

# Targeting the Second Transferrin Receptor as Emerging Therapeutic Option for $\beta$ -Thalassemia Major

Francesca Vinchi<sup>1,2</sup>, Muhammad Shahzad Ali<sup>1</sup>

**Correspondence:** Francesca Vinchi, *HemaSphere* Scientific Editor (fvinchi@nybc.org).

$\beta$ -thalassemia is an inherited genetic disorder of hemoglobin synthesis (hemoglobinopathy), with an overall carrier rate of 1.5% in the world population. It is caused by mutations either in the  $\beta$ -globin gene or its promoter that result in reduced  $\beta$ -globin chain expression leading to an imbalance between  $\alpha$ - and  $\beta$ -globin chains. Adult hemoglobin (HbA) primarily consists of 2  $\alpha$  chains and 2  $\beta$  chains which coordinate a heme group. In  $\beta$ -thalassemia the reduction or absence of  $\beta$ -globin chains leads to the accumulation of unstable  $\alpha$ -globin/heme complexes, called hemichromes, that easily precipitate and trigger reactive oxygen species (ROS) formation, thus contributing to ineffective erythropoiesis and anemia.<sup>1</sup> In  $\beta$ -thalassemia, expanded erythroid precursors produce elevated levels of the hormone erythropoietin (EPO), which negatively controls the production of the iron-regulatory hormone hepcidin. Reduced hepcidin increases intestinal iron absorption and release from macrophages, resulting in progressive iron accumulation in tissues, a major complication in  $\beta$ -thalassemia, as well as in erythroid cells, thus aggravating ineffective erythropoiesis through increased hemichrome production.

Anemia can range from mild to severe, based on which  $\beta$ -thalassemia patients are classified as nontransfusion-dependent (NTDT) or transfusion-dependent (TDT). Usually, patients with  $\beta$ -thalassemia major, due to almost complete absence of  $\beta$ -chains, have a greater and lifelong blood transfusion requirement along with iron chelation therapy, to limit complications associated with transfusional iron-overload.<sup>2</sup> So far, the only curative option for TDT patients is allogeneic bone marrow transplantation, which is often limited by the availability of HLA-matched donors and in some cases associated with severe post-transplant complications.<sup>3</sup> Although novel therapeutic approaches including gene therapy with autologous hematopoietic stem cells modified *ex vivo* to restore  $\beta$ -globin expression, and the administration of the activin receptor ligand trap luspatercept have been recently approved for  $\beta$ -thalassemia patients, their applicability in subsets of selected patients and incomplete effectiveness limit their use.<sup>4</sup> These considerations highlight the need of therapeutic options to improve the current treatments for  $\beta$ -thalassemia.

Recently, Di Modica and co-authors showed how the second transferrin receptor (TfR2) can be exploited for therapeutic purposes in TDT.<sup>5</sup> TfR2 is a transmembrane glycoprotein homologous to the classical transferrin receptor 1 (TfR1). While TfR1 is ubiquitously expressed and acts as main mechanism for cellular iron uptake through Tf-bound iron interaction, TfR2 is highly expressed in hepatocytes and rather involved in the regulation of hepcidin expression to modulate systemic iron levels.<sup>5</sup> Importantly, TfR2 was recently described as a sensor of circulating iron in erythroid cells, where it binds the erythropoietin (EPO) receptor (EPOR) and inhibits the activation of the EPO-EPOR signaling. Under condition of elevated Tf saturation, TfR2 is stabilized on the plasma membrane of both hepatocytes, increasing hepcidin to inhibit intestinal iron absorption, and erythroblasts, preventing excessive erythropoietic expansion. When Tf saturation decreases, membrane TfR2 is reduced, thus enhancing both iron absorption and erythropoiesis to avoid anemia. Thus, TfR2 plays a crucial role in balancing RBC production with systemic iron homeostasis, according to circulating iron levels.

Bone marrow TfR2 deletion was found to ameliorate anemia and ineffective erythropoiesis, and partially prevents hepatic iron loading in an NTDT mouse model.<sup>6</sup> Di Modica et al took advantage of a TDT mouse model obtained through the transplant of fetal liver cells of thalassemic embryos in myeloablated wild-type animals. This model recapitulates the phenotype of TDT  $\beta$ -thalassemia major, developing severe anemia and requiring red blood cell transfusions for survival.<sup>5</sup> Transplantation of fetal liver cells of thalassemic embryos carrying TfR2 deletion, resulting in the abrogation of erythroid TfR2 function, significantly ameliorated anemia and improved erythroid differentiation and RBCs morphology. Bone marrow TfR2-deleted mice had increased RBC count, hemoglobin levels, and hematocrit and decreased

