PA601

Evidence of Target Engagement and Modulation: Biomarker Analysis of the Phase 1b Inhaled Seralutinib Study

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BACKGROUND

- Abnormal signaling of PDGFα/β, CSF1R, and c-KIT as well as BMPR2 deficiency drive cellular overgrowth in the lung vasculature and play key roles in the development of PAH[1,2,3]
- Seralutinib is an inhaled small-molecule kinase inhibitor which selectively targets PDGFRα/β, CSF1R, and c-KIT signaling, and modulates BMPR2
- Studies of inhaled seralutinib in animal models support pharmacodynamic activity in the human lung at dose levels expected to have biologic and clinical activity:
  - ✓ 30-fold higher lung:plasma exposure (**Figure 1**)
  - ✓ Extended lung target engagement
  - ✓ Reversal of pulmonary vascular remodeling, improved hemodynamics, increased lung BMPR2 and reduced circulating NT-proBNP[4,5]

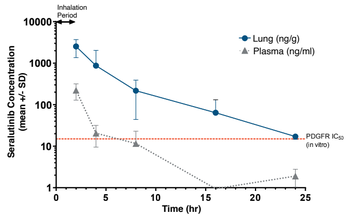

Figure 1. Seralutinib concentration in rat lung and plasma following treatment (4.3 mg/kg dose, 2 hr passive inhalation, n=4-8)

- Phase 1 studies in healthy volunteers and PAH subjects demonstrated that seralutinib was well tolerated at doses up to 90 mg BID[6,7]
- Here we use peripheral markers to measure target engagement and pharmacodynamic activity in circulation in PAH subjects

METHODS

- Phase 1b, multi-center, double-blind, randomized, placebo-controlled study (**NCT03926793**).[7] Eight subjects (PAH, FC II-III, on 2-3 background therapies) were randomized 3:1 to receive inhaled seralutinib 45 mg BID (escalating to 90 mg BID on day 8 at PI discretion) or placebo for 2 weeks
- Following informed consent, peripheral blood was collected for exploratory biomarker assessment at baseline and day 14 at three timepoints relative to inhalation (pre-dose, 5 min and 120 min)
- Percent inhibition of CSF1R receptor internalization was measured using a novel whole blood M-CSF induced CSF1R internalization FACS assay developed in-house and run at Primity Bio
- Whole blood gene expression mRNA profiling was performed using NovaSeq platform. Differential expression analysis was performed using DESeq2. Benjamini-Hochberg correction was used as the adjustment method for calculating p-values with significance cut-off at <0.05 level
- Epigenetic immunoprofiling assays were performed by Epiontis[8]

RESULTS

Target Engagement

- Seralutinib inhibits CSF1R receptor internalization in PAH subjects at 5 min post inhalation demonstrating successful target engagement at the dose levels studied (**Figure 2**)
- Consistent with rapid systemic clearance, CSF1R internalization is no longer inhibited at 120 minutes

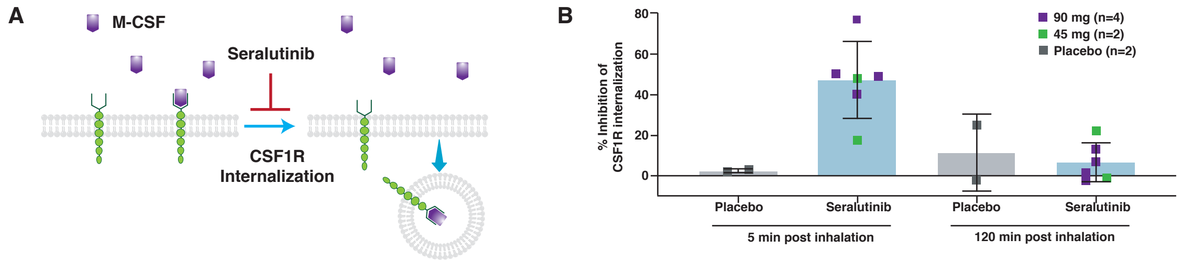

Figure 2. Seralutinib transiently inhibits CSF1R internalization. A. CSF1R assay schema; B. CSF1R activity in systemic circulation indicates target engagement at 5 minutes post-treatment (bars show mean and standard deviation)

