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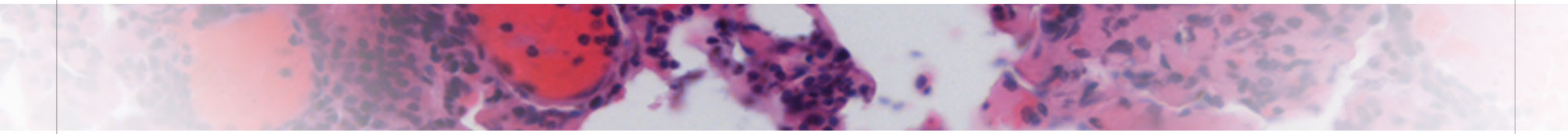
Abstract # 2736



# KER-050, a novel inhibitor of TGFβ superfamily signaling, induces red blood cell production by promoting multiple stages of erythroid differentiation

Marina Feigenson, Remya Nathan, Chris M Materna, Alana Gudelsky, Evan Lema, Claire C Tseng, ffolliott M Fisher, Jasbir Seehra, Jennifer L Lachey

Keros Therapeutics, Lexington, MA

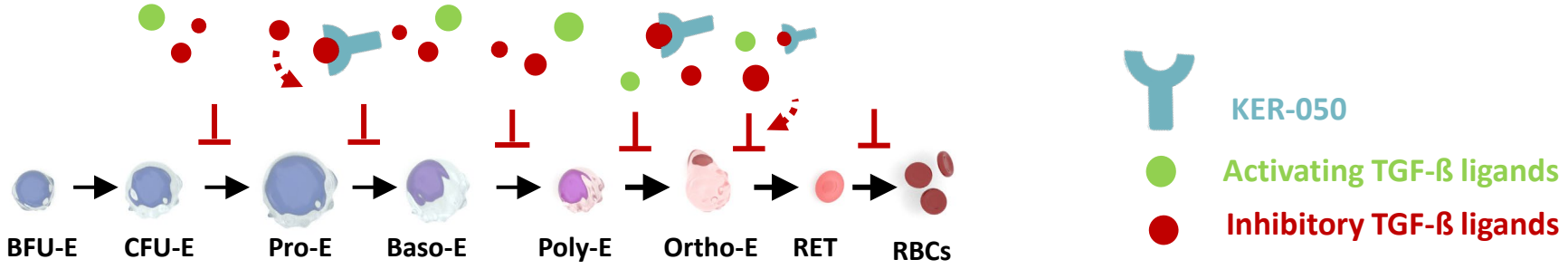
# Disclosures

All authors are employees of and shareholders of Keros Therapeutics, Inc

# Introduction

- Diseases such as myelodysplastic syndrome (MDS) and myelofibrosis (MF) are characterized by ineffective hematopoiesis, which can result in one or multiple cytopenias.
- Current treatment options to address anemia in these diseases target discreet stages in erythropoiesis, whereas defects leading to ineffective hematopoiesis can occur throughout the pathway. Therefore, a treatment that more globally modulates hematopoiesis has the potential to treat broad patient groups.
- The transforming growth factor beta (TGF-β) superfamily plays a key role in both activating and inhibiting differentiation of erythroid precursors to regulate erythropoiesis.
- KER-050, a modified ActRIIA ligand trap, is designed to promote erythropoiesis by blocking signaling of the inhibitory signals.
- In a Phase 1 clinical study, administration of KER-050 to healthy participants led to robust, rapid and sustained increases in red blood cells (RBCs), hemoglobin (HGB) and platelets, supporting an effect on the multiple stages of hematopoiesis.

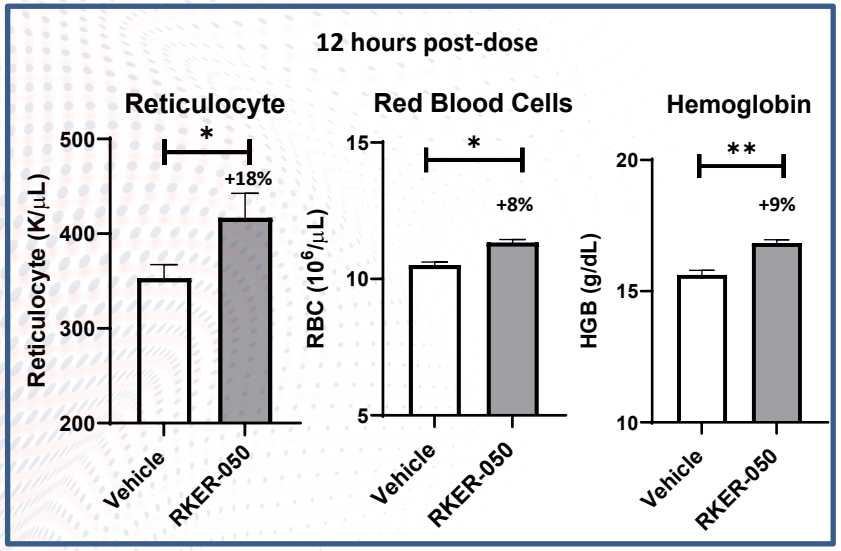
**The objective of this study is to characterize the mechanism and time course of KER-050-mediated effects on RBC production and changes in erythroid precursor cell populations in mice**

