PHARMACOLOGICAL INHIBITORS OF INTEGRIN $\alpha_V\beta_6$ THAT DIFFERENTIALLY MODULATE PROTEIN CONFORMATION ARE SIMILARLY EFFECTIVE AT INHIBITING TGF-β SIGNALING IN THE FIBROTIC LUNG

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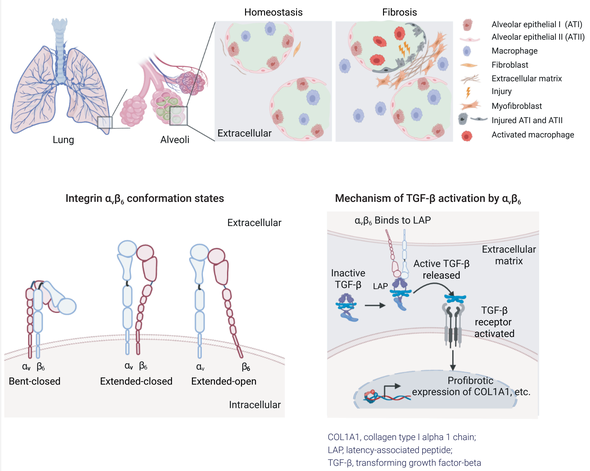
Research supported by Pliant Therapeutics, Inc.
Authors' relevant interests: EHB, PK, SH, VR, JS, ST, and MD are all employed by Pliant Therapeutics, Inc., and own stock. CC is a former employee of Pliant Therapeutics, Inc., and has stock options. PW has nothing to disclose

Poster no. 816

BACKGROUND AND RATIONALE

- Integrin $\alpha_v\beta_6$, expressed by lung epithelial cells, is a key regulator of transforming growth factor-beta (TGF-β) signaling in fibrotic lung tissue, representing a promising drug target in patients with idiopathic pulmonary fibrosis (IPF)



COL1A1, collagen type I alpha 1 chain; LAP, latency-associated peptide; TGF-β, transforming growth factor-beta

Figure 1. Conformations of $\alpha_v\beta_6$ and its role in the fibrotic lung

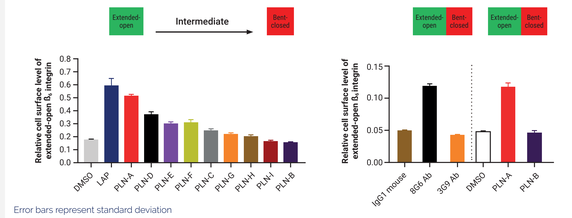
- Structurally, many integrins, including $\alpha_v\beta_6$, equilibrate between three main conformations: 'bent-closed', 'extended-closed', and 'extended-open'
- While a subset of pharmacological inhibitors of other integrins (e.g., $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$) have been found to paradoxically agonize their target by shifting integrin conformation and inducing outside-in-signaling,[1,2] little is known regarding outside-in-signaling induced by $\alpha_v\beta_6$ antagonists that block TGF-β signaling
- We analyzed the impact and differential effects of $\alpha_v\beta_6$ antagonists that modulate $\alpha_v\beta_6$ conformation and trafficking to/from the cell membrane on gene expression in primary lung epithelial cells and lung tissue explants from patients with IPF

METHODS

- Nine small molecule inhibitors by NanoString (PLN-A to I) and two antibody-based inhibitors (3G9 and 8G6) of integrin $\alpha_v\beta_6$ were evaluated in human epithelial cells for their impact on integrin conformation and trafficking to/from the cell surface, using In-Cell/On-Cell Western blotting
  - All inhibitors were evaluated at >10 × half-maximal inhibitory concentrations determined in ligand binding assay
- Stabilization of $\alpha_v\beta_6$ conformation was determined using an anti-ligand-induced binding site antibody
- Immunofluorescent staining of integrin $\alpha_v\beta_6$ was performed on human lung epithelial cells treated with 3G9 and 8G6 at 10 µg/ml to assess effects on integrin internalization
- Three small molecule inhibitors (PLN-A, PLN-B, and PLN-C; half-maximal inhibitory concentrations <50 nM) and two antibody inhibitors that differentially stabilized $\alpha_v\beta_6$ in conformations ranging from extended-open to bent-closed were evaluated by RNA sequencing for differential effects on gene expression in primary lung epithelial cells cultured on decellularized fibrotic lung extracellular matrix
  - Pathway enrichment analyses were performed using Enrichr[3,4]
- Follow-up NanoString evaluation of small molecule inhibitors that induce extended-open vs. bent-closed $\alpha_v\beta_6$ conformation was performed using precision-cut lung slices prepared from explanted lung tissue from patients with IPF

RESULTS

Small molecule and antibody inhibitors of $\alpha_v\beta_6$ have different effects on integrin conformation



