COMBINING DUAL $\alpha_V\beta_6/\alpha_V\beta_1$ INTEGRIN INHIBITOR, BEXOTEGRAST (PLN-74809), WITH STANDARD-OF-CARE THERAPIES HAS A SYNERGISTIC EFFECT ON REDUCING FIBROGENIC GENE EXPRESSION IN FIBROTIC HUMAN LUNG SLICES

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BACKGROUND

Idiopathic pulmonary fibrosis (IPF) is a rare fibrotic disease that progressively destroys lung structure and function and has a poor prognosis. The current standard-of-care drugs for IPF, nintedanib (a known inhibitor of FGFR, PDGFR, and VEGFR) and pirfenidone (mechanism not well understood), have modest effects on pulmonary function and life expectancy, but do not halt disease progression. Novel antifibrotic therapies with complementary mechanisms of action to nintedanib and pirfenidone are therefore required to further improve patient outcomes in IPF.

Bexotegrast (previously referred to as PLN-74809) is a dual-selective inhibitor of integrins $\alpha_V\beta_6$ and $\alpha_V\beta_1$ currently in development for the treatment of IPF. By blocking the interaction of latent-TGF-β with $\alpha_V\beta_6$ (on lung epithelial cells) and $\alpha_V\beta_1$ (on lung fibroblasts), bexotegrast reduces TGF-β signaling and fibrogenesis locally in the fibrotic lung. Using precision-cut lung slices (PCLS) generated from IPF lung explants, we investigated the individual and combined effects of bexotegrast and nintedanib or pirfenidone on the expression of genes related to the pathogenesis of IPF.

Previous quantitation of total mRNA extracted from fibrotic PCLS confirmed the anti-fibrotic effects of bexotegrast in the presence or absence of nintedanib and pirfenidone in 7 independent IPF tissues using a small focused gene panel (Fig.1)[1]. In this study, a subset of these PCLS samples were used to profile an expanded gene expression panel including markers of tissue damage, inflammation, fibroblast activation, and scar deposition.

Figure 1. Bexotegrast reduces fibrotic gene expression in IPF PCLS

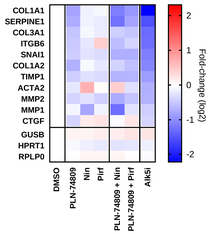


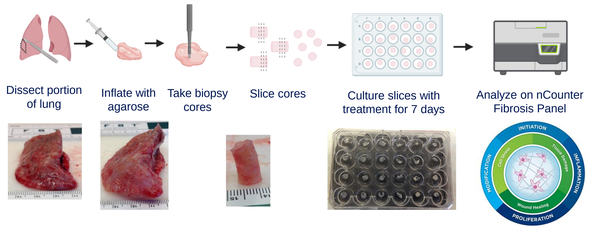
Figure 1 data represent mean (± SD) of 5–7 independent IPF tissues with ≥ 3 slices independently analyzed per patient tissue. Treatment effects were normalized to DMSO control for each tissue. Culture and treatment were for 7 days.
PLN-74809 = 200 nM; Nin = 75 nM; Pirf = 50 μM; ALK5i (R-268712) = 1 μM.

METHODS

Precision-cut lung slices

- Lung tissue was acquired from 4 patients with IPF undergoing lung transplantation
- PCLS generated from explants were cultured with inhibitors at clinically relevant concentrations: 200 nM bexotegrast, 75 nM nintedanib[2], 50 μM pirfenidone[2]

Figure 2. Flowchart of PCLS preparation, culture and analysis



- RNA containing lysate from n = 6 slices per treatment per lung were pooled for gene expression analysis versus vehicle (DMSO) using the 770-gene nCounter Fibrosis Panel (NanoString)
- Technical quality control and normalization of raw NanoString mRNA count data were performed using an R framework
- Differentially expressed genes were identified following multi-comparison correction (FDR<0.05)

