RKER-050, a novel inhibitor of TGF-β superfamily signaling, increased platelet production

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INTRODUCTION

Transforming growth factor-beta (TGF-β) superfamily signaling is important in regulating hematopoiesis, including normal platelet (PLT) production[1]. Megakaryocytes (MKs) are platelet producing cells that are controlled, in part, by this signaling pathway. Dysfunctional signaling can lead to abnormal PLT production causing thrombocytopenia as well as myeloproliferative diseases[2]. KER-050 is an investigational modified ActRIIA ligand trap designed to inhibit a subset of TGF-β superfamily ligands, including activin A, activin B, growth and differentiation factor (GDF) 8, and GDF11. In a clinical study in healthy volunteers, KER-050 increased red blood cell (RBC) and PLT counts. Furthermore, ongoing phase 2 clinical trials observed improved RBC and PLT counts with KER-050 treatment in both myelodysplastic syndrome (MDS) and myelofibrosis patients[3]. Understanding the mechanism of action of KER-050 in the context of PLT production is important to ascertain its potential clinical benefits.

OBJECTIVE

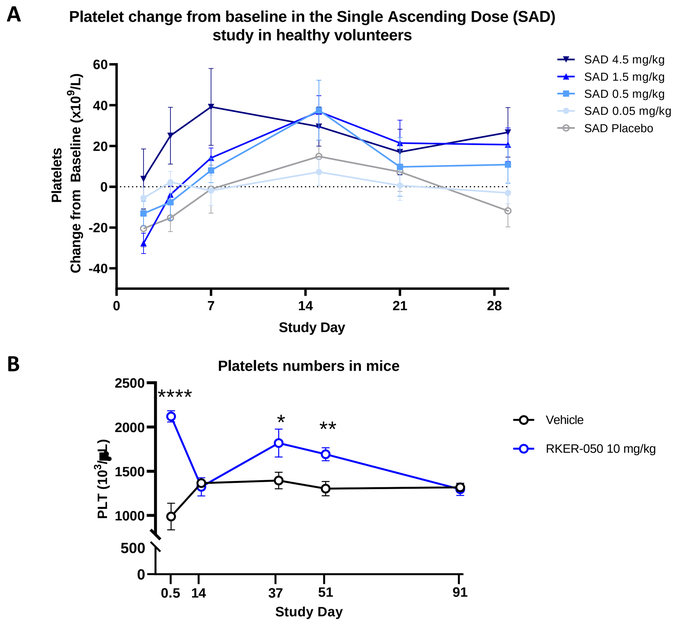
To investigate the effects of KER-050 and RKER-050 (a research form of KER-050) on platelet production

METHODS

In a Phase I study in healthy post-menopausal women, participants were randomized 4:1 to groups receiving a single subcutaneous injection of 0.05, 0.5, 1.5 or 4.5 mg/kg of KER-050 or Placebo; blood samples were collected at the time points indicated (**Fig. 1A**). Mouse studies used RKER-050 and utilized 8-14-week-old C57BL/6 mice. For PLT counts, % CD41+ (megakaryocyte marker) composition and ploidy assessment, a single intraperitoneal dose of RKER-050 (10 mg/kg) was given (**Fig. 1B and Fig. 2**). For gene expression analysis, qPCR was performed on CD41⁺ bone marrow cells from untreated mice (**Fig. 3**). For the activin A neutralizing antibody study, mice were treated with single dose of anti-activin A (5 mg/kg) or RKER-050 (10 mg/kg) and PLT counts assessed 24 hours post-dose (**Fig. 4**). For in vitro ploidy assays, bone marrow from untreated mice was cultured with vehicle (PBS), activin A, RKER-050, or RKER-050 + activin A for 6 days (**Fig. 5**). CD41⁺ cell ploidy was analyzed using a DNA stain. One-way ANOVA with Dunnett's multiple comparison or Student's t-test were used for statistical analysis.

