Dual $\alpha_V\beta_6/\alpha_V\beta_1$ inhibitor PLN-74809 blocks multiple TGF-β activation pathways associated with IPF

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Rationale

Integrin $\alpha_V\beta_6$- and $\alpha_V\beta_1$-mediated activation of TGF-β has been shown to promote fibrosis in the bleomycin (bleo) lung injury model, and elevated levels of $\alpha_V\beta_6$ are present in human IPF lung tissue.[1-4] We have developed PLN-74809, a dual selective $\alpha_V\beta_6/\alpha_V\beta_1$ small-molecule inhibitor (SMi), and compared its antifibrotic properties with single/pan-α_V integrin inhibitors and standard-of-care molecules (nintedanib and pirfenidone) across multiple IPF model systems.

Methods

Inhibitor potency and selectivity were characterized using ligand-binding, primary cell adhesion, and TGF-β reporter cell-based assays. A novel ELISA-based assay was developed to assess $\alpha_V\beta_1$ tissue levels. Gene expression analysis, hydroxyproline (OHP) quantitation, and/or second harmonic generation imaging of collagen fiber deposition were used to evaluate the antifibrotic properties of inhibitors in precision-cut lung slices (PCLS; generated from IPF patients and bleo-injured mouse explants) as well as in the standard murine bleo model of IPF.

Results

1) Integrin $\alpha_V\beta_6$ and $\alpha_V\beta_1$ were present at elevated levels in fibrotic human and mouse lung tissue

2) Dual $\alpha_V\beta_6/\alpha_V\beta_1$ inhibitor PLN-74809 blocked lung epithelial cell ($\alpha_V\beta_6$) and lung fibroblast ($\alpha_V\beta_1$) adhesion to latency-associated peptide (LAP)/latent TGF-β

3) Collagen gene expression levels in PCLS showed:

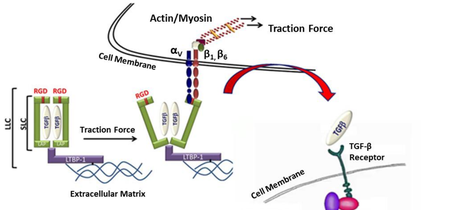
- Dual inhibition of $\alpha_V\beta_6/\alpha_V\beta_1$ is more effective than single inhibition of either integrin alone at reducing collagen gene expression
- Dual $\alpha_V\beta_6/\alpha_V\beta_1$ inhibition is equipotent to pan-α_V inhibition
- PLN-74809 is a more potent inhibitor of collagen gene expression than nintedanib or pirfenidone

4) Dual inhibition of $\alpha_V\beta_6/\alpha_V\beta_1$ significantly reduced pulmonary collagen deposition in the murine bleo model of IPF

Conclusions

Dual $\alpha_V\beta_6/\alpha_V\beta_1$ inhibitor PLN-74809 offers a targeted approach to block multiple avenues of TGF-β activation in the fibrotic lung.

Integrins $\alpha_V\beta_6$ and $\alpha_V\beta_1$ Activate Latent TGF-β Resulting in Profibrotic Gene Expression

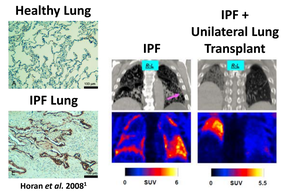


- Dual inhibition of $\alpha_V\beta_6$ (on epithelial cells) and $\alpha_V\beta_1$ (on fibroblasts) offers a targeted approach for blocking TGF-β signaling in the fibrotic lung

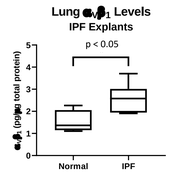
Adapted from: Chen *et al.* 2016[5]

1 TGF-β-Activating Integrins $\alpha_V\beta_6$ and $\alpha_V\beta_1$ Are Present at Elevated Levels in Fibrotic Lung Tissue

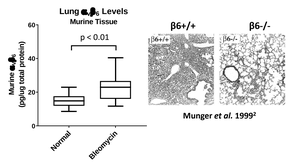
IHC staining and PET imaging show elevated $\alpha_V\beta_6$ levels in IPF lung tissue



