THE PYRUVATE KINASE ACTIVATOR AG-348 IMPROVES MURINE β-THALASSEMIC ANEMIA AND CORRECTS INEFFECTIVE ERYTHROPOIESIS

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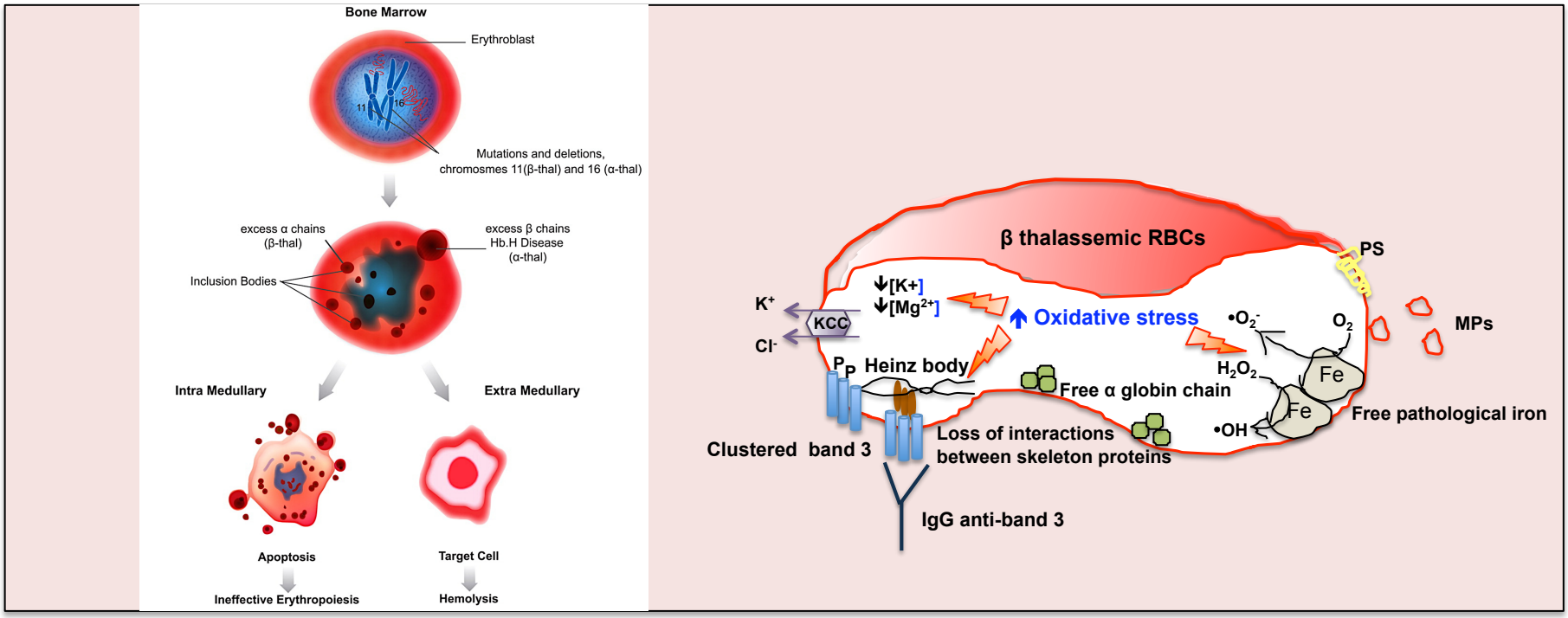
β-Thalassemic Syndromes are Worlwide Distributed Hereditary Red Cell Disorder and is a Model of Pathological Erythropoiesis

- **7% of global population is a carrier for severe hemoglobinopathies and β thalassemias are one of the most common inherited red cell disorders**

- **β thalassemias are characterized by absent or reduced synthesis of β globin chains, resulting in accumulation of free α-chain and free pathological heme.**

Weatherall DJ et al. Ann Human Biol 32: 117, 2005; Weatherall DJ Bull WHO 79: 704, 2001;
Rund D et al. NEJM 353: 1135, 2005

In β thalassemic Syndromes is due to both Ineffective Erythropoiesis and Reduced RBC Survival in the Peripheral Circulation



In β -thalassemias, the intra-cellular accumulation of free α -globin chains, free pathological iron and free heme result in high ROS production.

