REVERSE REMODELING AND ANTI-PROLIFERATIVE EFFECTS OF SERALUTINIB IN PAH PRECISION-CUT LUNG SLICES AND PULMONARY ARTERY SMOOTH MUSCLE CELLS

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BACKGROUND

- Pulmonary vascular remodeling in pulmonary arterial hypertension (PAH) involves abnormal muscularization of small pulmonary arteries
- Seralutinib is a novel, highly potent, and selective PDGFRα/β, CSF1R, and c-KIT tyrosine kinase inhibitor, targeting pathways that drive pulmonary artery vascular remodeling[1,2]
- BMPR2 deficiency is associated with a genetic disposition to develop PAH. Seralutinib induces BMPR2 and its downstream SMAD1/5/8 signaling in SuHx rat model of PAH[2]
- Study of pulmonary artery smooth muscle cells (PASMCs) from patients with idiopathic PAH (IPAH) allowed investigation of the anti-proliferative effects of seralutinib in these phenotypically distinct cells
- Use of precision-cut lung slices (PCLS) from patients with IPAH provided an opportunity to directly investigate the potential reverse remodeling effects of seralutinib

METHODS

- In IPAH PASMCs, proliferation (BrdU assay), protein (pPDGFR, pERK, pSMAD1/5/8), and mRNA (BMPR2, ID1/2/3) levels were assessed (**Figure 1**)
- In IPAH PCLS, seralutinib-induced changes in pulmonary artery muscularization (α-smooth muscle actin, αSMA), vessel thickness (histomorphometry), and apoptosis (TUNEL assay) were evaluated (**Figure 2**)
- Statistical analysis was performed using one-way analysis of variance (ANOVA), Dunnett's test

Figure 1. *In Vitro* Model: IPAH PASMCs.

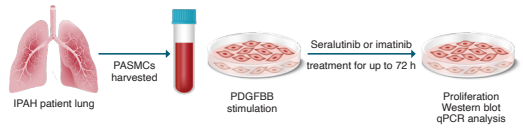
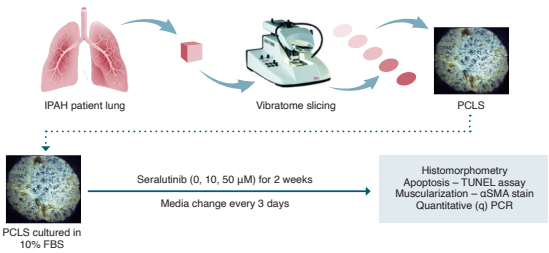
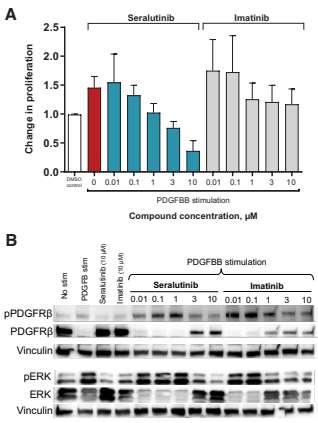


Figure 2. *Ex Vivo* Model: IPAH PCLS.



