PLN-74809, a dual $\alpha_V\beta_6/\alpha_V\beta_1$ integrin inhibitor, inhibits fibrosis in precision-cut liver tissue from PSC and PBC patients and the Mdr2 knockout mouse

S Turner[1], M Decaris[1], S Ho[1], C Chen[1], G Lee[1], V Rao[1], M Marlow[1], S Martin[1], T Chen[1], J Cha[1], M Munoz[1], T Hom[1], K Leftheris[1], Y Popov[2] and J Schaub[1]

[1]Pliant Therapeutics, South San Francisco, CA, [2]Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA

Rationale

Integrins $\alpha_V\beta_6$ and $\alpha_V\beta_1$ are heterodimeric proteins that bind and activate latent TGF-β. In primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC), $\alpha_V\beta_6$ and $\alpha_V\beta_1$ integrins are thought to play a role in the development and propagation of fibrosis through TGF-β activation by cholangiocytes and stellate cells, respectively. We have identified an orally available, dual-selective, small-molecule inhibitor of $\alpha_V\beta_6$ and $\alpha_V\beta_1$, PLN-74809, and tested its ability to block fibrosis through inhibition of integrin-mediated TGF-β activation.

Methods

IHC and ELISA-based assays were used to evaluate expression of $\alpha_V\beta_6$ and $\alpha_V\beta_1$ in human tissue samples from PSC and PBC patients. Anti-fibrotic properties of PLN-74809 and a similarly potent, dual-selective compound, PLN-75068, were evaluated *in vivo* in two mouse models of biliary fibrosis (Mdr2$^{-/-}$ and DDC diet) and in precision-cut liver tissue slices (PCLivS) prepared from PSC and PBC patient liver explants by IHC, hydroxyproline (OHP) quantitation, and gene expression analysis.

Results

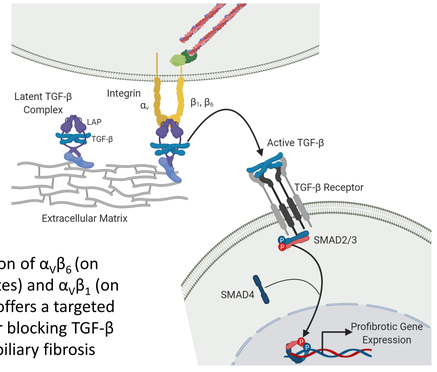
1) Integrins $\alpha_V\beta_6$ and $\alpha_V\beta_1$ were significantly elevated in fibrotic Mdr2$^{-/-}$ mice, and in PSC and PBC patient liver tissue

2) PLN-75068 reduced relative hepatic collagen levels and serum ALP levels in DDC diet-injured mice

3) PLN-74809 reduced hepatic TGF-β signaling, relative hepatic collagen levels, and serum ALP levels in Mdr2$^{-/-}$ mice

4) PLN-74809 reduced collagen gene expression in PCLivS prepared from PSC and PBC patient liver explants

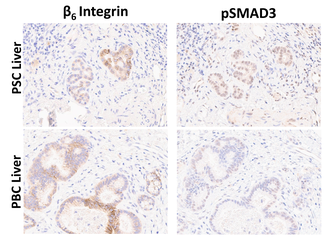
Integrins $\alpha_V\beta_6$ and $\alpha_V\beta_1$ Activate Latent TGF-β, Resulting in Profibrotic Gene Expression



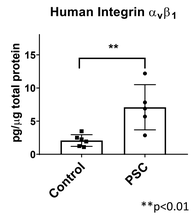
Dual inhibition of $\alpha_V\beta_6$ (on cholangiocytes) and $\alpha_V\beta_1$ (on fibroblasts) offers a targeted approach for blocking TGF-β signaling in biliary fibrosis

1 TGF-β-Activating Integrins $\alpha_V\beta_6$ and $\alpha_V\beta_1$ Are Present at Elevated Levels in Liver Tissue with Biliary Fibrosis

