Non-invasive imaging method demonstrates anti-fibrotic efficacy of a dual integrin alpha-v/beta-6 and alpha-v/beta-1 inhibitor in a rat model of biliary fibrosis

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BACKGROUND

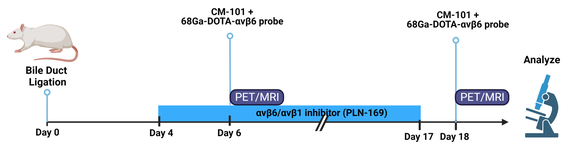
There is an unmet need for antifibrotic treatments which can prevent, halt or reverse hepatic fibrosis in chronic liver diseases; however, drug development efforts have been hampered by a lack of non-invasive methods to measure fibrosis. In primary sclerosing cholangitis, integrins overexpressed on injured cholangiocytes ($\alpha_v\beta_6$) and myofibroblasts ($\alpha_v\beta_1$) are attractive therapeutic targets because they bind and activate latent TGF-β, a key driver of liver fibrosis. Here, we used molecular MRI with CM-101, a type I collagen probe that directly measures fibrosis[1], and PET with ^{68}Ga-DOTA-R$_0$1-MG, an $\alpha_v\beta_6$ cysteine knot probe[2], to provide a non-invasive readout of $\alpha_v\beta_6$ and $\alpha_v\beta_1$ antagonism in a rat model of biliary fibrosis.

METHODS

In Vivo Analysis

Liver fibrosis was induced in rats by ligation of the common bile duct (BDL). Control rats underwent a sham surgery (Sham). The selective integrin $\alpha_v\beta_6/\alpha_v\beta_1$ antagonist PLN-169 was dosed orally at 300 mg/kg PO/QD (LD) or 500 mg/kg PO/BID (HD) 4-17 days post-BDL. MRI with the type I collagen probe CM-101 and PET with ^{68}Ga-DOTA-R$_0$1-MG, an $\alpha_v\beta_6$ cysteine knot probe, were performed on days 6 and 18 post-BDL.

Figure 1. Experimental Design

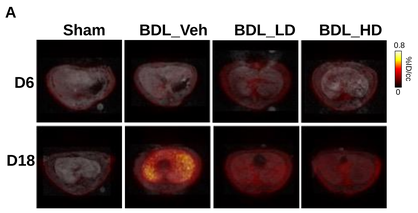


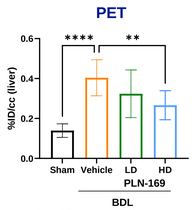
Ex Vivo Analysis

On day 18, livers were collected for histological and molecular analysis. Sirius red was used to stain collagen and fibrosis assessment was performed measuring collagen percent area (CPA) in ImageJ. $\alpha_v\beta_6$ expression was assessed by immunohistochemistry (IHC) and quantified in ImageJ. Gene expression changes were measured using the NanoString platform.

RESULTS Assessment of $\alpha_v\beta_6$ Expression

Figure 2. $\alpha_v\beta_6$ PET Imaging Identified Increased Target Expression with Injury and Reduction with Treatment





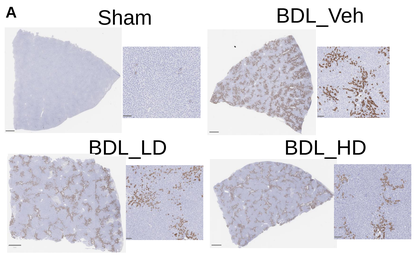
(A) Example PET images at 20 min post-injection of ^{68}Ga-DOTA-R$_0$1-MG on days 6 and 18 post-BDL. (B) Quantitation of %ID/cc in the liver at day 18 (D18).

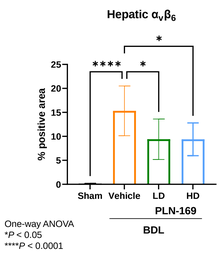
Veh = vehicle, LD = low dose PLN-169, HD = high dose PLN-169, %ID/cc = % injected dose per cubic centimeter

One-way ANOVA
**P < 0.01
****P < 0.0001

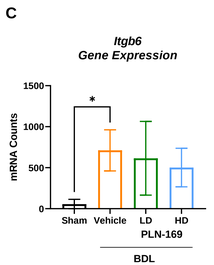
- No significant difference in $\alpha_v\beta_6$ levels was seen at Day 6 post-BDL
- At Day 18 post-BDL, vehicle-treated rats had significantly higher $\alpha_v\beta_6$ PET signal than both Sham and PLN-169 (HD) treated rats

Figure 3. Immunohistochemistry for Integrin β_6 Confirmed a Reduction in $\alpha_v\beta_6$ Levels with Treatment





One-way ANOVA
*P < 0.05
****P < 0.0001



(A) Example immunohistochemistry for integrin β6 in livers from rats 18 days post-BDL. (B) Quantification of % integrin β6-positive area in cross sections of liver 18 days post-BDL. (C) Expression of *Itgb6* gene in liver homogenate 18 days post-BDL.