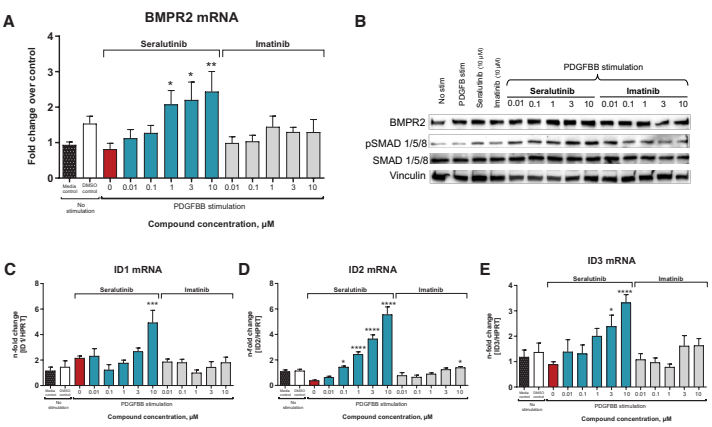
RESULTS

Seralutinib inhibited PDGFR signaling and proliferation of IPAH PASMCs



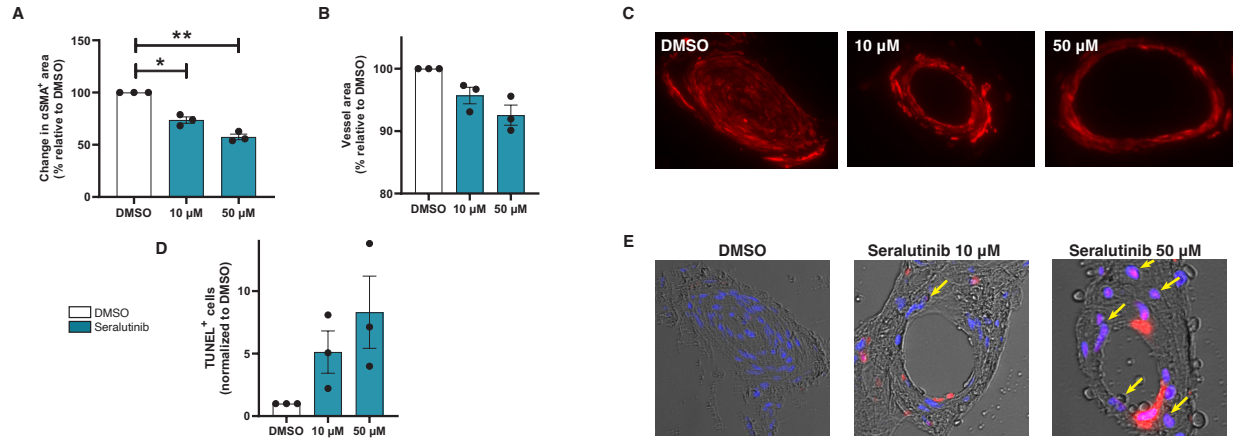
PASMCs were stimulated with PDGFBB (30 ng/mL) for 6 h for pPDGFR and pERK readout, and for 72 h for proliferation assay readout. Phosphorylation of PDGFRß and ERK was measured using Western blot analysis. Proliferation of cells was measured using BrdU assay. (**A**) Change in proliferation after PDGFBB stimulation with seralutinib or imatinib. (**B**) Western blot analysis showed decreased PDGFBB-induced phosphorylation of PDGFRβ and ERK following seralutinib treatment. Data represented as mean ± SEM (n=3 per treatment group).

Seralutinib treatment induced BMPR2 signaling and downstream ID genes in IPAH PASMCs



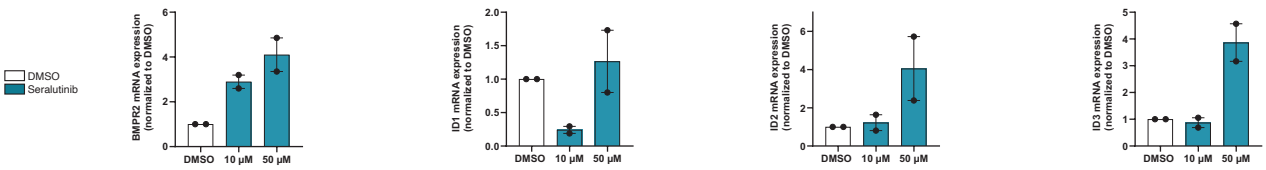
PASMCs were stimulated with PDGFBB (30 ng/mL) in presence of seralutinib or imatinib (0.01-10 μM) for 6 h, 24 h, and 48 h for measurement of pSMAD1/5/8, ID mRNA, and BMPR2 mRNA, respectively. At the end of the treatment, (**B**) phosphor and total SMAD1/5/8 and BMPR2 were measured using Western blot analysis. qPCR was performed to measure levels of (**A**) BMPR2 and its (**C–E**) downstream ID 1, 2, and 3 genes. Data represented as mean ± SEM (n=3 per treatment group). Statistical analysis was performed using one-way ANOVA with Dunnett's multiple comparisons test. *P<0.05, **P<0.01, ***P<0.001, and ****P<0.0001, seralutinib versus vehicle treatment group.

Seralutinib reverses pulmonary vessel remodeling in human IPAH PCLS



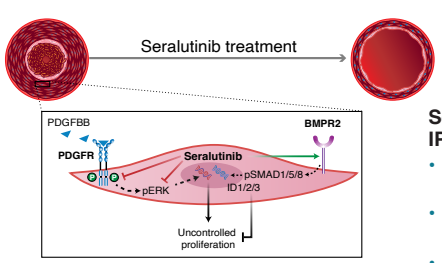
IPAH patient-derived PCLS were treated with seralutinib (10 μM, 50 μM) or DMSO for 14 days. Vascular remodeling was measured with change in the αSMA area and vessel area, and induction of apoptosis. Bar graph showing change in (**A**) αSMA+ area, (**B**) vessel area, and (**D**) TUNEL+ cells. Representative images of pulmonary artery (**C**) αSMA stain (red), and (**E**) TUNEL+ cells (red), nuclei stain (blue). Yellow arrows point to TUNEL+ cells. Data represented as mean ± SEM (n=3 for each treatment group). Statistical analysis was performed using one-way ANOVA with Dunnett's multiple comparisons test. *P<0.05, **P<0.01, seralutinib versus vehicle treatment group.

Seralutinib induces BMPR2 and its downstream ID genes expression in IPAH PCLS



RNA was isolated from PCLS after treatment with seralutinib (10 μM, 50 μM) or DMSO, and the expression analysis of BMPR2, ID1, ID2, and ID3 was performed using qPCR. Data represented as mean ± SEM (n=2 for each treatment group).

GRAPHICAL SUMMARY & CONCLUSIONS



Seralutinib treatment in IPAH-derived PCLS shows

- Decrease in muscularization
- Decrease in vessel wall thickness
- Increase in apoptosis

- In IPAH PASMCs, seralutinib inhibits PDGFR signaling and proliferation
- Seralutinib induces BMPR2 mRNA and downstream ID genes in PAH PASMCs
- Seralutinib demonstrated reverse remodeling in PCLS derived from PAH patients
- Inhaled seralutinib is in phase 3 development for PAH (PROSERA; NCT05934526)

References: 1 Pullamsetti SS, et al. *Int J Mol Sci.* 2023;24(16):12653.
2 Galkin A, et al. *Eur Respir J.* 2022;60(6):2102356.