Ginzburg Y et al. Blood 118: 4321, 2011; De Franceschi L et al. Oxidative Medicine and Cellular Longevity 2013, Matte A ARS 23: 1284, 2015

Potentiation of Endogenous Anti-oxidant Systems as Novel Therapeutic Strategy in β-thalassemia

- In β-thalassemia, exogenous anti-oxidants have been largely studied to limit ROS cytotoxity and ineffective erythropoiesis

- Potentiation of endogenous anti-oxidant systems might be a novel interesting therapeutic strategy to face chronic and severe oxidative stress such as in β-thalassemia.

Franco SS et al. Haematologica 99: 267, 2014; Matte A ARS 23: 1284, 2015; Rund D et al. NEJM 353: 1135, 2005; Macari ER et al. Blood 117: 5987, 2011; Schmidt HHHW et al. ARS 23:1130, 2015

ATP is important for activity of anti-oxidant systems and low ATP levels have been reported in β-thal RBCs

- In RBCs, ATP is generated through glycolysis and pyruvate kinase-R is involved in the final step of ATP production

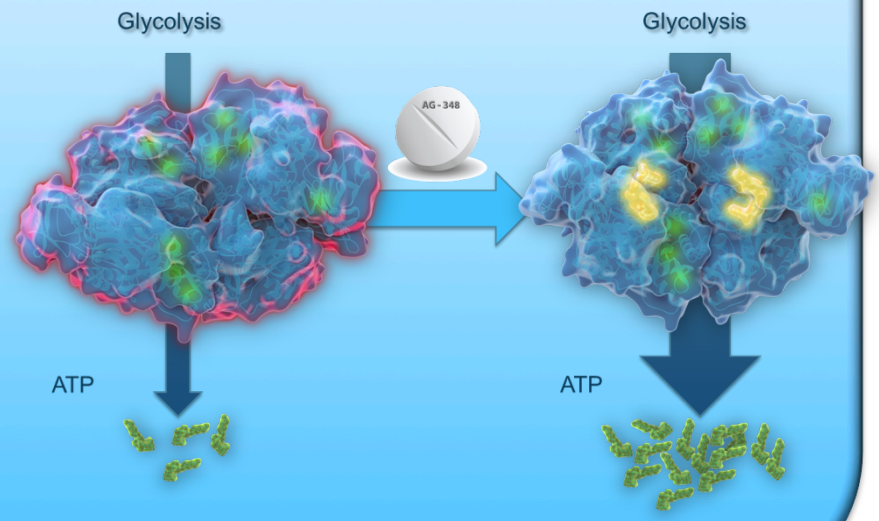
- β-thal intermedia RBCs show 3-fold faster glucose metabolism compared to controls

- Under oxygenation/deoxygenation condition, β-thal major and β-thal /HbE RBCs show reduced ATP content compared to healthy RBCs

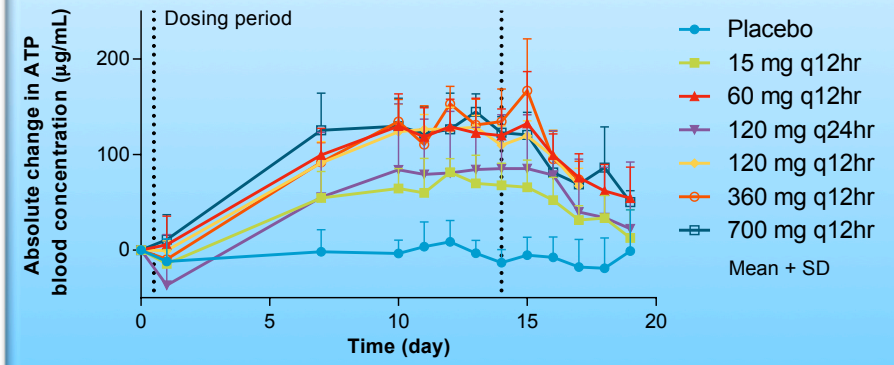
Ting YL et al. BJH 88: 547, 1994; Chakrabarty I et al. Arch Med Res 2012

