RKER-050, a novel inhibitor of TGF-β superfamily signaling, induced platelet production in healthy mouse megakaryocytes

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INTRODUCTION

Transforming growth factor-beta (TGF-β) superfamily signaling is vital 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, GDF8, and GDF11. In a clinical study in healthy volunteers, KER-050 increased red blood cell and PLT levels. 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 on platelet biology in mice.

METHODS

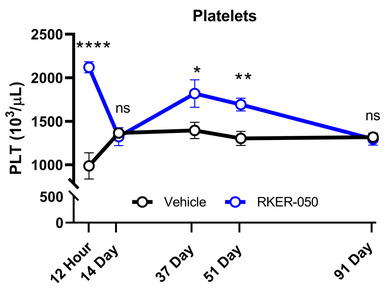
All studies used RKER-050, a research form of KER-050. Murine studies were carried out in 8-14-week-old C57BL/6 mice. For PLT number, % CD41+ (megakaryocyte marker) composition and ploidy assessment, a single intraperitoneal dose of RKER-050 (10 mg/kg) was given with multiple timepoints post-dose assessed. Ploidy levels of the CD41+ population were analyzed using a DNA stain.

For *in vitro* ploidy assays, bone marrow from untreated mice was isolated and cultured with vehicle, activin A, RKER-050, or RKER-050+activin A for 6 days and analyzed as described above. For gene expression analysis, qPCR was performed on RNA from bone marrow-derived CD41+ cells isolated from untreated mice.

One-way ANOVA with Dunnett's multiple comparison or Student's t-test were used for statistical analysis *p ≤0.05, **p ≤0.01, ***p ≤0.005, ****p ≤0.001.

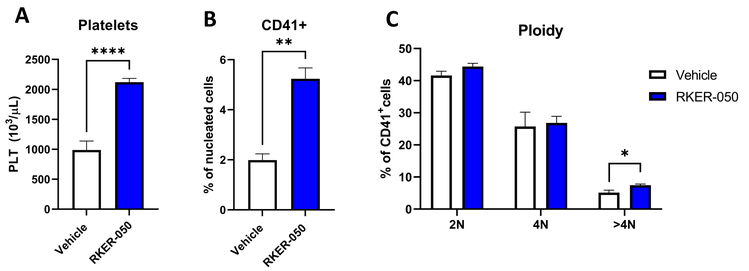
RESULTS

Figure 1: RKER-050 had a phasic effect on murine platelet counts



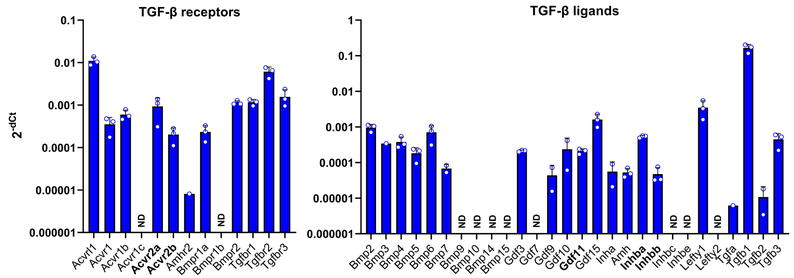
Mice treated with a single dose of RKER-050 had an increase in platelet numbers after 12 hours, 37 and 51 days, but counts normalized after 14 and 91 days demonstrating a phasic response. We hypothesize that RKER-050 may be affecting thrombopoiesis at multiple stages, including platelet formation and megakaryocyte progenitor renewal.

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



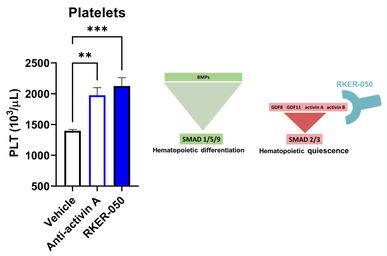
After 12 hours of RKER-050 treatment, both mouse (A) platelet counts and (B) CD41⁺ cell population were significantly increased. (C) After 24 hours, an increase in the percentage of CD41⁺ cells with higher ploidy was observed signifying a greater number of mature megakaryocytes after RKER-050 treatment.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

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



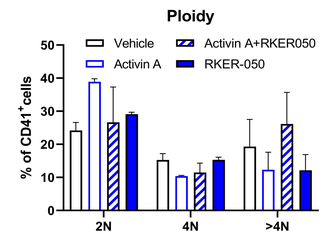
*In untreated mice, CD41⁺ bone marrow cells expressed many TGF-β family receptors and ligands demonstrating the capability of the TGF-β pathway to be involved in normal megakaryocyte function. Of note, the gene that codes for the activin A protein, INHBA, was moderately expressed compared to other family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

Figure 4: Anti-activin A treatment caused a similar effect as RKER-050 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[3]. RKER-050 is designed to bind 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.

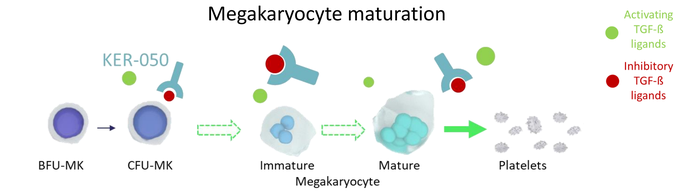
Figure 5: RKER-050 reversed activin A-mediated effects on megakaryocyte ploidy



Ex vivo treatment with activin A reduced the ploidy of murine CD41⁺ cells. RKER-050 reversed 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 preclinical studies, we determined that RKER-050 acted on multiple stages of thrombopoiesis resulting in increased platelets in mice. Concordant with the increase in platelet output, the percentage and ploidy of platelet progenitor CD41+ cells were also increased. The effects 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 role of TGF-β superfamily signaling in differentiation of megakaryocytes. We demonstrated that inhibition of activin A with a neutralizing antibody increased production of platelets, shifting the balance towards increased BMP signaling. Consistent with increased BMP signaling promoting thrombopoiesis, treatment with RKER-050 increased platelet production. These data support that RKER-050 promoted megakaryocyte maturation potentially by blocking inhibitory TGF-β ligands such as activin A. Previously, we have shown that RKER-050 treatment ameliorated the anemia in NUP98 mouse model of MDS[4]. Overall, these data support that KER-050 has the potential to treat diseases of thrombocytopenia.



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

¤ **Poster/abstract number P776 presents the effect of KER-050 on increasing platelets in MDS patients.**

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