Scale bars = 1 mm (whole section) and 100 µm (enlarged image)

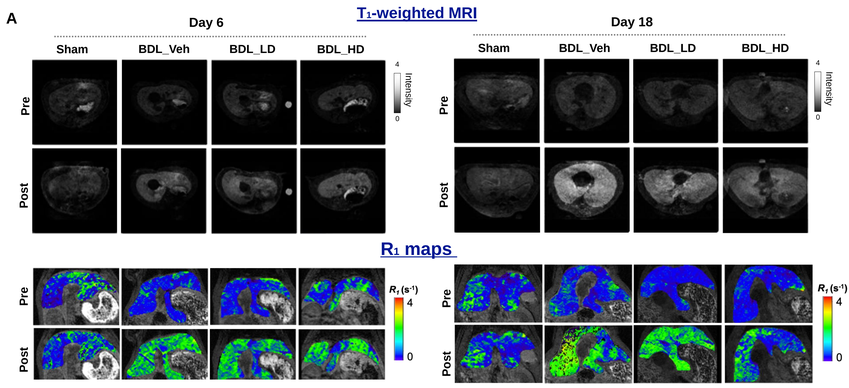
Veh = vehicle, LD = low dose PLN-169, HD = high dose PLN-169

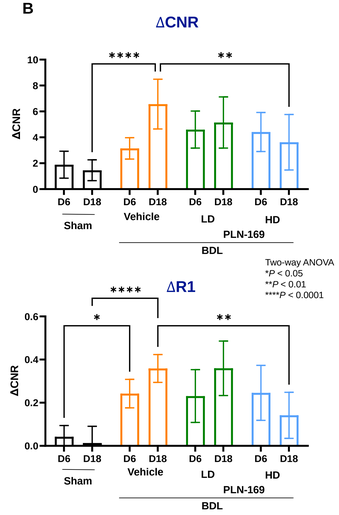
One-way ANOVA
*P < 0.05

- At Day 18 post-BDL, vehicle-treated rats had significantly higher integrin β_6 levels than sham rats as measured by gene expression and IHC
- PLN-169 treatment significantly reduced integrin β_6 protein levels in the liver

RESULTS Assessment of Hepatic Collagen

Figure 4. Type-I Collagen Imaging by CM-101 Identified Increased Hepatic Fibrosis with Injury and Reduction with Treatment



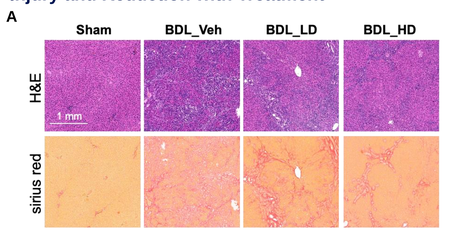


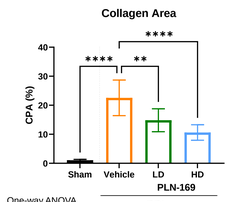
(A) Representative 3D T1W FLASH images (top) and R_1 maps (bottom) of rat livers acquired before and after CM-101 (15 min and 20 min post-injection, respectively). (B) Quantification of CM-101 ΔCNR (top) and ΔR_1 (bottom) of rat livers (15 min and 20 min post-injection, respectively) at day 6 (D6) and day 18 (D18) post-BDL. Statistical comparisons between vehicle and other groups at each timepoint are shown.

Veh = vehicle, LD = low dose PLN-169, HD = high dose PLN-169, CNR = liver-to-muscle contrast to noise ratio

- Vehicle-treated rats had significantly higher ΔCNR than sham rats 18 days post-BDL and significantly higher ΔR_1 6 and 18 days post-BDL
- High dose PLN-169 significantly reduced ΔCNR and ΔR_1 at day 18 post-BDL relative to vehicle. A trend down in ΔCNR was observed with low dose PLN-169.

Figure 5. Histological Assessment of Fibrosis Showed Increased Fibrosis with Injury and Reduction with Treatment



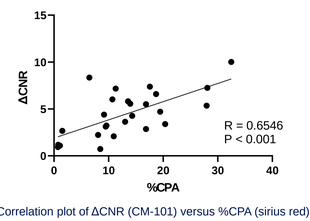


(A) Example H&E and sirius red images from rats 18 days post-BDL.
(B) Quantification of collagen percent area from sirius red staining

Veh = vehicle, LD = low dose PLN-169, HD = high dose PLN-169

One-way ANOVA
**P < 0.01
****P < 0.0001

Figure 6. Histological Assessment of Fibrosis Correlated with MRI-based Analysis



Correlation plot of ΔCNR (CM-101) versus %CPA (sirius red) in rat livers 18 days post-BDL

- Sirius red staining for collagen on liver samples from day 18 post-BDL showed a significant increase in fibrosis with BDL that is attenuated by treatment with PLN-169
- Collagen proportional area by sirius red stain correlates with ΔCNR from CM-101 MR imaging 18 days post-BDL

CONCLUSIONS

- **PET imaging for $\alpha_v\beta_6$ and molecular MRI of collagen non-invasively demonstrated changes in target expression and fibrosis in response to bile duct injury and treatment with a dual integrin $\alpha_v\beta_6/\alpha_v\beta_1$ antagonist in a rat model of biliary fibrosis**
- **Classic histological analysis confirmed the antifibrotic effect of dual integrin $\alpha_v\beta_6/\alpha_v\beta_1$ inhibition and the reduction in target expression**
- **Non-invasive imaging methods may support drug development in liver fibrosis by allowing assessment of target expression, target engagement, and treatment response to antifibrotic therapies**

References: 1. Farrar, C, et al. Radiology 2018; 287 (2). 2. Kimura, RH, et al. Nat Commun 2019; 10 (1).

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Disclosures: JS, JL, and ST are/were employees and shareholders of Pliant Therapeutics. PC is a consultant for Collagen Medical.