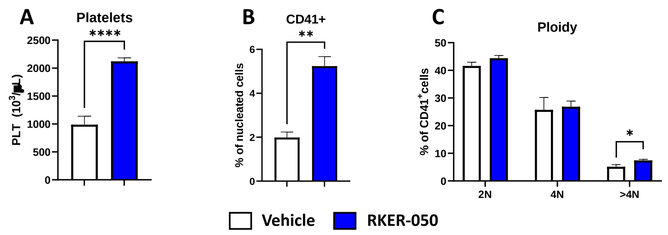
RESULTS

Figure 1: KER-050 and RKER-050 treatment induced an increase in platelets in humans and mice, respectively



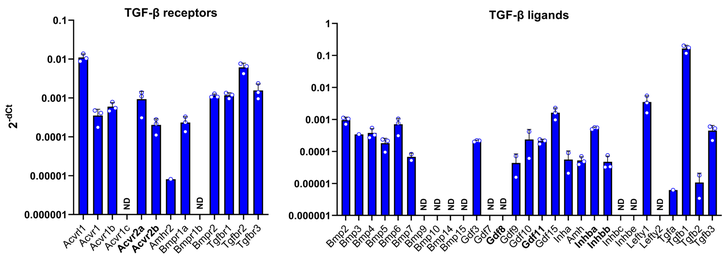
*(A) Dose-dependent increases in PLTs were observed in the SAD groups. The majority of participants (> 60%) in the two highest KER-050 dose groups exhibited a change from baseline in PLTs ≥ 30 x 10^9 cells/L at any timepoint during the study. (B) Single dose of RKER-050 in mice elevated PLTs 2-fold relative to vehicle within 12 hours of treatment. On day 14, PLT counts were no longer significantly different from Vehicle, but increased again on day 37. We suspect that the differences in KER-050 and RKER-050-induced PLT profiles in humans and mice are due to species-specific differences in PLT biology. More work will be done to delineate these differences to better characterize the mechanism of KER-050-induced MK maturation and PLT production. *p ≤0.05, **p ≤0.01 and ****p ≤0.0001.*

Figure 2: RKER-050 induced a rapid increase in murine platelet counts, megakaryocyte numbers and cell ploidy



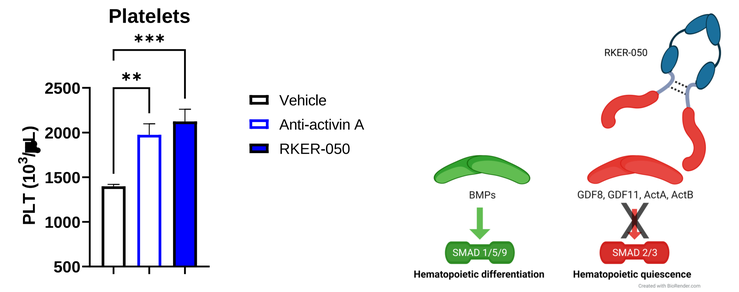
*After 12 hours of RKER-050 treatment, both mouse (A) platelet counts and (B) CD41⁺ cell population were significantly increased. (C) After 24 hours, an increase in the percentage of CD41⁺ cells with higher ploidy was observed signifying a greater number of mature megakaryocytes after RKER-050 treatment. These data support the effect of RKER-050 may occur on later stages of platelet development. *p ≤0.05, **p ≤0.01 and ****p ≤0.0001.*

Figure 3: Murine CD41⁺ cells expressed TGF-β family receptors and ligands



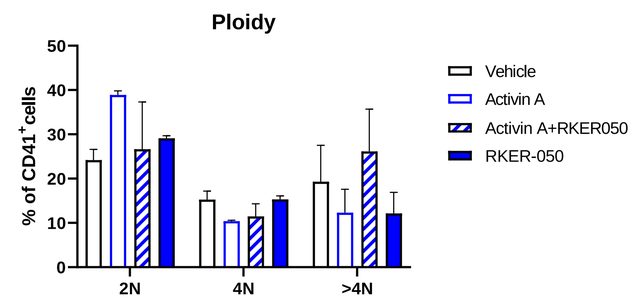
*In untreated mice, CD41⁺ bone marrow cells expressed many TGF-β family receptors and ligands demonstrating the capability of the TGF-β pathway to be involved in normal megakaryocyte function. Of note, the gene that can codes for the activin A protein, Inhba, was moderately expressed compared to other family member ligands. Ligands that bind to RKER-050 are in **Bold**. ND, not detected.*