RESULTS

Genes and Pathways Most Down-Regulated by Individual Treatments

Table 1. Top 20 Down-regulated Genes for Individual Treatments

Bexotegrast (PLN-74809)			Nintedanib			Pirfenidone		
Gene	log2FC	p-value	Gene	log2FC	p-value	Gene	log2FC	p-value
COL10A1	-2.78	<0.001	*FLT1*	-3.37	<0.001	*FGF19*	-1.14	0.005
POSTN	-0.96	0.016	*DLL4*	-2.79	<0.001	*KNG1*	-1.10	0.018
COL5A1	-0.93	0.007	*CDH5*	-2.33	<0.001	*CDH5*	-1.06	0.012
MARCO	-0.92	0.013	*COX4I2*	-2.07	<0.001	*MMRN1*	-0.98	0.021
MMP8	-0.88	0.016	*PECAM1*	-1.97	<0.001	*PECAM1*	-0.94	0.026
COL6A3	-0.83	0.034	*CETP*	-1.73	0.003	*CXCR4*	-0.89	0.021
GREM1	-0.83	0.016	*CXCR4*	-1.73	<0.001	*CD34*	-0.84	0.012
PECAM1	-0.83	0.046	*CXCL10*	-1.71	0.031	*RELN*	-0.83	0.037
COL1A2	-0.81	0.003	*NOTCH4*	-1.61	<0.001	*TEK*	-0.81	0.012
CXCR4	-0.78	0.035	*CD34*	-1.52	<0.001	*COL14A1*	-0.78	0.012
COL3A1	-0.74	0.037	*FLT4*	-1.48	0.003	*PDGFRB*	-0.75	0.004
LOX	-0.72	0.030	*MMP12*	-1.41	0.026	*COX4I2*	-0.75	0.037
MMP11	-0.67	0.016	*NOS3*	-1.36	0.002	*GREM1*	-0.74	0.032
FAP	-0.67	0.005	*ACVRL1*	-1.33	<0.001	*HAVCR1*	-0.73	0.019
PDGFRB	-0.67	0.004	*TEK*	-1.21	<0.001	*ACVRL1*	-0.73	0.021
FN1	-0.66	0.004	*TPSAB1/B2*	-1.19	<0.001	*NOTCH4*	-0.72	0.048
SERPINE1	-0.64	0.004	*COL10A1*	-1.17	0.017	*COL4A1*	-0.70	0.047
PLPP4	-0.64	0.030	*MMP9*	-1.14	0.016	*GAS1*	-0.64	0.009
LOXL1	-0.63	0.000	*MMP8*	-1.05	0.002	*CXCL12*	-0.59	0.035
TIMP1	-0.61	0.011	*MMP11*	-1.00	<0.001	*IFNG*	-0.59	0.044

log2FC (log2 fold change) relative to DMSO; p-values adjusted for multiple comparisons

- Genes most down-regulated by bexotegrast were related to fibrogenesis (e.g. *COL3A1*, *FAP*, *FN*, and *TIMP1*), as well as *SERPINE1*, a marker of TGF-β signaling.
- Genes most down-regulated by nintedanib and pirfenidone were related to vasculature development, including *CDH5*, *FLT1*, *TEK*, and *CD34*.

Table 2. Top Gene Ontology Biological Processes (Down)

Bexotegrast (PLN-74809)	Combined Score
extracellular structure organization (GO:0043062)	10837
extracellular matrix organization (GO:0030198)	10586
external encapsulating structure organization (GO:0045229)	8683
peptidyl-lysine oxidation (GO:0018057)	4898
collagen fibril organization (GO:0030199)	4453
endodermal cell differentiation (GO:0035987)	2912
endoderm formation (GO:0001706)	2459
positive regulation of fibroblast migration (GO:0010763)	2213
wound healing, spreading of epidermal cells (GO:0035313)	2213
CD40 signaling pathway (GO:0023035)	1848

Nintedanib	Combined Score
glomerulus vasculature development (GO:0072012)	4580
cellular response to UV-A (GO:0071492)	3216
vasculature development (GO:0001944)	2781
cellular component disassembly (GO:0022411)	2676
extracellular matrix disassembly (GO:0022617)	2676
wound healing, spreading of epidermal cells (GO:0035313)	2550
extracellular matrix organization (GO:0030198)	2256
branching involved in blood vessel morphogenesis (GO:0001569)	2107
response to UV-A (GO:0070141)	2107
extracellular structure organization (GO:0043062)	2079