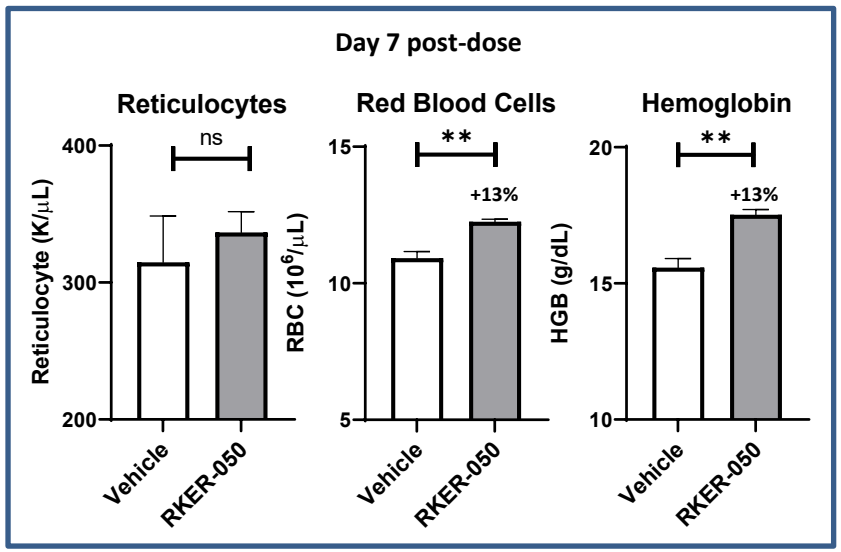


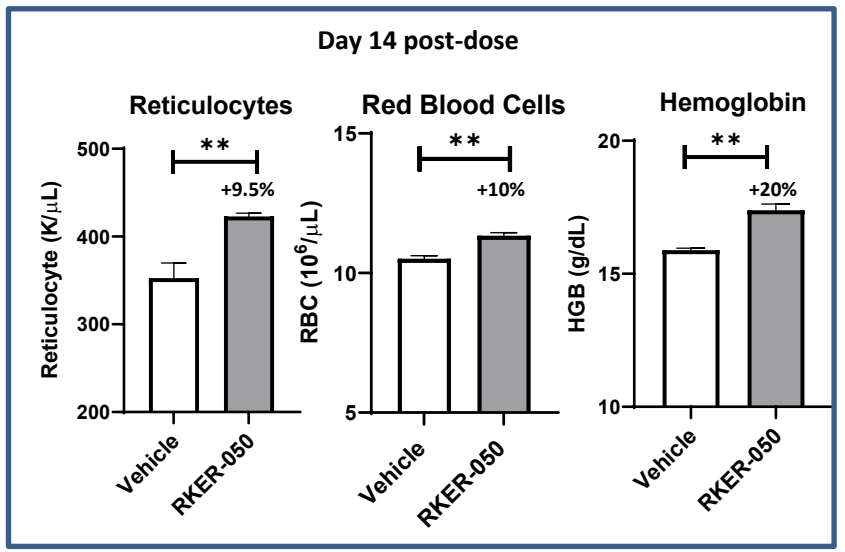
# Methods

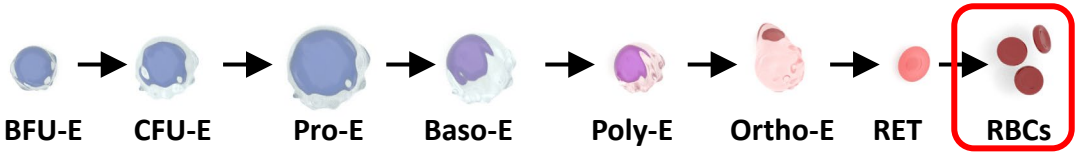
- Eleven-week-old mice were treated with a 10mg/kg dose of the research form of KER-050 (RKER-050) by a single intraperitoneal injection.
- Changes in hematological parameters in peripheral blood and in erythroid progenitors in bone marrow were measured at 12 hours, 2, 7, and 14 days after a single dose of RKER-050.
- Hematological parameters were measured in peripheral blood by IDEXX BioAnalytical.
- Erythroid progenitors were measured by harvesting bone marrow cells and staining with antibodies against Ter119 (PE-conjugated) and CD71 (FITC-conjugated) and analyzed by flow cytometry.
