A Modified Activin Receptor Type II Ligand Trap RKER-050 Restored Erythropoiesis in a Mouse Model of Myelofibrosis

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INTRODUCTION

Myelofibrosis (MF) is one of several myeloproliferative neoplasms most often associated with ineffective hematopoiesis, resulting in anemia. As a compensatory mechanism, extramedullary hematopoiesis is initiated in the spleen, resulting in splenomegaly. Abnormalities in megakaryocytes is thought to be one of the main drivers of MF disease progression[1]. Evidence suggests that the dysregulated TGF-β superfamily activity contributes to ineffective hematopoiesis[2].

KER-050 and its research form, RKER-050, are investigational modified activin receptor type IIA ligand traps designed to inhibit specific TGF-β superfamily ligands, including activin A, activin B, growth and differentiation factor (GDF) 8 and GDF11 to promote erythropoiesis and thrombopoiesis. Additionally, the KER-050 target ligands have been shown to promote bone resorption in preclinical studies[3,4]. Therefore, by inhibiting these ligands, KER-050 could potentially rebalance the bone marrow (BM) microenvironment, allowing restoration of hematopoiesis and alleviating extramedullary hematopoiesis and associated splenomegaly.

OBJECTIVE

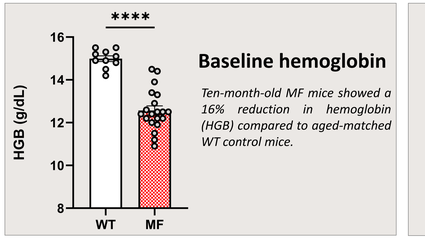
To investigate the potential of RKER-050 to reverse ineffective hematopoiesis in MF in a hypomorphic Gata1 MF mouse model (Gata1Low; MF mice) that presents similar characteristics to human MF, including ineffective hematopoiesis, cytopenias, extramedullary hematopoiesis and BM fibrosis.

METHODS

Using a therapeutic treatment paradigm, ten-month-old male MF mice with confirmed anemia were treated via intraperitoneal (IP) injection of vehicle (25mM Tris-HCl, 2.7mM KCl, 137mM NaCl; MF+VEH, n=10) or RKER-050 (10 mg/kg, MF+050, n=10) twice weekly for 12 weeks. Additionally, a wildtype control group was treated IP with vehicle (WT, n=10) twice weekly for 12 weeks.

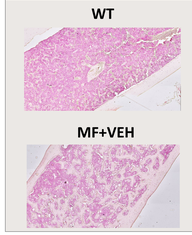
Blood for baseline hemoglobin was taken from a tail nick (~10 µl) and measured using a handheld hemoglobin analyzer. CBCs were measured from peripheral blood collected by terminal cardiac puncture under isoflurane and erythroid and myeloid progenitors were assessed via flow cytometry from cells isolated from femur and tibia bone marrow and spleen. Tibias were H&E stained for assessment of gross morphology and to assess BM space.

Student's t-test or One-way ANOVA with Tukey's multiple comparison was used for statistical analysis *p ≤0.05, **p ≤0.01, ***p ≤0.001, ****p ≤0.0001.



Baseline hemoglobin

Ten-month-old MF mice showed a 16% reduction in hemoglobin (HGB) compared to aged-matched WT control mice.

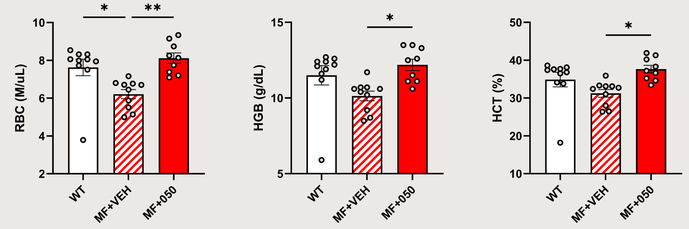


Reduced BM space

Marked trabecular bone growth was observed in marrow space of tibias isolated from MF mice 12 weeks after the initiation of treatment indicating the severity of the disease state and limited capacity for hematopoiesis within the BM compartment.

