**Introduction**

- Pulmonary arterial hypertension (PAH) is characterized by elevated pulmonary vascular resistance, impaired cardiac output, and right ventricle (RV) overload and hypertrophy. 
- PAH is associated with imbalanced TGF-β signaling, including insufficient activation of SMAD1/5/9 and/or inappropriately high SMAD2/3 signaling which is associated with inflammation, fibrosis, and eventual heart failure (HF).  
- Decreased BMPRII signaling is associated with the development of PAH.
  - Increasing BMPRII signaling through SMAD1/5/9 by administration of BMP9 reverses disease in rodent models of PAH.
  - Increased activin signaling through SMAD2/3 is associated with endothelial dysfunction.
  - Activin reduces levels of BMPRII in endothelial cells.
- In preclinical studies and clinical trials, treatment with an investigational ActRIIA ligand trap (ActRIIA-Fc) demonstrated beneficial treatment of PAH concomitant with an observed dose-limiting increase in red blood cells (RBCs).
- RKER-012 is a research form of KER-012, which is an investigational, modified ActRIIB ligand trap, designed to inhibit ActRII ligands, including activins, while sparing BMP9 activity, resulting in signaling that favors SMAD1/5/9.

**Aim:** To investigate the mechanism of RKER-012's prevention of PAH pathology.

**Methods**

- Sprague Dawley rats (24-29g) received either vehicle (DMSO; n=12/group) or 5%S4216 (200 mg/kg; n=12/group) 60 once and placed in either normoxic (41% O2) or hypoxic (13% O2) conditions.
- NK rats were treated with vehicle (TBS); while Hx rats were treated with vehicle (TBS), AcTRIIB-Fc (10 mg/kg) or RKER-012 (10 mg/kg). SQ twice weekly for 5 weeks.
- Rats were assessed terminally for RV and lung expression of markers of PAH pathology. Histopathology for lung inflammation, fibrosis, and smooth muscle hypertrophy was scored. RV histopathology is pending.
- Human Pulmonary Arterial Endothelial Cells (HPAECs) were treated with RKER-012 (10 ng/mL) and placed into normoxia (21% O2; n=4/group) or hypoxia (4% O2; n=4/group). After 48 hours, cell culture supernatant was collected for ActRIIB ELLA and RNA was extracted from cells for qPCR analysis.
- All data are presented as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns p>0.05.

**Results**

**RKER-012 reduced right ventricle (RV) hypertrophy and reduced pulmonary arterial pressure in the rat PAH model**

![Figure 1](image1.png)

**RKER-012 reduced lung inflammation, fibrosis, smooth muscle hypertrophy, and muscularization**

![Figure 2](image2.png)

**RKER-012 reduced markers of heart failure in the RV**

![Figure 3](image3.png)

**RKER-012 treatment reduced expression of markers of inflammation and fibrosis in the lung and heart, hallmarks of PAH pathology**

![Figure 4](image4.png)

**RKER-012/KER-012 targeted mediators of endothelial dysfunction**

![Figure 5](image5.png)

**Conclusions**

- RKER-012 is a modified ActRIIB ligand trap designed to inhibit SMAD2/3 signaling, favoring SMAD 1/5/9.
- In a Sugen hypoxia rat model of PAH, relative to vehicle, NK, RKER-012:  
  - reduced pulmonary arterial pressure and right ventricle hypertrophy;  
  - reduced lung fibrosis, inflammation, smooth muscle hypertrophy and muscularization;  
  - reduced markers of heart failure in RV;  
  - reduced changes in gene expression of markers of PAH-associated pathology; and  
  - consistently trended towards improved activity compared to ActRIIB-Fc.
- Binding studies demonstrate KER-012 or RKER-012:  
  - inhibited ligands associated with endothelial dysfunction, including activins A and B;  
  - could inhibit activin-mediated BMPRII internalization;  
  - spared BMP9 signaling;  
  - had a ligand binding profile consistent with promoting SMAD1/5/9 signaling over SMAD2/3;  
  - reversed hypoxia-mediated increase in activin expression in human endothelial cells.

These results provide early evidence that KER-012 has the potential to benefit lung and heart tissues in PAH, and support continued clinical development in patients.

**References**

1. Yang et al., 2020 (PMID: 32549945)
2. Andre et al., 2021 (PMID: 35141256)
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