DUAL INHIBITION OF INTEGRINS $\alpha_v\beta_6$ AND $\alpha_v\beta_1$ DECREASES PORTAL PRESSURE AND LIVER FIBROSIS IN RATS WITH BILIARY CIRRHOSIS

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INTRODUCTION

- Expression of $\alpha_v\beta_6$ and $\alpha_v\beta_1$ integrins is limited to epithelial and fibroblast cells, respectively. Both integrins activate latent transforming growth factor-beta (TGF-β), leading to phosphorylation of SMAD2/3 and transcription of fibrogenic genes, promoting liver fibrosis[1]
- Depletion of α_v subunit in hepatic stellate cells showed protection from fibrosis progression in a murine carbon tetrachloride (CCl_4) model of hepatic fibrosis[2]
- Elevated levels of $\alpha_v\beta_6$ were observed in livers of patients with primary sclerosing cholangitis (PSC) and in preclinical murine models of PSC, where loss of $\alpha_v\beta_6$ activity reduced fibrogenesis.[3–5] $\alpha_v\beta_1$ is expressed by hepatic stellate cells and is highly increased in livers of patients with PSC[6] (**see poster THU434**).[7] Inhibition of $\alpha_v\beta_1$ showed protective effects against liver fibrosis in a CCl_4 animal model[6]
- Localized TGF-β inhibition in the fibrotic liver, achieved by targeting integrins $\alpha_v\beta_6$ and $\alpha_v\beta_1$, may provide a novel approach to treat PSC, without affecting systemic TGF-β signalling
- PLN-74809 is an oral, once-daily, dual-selective inhibitor of $\alpha_v\beta_6$ and $\alpha_v\beta_1$ integrins in development for the treatment of PSC, with orphan medicinal product designation granted by the European Medicines Agency[8] and orphan drug designation granted by the United States Food and Drug Administration[9]
- Available safety and pharmacokinetic findings from participants with PSC enrolled in Part 1 of the ongoing Phase 2a INTEGRIS-PSC study (PLN-74809-PSC-203; NCT04480840) continue to support the favourable tolerability profile of PLN-74809[7]
- Phase 2a INTEGRIS-PSC Part 2 evaluation of PLN-74809, dosed at 80 mg or 160 mg once daily vs. placebo, is currently underway (**see poster THU434**)[7]
- PLN-75068 (PLi) is a dual $\alpha_v\beta_6/\alpha_v\beta_1$ inhibitor used as a tool in preclinical studies

AIM

- To investigate the effects of PLi, a dual $\alpha_v\beta_6/\alpha_v\beta_1$ inhibitor, on liver fibrosis and portal hypertension in rats with cholestatic biliary cirrhosis. Riociguat (RIO), a soluble guanylyl cyclase stimulator, was used in a positive control group[10]

METHODS

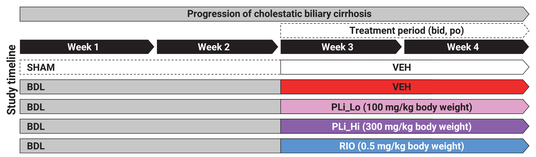

Figure 1. Study design

- Male Sprague–Dawley rats (strain: RjHan:SD) underwent bile duct ligation (BDL) or sham operation (SHAM) to induce cholestatic biliary cirrhosis
- Two weeks after surgery, PLi low dose (100 mg/kg) (PLi_Lo), PLi high dose (300 mg/kg) (PLi_Hi), RIO (0.5 mg/kg) or vehicle (VEH: 50% phosphate buffered saline [PBS], 50% propylene glycol) were administered twice daily via oral gavage for 2 weeks (**Figure 1**)
- At the end of the study timeline (i.e., 4 weeks after BDL/SHAM) the portal hypertensive syndrome was characterized under general anaesthesia by measurement of portal pressure (PP), mean arterial pressure (MAP), heart rate (HR) and splanchnic/portal blood flow.[11] Additionally, the hyperdynamic-index (HD-I: HR/MAP) was calculated as a marker for systemic vasodilation
- Blood was collected after haemodynamic assessment to measure key liver disease biomarkers (alanine transaminase [ALT], aspartate transaminase [AST], alkaline phosphatase [ALP] and bilirubin [BIL]) and plasma concentration of PLi
- Finally, liver tissue was harvested for further analyses:
  - Liver fibrosis was histologically quantified by automated morphometry of collagen proportionate area (CPA) on picrosirius red-stained full liver lobe sections and hepatic hydroxyproline content of a separate whole liver lobe
  - Active TGF-β signalling was assessed in bulk liver tissue by phosphorylated SMAD/SMAD2 ratio determined by a Meso Scale Discovery biomarker assay
  - Fibrogenic gene expression was assessed by a broad panel of genes using the NanoString platform