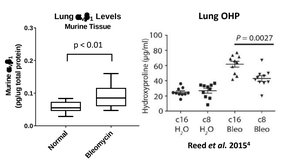
ELISA-based assay shows elevated $\alpha_V\beta_1$ levels in IPF lung tissue



$\alpha_V\beta_6$ levels are elevated in bleo-injured mouse lungs; Itgb6$^{-/-}$ mice are protected from fibrosis

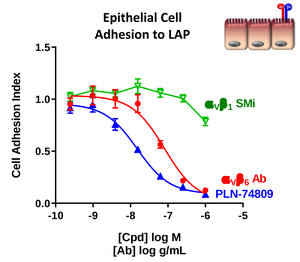


$\alpha_V\beta_1$ levels are elevated in bleo-injured mouse lungs; SMi blocks fibrosis



2 Dual $\alpha_V\beta_6/\alpha_V\beta_1$ Inhibitor PLN-74809 Blocks Lung Epithelial Cell and Fibroblast Adhesion to LAP

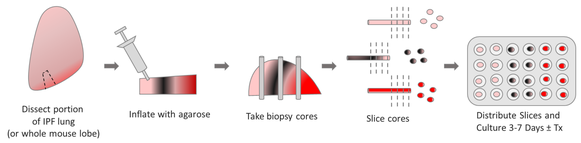
Anti-$\alpha_V\beta_6$ antibody – blocks epithelial cell adhesion only
$\alpha_V\beta_1$ SMi – blocks fibroblast adhesion only
PLN-74809 – blocks adhesion by both cell types



- Lung epithelial cells increase expression of $\alpha_V\beta_6$ upon injury, leading to elevated TGF-β activation
- PLN-74809 potently blocks epithelial cell adhesion to LAP, thereby preventing activation of TGF-β
- Fibroblasts also activate latent TGF-β via $\alpha_V\beta_1$ binding to LAP
- PLN-74809 potently blocks fibroblast adhesion to LAP, thereby preventing activation of TGF-β

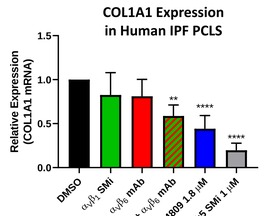
References: 1. Horan *et al. Am J Respir Crit Care Med* 2008;177:56-65; 2. Munger *et al. Cell* 1999;96:319-328; 3. Henderson *et al. Nat Med* 2013;19:1617-24; 4. Reed *et al. Sci Transl Med* 2015;7:288ra79; 5. Chen *et al. J Mol Cell Cardiol* 2016;93:162-174

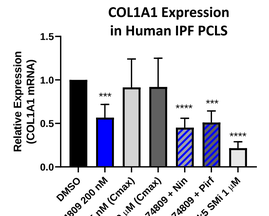
3 Dual $\alpha_V\beta_6/\alpha_V\beta_1$ Inhibition Reduces Collagen Expression in Precision-Cut Lung Slices (PCLS)



PCLS from IPF patient lung explants (n=5-7 patients) showed:

- Dual inhibition of $\alpha_V\beta_6/\alpha_V\beta_1$ with PLN-74809, or a combination of inhibitors, is more effective than single inhibition of either integrin at reducing collagen expression
- PLN-74809 inhibits collagen gene expression in the presence or absence of nintedanib or pirfenidone

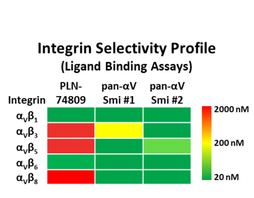


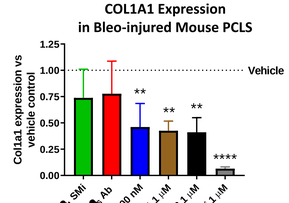


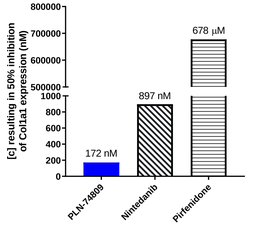
**p<0.01
***p<0.001
****p<0.0001

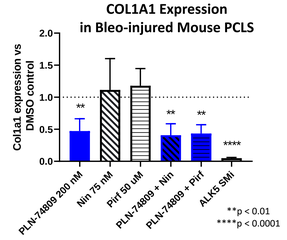
PCLS from bleomycin-injured mouse lungs showed:

- Dual inhibition of $\alpha_V\beta_6/\alpha_V\beta_1$ with PLN-74809, or a combination of inhibitors, is more effective than single inhibition of either integrin at reducing collagen expression
- Dual $\alpha_V\beta_6/\alpha_V\beta_1$ inhibition is equipotent to pan-α_V inhibition
- PLN-74809 is more potent than nintedanib or pirfenidone at blocking collagen gene expression in fibrotic lung tissue





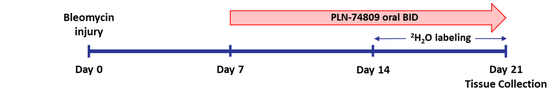


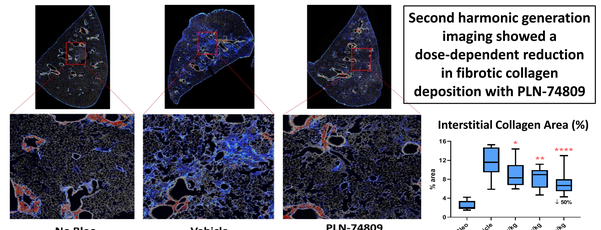


**p < 0.01
****p < 0.0001

Key abbreviations: Bleo, bleomycin; LAP, latency-associated peptide; OHP, hydroxyproline; PCLS, precision-cut lung tissue slices; SMi, small-molecule inhibitor

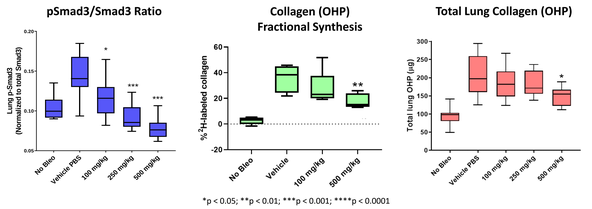
4 Dual $\alpha_V\beta_6/\alpha_V\beta_1$ Inhibition Reduces Pulmonary Collagen Deposition in the Murine Bleo Model





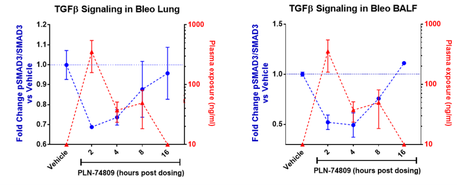
Second harmonic generation imaging showed a dose-dependent reduction in fibrotic collagen deposition with PLN-74809

Dual $\alpha_V\beta_6/\alpha_V\beta_1$ inhibition with PLN-74809 significantly and dose-dependently reduced pSmad3/Smad3 ratio and OHP deposition in bleo-injured mouse lungs



*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001

A clear PK/PD relationship between plasma levels of PLN-74809 (oral 250 mg/kg) and pSmad3/Smad3 ratio in mouse lung tissue/bronchoalveolar lavage cells was observed



Conclusions

- Levels of $\alpha_V\beta_6$ and $\alpha_V\beta_1$, two integrins that activate latent TGF-β through binding to LAP, are increased in fibrotic lung tissue
- PLN-74809 blocks lung epithelial cell ($\alpha_V\beta_6$) and lung fibroblast ($\alpha_V\beta_1$) adhesion to LAP, thereby preventing TGF-β activation
- Dual inhibition of $\alpha_V\beta_6/\alpha_V\beta_1$ in PCLS prepared from IPF patient explants or bleo-injured mouse lungs was more effective at reducing collagen gene expression than single integrin inhibition alone or standard-of-care drugs nintedanib and pirfenidone
- Dual inhibition of $\alpha_V\beta_6/\alpha_V\beta_1$ with PLN-74809 significantly reduced pulmonary collagen deposition in the bleo mouse model of IPF

Disclosures: All authors were employed by Pliant Therapeutics Inc. at the time of their contribution to the studies reported here.