpha_V\beta_1$ integrin-TGF-β activation pathway in fibrotic biliary disease**
- **These data support ongoing clinical studies evaluating the anti-fibrotic activity of bexotegrast in PSC (see abstract #5008)**

References: 1. Ramachandran P, et al. Nature 2019; 575(7783), 2. Andrews TS, et al. *Hepatol Commun* 2022; 6(4). 3. Chen EY, et al. *BMC Bioinformatics* 2013; 128(14).

Contact information: JSchaub@pliantrx.com

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