P1509 BONE MARROW TFR2 GENETIC DELETION ABROGATES BLOOD TRANFUSION REQUIREMENT IN THE HBBTH1/TH2 B-THALASSEMIC MURINE MODEL

**Topic:** 27. Thalassemias

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**Background:**

β-thalassemia is a genetic disorder caused by mutations in the β-globin gene, characterized by anemia, due to defective production of hemoglobin (Hb) and red blood cells (RBC), ineffective erythropoiesis (IE) and iron overload, whose management still requires improvement. People affected by the most severe transfusion-dependent (TDT) form of the disease require lifelong blood transfusions (BT), treatment associated to several complications. Allogenic bone marrow (BM) transplantation is the only definitive cure, but it is limited by the availability of suitable donors. Gene therapy and Luspatercept are recently approved therapeutic options applicable to selected patients.

Transferrin receptor 2 (TFR2) is an activator of the iron-regulatory hormone hepcidin in the liver and a brake of Erythropoietin signalling in erythroid cells, balancing RBC production according to iron availability. The potentiality of BM Tfr2 deletion for the treatment of β-thalassemia has been already proven in non-TDT mice, both as a monotherapy (Artuso et al., Blood 2018) and, even more strikingly, in combination with iron-restricting approaches (Casu, Pettinato et al., Blood 2020).

**Aims:**

Here, we investigate whether BM Tfr2 targeting might represent a therapeutic option for TDT.

**Methods:**

TDT mice (Hbbth1/th2) with heterozygous (Tfr2BMhetero/Hbbth1/th2) and homozygous (Tfr2BMKO/Hbbth1/th2) BM Tfr2 deletion were generated by transplantation of fetal liver cells (FLT) from day E14.5 Hbbth1/th2, Tfr2-hetero/Hbbth1/th2 and Tfr2-ko/Hbbth1/th2 embryos. A cohort of mice was sacrificed 8 weeks after FLT for complete phenotypic analysis before the onset of transfusion-dependence. Blood transfusion requirement was monitored on a second cohort of mice through a weekly evaluation of Hb levels performed from 12 to 30 weeks after FLT. Animals were transfused when Hb dropped ≤5.5 g/dL.

**Results:**

Eight weeks after FLT, BM Tfr2 deletion improved anemia of Hbbth1/th2 mice with a gene-dosage effect, increasing RBC count and Hb levels and reducing the percentage of circulating reticulocytes. In addition, Tfr2BMKO/Hbbth1/th2 animals had a more effective erythropoiesis both in the BM and in the spleen, as shown by an increased proportion of the most mature erythroblasts (orthochromatic, reticulocytes and RBCs). The expression of α-globin was reduced in the BM and spleen of Tfr2-deficient mice: this partially rescued the unbalance with β-globin, contributing to the more effective erythropoiesis and leading to an improved RBCs morphology.

BM Tfr2 deficient mice maintained RBC count and Hb levels higher than controls until 30 weeks after FLT without any transfusion. On the contrary, Hbbth1/th2 mice became transfusion-dependent 14 weeks after FLT, with some animals suffering from very severe anemia and requiring weekly BT. Among these, 2 out 7 mice died, while no mortality was recorded among BM Tfr2 deficient mice. At the end of the follow-up Tfr2BMKO/Hbbth1/th2 animals had
lower hepatic iron content than Hbβδ1/βδ2 controls, showing that BM Tfr2 deletion may prevent secondary iron-loading in these mice.

**Summary/Conclusion:**

Hematopoietic Tfr2 deletion increased the levels of functional Hb and RBCs and improved IE in TDT mice, thus mitigating the severity of the disease. This effect was sufficient to avoid long-term blood transfusions, to abolish mortality due to severe chronic anemia or BT complications, and to strongly reduce iron loading, showing that BM TFR2 targeting might represent a therapeutic option also for TD β-thalassemia. Further studies are ongoing to elucidate the exact mechanism.