- BFU-E and CFU-E numbers were analyzed by counting the number of colonies formed by culturing bone marrow cells in a semi-solid media.
- Enucleated erythroid progenitors were measured by staining the bone marrow cells with DRAQ5 nuclear fluorescent dye and evaluating by flow cytometry the number of DRAQ5+ cells in Ter119+ (erythroid cells) population.
- Erythropoietin serum levels were measured by mouse EPO Immunoassay (ELISA).

# A single dose of RKER-050 resulted in rapid and sustained increases in hematological parameters



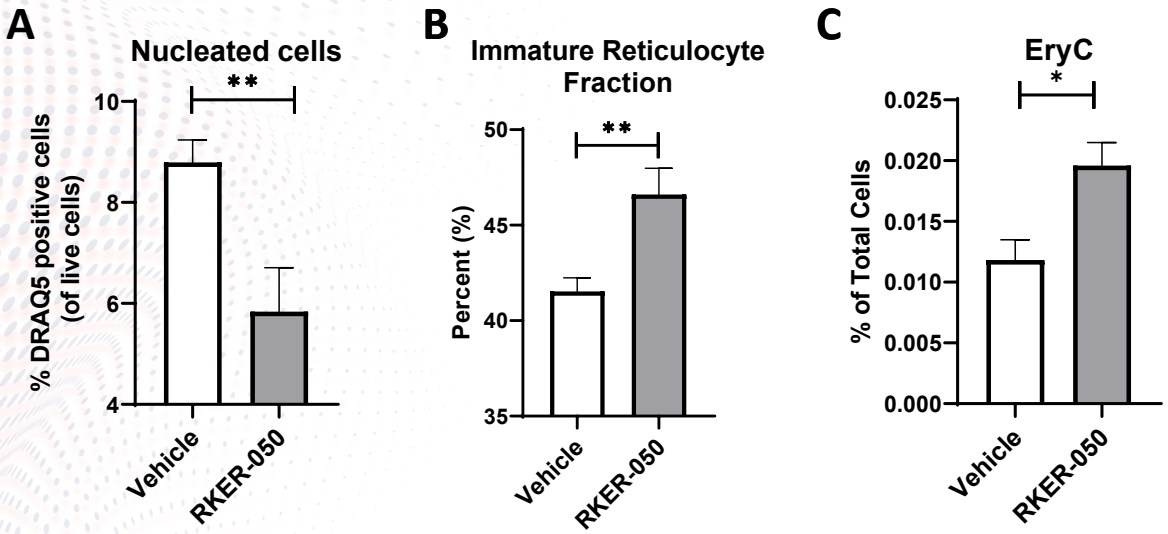




- Mice treated with RKER-050 exhibited 18% increase in RET, 8% in RBCs and 9% in HGB 12 hours after treatment. This timing is consistent with maturation of late-stage progenitors.
- These effects were maintained till at least 14 days post a single dose of RKER-050.