AG-348: Allosteric activator of the red cell isoform of pyruvate kinase (PK-R)

AG-348 binds at PK-R enzyme dimer-dimer interface to promote active tetramer



AG-348 increases ATP levels in healthy volunteers (NCT02149966)



AG-348 is currently in Phase 2 testing in patients with pyruvate kinase deficiency (NCT02476916)

Ineffective erythropoiesis is present in PK Deficiency

- **PK deficiency is the most common hereditary abnormality in glycolytic pathway, resulting in a large clinical spectrum of hemolytic anemia**

- **Abnormalities in erythropoiesis,** (dyserythropoiesis and ineffective erythropoiesis) **have been described in <u>few patients with PK deficiency</u> as well as in a <u>PK mutated mouse model</u>, suggesting a possible role of PK in erythroid maturation events**

Zanella A et al. 13: 57, 2000; Aizawa S et al. AJH 74: 68, 2003; Aizawa S et al. Exp Hematol 33: 1292, 2005; Haija MA et al Ped Blood Cancer 61: 1463, 2014

Study Aim

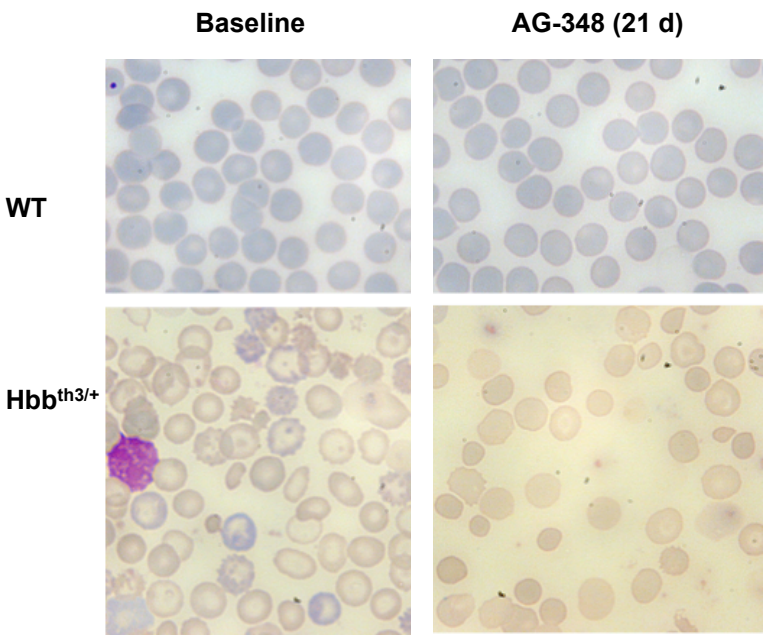
To evaluate the impact of AG-348 on anemia and ineffective erythropoiesis in a mouse model of β thal intermedia