IHC staining showed $\alpha_V\beta_6$ expression and SMAD3 phosphorylation in PSC and PBC liver tissue

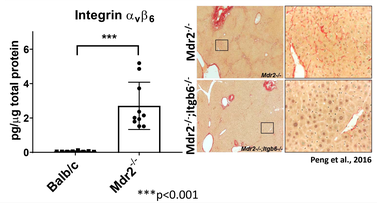


ELISA-based assay showed elevated $\alpha_V\beta_1$ levels in PSC liver tissue



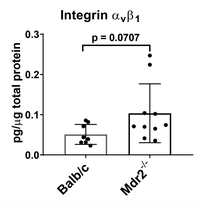
**p<0.01

$\alpha_V\beta_6$ levels were elevated in Mdr2$^{-/-}$ mouse livers; Itgb6$^{-/-}$ mice were protected from fibrosis

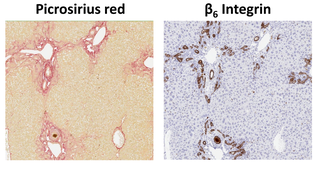


***p<0.001

$\alpha_V\beta_1$ levels were elevated in Mdr2$^{-/-}$ mouse livers

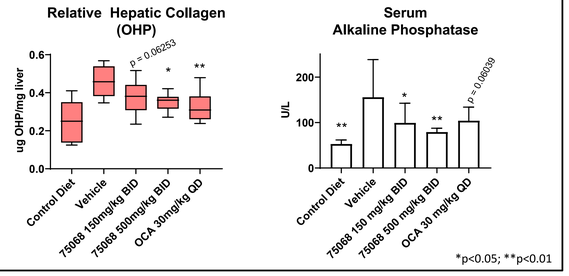


2 Dual $\alpha_V\beta_6/\alpha_V\beta_1$ Inhibition Reduced Hepatic Collagen Deposition in the DDC Biliary Fibrosis Model



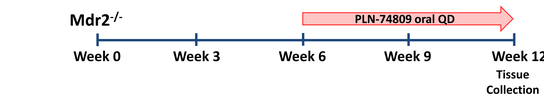
4 weeks of 0.03% DDC feeding induced collagen deposition and integrin $\alpha_V\beta_6$ expression in the liver

Dual $\alpha_V\beta_6/\alpha_V\beta_1$ inhibition with PLN-75068 significantly and dose-dependently reduced collagen deposition and serum ALP in DDC-injured mice

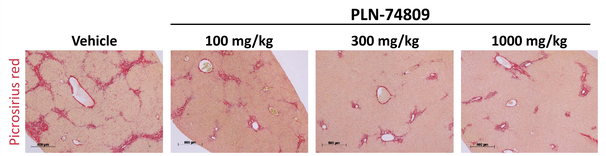


*p<0.05; **p<0.01

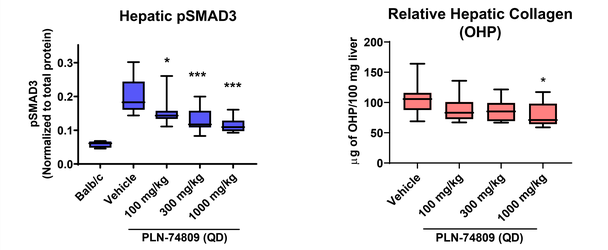
3 Dual $\alpha_V\beta_6/\alpha_V\beta_1$ Inhibition Reduced Hepatic Collagen Deposition in the Mdr2$^{-/-}$ Biliary Fibrosis Model



Picrosirius red staining showed a dose-dependent decrease in hepatic collagen deposition with PLN-74809

