RATIONALE FOR EVALUATION OF PLN-74809 TREATMENT IN PARTICIPANTS WITH PRIMARY SCLEROSING CHOLANGITIS IN PHASE 2A STUDY INTEGRIS-PSC

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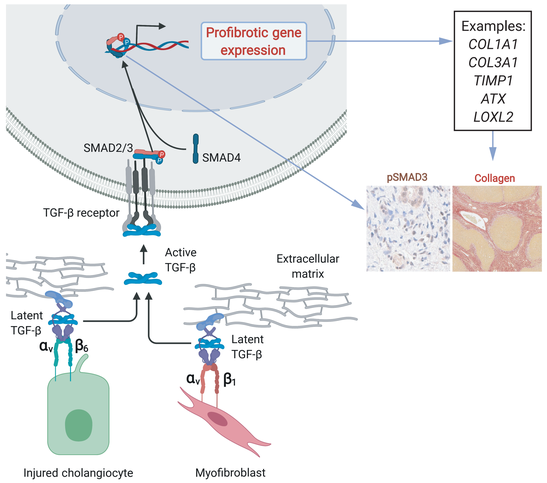
INTRODUCTION

- Primary sclerosing cholangitis (PSC) is a rare, idiopathic, cholestatic liver disease characterized by biliary inflammation and progressive fibrosis[1,2]
  - Over time, biliary and hepatic inflammation progresses to serious, and often fatal, liver complications, such as cirrhosis, portal hypertension and end-stage liver disease[1,3]
- Transforming growth factor-beta (TGF-β) signalling activated by α_v integrins is a key driver of fibrosis in the liver[4]
- In PSC, integrins expressed on injured cholangiocytes ($\alpha_v\beta_6$) and myofibroblasts ($\alpha_v\beta_1$) regulate TGF-β activity[5] (**Figure 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[6,7] (**Figure 2**)

Figure 1. Roles of $\alpha_v\beta_6$ and $\alpha_v\beta_1$ integrins in biliary fibrosis



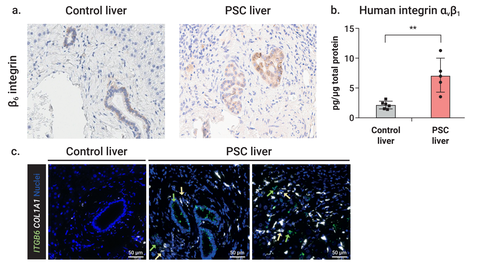
This diagram has been developed by Pliant Therapeutics, Inc.
ATX, autotaxin; *COL1A1*, collagen type I alpha 1 chain; *COL3A1*, collagen type 3 alpha 1 chain; *LOXL2*, lysyl oxidase homolog 2; pSMAD, phosphorylated SMAD; SMAD, family of proteins similar to the gene products of the Drosophila gene 'mothers against decapentaplegic' (*Mad*) and the *C. elegans* gene *Sma*; TGF-β, transforming growth factor-beta; *TIMP1*, tissue inhibitor matrix metalloproteinase 1

- There are no widely approved medical treatments for PSC; disease management is confined to supportive measures, which fail to address disease progression. Thus, there remains a significant unmet medical need for effective therapies for PSC
- 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]
- PLN-74809 has been administered to over 450 study participants (including healthy volunteers and patients), achieved high target engagement of $\alpha_v\beta_6$ and reduced TGF-β signalling in the human lung. PLN-74809 has a favourable safety and tolerability profile with no drug-related serious adverse events (AEs) or drug-related severe AEs reported to date[10]
- Localized TGF-β inhibition in the fibrotic liver, achieved by targeting $\alpha_v\beta_6$ and $\alpha_v\beta_1$ integrins with PLN-74809, may provide a novel approach for treating PSC, without affecting systemic TGF-β signalling

AIM

- To summarize the preclinical studies investigating the antifibrotic activity resulting from dual integrin inhibition by PLN-74809 in precision-cut liver slices (PCLivS) from patients with PSC and *in vivo* models of PSC that support the Phase 2a clinical evaluation of PLN-74809 in participants with large-duct PSC in the ongoing INTEGRIS-PSC study (PLN-74809-PSC-203; NCT04480840)

Figure 2. Determination of $\alpha_v\beta_6$ and $\alpha_v\beta_1$ integrin expression in explants from patients with PSC



Protein expression of integrins (**a.**) $\alpha_v\beta_6$ and (**b.**) $\alpha_v\beta_1$ was higher in explants from patients with PSC compared with control human liver tissue, as determined by IHC and the Meso Scale Discovery custom electrochemiluminescence-based assay, respectively (error bars represent standard deviation; **p<0.01 vs. control). *In situ* hybridization of explants from patients with PSC and control human liver tissue (**c.**) for expression of *ITGB6* (green) and *COL1A1* (white) demonstrates that cells expressing *ITGB6* (green arrows) are adjacent to those expressing collagen (yellow arrows) in regions of classic onion skin fibrosis (middle) and cirrhosis (right)
COL1A1, collagen type I alpha 1 chain; IHC, immunohistochemistry; *ITGB6*, integrin subunit beta 6; PSC, primary sclerosing cholangitis

