EVALUATION OF THE ANTIFIBROTIC EFFECT OF A DUAL $\alpha_V\beta_6/\alpha_V\beta_1$ INTEGRIN INHIBITOR IN A MOUSE MODEL OF RHEUMATOID ARTHRITIS-ASSOCIATED INTERSTITIAL LUNG DISEASE

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BACKGROUND AND RATIONALE

Idiopathic pulmonary fibrosis (IPF) is the prototypical progressive fibrosing interstitial lung disease (ILD); however, progressive fibrosis can also be observed in other ILDs. ILD with radiological signs of fibrosis and progression over time is referred to as progressive pulmonary fibrosis (PPF) and includes a range of underlying diagnoses, including rheumatoid arthritis-associated ILD (RA-ILD).[1]

TGF-β signaling is a key driver of fibrotic disease, including pulmonary fibrosis. Integrins $\alpha_V\beta_6$ (epithelial cells) and $\alpha_V\beta_1$ (fibroblasts) promote pulmonary fibrosis through the activation of latent TGF-β, which leads to myofibroblast activation and new collagen synthesis (Figure 1).

Preclinical evaluation of a dual-selective inhibitor of integrins $\alpha_V\beta_6$ and $\alpha_V\beta_1$ demonstrated antifibrotic activity in vivo in the bleomycin mouse model of pulmonary fibrosis and in precision-cut lung slices (PCLS) from IPF and RA-ILD patient explants (Figure 2). [2,3]

Therefore, in this study, we evaluated the antifibrotic activity of a dual $\alpha_V\beta_6/\alpha_V\beta_1$ integrin inhibitor in vivo in a zymosan-induced mouse model of RA-ILD.

Figure 1. Dual $\alpha_V\beta_6/\alpha_V\beta_1$ integrin inhibition blocks activation of latent TGF-β and reduces fibrosis

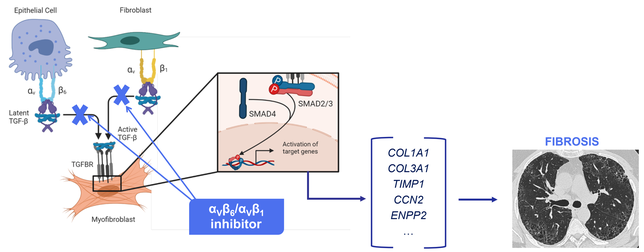
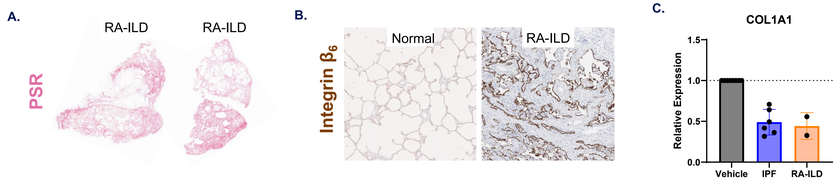


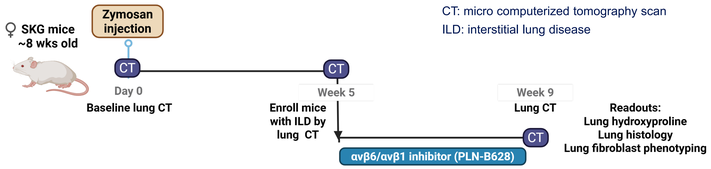
Figure 2. Inhibition of $\alpha_V\beta_6/\alpha_V\beta_1$ integrin reduces collagen gene expression in PCLS from RA-ILD explants



(A) Staining for collagen via picrosirus red (PSR) and **(B)** integrin β6 (brown) in RA-ILD lung explants. **(C)** Relative expression of collagen, type I, alpha 1 (COL1A1) in $\alpha_V\beta_6/\alpha_V\beta_1$ inhibitor-treated fibrotic PCLS from IPF or RA-ILD explants.