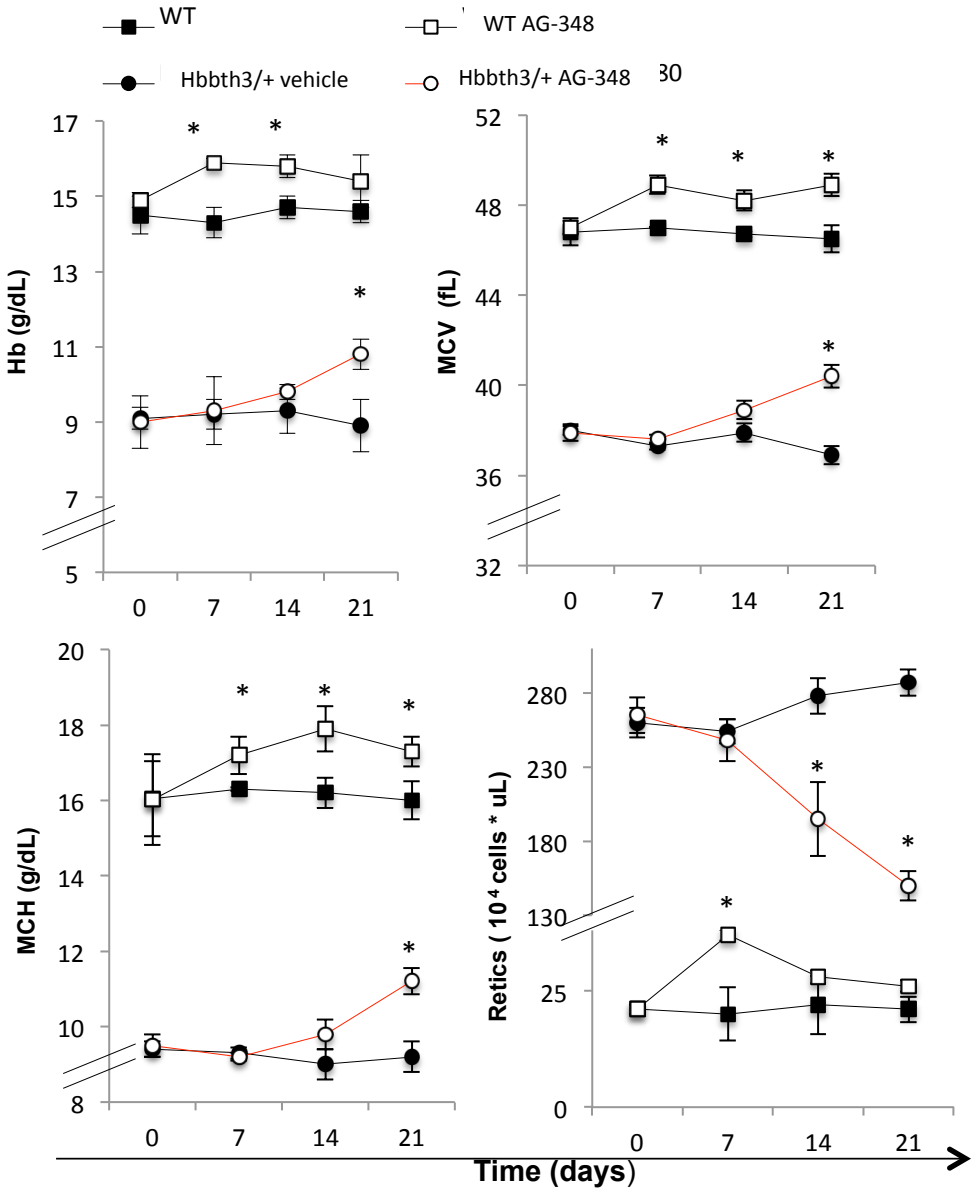
Study Design

- **$Hbb^{3th/+}$ mice were used as model of b-thalassemia intermedia and C57B6/2J as WT controls. Two month-old females were treated with either vehicle or AG-348 at 50 mg/kg bid by oral gavage.**
- **Hematological parameters, red cell indices and reticulocytes were determined.**
- **Flow cytometric analysis of erythroid precursors from bone marrow and spleen was performed using the CD44 and TER-119.**
- **Annexin-V positivity of orthochromatic erythroblasts was assessed.**
- **Histological analysis of spleen and liver was carried independently by two pathologists.**
- **RT-PCR analysis (including hepcidin and erythroferrone) and immunoblot analysis were carried out on sorted erythroid precursors and on liver cells.**