# RKER-050 accelerated maturation of late-stage erythrocyte precursors and outflux of reticulocytes into circulation

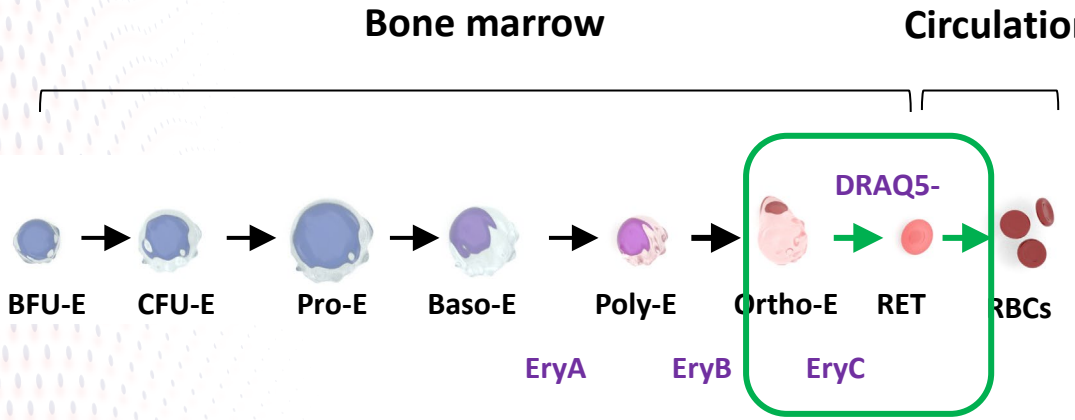



**Treatment with RKER-050:**

A) Reduced the number of nucleated erythroid cells in the bone marrow

B) Increased the percent of immature reticulocytes in peripheral blood, supporting an increased outflux of reticulocytes into circulation

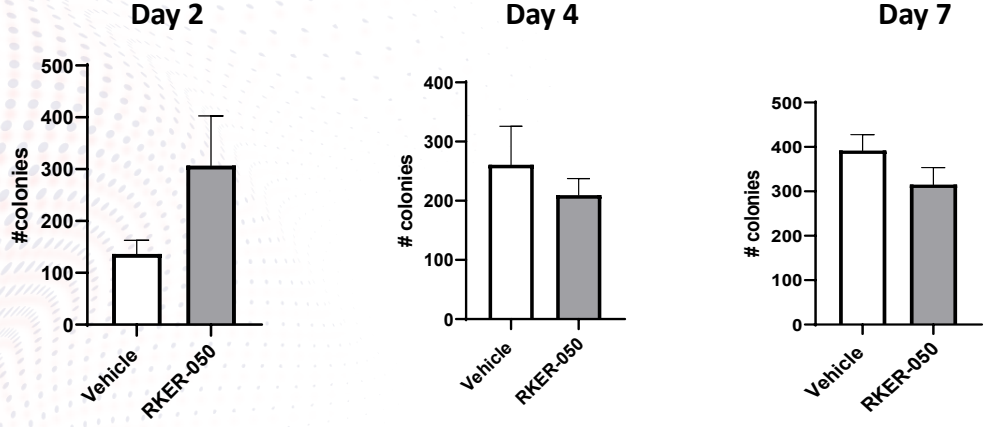
C) Led to increases in late orthochromatic erythroblasts (EryC) at day 7



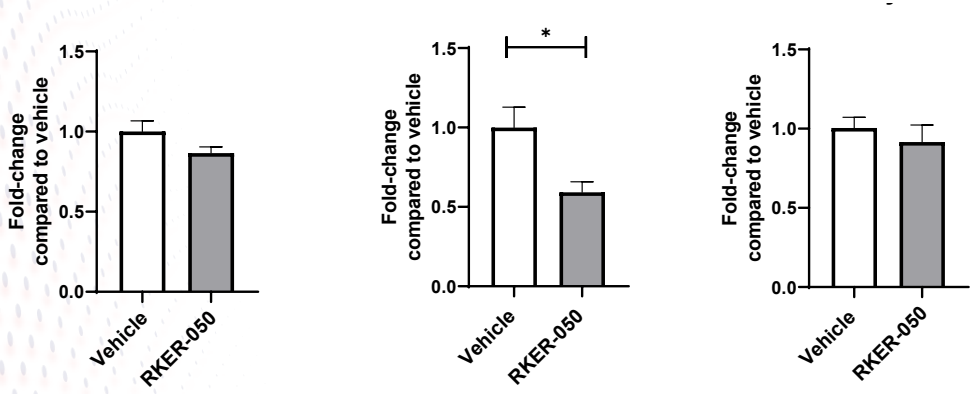

**These data demonstrate increased differentiation and outflux of late-stage erythroid progenitors from the bone marrow to the circulation**