RESULTS

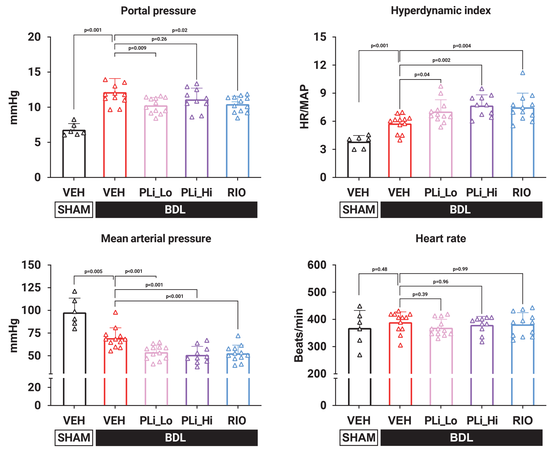

Figure 2. Determination of PP, HD-I, MAP and HR

- Cirrhotic BDL-VEH rats showed significantly higher (mean ± standard deviation) PP (12.2±1.9 vs. 6.8±0.9 mmHg; p<0.001) than healthy controls (SHAM)
- PP was lowered by PLi_Lo (-10%; p=0.009), PLi_Hi (-9%; p=0.26) and RIO (-10%; p=0.02)
- HD-I was increased in all treated groups (PLi_Lo: p=0.04; PLi_Hi: p=0.002; RIO: p=0.004) compared with diseased controls (BDL-VEH), most likely due to significantly decreased MAP in the treated groups compared with diseased controls (PLi_Lo: -22%; PLi_Hi: -27%; RIO: -24%; all p<0.001) while the HR remained unchanged across all groups (**Figure 2**)

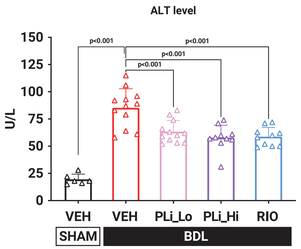

Figure 3. Liver injury by plasma levels of ALT

- Cirrhotic BDL-VEH rats exhibited significantly elevated ALT levels compared with healthy controls (85±17 U/L vs. 20±5 U/L; p<0.001), indicating relevant hepatocellular injury
- There was a significant decrease in ALT levels with PLi_Lo (63±10 U/L; p<0.001), PLi_Hi (58±12 U/L; p<0.001) and RIO (59±9 U/L; p<0.001) vs. BDL-VEH (**Figure 3**)
- There was a significant decrease in AST levels with RIO (p=0.03), but ALP and BIL levels in all treated rats were similar to those in cirrhotic BDL-VEH rats

FIGURE FOOTNOTES

Error bars represent standard deviation
ALT, alanine transaminase; BDL, bile duct ligation; bid, twice daily; *Col1a1*, collagen type I alpha 1; CPA, collagen proportionate area; *Ctgf*, connective tissue growth factor; HR, heart rate; MAP, mean arterial pressure; min, minute; mRNA, messenger ribonucleic acid; p.a.IC_{90}, protein-binding adjusted 90% maximal inhibitory concentration; PLi, Hi: PLN-75068 high dose (300 mg/kg); PLi_Lo, PLN-75068 low dose (100 mg/kg); po, *per os* (by oral gavage); pSMAD, phosphorylated SMAD; RIO, riociguat; SHAM, sham operation; SMAD, family of proteins similar to the gene products of the Drosophila gene 'mothers against decapentaplegic' (*Mad*) and the *C. elegans* gene *Sma*; VEH, vehicle

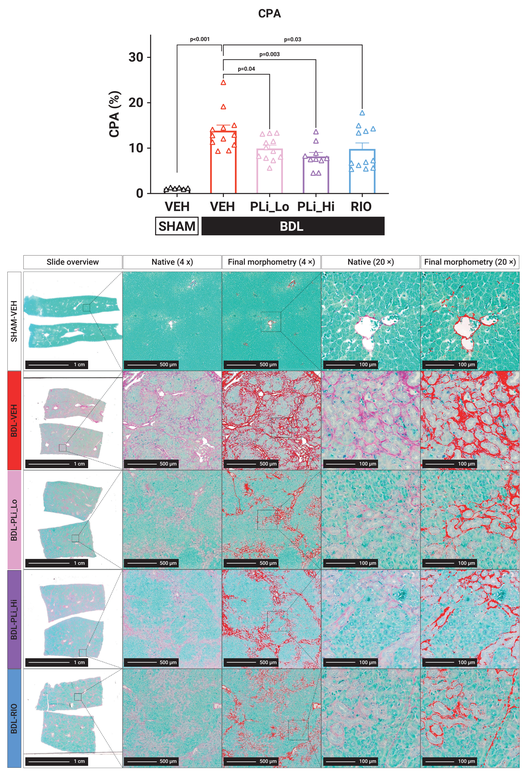

Figure 4. Quantification of hepatic fibrosis as CPA by picrosirius-red/fast-green staining