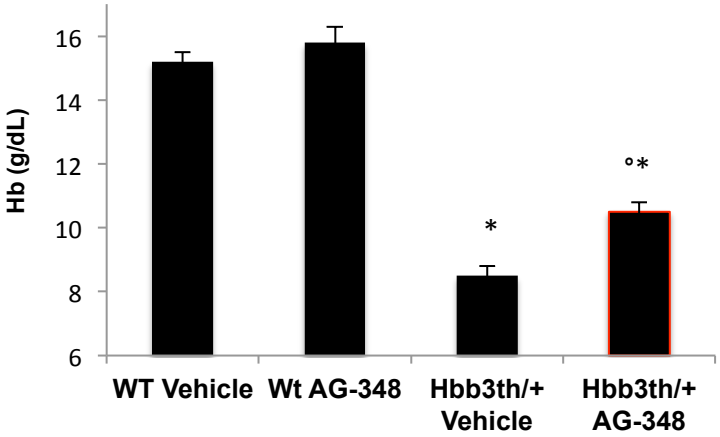
Franco SS et al. Haematologica 99: 267, 2014; Liu J et al. Blood 121: e43, 2013;
Matte A et al. Antioxid Redox Signal 23: 1284, 2015

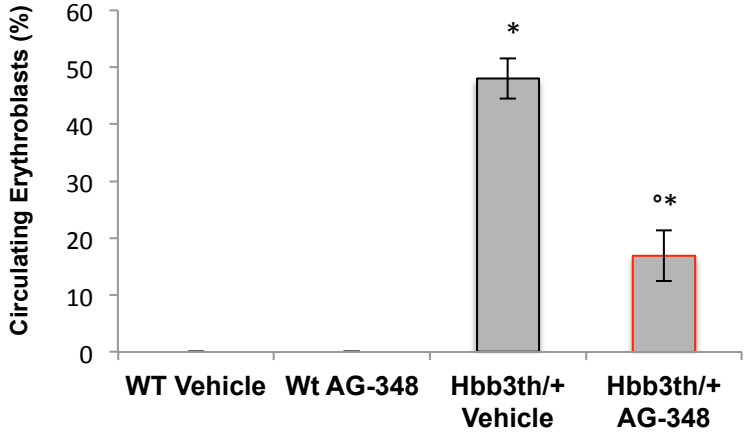
AG-348 treatment significantly ameliorates anemia in a mouse model of β-thalassemia





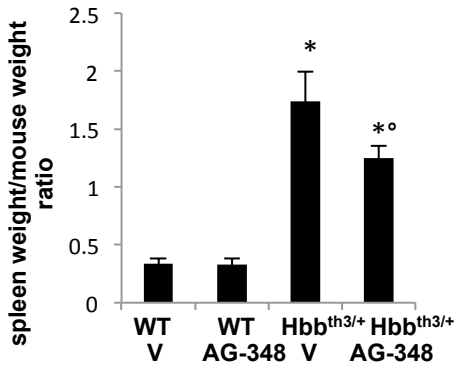
Long-term (7 weeks treatment) with AG-348 showed increased Hb and reduction in circulating erythroblasts