PRECLINICAL EVIDENCE

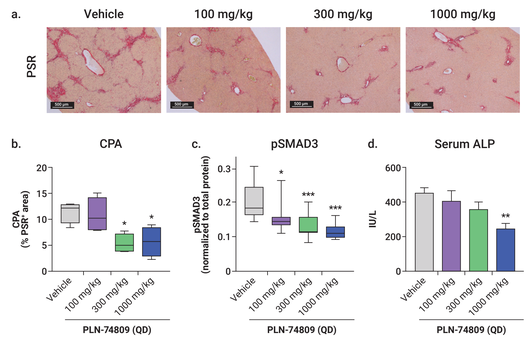
In vivo methodology

- PLN-74809 or vehicle were administered orally for 6 weeks in BALBc.Mdr2$^{-/-}$ mice with established fibrosis
- A tool $\alpha_v\beta_6$ and $\alpha_v\beta_1$ inhibitor compound, PLN-75068, was tested therapeutically in a diet-induced mouse model of biliary fibrosis using 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC)
- Hepatic collagen was quantified by hydroxyproline (OHP) and collagen proportionate area (CPA), TGF-β signalling by phosphorylated SMAD3 (pSMAD3) levels and cholestasis by serum alkaline phosphatase (ALP) levels

In vivo results

- PLN-74809 dose-dependently reduced OHP vs. vehicle (up to ~30%; p<0.05, data not shown), CPA (up to ~50%; p<0.05), pSMAD3 (up to ~40%; p<0.001) and serum ALP (46%; p<0.01) in the BALBc.Mdr2$^{-/-}$ mouse model (**Figure 3**)
  - PLN-75068 reduced OHP vs. vehicle (up to ~20%; p<0.05) in DDC-injured mice in a dose-dependent manner (data not shown)

Figure 3. Effect of dual $\alpha_v\beta_6$ and $\alpha_v\beta_1$ inhibition with PLN-74809 on collagen deposition and cholestasis in the BALBc.Mdr2$^{-/-}$ model

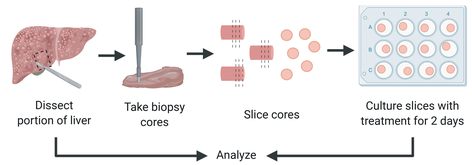


BALBc.Mdr2$^{-/-}$ mice were administered vehicle or PLN-74809 100 mg/kg, 300 mg/kg or 1000 mg/kg QD for 6 weeks. Reduced collagen deposition was determined by (**a.**) PSR staining, with (**b.**) quantification as CPA. Reduced TGF-β signalling was determined by (**c.**) pSMAD3 quantification using the Meso Scale Discovery custom electrochemiluminescence assay. Reduced cholestasis was determined with an assay to assess (**d.**) serum ALP. Error bars represent standard deviation; *p<0.05, **p<0.01, ***p<0.001 vs. vehicle (PBS) *, positive; ALP, alkaline phosphatase; CPA, collagen proportionate area; PBS, phosphate buffered saline; pSMAD, phosphorylated SMAD; PSR, picrosirius red; QD, once daily; SMAD, family of proteins similar to the gene products of the Drosophila gene 'mothers against decapentaplegic' (*Mad*) and the *C. elegans* gene Sma; TGF-β, transforming growth factor-beta

Ex vivo (PCLivS) methodology

- An *ex vivo* study evaluated the effects of 2-day treatment with PLN-74809 on the expression of profibrotic genes, collagen type I alpha 1 chain (*COL1A1*) and collagen type I alpha 2 chain (*COL1A2*), in PCLivS from patients with biliary fibrosis (n=4 PSC; n=3 primary biliary cholangitis [PBC]) (**Figure 4**)

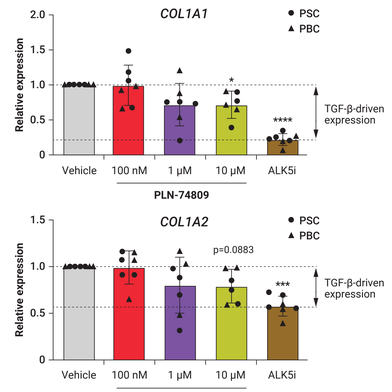
Figure 4. Schematic diagram showing the procedure to generate PCLivS from liver explants



Ex vivo (PCLivS) results

- After 2 days in culture and incubation with PLN-74809, TGF-β-driven *COL1A1* and *COL1A2* gene expression was reduced in a dose-dependent manner (up to ~35%; p<0.05 and up to ~50%; p=0.0883, respectively) in PCLivS generated from explants from patients with PSC and PBC compared with vehicle (dimethylsulfoxide) (**Figure 5**)

Figure 5. Effect of PLN-74809 and TGF-β receptor I kinase inhibitor (ALK5i; positive control) on collagen gene expression in PCLivS generated from liver explants from patients with biliary fibrosis (n=4 PSC; n=3 PBC)