Figure 4: Anti-activin A and RKER-050 treatment had similar effects on platelet counts



*Both anti-activin A and RKER-050 significantly increased platelet numbers in mice. Activin A is highly expressed in the bone marrow stroma[4] and competes with bone morphogenetic protein (BMP) signaling. BMPs, specifically BMP4, are known to mediate differentiation of megakaryocytes[5]. RKER-050 is designed to bind and sequester activin A with high affinity suggesting inhibiting activin A may be a partial driver for RKER-050's observed effects on platelets, potentially by blocking SMAD 2/3-driven hematopoietic quiescence signaling. **p ≤0.01 and ****p ≤0.001.*

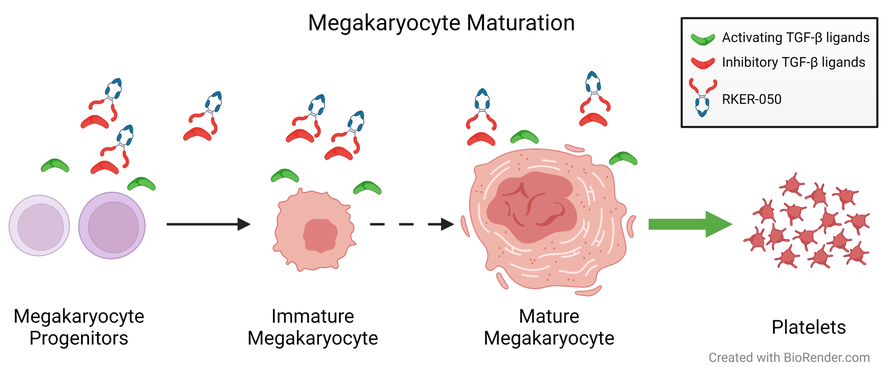
Figure 5: RKER-050 mitigated activin A-mediated effects on megakaryocyte ploidy



Ex vivo treatment with activin A reduced the ploidy of murine CD41⁺ cells. RKER-050 mitigated the reduction of CD41⁺ cell ploidy mediated by activin A, suggesting that RKER-050 may block the effects of activin A as well as restore normal megakaryocyte maturation.

CONCLUSIONS

In these studies, we observed that both KER-050 and RKER-050 increased platelet production in healthy volunteers and mice, respectively. Concordant with the increase in platelet output in mice, the percentage and ploidy of platelet progenitor CD41+ cells were also increased. The observed effects in mice on platelet counts from one dose of RKER-050 were phasic and long lasting. CD41+ cells express activins, GDFs, BMPs and TGF-β ligands and their cognate receptors, supporting the presence of TGF-β superfamily members and potential role in megakaryocyte differentiation. We demonstrated that inhibition of activin A with a neutralizing antibody increased production of platelets, potentially by shifting the balance towards increased BMP signaling. Likewise, RKER-050 treatment resulted in increased platelet production which is consistent with its activin inhibiting activity and potentially also mediated through increased BMP signaling. The data presented here is consistent with improvement in RBC and PLT counts observed in ongoing phase 2 clinical trials in MDS and myelofibrosis patients[3]. Therefore, KER-050 represents a potentially promising approach for patients with MDS, myelofibrosis and other hematological diseases where ineffective hematopoiesis occurs.



KER-050 is designed to bind and block select inhibitory TGF-β superfamily ligands, potentially causing an increase in thrombopoiesis

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