# RKER-012, a Novel Activin Receptor Type IIB (ActRIIB) Ligand Trap, Reduced Cardiac and Pulmonary Pathology in a Sugen/Hypoxia (SH) Model of Pulmonary Arterial Hypertension (PAH)

Keith Babbs, Chris Materna, Alana Gudelsky, Evan Lema, Emily Ledoux, Claire Tseng, ffolliott M Fisher, Jasbir Seehra, Jennifer Lachey

Keros Therapeutics, Lexington, MA, USA

## Introduction

- Pulmonary arterial hypertension (PAH) is characterized by elevated pulmonary vascular resistance, impaired cardiac output, and right ventricle (RV) overload and hypertrophy[1].
- PAH is associated with imbalanced TGF-ß 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)[1].
- Decreased BMPRII signaling is associated with the development of PAH[2].
  - Increasing BMPRII signaling through SMAD1/5/9 by administration of BMP9 reverses disease in rodent models of PAH[3].
  - Increased activin signaling though SMAD2/3 is associated with endothelial dysfunction[2].
  - Activin reduces levels of BMPRII in endothelial cells[4].
- 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)[5,6].
- 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 (241-295g) received either vehicle (DMSO; n=6/group) or SU54216 (200 mg/kg; n=12/group) SQ once and placed in either normoxic (Nx; ~21% $O_2$) or hypoxic (Hx; ~13% $O_2$) conditions.
  - Nx rats were treated with vehicle (TBS), while Hx rats were treated with vehicle (TBS), ActRIIA-Fc (10 mg/kg) or RKER-012 (10 mg/kg) SQ twice weekly for 3 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 µg/mL) and placed into normoxia (21% $O_2$) or hypoxia (1% $O_2$). After 48 hours, cell culture supernatant was collected for Activin A ELISA 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

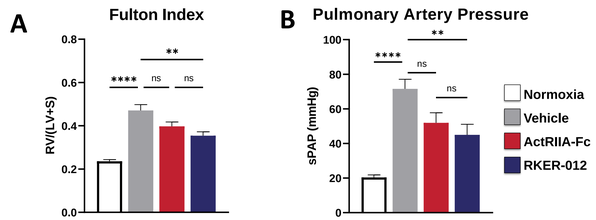


**Figure 1**. Vehicle-treated Hx rats had (A) RV hypertrophy and (B) elevated pulmonary arterial pressure. Treatment with RKER-012 significantly reduced both of these pathologies.

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

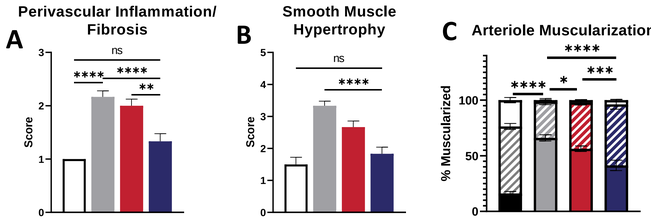


**Figure 2**. Veh-Hx rats had increased (A) lung inflammation, (B) smooth muscle hypertrophy, and (C) arteriole muscularization. Relative to veh-Hx, treatment with RKER-012 prevented these pathologies. In Figure 2C, solid bars = fully muscularized, hatched bars = partially muscularized, and white bars = nonmuscularized.

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

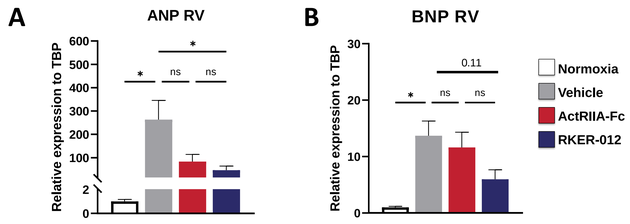


**Figure 3**. Vehicle-treated Hx rats had elevated RV expression of (A) atrial natriuretic peptide (ANP) and (B) brain natriuretic peptide (BNP), which are markers of heart failure. Treatment with RKER-012 significantly reduced expression of ANP and trended to a reduction in BNP.

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

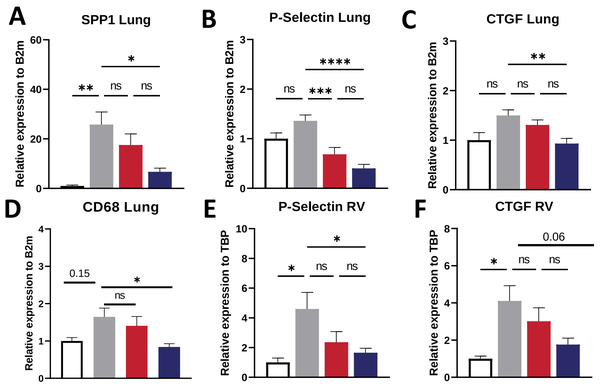


**Figure 4**. Vehicle-treated Hx rats had elevated expression of lung and RV expression of genes associated with PAH pathology, including lung secreted phosphoprotein 1 (SPP1; A), P-selectin (B) connective tissue growth factor (CTGF; C), and a trend for increased CD68 (D), and RV P-selectin (E) and CTGF (F). Treatment with RKER-012 significantly reduced expression of SPP1, P-Selectin, CTGF, and CD68 in lung, and P-Selectin in RV, with a trend for reduced CTGF in RV. Expression was normalized to B2M (bet-2-macroglobulin) or TBP (tata-box binding protein)

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

A

| | $K_D$ (pM) | | | |
|---|---|---|---|---|
| | Activin A | Activin B | GDF-11 | BMP-9 |
| KER-012 | 120 | 132 | 77 | 30,000 |
| ActRIIA | 92 | 47 | 47 | 7,500 |

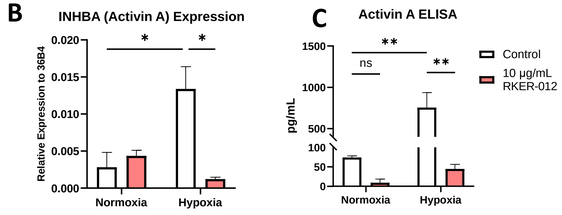


**Figure 5.** (A) $K_D$ determined by Surface Plasmon Resonance on Biacore T200. KER-012 had high affinity for SMAD2/3 ligands and reduced affinity for BMP-9 compared to ActRIIA. HPAECs exposed to 1% $O_2$ for 48 hours had (B) significantly elevated expression of Activin A and (C) increased levels of free Activin A in cell culture supernatant relative to cells in normoxia. Treatment with RKER-012 mitigated these responses.

## Conclusions

- **RKER-012 is a modified ActRIIB ligand trap designed to inhibit SMAD 2/3 signaling, favoring SMAD 1/5/9.**
- **In a Sugen hypoxia rat model of PAH, relative to vehicle Nx, 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 ActRIIA-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. Yung et al., 2020 (PMID: 32404506)
2. Andre et al., 2021 (PMID: 35141256)
3. Long et al., 2015 (PMID: 26076038)
4. Ryanto et al., 2021 (PMID: 33741934)
5. Sherman et al., 2012 (PMID: 23939631)
6. Humbert et al., 2021 (PMID: 33789009)

## Contact

Justin Frantz
jfrantz@soleburytrout.com
(617) 221-9100