Improvement in Cardiopulmonary Function in a Rat Model of Pulmonary Arterial Hypertension Observed with RKER-012, a Novel Activin Receptor Type II Ligand Trap, was Associated with Reduced Markers of Inflammation and Fibrosis in the Right Ventricle

Chris Materna[1], Keith Babbs[1], ffolliott Fisher[1], Joe Worrall[1], Jasbir Seehra[1], Jennifer Lachey[1]

[1]*Keros Therapeutics, Inc., Lexington, MA, USA*

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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-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)[2,3].
- In preclinical studies and clinical trials, treatment with an investigational ActRIIA ligand trap (ActRIIA-Fc) demonstrated benefits in cardiopulmonary function concomitant with an observed dose-limiting increase in red blood cells (RBCs)[4].
- RKER-012 is a research form of KER-012, which is an investigational modified ActRIIB ligand trap designed to reduce SMAD2/3 signaling without affecting RBCs.
- **Our goal was to investigate the mechanism of RKER-012's prevention of PAH cardiac pathology by exploring changes in functional measures with changes in biomarkers of inflammation and fibrosis.**

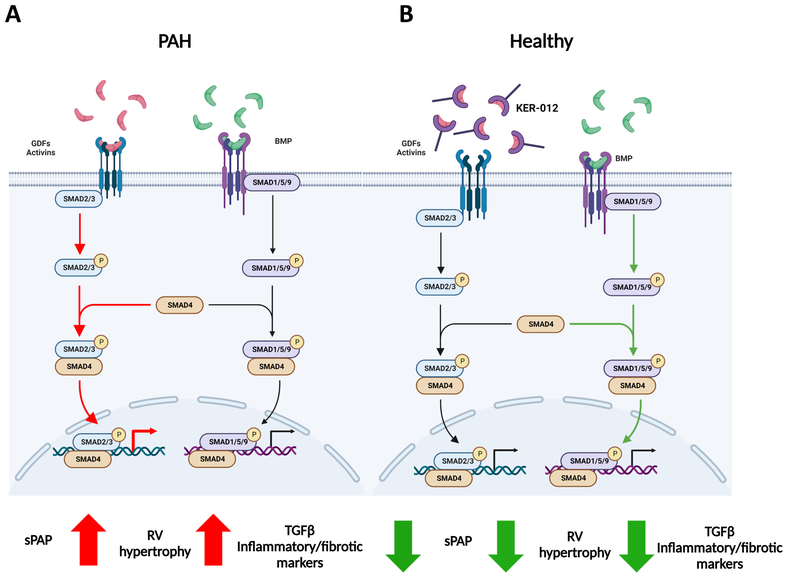

Figure 1. KER-012 is designed to inhibit SMAD2/3 signaling. (A) TGF-β ligands, including activin A, B, and GDF11, bind ActRII resulting in phosphorylation of SMAD2/3 causing it to complex with SMAD4 and regulate gene expression. The regulation of target genes in this manner leads to increased systolic pulmonary arterial pressure (sPAP) and RV hypertrophy. (B) KER-012 is designed to bind TGF-β superfamily ligands, including activins A and B, and to inhibit SMAD2/3 signaling. This inhibition of SMAD2/3 may prevent increased sPAP and RV hypertrophy. Figure made using BioRender.

METHODS

- Sprague Dawley rats (241-295 g) were administered SU54216 (200 mg/kg; n=12/grp) SQ once and placed in hypoxic (Hx; ~13%) conditions. A control cohort was treated with vehicle (DMSO) and maintained under normoxic (Nx; ~21%) conditions.
- Nx rats were treated with vehicle (TBS), while Hx rats were treated with vehicle (TBS) or RKER-012 (10 mg/kg) SQ twice weekly for 3 weeks.
- Systolic pulmonary artery pressure (sPAP) was measured using a Miller pressure catheter and RV hypertrophy was assessed using Fulton Index.
- Rats were assessed terminally for markers of inflammation and fibrosis in the right ventricle by RT-qPCR.
- All data represented as mean + SEM.

