Dual alpha-v/beta-6 and alpha-v/beta-1 integrin inhibitor bexotegrast targets TGF-beta inhibition to specific cell types in human liver explant tissue with biliary fibrosis

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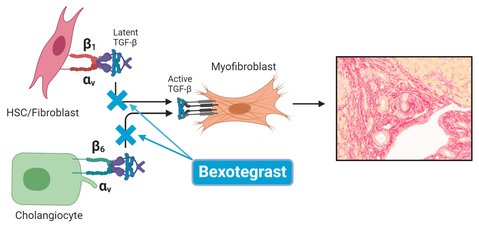
BACKGROUND

TGF-β is a master regulator of fibrotic disease, however systemic inhibition of TGF-β signaling has limited therapeutic utility due to the pleiotropic nature of TGF-β in regulating homeostatic cellular pathways. Bexotegrast (PLN-74809) is a dual inhibitor of the TGF-β-activating integrins $\alpha_v\beta_6$ (expressed by injured cholangiocytes) and $\alpha_v\beta_1$ (expressed by myofibroblasts) currently in clinical development for the treatment of primary sclerosing cholangitis (PSC) in the INTEGRIS-PSC study (NCT04480840).[1] The aim of this study was to combine 10x single nuclei RNA sequencing (snRNA-seq) with the precision-cut liver slice (PCLivS) platform to test the hypothesis that bexotegrast targets reduction of TGF-β signaling to specific liver cell types in biliary fibrosis.

Figure 1. Bexotegrast Reduces Biliary Fibrosis by Inhibiting Integrin $\alpha_V\beta_6$- and $\alpha_V\beta_1$-mediated TGF-β Activation



METHODS

snRNA-seq Analysis of Precision-Cut Liver Slices

PCLivS generated from PSC (n = 3) and PBC (n = 1) liver explants were cultured for 2 days in the presence of bexotegrast, TGF-β receptor 1 kinase inhibitor (ALK5i; R-268712), or vehicle (DMSO). Nuclei were isolated from 2 pooled slices per treatment and processed for single nuclear barcoding using 10x Chromium Next GEM 3' HT kits. Comparative differential gene expression and pathway enrichment analysis were performed on annotated cell subpopulations. Differentially expressed genes (DEGs) were defined as genes with absolute log2 fold-change ≥ 0.5 and an FDR ≤ 0.05.

Figure 2. Generation and Culture of PCLivS

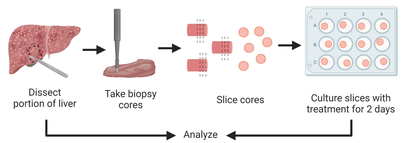
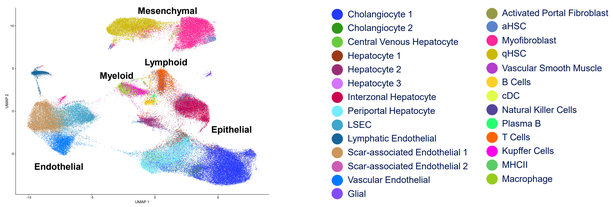


Figure 3. snRNA-seq Analysis of Hepatic Cell Populations in PCLivS



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. aHSC, activated hepatic stellate cell; cDC, conventional dendritic cell; LSEC, liver sinusoidal endothelial cell; MHCII, major histocompatibility complex class II; qHSC, quiescent hepatic stellate cell

- Major hepatic cell populations were identified in PCLivS, including myofibroblasts, cholangiocytes, hepatocytes, Kupffer cells and endothelial cells

RESULTS

- A comparison of the number of DEGs across cell populations and treatments revealed distinct response profiles from bexotegrast- and ALK5i-treated PCLivS **(Figure 4)**
- Target cells expressing $\alpha_v\beta_6$ and $\alpha_v\beta_1$, as well as endothelial cells showed a similar response to bexotegrast and ALK5i, reducing expression of genes related to extracellular matrix organization **(Figure 5)**
- In response to ALK5i, immune cells upregulated genes related to immune signaling, consistent with an immunomodulatory role for TGF- β. In contrast, bexotegrast had an attenuated effect on immune cell gene expression **(Figure 6)**

Figure 4. Differentially Expressed Genes Across Cell Types and Treatments

# DEG	
Up	Down
400	400
300	300
200	200
100	100
50	50
1	1
0	0

	Bexo Down	ALK5i Down	Bexo Up	ALK5i Up	Ratio
Vascular Endothelial					1.28
Cholangiocyte 1					1.18
LSEC					0.94
Interzonal Hepatocyte					0.92
Scar-associated Endothelial 1					0.91
Myofibroblast					0.72
Lymphatic Endothelial					0.59
qHSC					0.57
Periportal Hepatocyte					0.44
T Cells					0.41
Kupffer Cells					0.36
Macrophage					0.33
Hepatocyte 2					0.22
Hepatocyte 1					0.15
B Cells					0.09
Natural Killer Cells					0.01

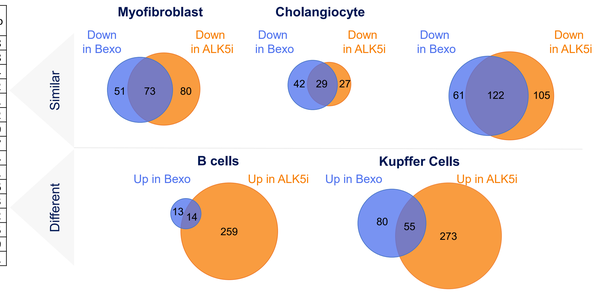
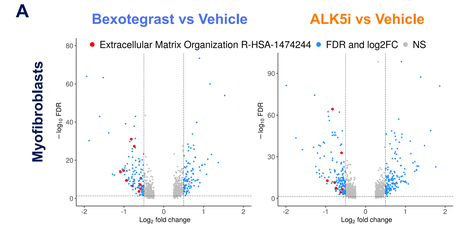
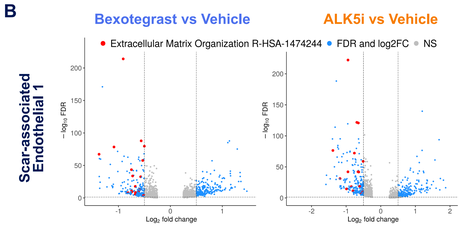


