LPA1 Antagonism by PIPE-791 Reduces Alveolar Epithelial Cell Apoptosis *In Vitro*

Michael M Poon, Alexander R Broadhead, Christopher S Baccei, Didier Bagnol, Jeffrey R Roppe, Thomas O Schrader, Lino Valdez, Yifeng Xiong, Austin C Chen, Timothy Watkins, Daniel S Lorrain.
Contineum Therapeutics, San Diego, CA

Introduction

Alveolar epithelial cell loss is a hallmark of idiopathic pulmonary fibrosis (IPF). PIPE-791 is an LPA1 selective, small molecule antagonist currently in a Phase 2 clinical trial for IPF.

Type I alveolar epithelial cells (AT1) line the lung alveoli and facilitate gas exchange. Repeated injury leads to AT1 cell loss and pro-inflammatory factor release possibly contributing to IPF etiology.

AT1 cells are lost via apoptosis, an effect that is diminished in LPA1$^{-/-}$ mice (Funke et al., 2012). Here we show:

- *In vitro* - PIPE-791 reduces LPA mediated human AT1 apoptosis
- *In vivo* - PIPE-791 reduces bleomycin induced AT1 apoptosis in mice
- Conditioned media from human IPF-derived precision cut lung slice (PCLS) cultures induces apoptosis in an LPA1-sensitive manner (inhibited by PIPE-791).

Methods

***In vitro* AT1 apoptosis assay.** Human primary epithelial cells (HPAEpiCs, ScienCell) were plated at 15k/96-well and adhered overnight. In culture, HPAEpiCs terminally differentiate into AT1 cells. Cells were pretreated with vehicle or 300 nM PIPE-791 for 8h. LPA was added to the cells, incubated for 24h, then immunostained for podoplanin (Pdpn), cleaved caspase 3 (Asp175), and Hoechst. Apoptosis was measured as a percent cleaved caspase 3$^+$/Pdpn$^+$/Hoechst$^+$ of total Pdpn$^+$/Hoechst$^+$.

***In vivo* bleomycin model.** Female C57B6 mice were dosed with PIPE-791 (3 mg/kg) 24h prior to intratracheal bleomycin (3u/kg) administration. Lungs were harvested 3 days and inflated with formalin. Left and inferior lobes were sectioned and immunostained for Pdpn, TUNEL, and Hoechst. Cells quantified as a percentage of TUNEL$^+$/Pdpn$^+$ total Pdpn cells.

***Ex vivo* conditioned media studies.** Cryopreserved precision cut lung slices (PCLS; Anabios) were cultured in vehicle or 300 nM PIPE-791 for 6 days. Conditioned media was collected at study end and frozen (-80 ºC). Cultured human AT1 cells were preincubated with vehicle or 300 nM PIPE-791 for 8 h, then 50% of culture media exchanged for conditioned media from IPF or healthy donor PCLS. In some cases PIPE-791 was added to reach a 300 nM. After 1 day, cells were fixed and stained as described for the *in vitro* AT1 apoptosis assay.

AT1 cells line the alveolar wall

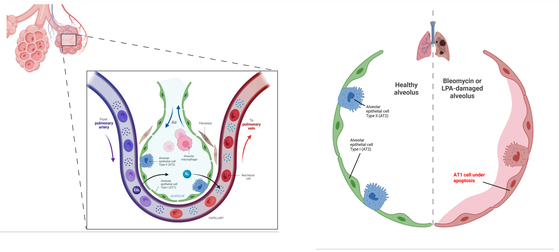


Figure 1. Alveolar epithelial cells line the wall of the aveolus and are comprised of Type I (AT1) and Type II cells (AT2). AT1 cells are generally responsible for enabling gas exchange whereas ATII cells can differentiate into and replace AT1 cells in the event of loss. Upon acute injury, AT1 cells undergo apoptosis. Upon repeated injury, loss of these cells can contribute to more chronic indications such as pulmonary fibrosis.