Error bars represent standard deviation

Figure 2. Effect of small molecule and antibody inhibitors on $\alpha_v\beta_6$ conformation. All compounds were tested at 1 µM. DMSO and IgG1 (mouse) were used as controls. DMSO, dimethylsulfoxide; LAP, latency-associated peptide

- The small molecule inhibitors stabilized $\alpha_v\beta_6$ in a range of conformations from extended-open (PLN-A) to bent-closed (PLN-B)
- Inhibitory antibody 8G6 stabilized $\alpha_v\beta_6$ in an extended-open conformation while inhibitory antibody 3G9 stabilized $\alpha_v\beta_6$ in a bent-closed conformation
- Latency-associated peptide, an endogenous ligand for $\alpha_v\beta_6$, evaluated as a control, induced an extended-open conformation

$\alpha_v\beta_6$ inhibitors that stabilized an extended-open conformation resulted in $\alpha_v\beta_6$ internalization from the cell surface

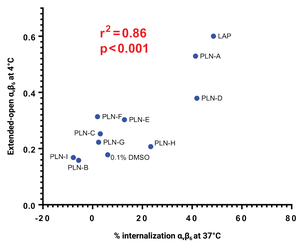


Figure 3. Plot comparing induction of extended-open $\alpha_v\beta_6$ conformation at the cell surface with subsequent $\alpha_v\beta_6$ internalization (all compounds were tested at 1 µM)

- Quantitative analysis of receptor internalization was measured by On-Cell Western blotting that compared integrin cell surface levels from 4°C (internalization prevented) to 37°C (internalization allowed)
- Endogenous ligand (latency-associated peptide), PLN-A, and PLN-D induced >40% $\alpha_v\beta_5$ internalization, while PLN-I and PLN-B showed minimal internalization

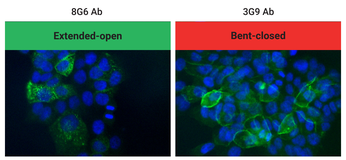


Figure 4. Immunofluorescence staining of $\alpha_v\beta_6$ in human lung epithelial cells treated with $\alpha_v\beta_6$ antibody inhibitors

- Consistent with small molecule inhibitors, treatment with an inhibitory antibody that stabilized an extended-open conformation (8G6) induced internalization of $\alpha_v\beta_6$, while treatment with an inhibitory antibody that stabilized a bent-closed conformation (3G9) did not

$\alpha_v\beta_6$ small molecule and antibody inhibitors that induce different $\alpha_v\beta_6$ integrin conformations were evaluated for downstream effects on gene expression using human lung epithelial cells cultured on extracellular matrix isolated from fibrotic lungs

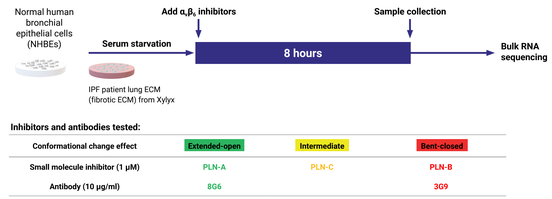


Figure 5. RNA sequencing study design

- No genes or gene expression pathways linking different $\alpha_v\beta_6$ integrin-conformations with differential gene expression were found to be consistent across small molecule and antibody inhibitor-treated primary lung epithelial cells
- $\alpha_v\beta_6$ inhibitors that stabilized different $\alpha_v\beta_6$ conformations were found to have similar effects on downstream gene expression in primary lung epithelial cells (attenuation of TGF-β signaling pathway and TGF-β regulation of extracellular matrix)
- Similar patterns of fibrosis-related gene expression were observed in lung epithelial cells treated with $\alpha_v\beta_6$ inhibitors and antibodies inducing closed and open conformations

Table 1. Common differentially expressed genes observed across all small molecule and antibody inhibitors of $\alpha_v\beta_6$ vs. relative DMSO and IgG1 controls by RNA sequencing

