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Seralutinib demonstrated *in vitro* reduction of vascular inflammatory drivers underlying pulmonary hypertension

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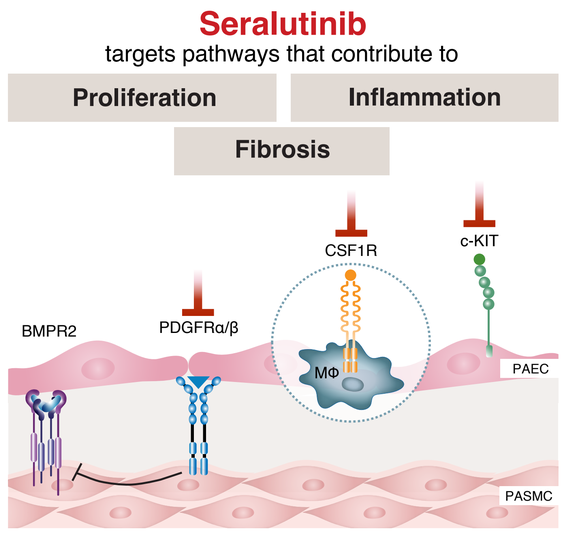
BACKGROUND

- Inflammation and immune dysregulation contribute to vascular remodeling in group 1 and 3 pulmonary hypertension subtypes[1,2]
- The degree of perivascular immune infiltration correlates with vascular remodeling[2-5]
- Cytokines and chemokines produced by infiltrating immune cells contribute to endothelial injury, smooth muscle cell proliferation, and fibrogenesis/ fibroblast activation[6-8]
- Seralutinib, a potent inhaled PDGFR, CSF1R, and c-KIT tyrosine kinase inhibitor (**Figure 1**), significantly reduced pulmonary vascular resistance in the phase 2 TORREY study in pulmonary arterial hypertension (PAH; NCT04456998)[9]
- Seralutinib treatment was associated with an anti-inflammatory proteomic signature after 24 weeks of treatment in TORREY[10] and in the SU5416/Hypoxia model of PAH[11]
- This study assessed seralutinib's impact on inflammation in primary human cellular immune assays

Figure 1. Seralutinib mechanism of action



Blunted arrows indicate inhibition.
BMPR2, bone morphogenetic protein receptor type 2; c-KIT, mast/stem cell growth factor receptor kit; CSF1R, colony stimulating factor 1 receptor; MΦ, macrophage; PAEC, pulmonary artery endothelial cell; PASMC, pulmonary artery smooth muscle cell; PDGFR, platelet-derived growth factor receptor.