Podoplanin is a marker for AT1 cells

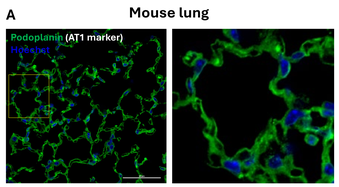


Figure 2. Podoplanin is a marker for AT1 cells. A. Podoplanin (Pdpn) is a marker for AT1 cells. Normal mouse lungs were dissected and inflated with formalin then sectioned and immunostained with an antibody for Pdpn, counterstained with Hoechst. Yellow inset magnified (right) B. Human HPAEpiCs differentiated into AT1 cells stain positive for Pdpn. C. Relative *LPA* receptor expression in the cultured cells.

PIPE-791 prevents LPA-induced AT1 apoptosis

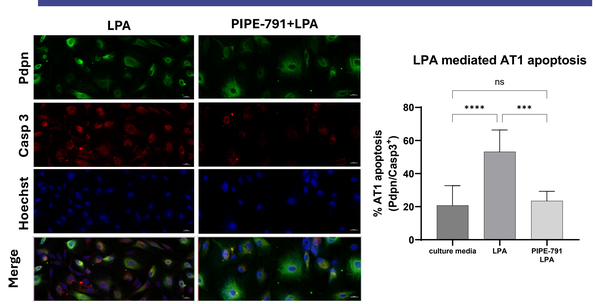
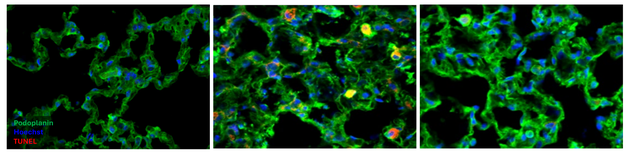


Figure 3 PIPE-791 prevents LPA-induced AT1 apoptosis. Cultured AT1 cells were treated with vehicle or PIPE-791 (300 nM) 8 h, then LPA (10 µM) added for an additional 24 h. Apoptosis was evaluated using an antibody against cleaved caspase 3 and percent apoptosis calculated. Left, representative images, Pdpn (green), cleaved caspase (red), Hoechst, (blue). Right, PIPE-791 significantly inhibits LPA induced apoptosis (**** p < 0.0001, n>7, one way ANOVA, Tukey's)

PIPE-791 prevents bleomycin induced AT1 apoptosis *in vivo*



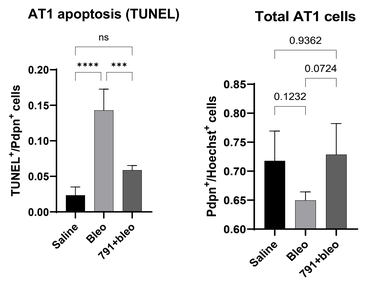
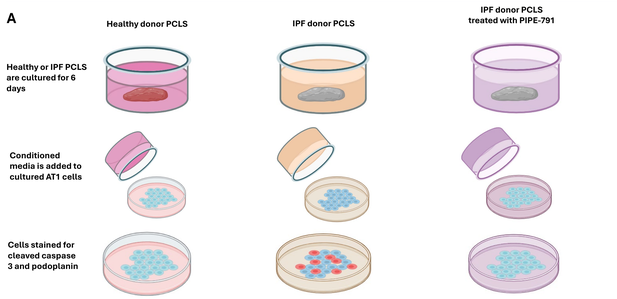


Figure 4. PIPE-791 prevents bleomycin induced AT1 apoptosis *in vivo*. Mice were treated with 3 mg/kg PIPE-791 24 h prior to intratracheal delivery of bleomycin (3u/kg). Lungs were harvested 3 d later and inflated with formalin. Lungs were then removed and inferior and left lobes were sectioned and stained for Pdpn, TUNEL, and Hoechst. Images were (*** p < 0.001, **** P < 0.0001, One way ANOVA, Tukey's, n=4)

Conditioned media from IPF-PCLS induces AT1 apoptosis in an LPA1-sensitive manner