Figure 5. Comparison of Differential Gene Expression in Key Pathologic Cell Types



Pathways associated with genes downregulated by both bexotegrast and ALK5i in myofibroblasts

Reactome 2022 Terms	Adj. P-Value
Extracellular Matrix Organization R-HSA-1474244	0.02496
Collagen Formation R-HSA-1474290	0.03504
Elastic Fibre Formation R-HSA-1566948	0.03504

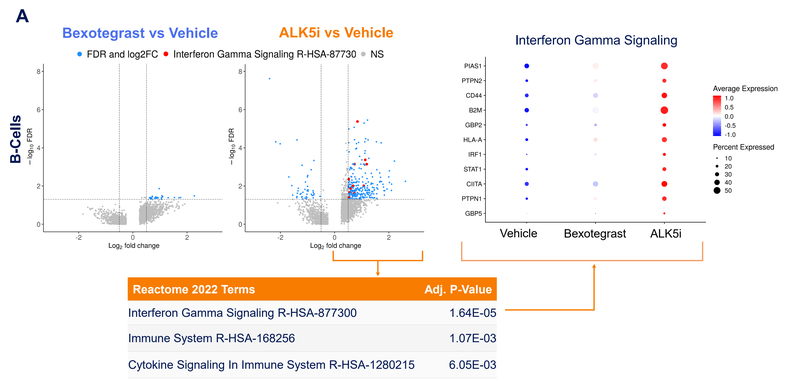


Pathways associated with genes downregulated by both bexotegrast and ALK5i in scar-associated endothelial cells

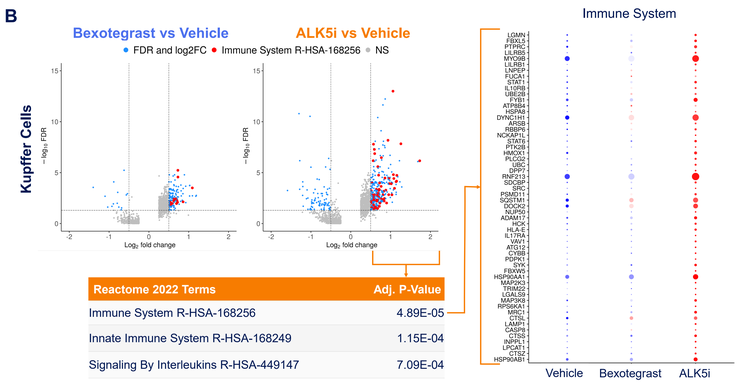
Reactome 2022 Terms	Adj. P-Value
Extracellular Matrix Organization R-HSA-1474244	1.22E-08
Non-integrin membrane-ECM Interactions R-HSA-3000171	3.81E-05
Signaling By PDGF R-HSA-186797	1.78E-03

Volcano plots of differentially expressed genes in bexotegrast-treated or ALK5i-treated vs vehicle-treated comparison in myofibroblasts (A) and scar-associated endothelial cells (B).
Genes indicated in blue are | log2FC | > 0.5 and FDR < 0.05. Selected Reactome 2022 terms for significantly downregulated genes overlapping between bexotegrast and ALK5i are shown in accompanying tables. DEGs in the significantly downregulated extracellular matrix organization term are highlighted in the volcano plots (red).

Figure 6. Comparison of Differential Gene Expression in Immune Cell Types



Reactome 2022 Terms	Adj. P-Value
Interferon Gamma Signaling R-HSA-877300	1.64E-05
Immune System R-HSA-168256	1.07E-03
Cytokine Signaling In Immune System R-HSA-1280215	6.05E-03



Reactome 2022 Terms	Adj. P-Value
Immune System R-HSA-168256	4.89E-05
Innate Immune System R-HSA-168249	1.15E-04
Signaling By Interleukins R-HSA-449147	7.09E-04

Volcano plots of differentially expressed genes in bexotegrast-treated or ALK5i-treated vs vehicle-treated comparison in B-cells (A) and Kupffer cells (B). Genes indicated in blue are | log2FC | > 0.5 and FDR < 0.05. Selected Reactome 2022 terms for significantly upregulated genes unique to ALK5i-treated comparison are shown in accompanying tables. DEGs in the significantly upregulated Interferon Gamma Signaling (A) or Immune System (B) terms in ALK5i-treated comparison are highlighted in the volcano plots (red) and expression across treatment groups is shown in the dot plots.

CONCLUSIONS

- **The antifibrotic activity of bexotegrast was similar to ALK5i in cells expressing the integrin targets, $\alpha_v\beta_6$ and $\alpha_v\beta_1$, and other pathologic cell types**
- **In immune cells, ALK5i upregulated immune response genes while bexotegrast had an attenuated effect, highlighting the differentiated activity of bexotegrast on TGF-β signaling**
- **These data demonstrate the utility of snRNA-seq of PCLivS for distinguishing the cell-specific effects of anti-fibrotic therapies**

Reference: 1. EASL ILC 2024 LBP-039

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