

P1520 AN ACTIVIN RECEPTOR IIB LIGAND TRAP, IN COMBINATION WITH TMPRSS6 INDUCED IRON-RESTRICTION, IS A SUPERIOR TREATMENT FOR CORRECTING B-THALASSEMIA IN MICE

Topic: 27. Thalassemias

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Background: The hallmarks of β -thalassemia (BT) include ineffective erythropoiesis (IE), splenomegaly and iron overload (IO). There are several new promising therapeutics to treat BT under clinical investigation. One strategy employs iron restriction (IR) using antisense oligonucleotide technology and targets the matriptase-2 (*TMPRSS6*) gene (T-ASO). This mode of IR has the potential to be more tolerated by BT patients than oral chelators. In mouse studies, T-ASO has been shown to improve anemia, red blood cell (RBC) life span, splenomegaly, and several IO markers (Casu et al., 2016, 2020; Guo et al., 2013). Another exciting therapeutic approach targets the TGF- β pathway. Luspatercept, an FDA approved TGF- β ligand trap designed against the extracellular binding domain of activin receptor IIB. It has been shown to improve anemia in transfusion dependent BT (Cappellini and Taher, 2021). Mouse studies using the murine analog (RAP-536) was shown to promote erythropoietin (EPO)-independent maturation of late-stage erythroid cells and to increase RBC and hemoglobin (Hb) in a dose-dependent manner (Suragani et al., 2014).

Aims: Our aim was to investigate if this strategy would successfully target distinct morbidities associated with BT.

Methods: We generated a Luspatercept-like protein (RAP-GRL) and treated *th3/+* mice in combination with T-ASO (RAP-GRL+T-ASO). First, we designed, cloned, and purified RAP-GRL from a mammalian cell line. Then we tested the efficacy of our design, using RBC and Hb as our ready-out, by injecting wild-type (WT) and *th3/+* mice with 10mg/kg of RAP-GRL subcutaneously (s.c.) for 3 weeks. For our combination therapy experiments we administered RAP-GRL s.c. along with T-ASO (5mg/kg) via intraperitoneal (i.p.) injection to *th3/+* mice. We evaluated IE and splenomegaly by flow cytometry using CD71, Ter119 and CD44 analysis of bone marrow (BM) and spleen (SPL). Serum iron, EPO, hepcidin and erythroferrone (ERFE) measurements were done by ELISA.

Results: Our results showed that treatment of WT or *th3/+* with RAP-GRL increased RBC and Hb in both WT and *th3/+* mice (n=3-9), evidence that our construct and purification methods were viable to study the combination therapy strategy. In our combination therapy experiments, RBC and Hb RBC parameters were increased in all treatment groups except vehicle controls, with the best results achieved in the RAP-GRL+T-ASO treated group (**Figure 1**). Serum assessments of iron (**Figure 1**), transferrin saturations (TfSAT), and ERFE showed that only animals treated with the T-ASO exhibited significant changes in iron metabolism. Additionally, flow cytometric analysis showed improved IE in *th3/+* treated with single agents (T-ASO or RAP-GRL) in the BM and SPL, but additive improvements in the combination groups. Interestingly, EPO levels were increased only in the group treated with RAP-GRL only. Messenger RNA analysis of the kidney did not correlate with our EPO serum findings.

Image:

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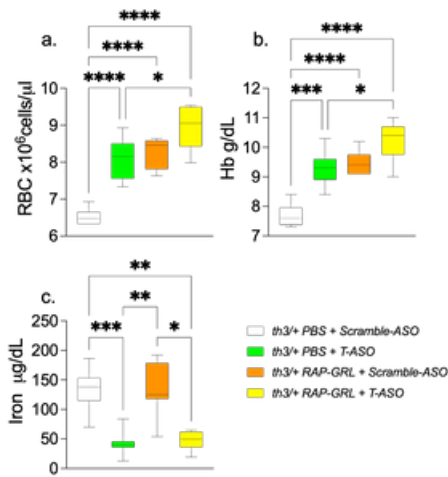


Figure 1 The combination of T-ASO and RAP-GRL is more effective at improving anemia and lowering serum iron levels in *th3/+* mice. Combination treated mice showed additive improvements in RBC (a) and Hb (b) compared to singly treated T-ASO and RAP-GRL mice or vehicle controls. Only mice treated with T-ASO, both singly or in combination with RAP-GRL (c), displayed lowered serum iron levels. Animals were treated for 6 weeks. Male and female mice were used in this study (n=6-7 per group). Statistical analysis was performed using a One-way ANOVA with Dunnett's multiple comparisons test * $p > 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Summary/Conclusion: In conclusion, our results provide pre-clinical support for combining IR and TFG- β ligand-trap strategies for the treatment of BT, and also evidence that the modes of action of these two drugs act independently. How activin ligand traps are able to increased RBCs remains a mystery. However, our data provides evidence that IR, in conjunction with the erythroid maturation action of drugs like Luspatercept, may offer an additive, more effective and more tolerable therapeutic strategy for BT patients.

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