KER-050, a Novel Inhibitor of TGFβ Superfamily Signaling, Induces Red Blood Cell Production and is a Potential Candidate for the Treatment of Ineffective Hematopoiesis

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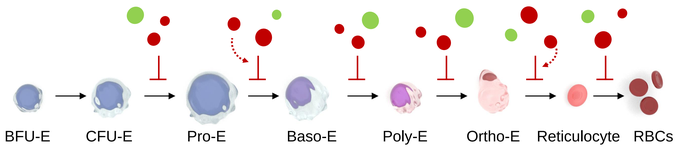
Introduction

- The transforming growth factor beta (TGFβ) superfamily consists of over 30 ligands, including growth differentiation factors (GDFs), bone morphogenetic proteins (BMPs), and activins, that function to regulate several physiological processes, including muscle function, bone growth, vascular remodeling, and hematopoiesis.
- The action of TGFβ-like ligands is transduced through the cells by a class of molecules known as the mothers against decapentaplegic homologs (SMADs). Canonical TGFβ-like signaling is mediated by phosphorylation of either SMAD 1/5/8 or SMAD 2/3 (Bruce *et al*. FEBS Lett, 2012).
- Studies have found that certain TGFβ-like ligands regulate red blood cell (RBC) production by promoting progression of precursor cells through the stages of hematopoiesis, while others delay progression and maintain precursor cells in a quiescent state (see cartoon below).
- Generally, BMPs signal through SMAD 1/5/8 to promote progression, while GDFs, such as GDF11, and activins signal through SMAD 2/3 to act as quiescence factors (Komrokji *et al*. Lancet Haematol, 2018).
- Erythropoietin (EPO) is a known erythroid differentiation factor that increases RBC production by stimulating early stages of erythropoiesis. While EPO is commonly used to treat anemias, many patients are refractive or become resistant to the treatment
- ***KER-050, a novel activin type 2A receptor (ActRIIA) ligand trap comprised of a modified ActRIIA extracellular domain fused to the Fc of a human IgG, is designed to inhibit GDF8, GDF11, activin A, and activin B, thereby reducing SMAD 2/3 activation and resulting in increased RBC production.***
- In a Phase 1 clinical study, KER-050 administration led to robust increases in RBCs, hemoglobin, and platelets (***poster EHA-2788***). Similar results were shown in cynomolgus monkeys (***poster EP-782***).

The objective of this study is to elucidate the mechanisms underlying the pharmacological action of KER-050 on erythropoiesis by determining the stages of red blood cell differentiation and maturation affected by KER-050 treatment.

Cartoon depicting erythroid maturation process and the role of TGFβ signaling in this process

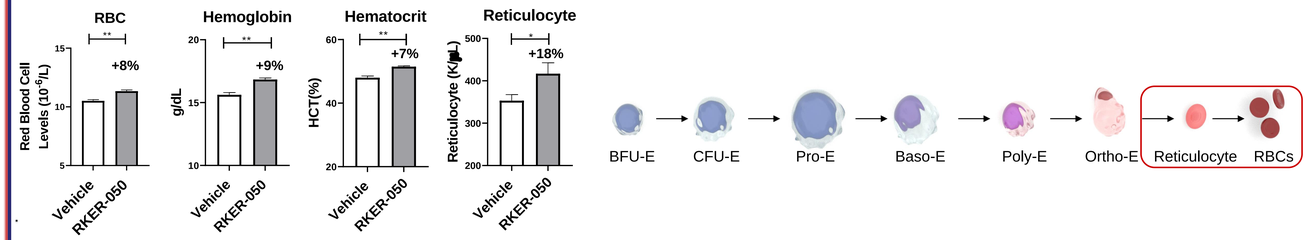
Stages of RBC maturation:
BFU-E = Burst Forming Unit-Erythroid
CFU-E = Colony Forming Unit –Erythroid
Pro-E = Proerythroblast
Baso-E = Basophilic Erythroblast
Poly-E = Polychromatic Erythroblast
Ortho-E = Orthochromatic Erythroblast



Methods

- 10-week-old C57BL/6 mice were used for all experiments.
- Mice were injected intraperitoneally with either vehicle (TBS pH 7.4) or RKER-050 (version of KER-050 with a murine IgG) at 10 mg/kg.
- Peripheral blood was assessed for hematological parameters at 12 hours after treatment (Figure 1).
- Flow cytometry analysis was performed on cells isolated from bone marrow 4 days after treatment. The Pro-E population was determined as Ter119 dim;CD71^{+} cells. BFU-E and CFU-E assays were performed on bone marrow isolated cells cultured in appropriate semi-solid media (Figure 2).
- In addition, mice were treated with RKER-050 (10 mg/kg), with a neutralizing EPO antibody (Epo mAb, 5.5mg/kg, days 0 and 2), or with RKER-050 and Epo mAb in combination. Hematological parameters were measured 3 days after treatment (Figure 3).