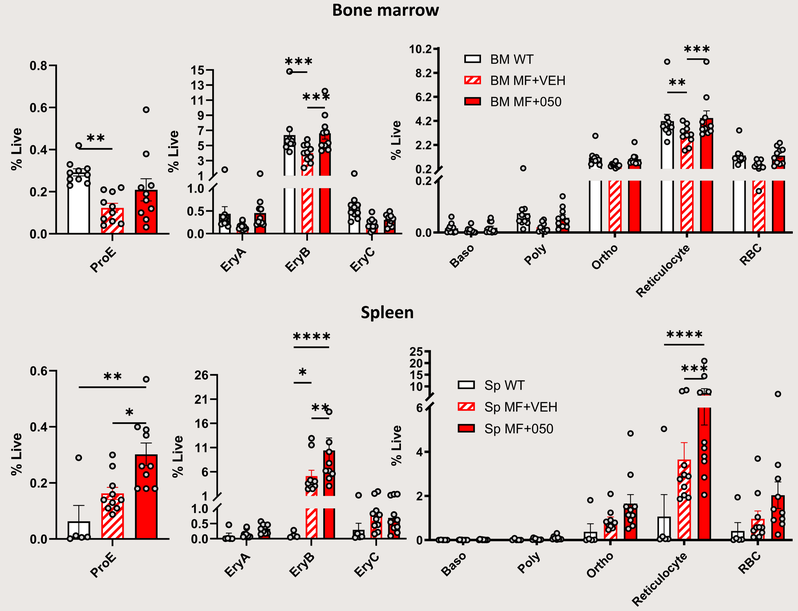
RESULTS

Figure 1. Red blood cell parameters recovered in MF mice treated with RKER-050



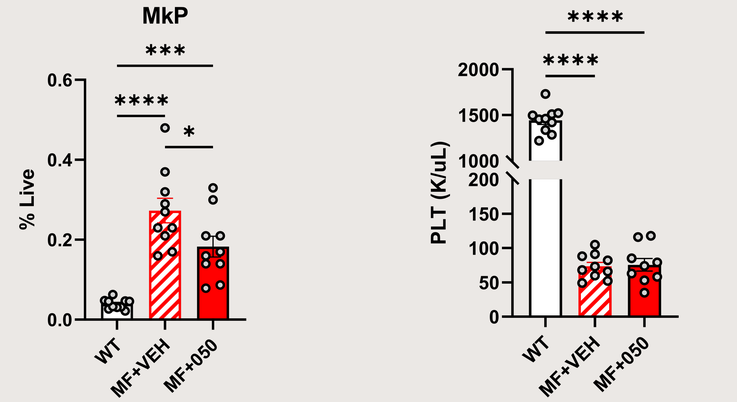
After 12 weeks of treatment, MF+VEH mice continued to exhibit a significant or trending decrease in red blood cell parameters, including red blood cells (RBCs; -19%), HGB (-20%), and hematocrit (HCT; -10%) compared to WT mice. In contrast, MF+050 mice had a significant recovery of RBCs (+31%), HGB (+24%) and HCT (+20%), compared to MF+VEH mice, demonstrating that RKER-050 reversed anemia in this advanced disease MF model.

Figure 2. RKER-050 mitigated MF erythroid precursor deficits in the BM of MF mice but further increased progenitors in the spleen



In bone marrow, MF+VEH mice showed a significant reduction in erythroid progenitors compared to WT mice. Treatment with RKER-050 (MF+050) resulted in significant increases in EryB (+66%) and reticulocytes (+46%) compared to MF+VEH mice; suggesting increased bone marrow erythropoiesis with RKER-050 treatment. Increased erythroid progenitors in the spleen of MF+050 compared to MF+VEH mice may be due to a severe disease state and marked reduction in the bone marrow compartment in MF mice.

Figure 3. RKER-050 reduced megakaryocyte progenitors in MF BM toward WT levels, but no evidence of improvement in circulating PLT



RKER-050 significantly reduced megakaryocyte progenitors (MkP) (-33%; MF+050) in the bone marrow compared to MF+VEH, suggesting RKER-050 may positively influence the megakaryocyte lineage. However, platelets (PLTs) in MF+050 mice were not significantly increased compared to the MF+VEH treatment group.

CONCLUSIONS

Our results show that RKER-050 can promote erythropoiesis and reduce aberrant MkP proliferation in the BM in a severe MF disease mouse model, as evidenced by the reversal of anemia and a decrease in the MkP subpopulation. Reestablishing BM hematopoiesis could obviate the need for compensatory extramedullary hematopoiesis in the spleen, the major driver of splenomegaly in MF patients. 10-month-old MF mice at the initiation of treatment had severely compromised BM as evidenced histologically. While treatment reversed the anemia, the severely compromised BM may be unable to support the normal hematopoietic BM niche. Further detailed analyses of bone morphology will elucidate the potential of RKER-050 to treat ectopic bone growth, and future studies will evaluate the potential of RKER-050 to treat an earlier stage of disease where the BM compartment is less severely compromised. In conclusion, KER-050 represents a potentially promising approach for patients with MF and other hematological diseases where ineffective hematopoiesis occurs.

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