- Fibrosis of liver tissue was histologically assessed by CPA. Cirrhotic BDL-VEH rats showed significantly higher CPA than healthy controls (13.8±4.3 vs.1.1±0.2%; p<0.001)
- Fibrosis of liver tissue was decreased by PLi_Lo (-28%; p=0.04), PLi_Hi (-41%; p=0.003) and RIO (-29%; p=0.03) compared with BDL-VEH (**Figure 4**)
- Hepatic hydroxyproline levels showed no significant differences across PLi- and RIO-treated vs. untreated BDL rats (VEH: 489±163 µg/g; PLi_Lo: 508±111 µg/g; PLi_Hi: 473±114 µg/g; RIO: 476±174 µg/g), suggesting that the reductions in CPA may reflect changes to collagen architecture or loss of a subset of collagen structures rather than changes to total collagen content

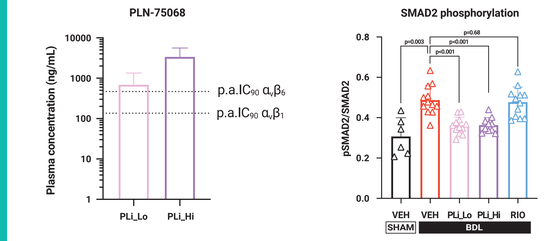

Figure 5. Plasma concentration of PLi

Figure 6. TGF-β signalling by SMAD2 phosphorylation

- Both doses of PLi reached >90% maximal inhibitory concentration for $\alpha_v\beta_6$- and $\alpha_v\beta_1$-mediated TGF-β activation at ~maximum concentration in blood plasma and the PLi_Hi dose achieved approximately 4–5 times higher plasma concentration than the PLi_Lo dose (2860 ng/L vs. 702 ng/L, respectively) (**Figure 5**)
- PLi_Hi treatment induced weight loss (-3.44%) during the treatment period compared with treatment start, but this was not statistically significant
- Rats treated with PLi showed significantly decreased SMAD2 phosphorylation compared with BDL-VEH (PLi_Lo: 0.36±0.04, p<0.001; PLi_Hi: 0.36±0.04, p<0.001; vs. 0.49±0.07), while RIO had no effect on SMAD2 phosphorylation, indicating a reduction in TGF-β signalling with inhibition of $\alpha_v\beta_6$ and $\alpha_v\beta_1$ integrins specifically (**Figure 6**)

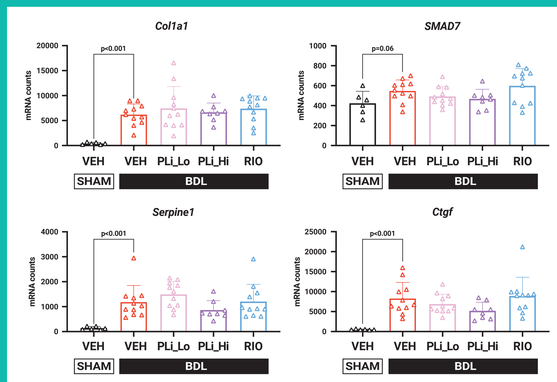

Figure 7. Fibrogenic gene expression

- Regarding fibrogenic gene expression, at the terminal timepoint there were no significant changes in collagen type I alpha 1 (*Col1a1*) and a non-significant trend towards a PLi-dose-dependent reduction of TGF-β target genes *SMAD7*, *Serpine1* and connective tissue growth factor (*Ctgf*) compared with BDL-VEH (**Figure 7**)

CONCLUSIONS

- Dual $\alpha_v\beta_6/\alpha_v\beta_1$ inhibition by PLi ameliorated portal hypertension in rats with biliary cirrhosis
- Dose-dependent plasma pharmacokinetics and inhibition of SMAD2 phosphorylation suggest effective target engagement of PLi
- Liver fibrosis assessed by CPA was decreased in rats treated with PLi_Lo and PLi_Hi
- Some dose-dependent inhibitory effects on the TGF-β gene targets *Ctgf*, *Serpine1* and *SMAD7* were observed; however, other fibrosis readouts remained unchanged by integrin inhibition by PLi or RIO
- Additional mechanistic studies are warranted to explore the underlying mechanism of the PP-lowering effects of integrin $\alpha_v\beta_6/\alpha_v\beta_1$ inhibition of biliary cirrhosis

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