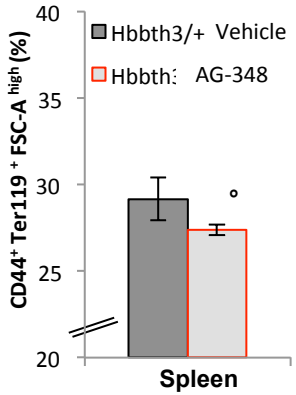


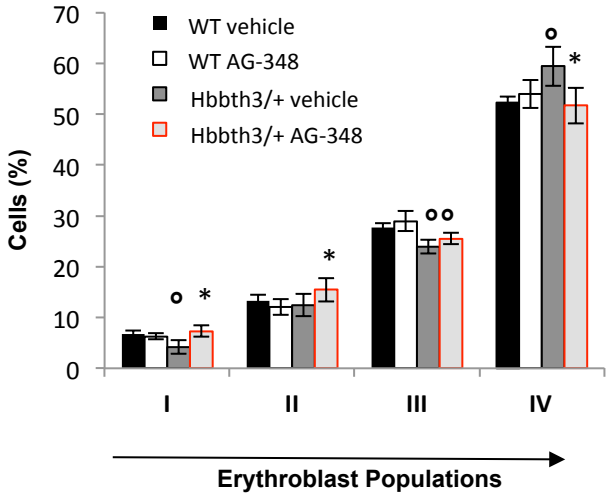


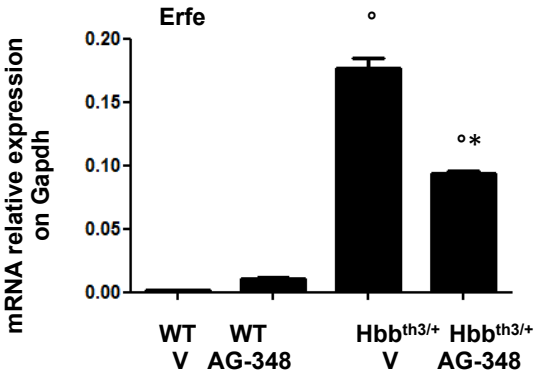
*p< 0.05 compared to WT
°p<0.05 compared to vehicle (V)

In β-thalassemic mice, AG-348 treatment ameliorates the ineffective erythropoiesis and is associated with decreased *Erfe* expression

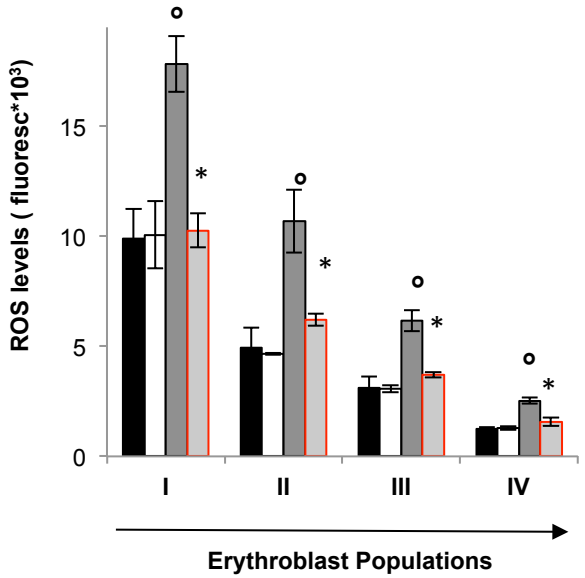


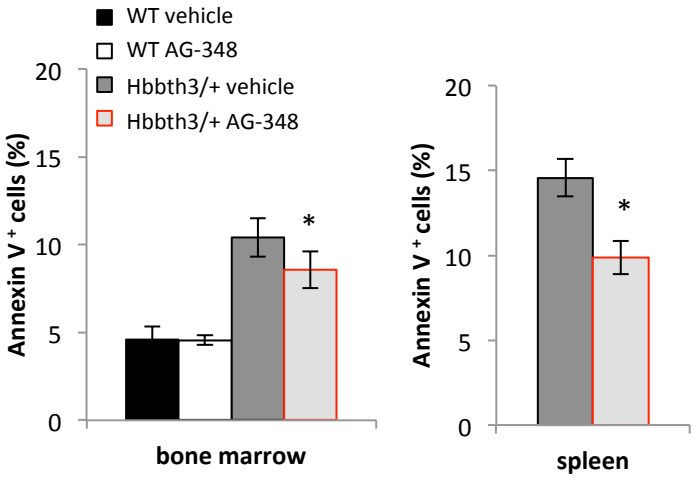






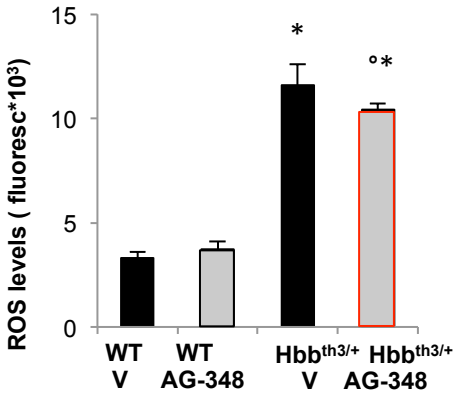
In β-thalassemic Mouse Erythroid Precursors, AG-348 Treatment Reduces ROS Levels and the Amount of Apoptotic Orthochromatic E.