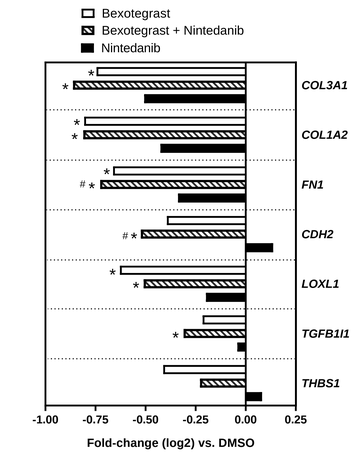
Pirfenidone	Combined Score
glomerulus vasculature development (GO:0072012)	9059
fusion of virus membrane with host plasma membrane (GO:0019064)	5084
membrane fusion involved in viral entry into host cell (GO:0039663)	5084
retina vasculature development in camera-type eye (GO:0061298)	3792
chemokine (C-X-C motif) ligand 12 signaling pathway (GO:0038146)	3792
vasculature development (GO:0001944)	3154
wound healing, spreading of epidermal cells (GO:0035313)	1706
endothelial cell proliferation (GO:0001935)	1390
endothelium development (GO:0003158)	1214
negative regulation of hemostasis (GO:1900047)	929

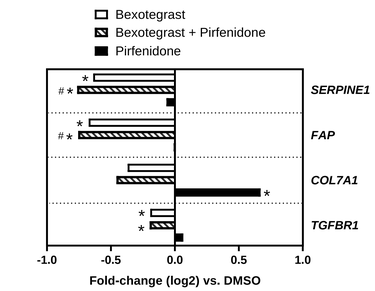
All genes down-regulated with an adjusted p-value <0.05 were entered into Enrichr[3]. GO Biological Processes with adjusted p-value <0.05 were sorted by combined score (Fisher exact test corrected by expected rank test)

- Bexotegrast primarily reduced gene expression pathways associated with extracellular matrix
- Pirfenidone primarily reduced gene expression pathways associated with vasculature
- Nintedanib reduced gene expression pathways associated with vasculature and extracellular matrix

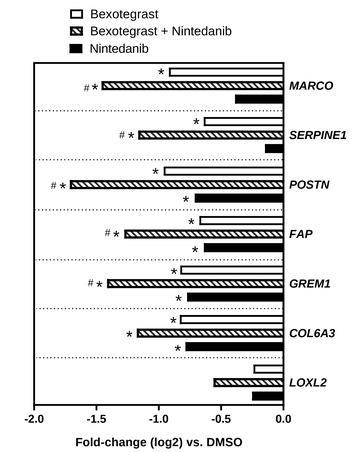
Combining Bexotegrast (PLN-74809) with Standard-of-Care Therapies Identified:

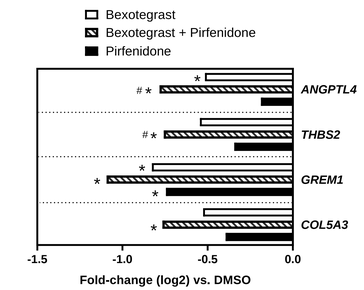
1. Genes downregulated to a similar degree by bexotegrast tested alone or in combination with standard-of-care





2. Genes further downregulated by combining bexotegrast with standard-of-care (i.e., additive effect)





* = p-value <0.05 vs vehicle (DMSO)
# = p-value <0.05 vs standard-of-care alone (adjusted for multiple comparison of genes down-regulated by bexotegrast + standard-of-care vs vehicle)

CONCLUSIONS

- Differential gene expression analysis of fibrotic human lung slices treated with bexotegrast (PLN-74809) and/or previously approved therapies nintedanib/pirfenidone show standalone and combinatorial treatment effects
- These results suggest the mechanism of action of bexotegrast (currently being evaluated in Phase 2 clinical trials for patients with IPF) may be independent of and complementary to that of currently approved therapies

References: 1. Decaris ML, et al. Respir Res 2021;22(1):265; 2. Ogura T, et al. Eur Respir J. 2015;45(5):1382-92; 3. Chen EY, et al. BMC Bioinformatics 2013; 128(14).

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