Pharmacodynamics: Gene Expression

- Gene expression profiles at day 14 relative to baseline are supportive of biological activity by seralutinib (**Figure 3**)
- Differential expression analysis comparing Day 14 to baseline identified treatment-associated shifts in 779 genes, after adjusting for false discovery
- The seralutinib-associated pharmacodynamic signature was most prominent in subjects receiving the higher seralutinib dose in week 2 of the study
- Seralutinib signature will be measured and related to efficacy in an ongoing phase 2 study

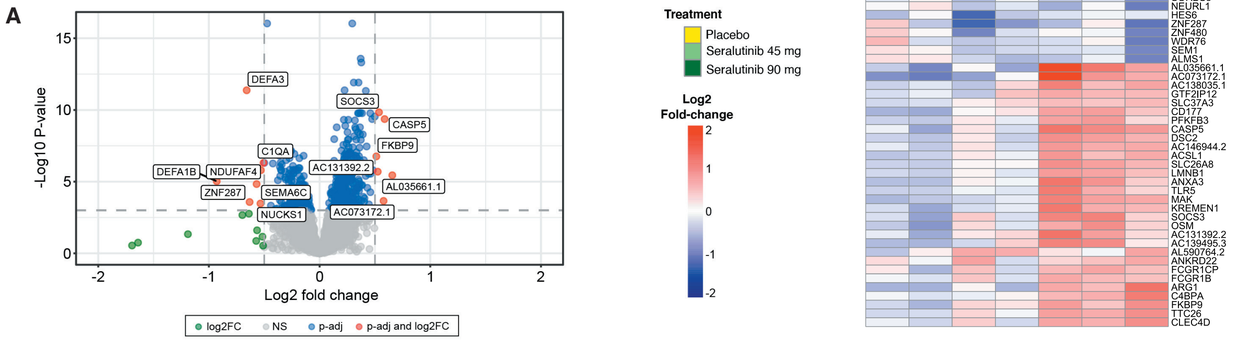

Figure 3. Gene expression profiles at day 14 relative to baseline are supportive of pharmacodynamic modulation by seralutinib (N=7, evaluable). A. Volcano plot shows differentially expressed genes from baseline to day 14. Genes are colored if they meet criteria for significance (FDR-adjusted p-value <0.05) or effect size (absolute log2 fold-change (FC) >0.5). Genes meeting both p-value and FC criteria are colored red and labeled, whereas genes meeting only the p-value or FC criteria are colored blue or green, respectively. B. Heatmap shows mean relative expression change from baseline to Day 14 (log2 fold-change) of top 30 up- and downregulated genes for each subject. Color bars above heatmap indicate treatment group (yellow = placebo, light green = seralutinib 45 mg BID, dark green = seralutinib 90 mg BID)

Pharmacodynamics: Epigenetic Immunoprofiling

- Preclinical studies implicate FOXP3+ Treg deficiency in development and severity of PAH[9]
- FOXP3/CD4 ratio is elevated in all patients treated with seralutinib (median 17% increase)
- FOXP3/CD4 is a novel candidate peripheral marker for disease-modifying activity

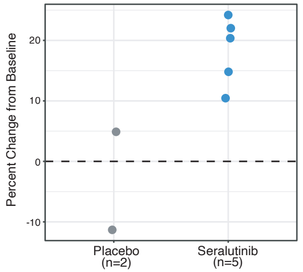

Figure 4. Epigenetic immunoprofiling assay shows percent change from baseline in FOXP3/CD4 ratio (N=7, evaluable)

SUMMARY

- Preliminary biomarker findings suggest seralutinib demonstrates biological activity in PAH patients after 2 weeks of treatment:
  - Target engagement and modulation of gene expression in the periphery suggest pharmacodynamic activity
  - FOXP3:CD4 T-cell ratio may represent a biomarker of therapeutic effect; requires further validation
- A randomized, double-blind, placebo-controlled, multicenter, phase 2 clinical study (**TORREY; NCT04456998**) to evaluate efficacy and safety of seralutinib for the treatment of WHO Group 1 PH is currently recruiting subjects
- Candidate biomarkers will be measured in the phase 2 study to identify predictive and pharmacodynamic markers of treatment response, with the aim of advancing personalized medicine in PAH

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