RKER-012 prevented Sugen+hypoxia mediated PAP and RV hypertrophy

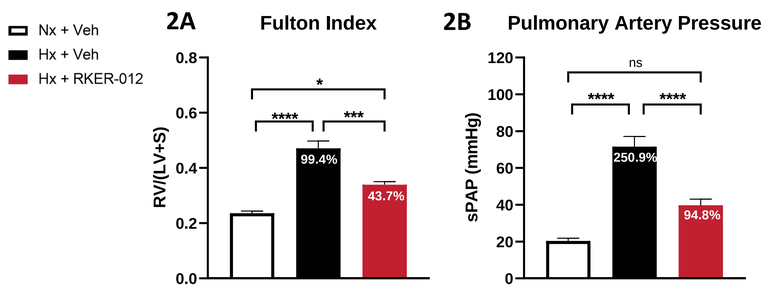

Figure 2. Relative to normoxia, hypoxia + vehicle rats had significantly increased RV hypertrophy and systolic pulmonary arterial pressure. Hypoxia + RKER-012 rats had significantly reduced RV hypertrophy and systolic pulmonary arterial pressure relative to hypoxia + vehicle. One-way ANOVA followed by Sidak post-hoc test. ns – not significant; *p ≤ 0.05; ***p ≤ 0.001 ; ****p ≤ 0.0001. Percent change compared to Nx + Veh.

3 RKER-012 prevented increase in markers of inflammation in RV

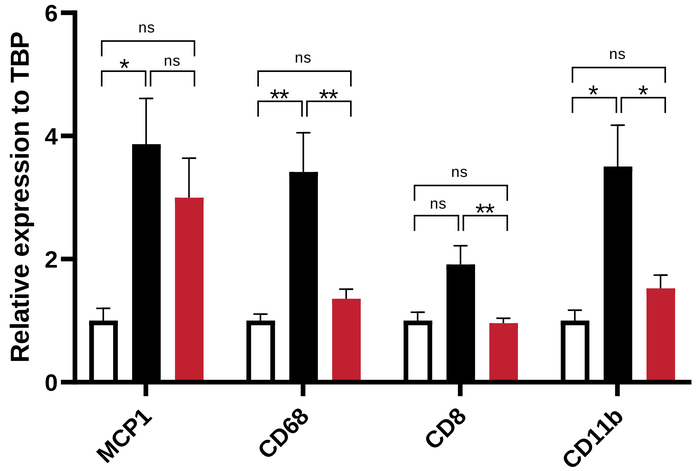

Figure 3. Relative to normoxia, hypoxia + vehicle had significantly increased monocyte chemoattractant protein 1 (MCP1), cluster of differentiation (CD68) and cluster of differentiation 11b (CD11b) expression in RV tissue. No significant change in expression was observed for cluster of differentiation 8 (CD8). Relative to hypoxia + vehicle, hypoxia + RKER-012 had significantly reduced expression of CD68, CD8, and CD11b in RV tissue. No significant changes were observed in MCP1. One-way ANOVA followed by Sidak post-hoc test. ns – not significant; *p ≤ 0.05; **p ≤ 0.01. TBP – TATA-Box Binding protein, CD68 – macrophage marker, CD8 – cytotoxic T cell marker, CD11b – leukocyte specific receptor.