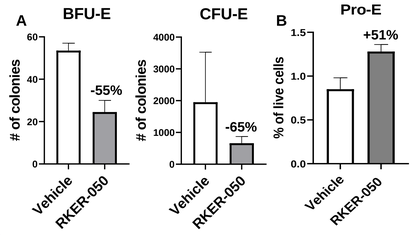
Figure 1: RKER-050 had a rapid effect on RBC differentiation, potentially originating from direct reticulocyte maturation



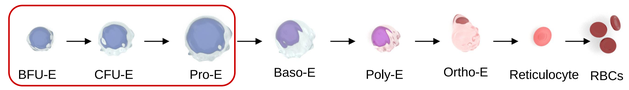
[Student's T-test. *p<0.05, **p<0.001; N=10; Error bars=SEM]

The rapid increase in hematological parameters at 12 hours after RKER-050 treatment, suggests that RKER-050 promotes the later stages of erythroid differentiation by increasing reticulocyte maturation and release to contribute to the overall increase in RBC number.

Figure 2: Changes in BFU-Es, CFU-Es and Pro-Es with RKER-050 treatment suggest an effect on early stages of erythroid maturation

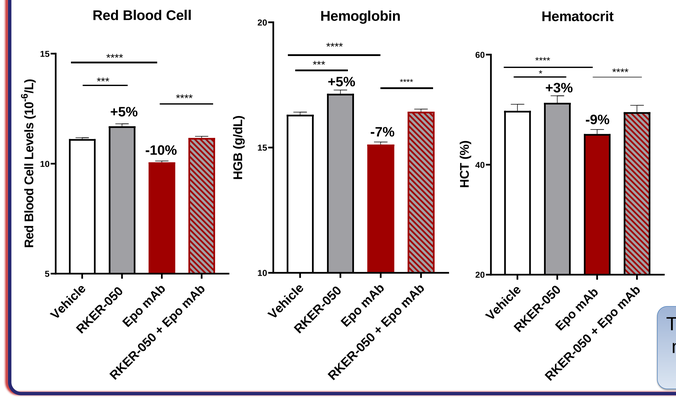


Four days after treatment with RKER-050, there was a decrease in BFU-E and CFU-E early progenitors (A), as well as an increase in Pro-Es (B). [N=2; Error bars=SEM]



These data suggest a dynamic process in which RKER-050 drives RBC maturation by promoting the differentiation of early-stage progenitors

Figure 3: RKER-050 increased hematological parameters by a separate mechanism than EPO



To test whether RKER-050 acts independently of EPO, mice were treated with RKER-050, Epo mAb, or both.

- RKER-050 treatment increased hematological parameters, consistent with previous experiments.
- Epo mAb treatment resulted in a decrease in hematological parameters, as expected.
- Treatment with RKER-050 and Epo mAb in combination prevents the decrease in hematological parameters seen with Epo mAb, returning the parameters to vehicle-treated levels.

[One-way ANOVA, Tukey's test. **** p<0.0001, * p<0.05; N=8; Error bars=SEM]

These data suggest that RKER-050 and EPO have separate mechanisms of action, potentially acting on different stages of RBC maturation.

Conclusions

- KER-050 is a novel modified ActRIIA ligand trap that is designed to target SMAD 2/3-signaling ligands and increase RBCs.
- Overall, KER-050 is a hematologic agent that potentially has a dual mechanism on both early erythroid progenitor differentiation and late precursor maturation
  - Increases in RBC and reticulocytes evident 12 hours after single administration support an effect on terminal maturation
  - RKER-050 treatment reduced BFU-Es and CFU-Es concomitantly increased Pro-E precursors, demonstrating an effect on early stages of erythropoiesis
- Our data suggest that the effect of RKER-050 on erythroid maturation is through a mechanism that is distinct from EPO, potentially providing a new approach to treat anemias.
- KER-050 had clinical effects on RBCs, HGB and reticulocytes in a Phase 1 clinical trial (***Poster EHA-2788***). These data suggest that KER-050 has the potential to alleviate symptoms of anemia, supporting its development for the treatment of cytopenias that occur due to ineffective erythropoiesis, such as in patients with myelodysplastic syndromes and myelofibrosis.