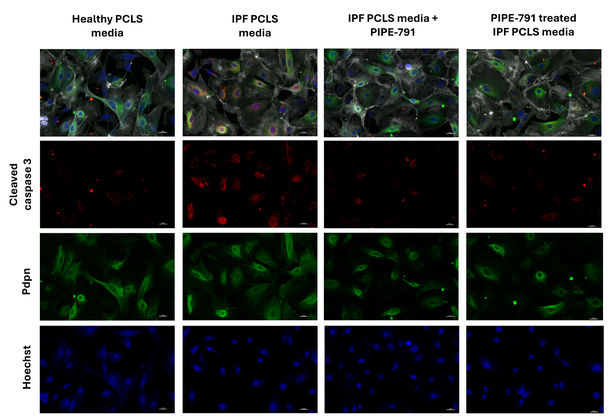


Figure 5A. Experimental Design. Conditioned media (after 6 *div*) from healthy or IPF-donor precision cut lung slices was collected and applied to cultured AT1 cells. Cells were cultured in conditioned media for 24 h, then stained for Pdpn, cleaved caspase 3, and Hoechst.

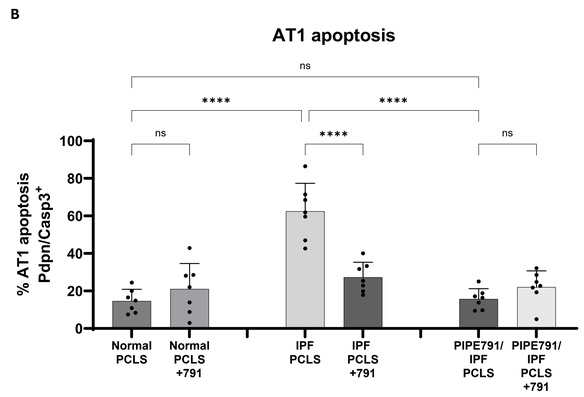


Figure 5B. IPF-donor conditioned media induces apoptosis, whereas normal PCLS conditioned media does not. Apoptosis was inhibited when PIPE-791 (300 nM) was added to AT1 cells prior to IPF conditioned media addition or when PIPE-791 (300 nM) was added to IPF-PCLS during media conditioning. Mass spec analysis indicates that PIPE-791 in the IPF-PCLS conditioned media was below limit of quantification (<1.05 nM, n=4).

PIPE-791 reduces fibrosis transcripts in IPF PCLS

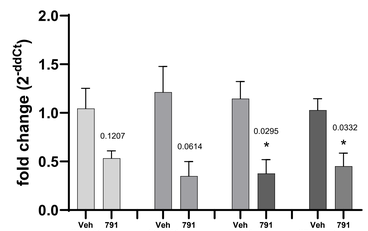


Figure 6. PIPE-791 treatment of PCLS reduces fibrosis-related transcripts including *COL1A1*, *COL3A1*, *TIMP1*, and *SERPINE1*. Conditioned media from IPF-PCLS were used in the experiments described in Figure 5.

Donor: 64yo, Caucasian female, idiopathic pulmonary fibrosis; symmetric bronchiectasis. Coronary artery disease. Hypertension 0-5yrs; Skin - squamous basal cell carcinoma on nose, removed in 2020. Wine, champagne, beer 2-3 occasionally 2x/mo x 40yrs. Patient worked up for lung transplant. PCLS collected from left superior R1.

Conclusions

- Alveolar epithelial cells Type I (AT1) undergo apoptosis following LPA (*in vitro*) or bleomycin (*in vivo*) insult.
- Apoptosis is prevented by PIPE-791, a selective LPA1 antagonist.

PIPE-791, via LPA1, prevents AT1 apoptosis, adding another facet of addressing IPF in addition to its established role as an antifibrotic.

1. Camelo A, Dunmore R, Sleeman MA, Clarke DL. The epithelium in idiopathic pulmonary fibrosis: breaking the barrier. Front Pharmacol. 2014 Jan 10;4:173.
2. Funke M, Zhao Z, Xu Y, Chun J, Tager AM. The lysophosphatidic acid receptor LPA1 promotes epithelial cell apoptosis after lung injury. Am J Respir Cell Mol Biol. 2012 Mar;46(3):355-64. doi: 10.1165/rcmb.2010-0155OC.
3. Poon M, Lorrain K, Broadhead A, Stebbins K, Bagnol D, Edu G, Joseph G, Baccei C, Roppe J, Schrader T, Valdez L, Xiong Y, Chen A, Lorrain D. The LPAR1 antagonist, PIPE-791 produces antifibrotic effects in models of lung fibrosis. Respir Res. 2025 Aug 31;26(1):265.