METHODS

In vitro system of primary human vascular inflammation

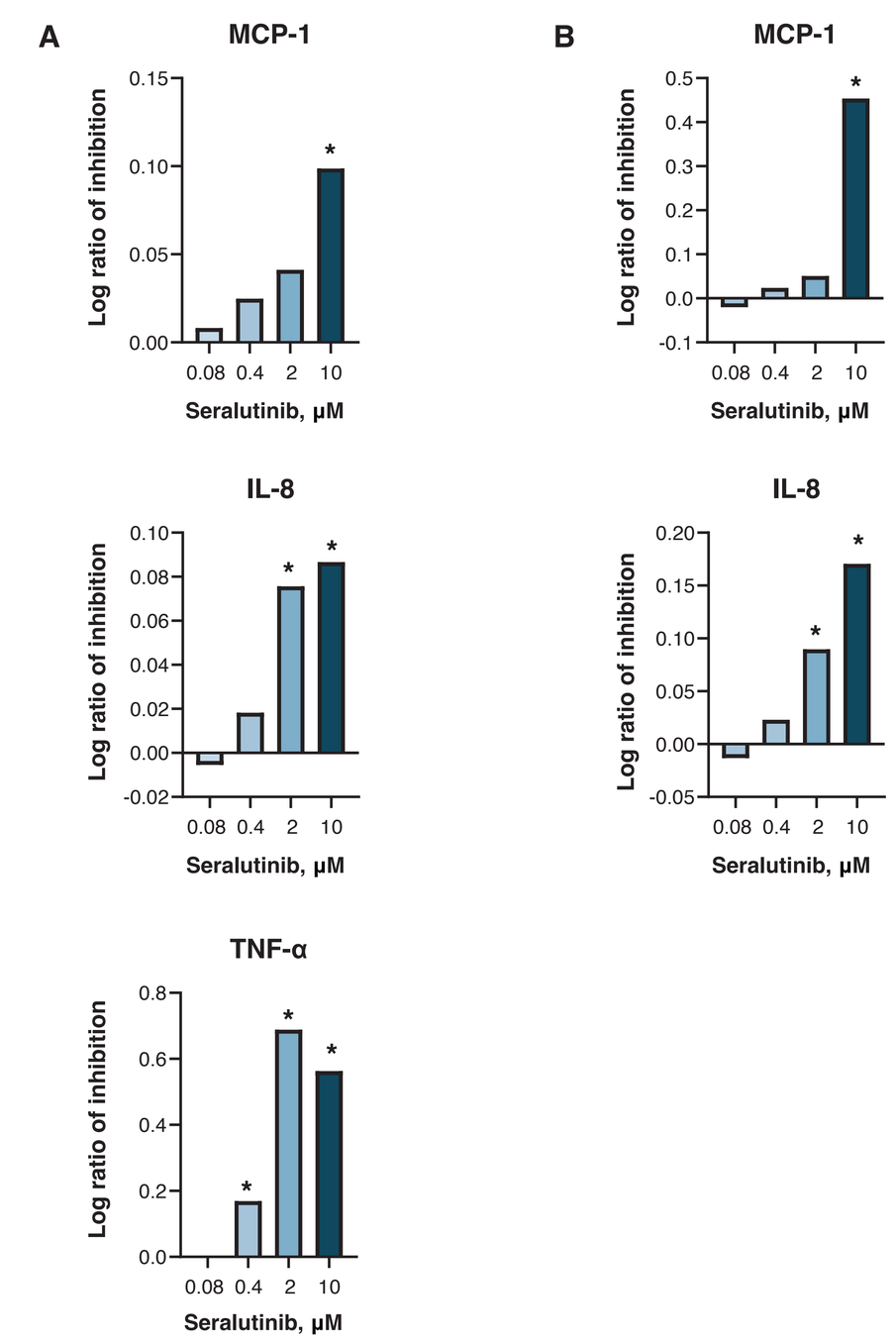
- Healthy primary human cell-based BioMAP® systems were used to assess immune and tissue-specific responses. Cells from 2-6 donors were pooled for each assay
  - LPS System: Peripheral blood mononuclear cells (PBMCs) co-cultured with human umbilical vein endothelial cells (HUVECs), stimulated with lipopolysaccharide, LPS (a TLR4 ligand) for 24 h
  - SAg System: PBMCs co-cultured with HUVECs, stimulated with super antigen, SAg (a T-cell receptor [TCR] cross-linker) for 96 h
- Soluble biomarkers were quantified using Homogeneous Time Resolved Fluorescence (HTRF®)

In vitro system of primary human immune cell activation

- Freshly isolated PBMCs from 4 healthy donors were pretreated with seralutinib for 1 h and stimulated with:
  - LPS from *E. coli* O111:B4 (Invivogen) for 18 h (innate immune activation)
  - T Cell TransAct™ (Miltenyi Biotec) (activation via CD3 and CD28) for 48 h (adaptive immune activation)
- Soluble markers were measured by Luminex® assays (R&D Systems)