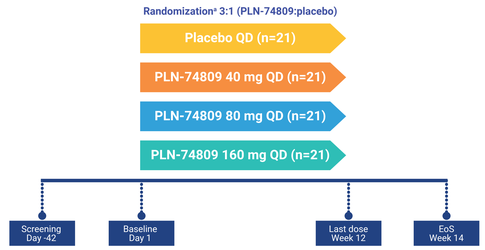


Error bars represent standard deviation; *p<0.05; ***p<0.001; ****p<0.0001 vs. vehicle (DMSO)
ALK5i, TGF-β receptor I kinase inhibitor; *COL1A1*, collagen type I alpha 1 chain; *COL1A2*, collagen type I alpha 2 chain; DMSO, dimethylsulfoxide; PBC, primary biliary cholangitis; PCLivS, precision-cut liver slices; PSC, primary sclerosing cholangitis; TGF-β, transforming growth factor-beta

INTEGRIS-PSC CLINICAL STUDY

- INTEGRIS-PSC (PLN-74809-PSC-203; NCT04480840) is a Phase 2a, multinational, randomized, double-blind, dose-ranging, placebo-controlled study to evaluate the safety, tolerability and pharmacokinetics (PK) of once-daily (QD) treatment with oral PLN-74809 over 12 weeks in approximately 84 adult participants (**Figure 6**)
- Key eligibility criteria include:
  - 18–75 years of age
  - Established clinical diagnosis of large-duct PSC
  - Normal or elevated serum ALP concentration ≤10 × upper limit of normal (ULN)
  - Serum aspartate aminotransferase and serum alanine aminotransferase concentrations ≤5 × ULN
  - Serum total bilirubin ≤1.5 × ULN
  - Suspected liver fibrosis without cirrhosis by liver stiffness measurement, enhanced liver fibrosis score, historical liver biopsy demonstrating fibrosis or magnetic resonance elastography
  - Participants are allowed to receive treatment with ursodeoxycholic acid, provided it has been administered at a stable dose of <25 mg/kg/day for ≥3 months before screening and is expected to remain unchanged during the study
  - Treatment with inflammatory bowel disease therapies is allowed, provided the dose(s) has been stable for 12 weeks
- Safety assessments include type, incidence and severity of AEs, laboratory tests, vital signs, safety electrocardiograms (ECGs) and physical examinations
- PK samples are collected pre-dose and at least 2 hours post-dose on Day 1 (Baseline/randomization), Week 4 and Week 12

Figure 6. INTEGRIS-PSC study design



[a]Randomization stratified by use of UDCA (yes/no) at Baseline
EoS, end of study; QD, once daily; UDCA, ursodeoxycholic acid

Interim safety and PK findings

- Here, we present data from a review of blinded safety and PK data from the fully enrolled Part 1 (i.e., PLN-74809 40 mg vs. placebo, QD; 3:1 randomization) of the ongoing INTEGRIS-PSC study performed by the Data Safety Monitoring Board (DSMB) to support evaluation of higher daily doses (i.e., Part 2, PLN-74809 80 mg and 160 mg vs. placebo, QD)
  - Safety data from blinded study drug (i.e., PLN-74809 or placebo) were evaluated and compared with the known safety and tolerability profile of PLN-74809
  - PK data from participants with PSC were compared with PK data from healthy volunteers
- A total of 29 participants with PSC were randomized to Part 1 (i.e., PLN-74809 40 mg vs. placebo, QD; 3:1 randomization ratio)
  - At the time of the DSMB analysis cut-off date, 26 participants received treatment with blinded study drug and 10 had completed 12-week treatment
- PLN-74809 was generally well tolerated in participants with PSC
  - Most AEs were mild; none were severe. The most common AE was headache, reported in 3 participants; all other AEs were reported in ≤2 participants
  - One participant prematurely discontinued due to AEs of dyspnoea and congestion associated with coronavirus disease 2019
  - No notable changes in haematology and biochemistry parameters, vital signs or ECG parameters were observed
- One participant who completed 12-week treatment with blinded study drug experienced 3 serious AEs (Grade 3 abdominal pain, cholecystitis and pancreatitis) at least 20 days after the last dose; all events resolved and were deemed not related to the study drug by the Investigator (pancreatitis complication from endoscopic retrograde cholangiopancreatography)
- PLN-74809 PK in participants with PSC were consistent with those of healthy volunteers, based on estimation of area under the plasma concentration-time curve between 0 and 24 hours (AUC_{0-24} using a population PK model[11]

CONCLUSIONS

- **Pharmacological inhibition of $\alpha_v\beta_6$ and $\alpha_v\beta_1$ integrins demonstrated antifibrotic activity in two models of biliary fibrosis and in PCLivS from patients with PSC or PBC**
- **Favourable tolerability profile for PLN-74809 was observed based on available blinded safety data and PK findings from participants with PSC enrolled in Part 1 of the ongoing Phase 2a INTEGRIS-PSC study**
- **Evaluation of PLN-74809 doses of 80 mg and 160 mg QD vs. placebo is currently underway in Part 2 of the INTEGRIS-PSC study**

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