STUDY DESIGN AND METHODS

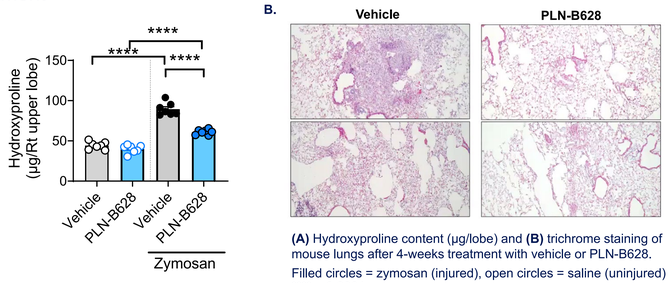
Figure 3. In vivo study design



- Female SKG mice were dosed with zymosan to induce an RA-ILD phenotype
- Mice with evidence of lung fibrosis by CT scan at week 5 were treated with PLN-B628, a dual $\alpha_V\beta_6/\alpha_V\beta_1$ inhibitor, or vehicle for 4 weeks
- Lung fibrosis was evaluated longitudinally by CT scan and at week 9 by hydroxyproline analysis, histology, and flow analysis for fibroblast populations

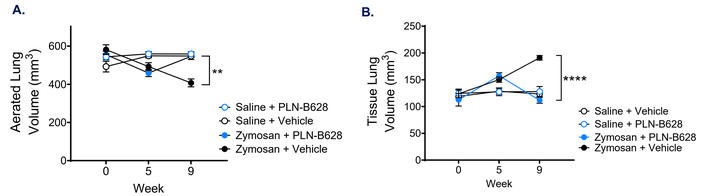
RESULTS

Figure 4. Dual $\alpha_V\beta_6/\alpha_V\beta_1$ integrin inhibitor reduced lung fibrosis after 4-weeks treatment



(A) Hydroxyproline content (µg/lobe) and **(B)** trichrome staining of mouse lungs after 4-weeks treatment with vehicle or PLN-B628.
Filled circles = zymosan (injured), open circles = saline (uninjured)
****p<0.0001

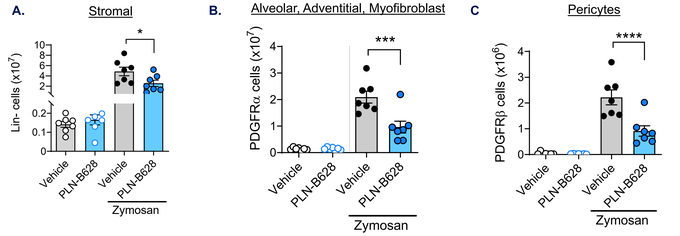
Figure 5. Dual $\alpha_V\beta_6/\alpha_V\beta_1$ inhibitor improved lung CT parameters



(A) Longitudinal analysis of aerated lung volume (air space) and **(B)** tissue lung volume at baseline (week 0), initiation of treatment (week 5) and study end (week 9) by micro-CT
Filled circles = zymosan (injured), open circles = saline (uninjured)
**p<0.01, ****p<0.0001

- Dual $\alpha_V\beta_6$ and $\alpha_V\beta_1$ integrin inhibition significantly reduced lung hydroxyproline relative to vehicle in SKG + zymosan mice (32%, p<0.0001)
- CT scans of the lungs at the initiation of treatment showed similar zymosan-induced changes; however, at week 9, PLN-B628 treated mice showed significantly greater aerated volume (34%, p<0.01) and lower tissue volume (42%, p<0.0001) than vehicle-treated mice
- Changes in fibrosis were accompanied by significant reductions in the number of fibroblasts in the lung (PDGFRα⁺, 54%, p<0.001)

Figure 6. Dual $\alpha_V\beta_6/\alpha_V\beta_1$ inhibitor reduced lung fibroblast populations



Flow analysis of **(A)** stromal cells (Lin⁻, CD45⁻CD31⁻Epcam⁻), **(B)** alveolar fibroblasts, adventitial fibroblasts, and myofibroblast (Lin⁻PDGFRα⁺) and **(C)** pericytes (Lin⁻PDGFRβ⁺) in mouse lungs after 4-weeks treatment with vehicle or PLN-B628.
Filled circles = zymosan (injured), open circles = saline (uninjured)
*p<0.05, ***p<0.001, ****p<0.0001

CONCLUSIONS

- **Dual inhibition of $\alpha_V\beta_6/\alpha_V\beta_1$ integrins is antifibrotic in a mouse model of RA-ILD**
- **These data support further investigation into the potential antifibrotic activity of targeting TGF-β signaling through integrins as a therapy in fibrosing ILDs**

References: 1. Rajan et al. Eur Respir J (2023). 2. Decaris et al. Respir Res (2021). 3. An et al., ATS (2024)

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Disclosures: JS, EB and MD are employees and shareholders of Pliant Therapeutics