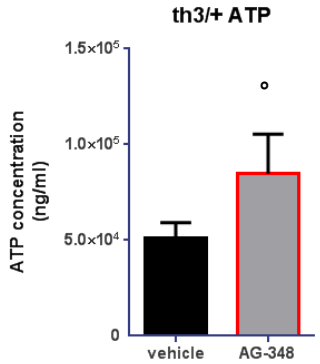


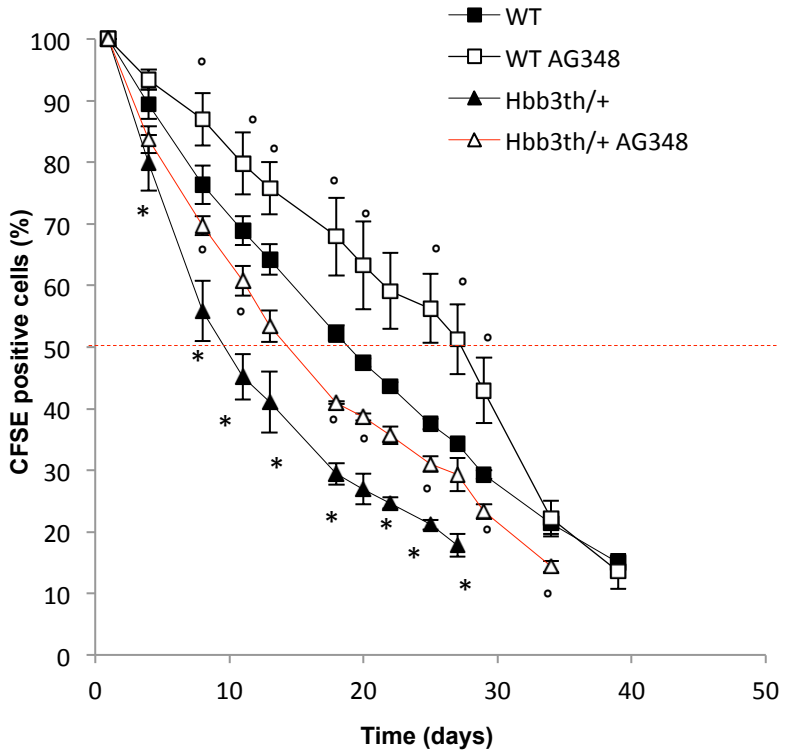


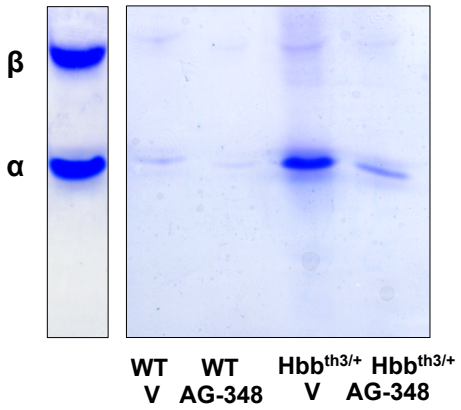
*p< 0.05 compared to WT
°p<0.05 compared to vehicle (V)