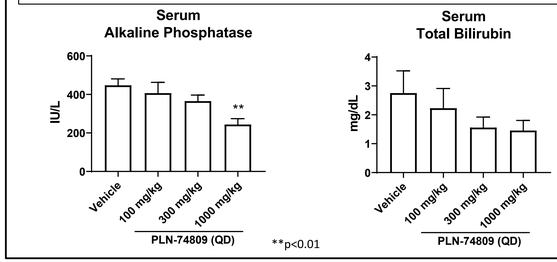


Dual $\alpha_V\beta_6/\alpha_V\beta_1$ inhibition with PLN-74809 significantly and dose-dependently reduced SMAD3 phosphorylation and collagen deposition in Mdr2$^{-/-}$ livers



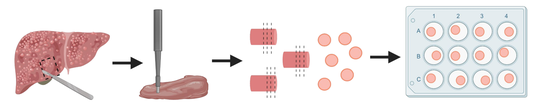
*p<0.05; **p<0.01; ***p<0.001

Dual $\alpha_V\beta_6/\alpha_V\beta_1$ inhibition with PLN-74809 dose-dependently reduced serum markers of biliary injury



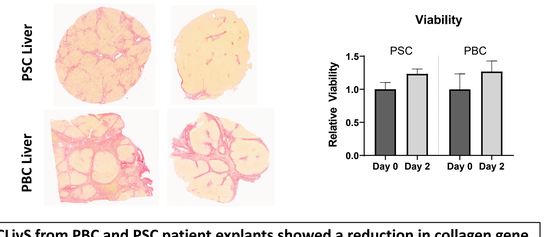
**p<0.01

4 Dual $\alpha_V\beta_6/\alpha_V\beta_1$ Inhibition Reduced Collagen Expression in PBC/PSC Patient Tissue

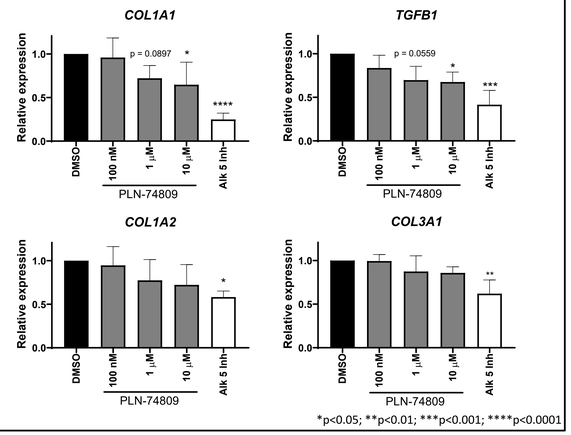


Picrosirius red staining for collagen showed extensive fibrosis in PCLivS tissue

PCLivS were viable for 48 hours in culture



PCLivS from PBC and PSC patient explants showed a reduction in collagen gene expression with PLN-74809 treatment



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

Conclusions

- Levels of $\alpha_V\beta_6$ and $\alpha_V\beta_1$, two integrins that activate latent TGF-β through binding to LAP, were increased in biliary fibrosis in human and mouse samples
- Dual inhibition of $\alpha_V\beta_6/\alpha_V\beta_1$ with PLN-74809 or PLN-75068 significantly reduced hepatic TGF-β signaling, hepatic collagen deposition, and serum ALP in either the Mdr2$^{-/-}$ or DDC mouse models of biliary fibrosis
- Dual inhibition of $\alpha_V\beta_6/\alpha_V\beta_1$ with PLN-74809 reduced collagen gene expression in PCLivS prepared from PSC and PBC patient tissue explants
- PLN-74809 warrants further investigation as a direct anti-fibrotic in PSC and PBC clinical trials

References: Peng Z, *et al. Hepatology* 2016,6(1):217–232

Key abbreviations: ALP, alkaline phosphatase; DDC, 3.5-Diethoxycarbonyl-1.4-dihydrocollidine; ELISA, enzyme-linked immunosorbent assay; IHC, immunohistochemistry; LAP, latency-associated peptide; OCA, obeticholic acid; OHP, hydroxyproline; PBC, primary biliary cirrhosis; PCLivS, precision-cut liver slices; PSC, primary sclerosing cholangitis; QD, once daily

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