# RKER-050 increased early-stage precursors and numbers of cells progressing through erythropoiesis

## CFU-E

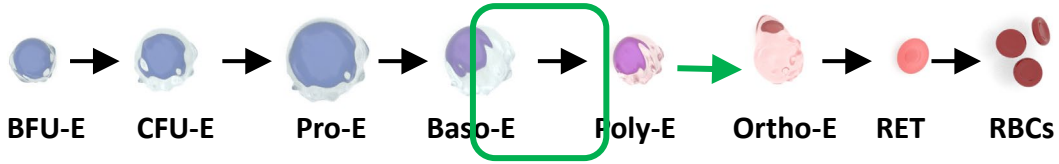



## EryB

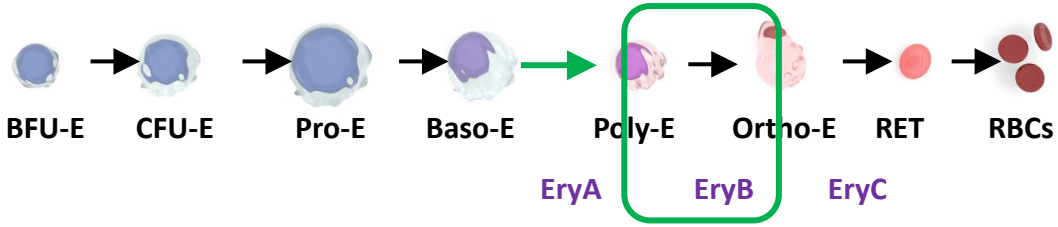



**Day 2 – RKER-050 increased early-stage progenitor cells**

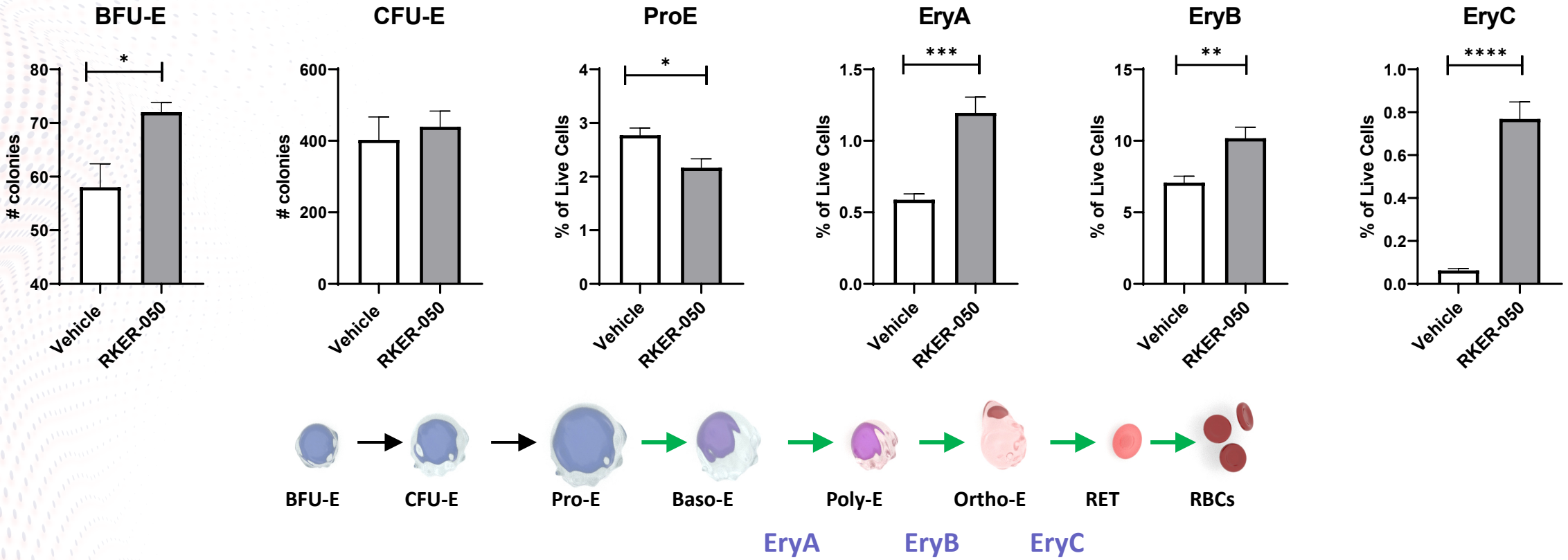

**Day 4 – EryB population matured to EryC, depleting its numbers**




**Day 7 – EryB cell pool repleted, demonstrating that the early precursors mobilized on Day 2 differentiate to replenish the progenitor pools**