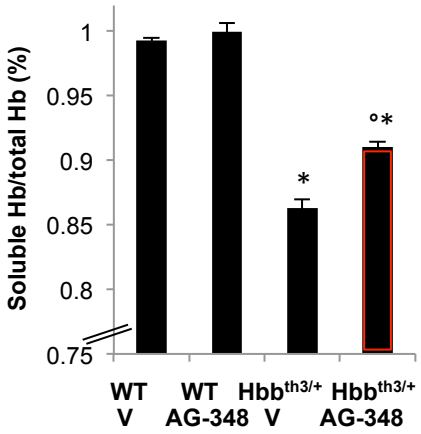
In β-thalassemic mice, AG-348 treatment reduces ROS production and alpha chain aggregates in RBCs and increased RBC survival





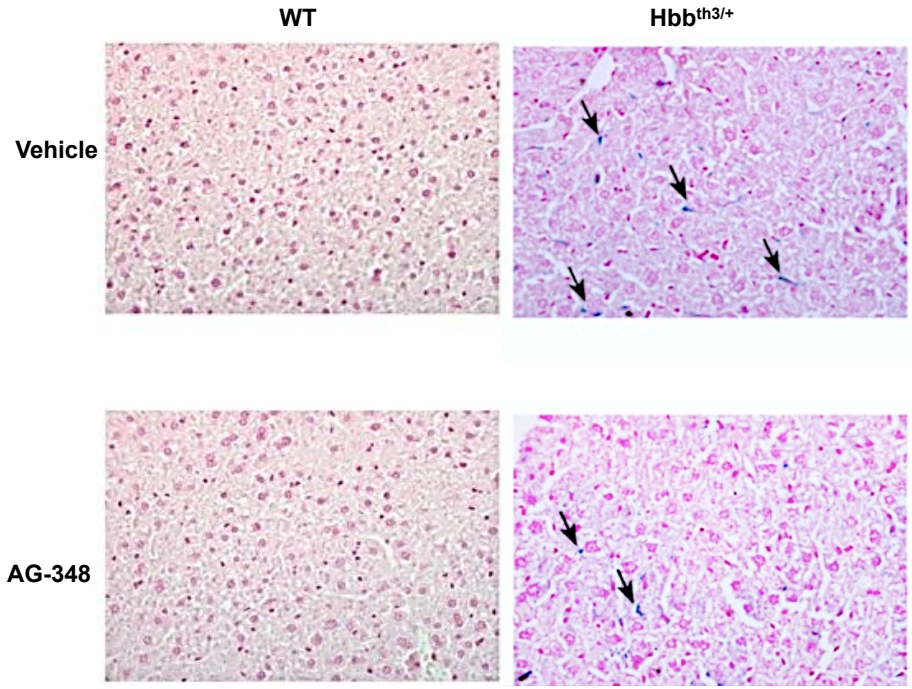


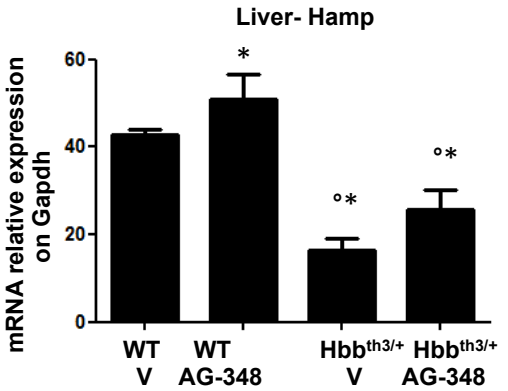




*p< 0.05 compared to WT
°p<0.05 compared to vehicle (V)

β-thalassemic AG-348 treated mice showed reduced liver iron overload and increased Hepcidin expression





*p< 0.05 compared to WT
°p<0.05 compared to vehicle (V)

Conclusions

✧ **In β thalassemic mice, AG-348:**

- **Reduces ineffective erythropoiesis, extramedullar erythropoiesis, Erfe expression and ROS levels**
- **Increases Hb levels, reduces reticulocyte count and circulating erythroblasts**
- **Significantly increases RBC survival**
- **Reduces liver iron overload and increases Hamp**

✧ **AG-348 might represent a novel therapeutic approach in clinical management of anemia in β thalassemic syndromes.**

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