	Extended-open PLN-A	Intermediate PLN-C	Bent-closed PLN-B	Extended-open 8G6 Ab	Bent-closed 3G9 Ab
Gene	Lung fibrotic ECM			Lung fibrotic ECM	
SRGAP3	1.62	1.46	1.44	0.83	0.86
PEG10	1.26	1.42	1.12	0.73	0.60
SDPR	0.65	0.60	0.49	0.49	0.59
ANKRD1	-2.73	-2.14	-1.56	-1.76	-1.25
MSC	-2.64	-2.46	-1.63	-1.87	-1.47
ADAMTS15	-2.63	-2.11	-2.04	-1.37	-1.16
NEURL1B	-2.20	-1.85	-1.64	-1.28	-1.24
C4orf26	-2.21	-1.93	-1.54	-1.80	-1.37
NKILA	-2.58	-2.11	-1.79	-1.37	-1.27
PMEPA1	-2.12	-1.75	-1.27	-1.03	-0.82
SERPINE1	-1.88	-1.75	-1.42	-1.35	-1.19
IVNS1ABP	-2.05	-1.95	-1.53	-1.12	-0.89
KANK4	-1.87	-1.56	-1.34	-1.17	-1.11
AKAP12	-1.63	-1.70	-1.38	-1.03	-0.88
PDGFB	-1.49	-1.68	-1.34	-1.27	-1.11
NEDD9	-1.47	-1.21	-1.06	-0.90	-0.79
ATF3	-1.93	-1.62	-1.67	-1.29	-1.21
CTGF	-1.38	-1.07	-0.66	-0.65	-0.59
BPGM	-1.48	-1.34	-1.16	-0.75	-0.60
SEMA7A	-1.33	-1.31	-1.06	-0.83	-0.76
SMAD7	-1.29	-1.25	-0.86	-0.87	-0.68
PODXL	-0.60	-1.02	-0.88	-1.01	-0.97
TUBB3	-1.12	-1.13	-0.90	-0.81	-0.70
WNT5B	-1.42	-1.39	-1.04	-0.80	-0.75
FZD10	-0.93	-0.91	-0.81	-0.61	-0.64
GADD45B	-1.03	-0.87	-0.92	-0.71	-0.66

Cut off: False Discovery Rate <=0.01 and |change in expression|>=33%

Log_2 fold change vs. DMSO

Table 2. Top pathway enrichment analysis of shared differentially expressed genes between small molecule inhibitors and antibodies

Term	Adjusted p value
TGF-β signaling pathway	<0.001
TGF-β regulation of extracellular matrix	0.003
RAGE pathway	0.003
SMAD2/3 nuclear pathway	0.004
Hypertrophy pathway	0.006
Hippo signaling pathway	0.017
Basal cell carcinoma	0.029
Carcinoma	0.029
Oncostatin M	0.053

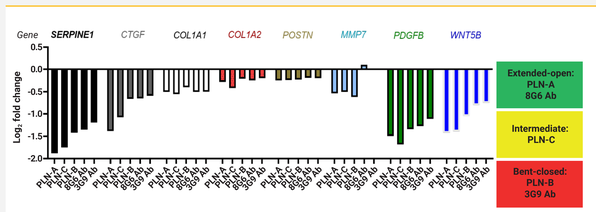


Figure 6. Fibrosis-related gene expression in lung epithelial cells treated with small molecule and antibody inhibitors of $\alpha_v\beta_6$

Pharmacological $\alpha_v\beta_6$ inhibitors that differentially modulate integrin conformation were equally effective at blocking TGF-β gene expression in slices prepared from IPF explants

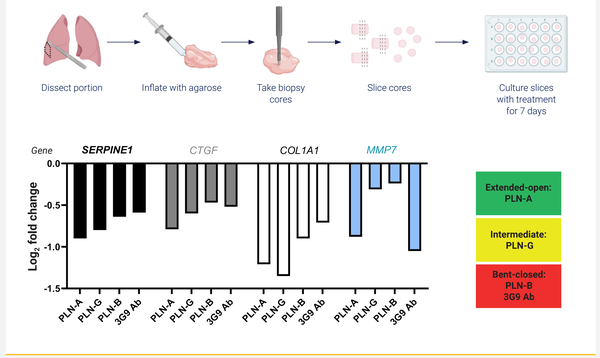


Figure 7. Preparation of precision-cut lung slices and effect of small molecule and antibody inhibitors of $\alpha_v\beta_6$ on fibrosis-related gene expression in precision-cut lung slices by NanoString

- Similar to data obtained from primary cells, small molecule and antibody inhibitors of $\alpha_v\beta_6$ inducing different integrin conformations were each effective at reducing fibrosis-related genes in precision-cut lung slices prepared from IPF explants

CONCLUSIONS

- $\alpha_v\beta_6$ small molecule inhibitors that differentially modulate integrin $\alpha_v\beta_6$ conformation are equally effective at blocking $\alpha_v\beta_6$-mediated regulation of TGF-β signaling in bronchial cell- and fibrotic lung tissue-based assays, with no $\alpha_v\beta_6$ conformation-related changes in gene expression observed
- This study supports the ongoing evaluation of $\alpha_v\beta_6$ inhibitors for the treatment of IPF

ACKNOWLEDGMENTS

Editorial assistance was provided by Alpharmaxim Healthcare Communications and funded by Pliant Therapeutics, Inc.

REFERENCES

1. Van Agthoven JF, et al. *Nat Struct Mol Biol* 2014;21(4):383–388
2. Huang J, et al. *J Hematol Oncol Pharm* 2019;12(1):26
3. Chen EY, et al. *BMC Bioinformatics* 2013;14:128
4. Kuleshov MV, et al. *Nucleic Acids Res* 2016;44(W1):W90–W97

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