<sup>1</sup>Iron Research Laboratory, Lindsley Kimball Research Institute, New York Blood Center, New York, NY, USA

<sup>2</sup>Department of Pathology and Laboratory Medicine, Weill Cornell Medicine, New York, NY, USA  
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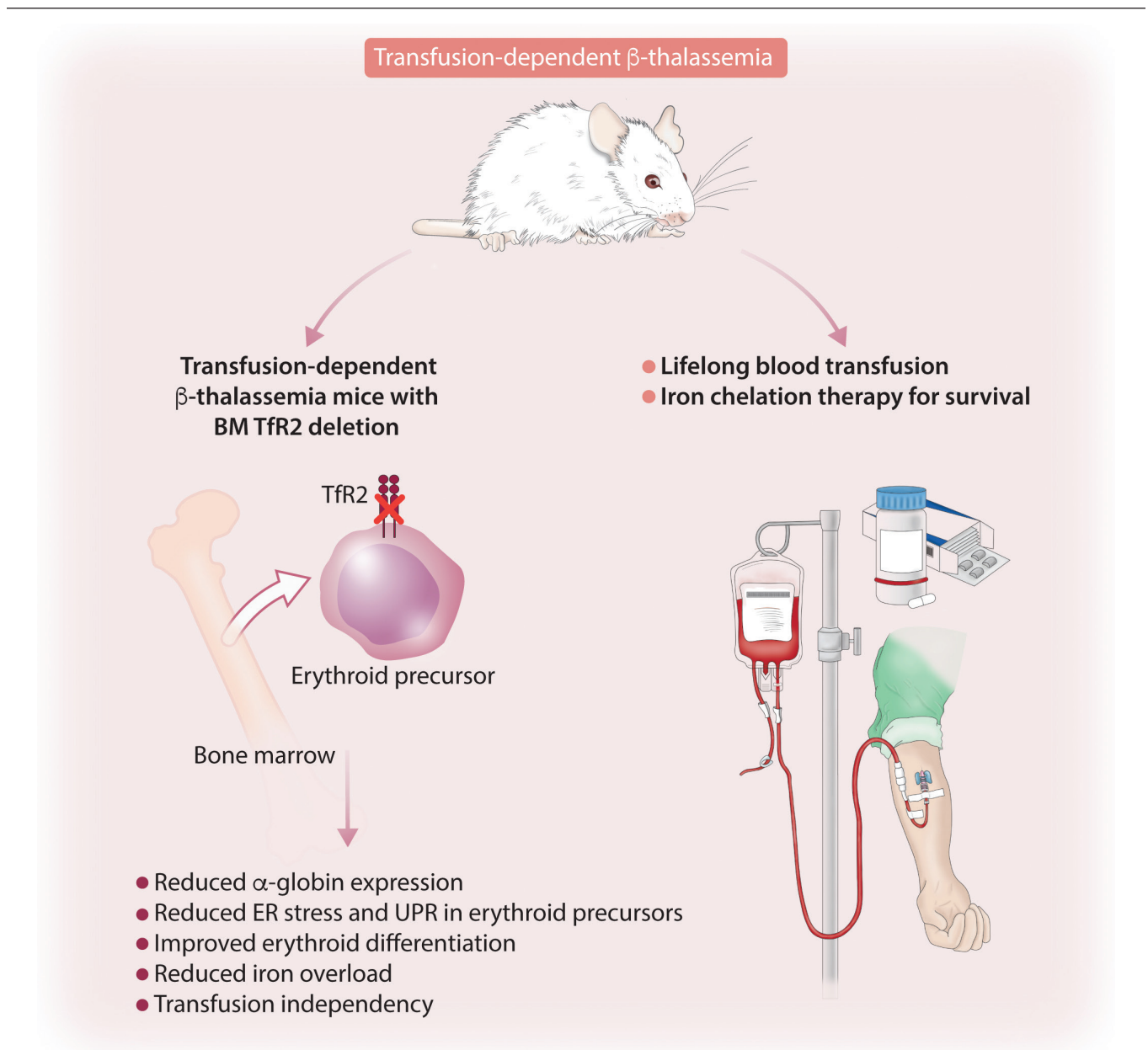
*HemaSphere* (2022) 6:11(e799).  
<http://dx.doi.org/10.1097/H59.0000000000000799>.