RKER-012 reduced increase in markers of fibrosis in RV

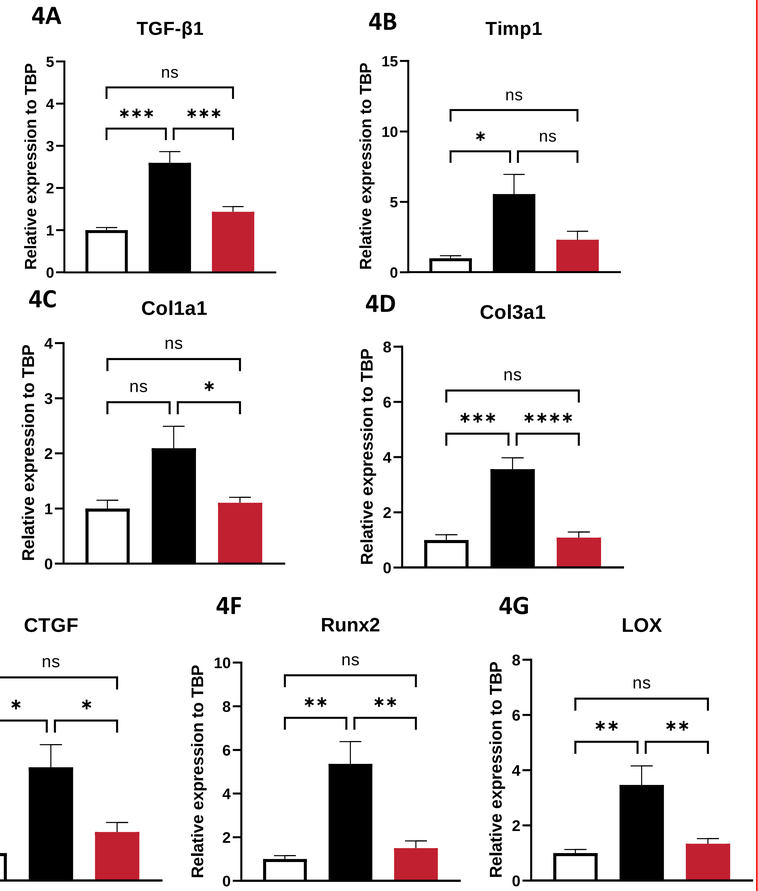

Figure 4. Relative to normoxia, hypoxia + vehicle had significantly increased transforming growth factor-β1 (TGF-β1), TIMP Metallopeptidase Inhibitor 1 (Timp1), collagen type III alpha chain 1 (Col3a1), connective tissue growth factor (CTGF), Runt-related transcription factor 2 (Runx2), and lysyl oxidase (LOX) expression in RV tissue. No significant change in expression was observed for collagen type I alpha chain 1 (Col1a1). Relative to hypoxia + vehicle, hypoxia + RKER-012 had significantly reduced expression of TGF-β1, Col1a1, Col3a1, CTGF, Runx2, and LOX in RV tissue. No significant changes were observed in Timp1. One-way ANOVA followed by Sidak post-hoc test. ns – not significant; *p ≤ 0.05; **p ≤ 0.01 ; ***p ≤ 0.001 ; ****p ≤ 0.0001. TBP – TATA-Box Binding protein.

CONCLUSIONS

- Sugen/hypoxia rat model (Hx + Veh) showed increased sPAP and RV hypertrophy compared to control animals (Nx + Veh).
- RKER-012 treatment reduced increased sPAP and RV hypertrophy in Hx rats (Hx+ RKER-012).
- RV cardiac tissue of hypoxic rats (Hx+Veh) showed increased expression of markers of macrophage, cytotoxic T cells and monocyte/neutrophil infiltration.
- Hypoxia resulted in increased expression of multiple molecular pathways of fibrosis including TGFβ1, CTGF and lysyl oxidase (LOX)
- Treatment with RKER-012 reduced the development of inflammation in right ventricle.
- RKER-012 treatment decreased known drivers of fibrosis, including TGF-β1 and CTGF in the right ventricle.
- These results support that RKER-012 has potent antifibrotic and anti-inflammatory properties
- **These results provide preclinical evidence that KER-012 has the potential to prevent the increase of PAP and benefit the right ventricle in pulmonary arterial hypertension patients, and support continued clinical development in patients.**

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CONTACT INFORMATION

We're hiring!

Deepankar Roy
droy@kerostx.com