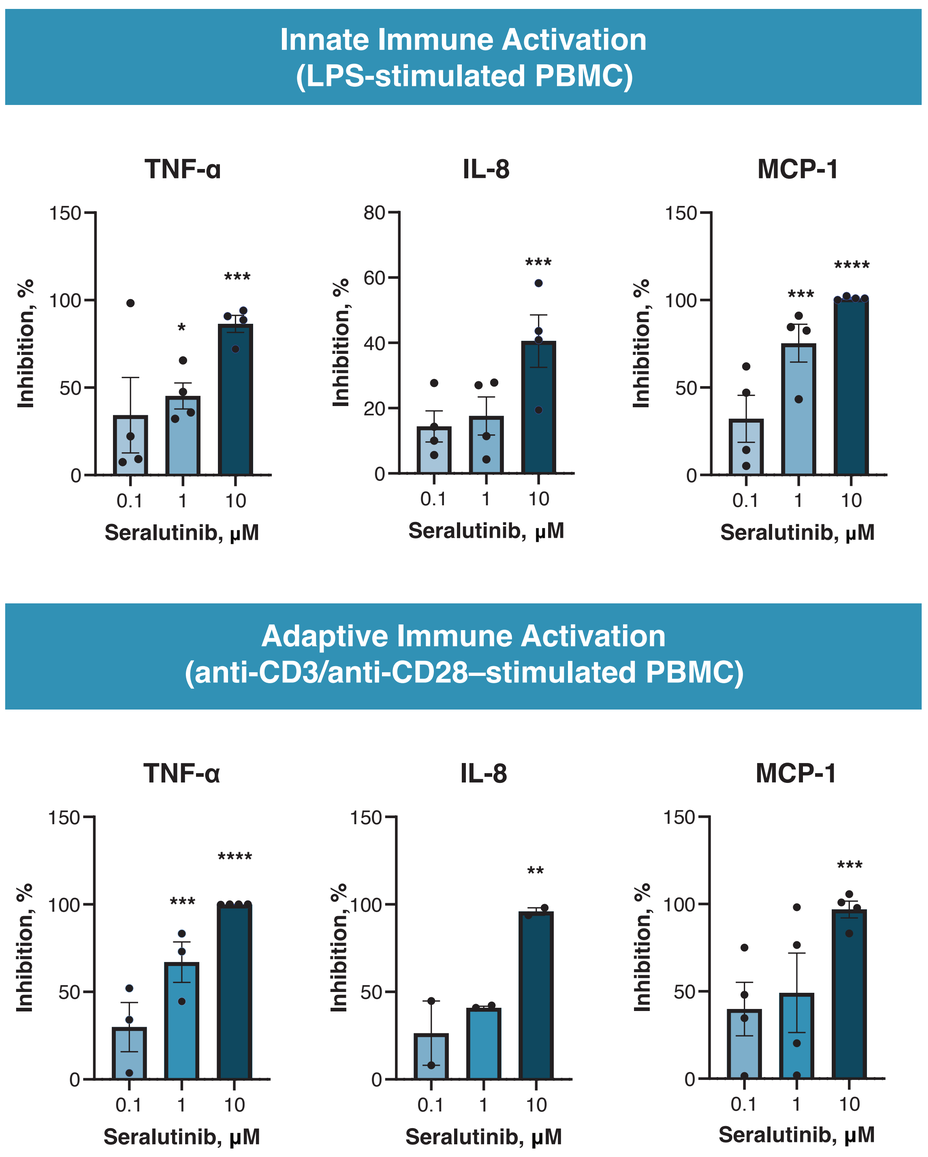
RESULTS

Figure 2. Seralutinib reduced production of inflammatory cytokines and chemokines in *in vitro* models of primary human vascular inflammation



A single pool of human PBMCs (n=2-6) co-cultured with HUVEC were treated with seralutinib at noted concentrations and stimulated with either (**A**) LPS or (**B**) SAg. MCP-1, IL-8, and TNF-α were measured from supernatant at 24 h (LPS) or 96 h (SAg). Significant (*) biomarker activity cut-off was determined when ≥2 consecutive concentrations produced directional changes outside the significance envelope and exceeded a ≥20% effect size ($Ilog_{10}$ ratiol ≥0.1). HUVEC, human umbilical vein endothelial cell; IL-8, interleukin 8; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein 1; PBMC, peripheral blood mononuclear cell; SAg, super antigen; TNF-α, tumor necrosis factor α.

Figure 3. Seralutinib reduced TNF-α, IL-8, and MCP-1 production from activated primary human innate and adaptive immune cells



Freshly isolated human PBMCs (n=4) were stimulated with either LPS (innate) or anti-CD3/anti-CD28 (adaptive; TCR) and treated with seralutinib at noted concentrations. MCP-1, IL-8, and TNF-α were measured from supernatant at 18 h (LPS) or 48 h (TCR). Percent inhibition was calculated compared to average of vehicle control per analyte measured. Data are expressed as mean ± SEM (n=4). 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 versus stimulation control. ANOVA, analysis of variance; IL-8, interleukin 8; MCP-1, monocyte chemoattractant protein 1; PBMC, peripheral blood mononuclear cell; SEM, standard error of the mean; TCR, T-cell receptor; TNF-α, tumor necrosis factor α.

- In co-culture of PBMCs and HUVEC, seralutinib treatment significantly inhibited the production of chemotactic factors (IL-8/CXCL8 and MCP-1/CCL2) and pro-inflammatory mediator TNF-α in a dose-dependent manner under innate (LPS) and adaptive (SAg) activation conditions
- Seralutinib treatment dose-dependently reduced the induction of innate (LPS) and adaptive (TCR) immune-mediated production of IL-8/CXCL8, MCP-1/CCL2, and TNF-α by PBMCs alone

CONCLUSIONS

- Seralutinib demonstrated anti-inflammatory effects across different human models of *in vitro* vascular inflammation and innate and adaptive immune activation
- In combination with clinical biomarker data, these findings highlight the potential of seralutinib to reduce the hyperactive vascular inflammation underlying pulmonary hypertension
- Further investigation of these mechanisms is planned

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