reticulocytes. The authors observed overall reduction of transferrin saturation and serum iron in bone marrow Tfr2-deleted  $\beta$ -thalassemia mice, likely due to increased iron consumption by improved erythropoiesis. However, iron content in the liver, spleen, kidney, and heart, along with hepatic expression of Tfr1 and iron-responsive bone morphogenetic protein 6 remained unchanged. In line with increased hemoglobin, serum EPO levels were decreased in Tfr2-deleted  $\beta$ -thalassemia mice compared to controls. EPO reduction (Figure 1), together with reduction of immature erythroid precursors, limited the production of ERFE in the bone marrow, likely accounting for increased hepatic hepcidin production, which in turn contributed to lowering transferrin saturation. Overall, these observations indicate that Tfr2 deletion boosts EPO sensitivity in TDT, as well as previously demonstrated in NTD.

Interestingly, bone marrow Tfr2 deletion was associated with reduced  $\alpha$ -globin levels and more appropriate levels of the  $\alpha$ -hemoglobin stabilizing protein, a chaperone required to prevent the

harmful aggregation of  $\alpha$ -globin.<sup>7</sup> This suggests that, in the absence of Tfr2,  $\alpha$ -globin production was decreased, pointing to a restored balance between  $\alpha$  and  $\beta$ -globin chains. Accordingly, the transcription factor, Kruppel-like factor 4, a key player in the transcriptional regulation of  $\alpha$ -globin expression, was reduced in the bone marrow Tfr2-deleted  $\beta$ -thalassemic mice relative to controls. Whether TFR2 directly modulates  $\alpha$ -globin via KLF4 remains to be clarified.

The molecular analysis of thalassemic Tfr2-deficient bone marrow cells of  $\beta$ -thalassemic mice revealed an alleviation of endoplasmic reticulum (ER) stress, which is usually elevated as a consequence of free  $\alpha$ -globin and hemichrome precipitation. Indeed, the expression of several genes that control the UPR pathways was reduced in bone marrow TFR2-deleted  $\beta$ -thalassemic mice. Overall, these findings suggest that, in the absence of TFR2, thalassemic erythroid cells have a reduced abundance of toxic free  $\alpha$ -globin chains, which lowers ER stress. Importantly, blood transfusion requirement was completely abolished in association with improved erythropoiesis and reduction in liver



**Figure 1. Tfr2 deletion in TDT murine model ameliorates ineffective erythropoiesis, anemia and iron-overload, leading to increased transfusion-free survival.** TDT = transfusion-dependent; Tfr2 = second transferrin receptor.

iron content in bone marrow Tfr2-deleted  $\beta$ -thalassemic mice, whereas controls mice maintained transfusion requirement throughout their lifespan.

In summary, Tfr2 deletion in a TDT murine model ameliorates ineffective erythropoiesis, anemia, and iron-overload, leading to transfusion independency and increased transfusion-free survival. These studies highlight TFR2 targeting as a promising therapeutic option for  $\beta$ -thalassemia, with potential both as a monotherapy and combined therapy with available treatments, and open up the doors for the discovery of pharmacological inhibitors that specifically target TFR2 for clinical applications.

#### AUTHOR CONTRIBUTIONS

FV and MSA wrote the article.

#### DISCLOSURES

The authors have no conflicts of interest to disclose.

#### REFERENCES

1. Grech L, Borg K, Borg J. Novel therapies in  $\beta$ -thalassaemia. *Br J Clin Pharmacol*. 2022;88:2509–2524.
2. Viprakasit V, Ekwattanakit S. Clinical classification, screening and diagnosis for thalassemia. *Hematol Oncol Clin North Am*. 2018;32:193–211.
3. Cappellini MD, Marcon A, Fattizzo B, et al. Innovative treatments for rare anemias. *HemaSphere*. 2021;5:e576.
4. Ferrari G, Cavazzana M, Mavilio F. Gene therapy approaches to hemoglobinopathies. *Hematol Oncol Clin North Am*. 2017;31:835–852.
5. Di Modica SM, Tanzi E, Olivari V, et al. Transferrin receptor 2 (Tfr2) genetic deletion makes transfusion-independent a murine model of transfusion-dependent  $\beta$ -thalassemia. *Am J Hematol*. 2022;97:1324–1336.
6. Artuso I, Lidonnici MR, Altamura S, et al. Transferrin receptor 2 is a potential novel therapeutic target for  $\beta$ -thalassemia: evidence from a murine model. *Blood*. 2018;132:2286–2297.
7. Feng L, Gell DA, Zhou S, et al. Molecular mechanism of AHSP-mediated stabilization of  $\alpha$ -hemoglobin. *Cell*. 2004;119:629–640.