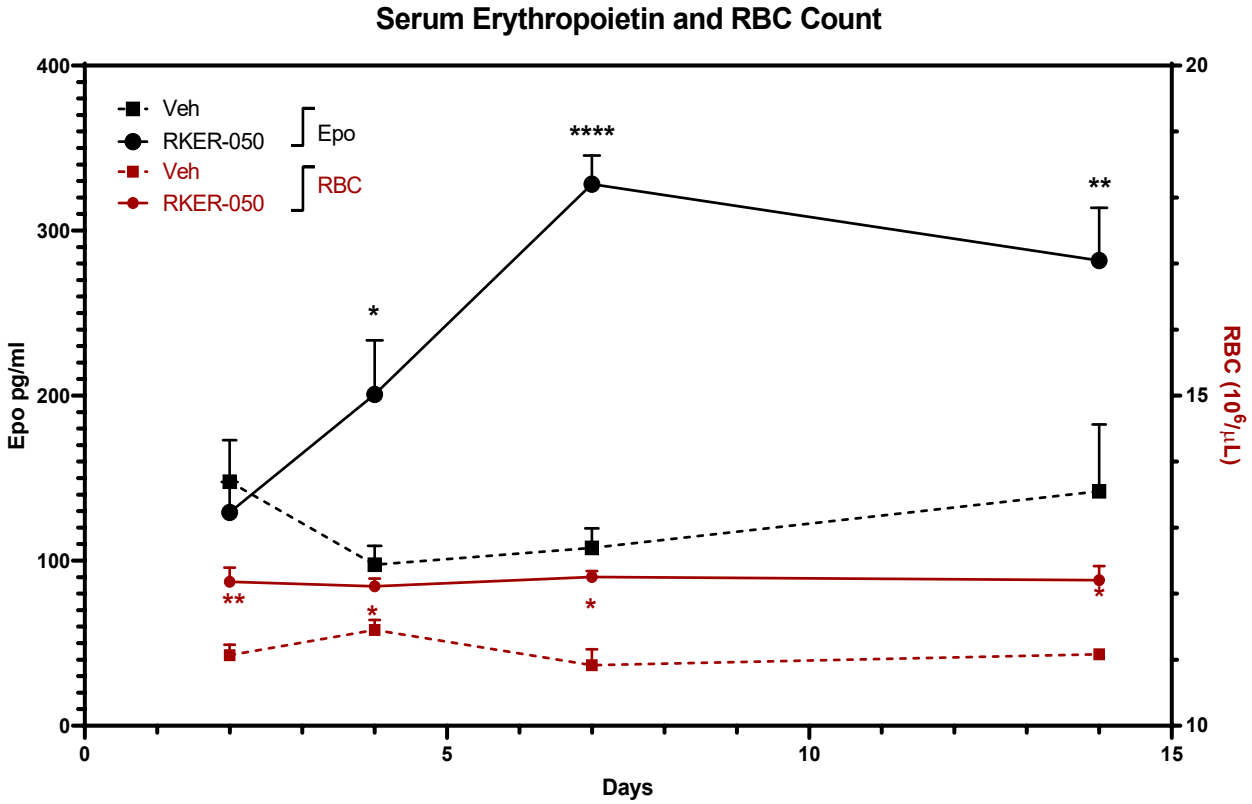
# Erythropoietic tone is upregulated 14 days after a single RKER-050 injection




**Observed increases in early and late-stage progenitors at day 14 post-single dose support the hypothesis that treatment with RKER-050 expands the early progenitor pool that continue to mature and contribute to the overall upregulation of erythropoiesis**

# RKER-050 treatment resulted in increase in circulating erythropoietin levels even in the context of increased RBCs

- RKER-050-treated mice exhibited increases in RBCs and EPO levels increased by 2-3-fold starting at day 4 compared to the vehicle-treated mice.
- Elevated serum EPO potentially enhances the RKER-050 effect of increasing early progenitor cells, promoting their differentiation and contributing to a sustained effect on erythropoiesis.



# Summary and Conclusions

- Our studies demonstrate that RKER-050 potentially affects erythropoiesis at several stages:
  - *Stimulated terminal maturation of late-stage erythroid precursors and increased the outflux of late-stage reticulocytes into circulation*
  - *Expanded the early-stage precursor population that differentiate to replenish the late-stage erythroblast pools*
  - *Increased erythropoietin levels*
- Our results suggest that the combination of RKER-050 effects on erythropoiesis contributes to a rapid increase in RBCs and a continuous supply of progenitors that allows for sustained upregulation of erythropoiesis.
- The ability of KER-050 to potentially target multiple stages along the erythropoiesis cascade makes it a potential therapeutic candidate for diseases that cause anemia due to ineffective erythropoiesis, including myelodysplastic syndrome and myelofibrosis, where defects can arise throughout the erythropoietic pathway.