**PROJECT 2 (optional)**

**DoS**: Antonella Nai

Title: Transferrin receptor 2: a novel therapeutic target for beta-thalassemia

Curriculum: Experimental and Clinical Medicine

Link to OSR/UniSR personal page: https://research.hsr.it/en/divisions/genetics-and-cell-biology/regulation-of-iron-metabolism.html

**Project description** (*Number of characters*, *including spaces*: 2.000 - 3.000):

β-thalassemia is a disorder caused by mutations in the β-globin gene, characterized by ineffective erythropoiesis (IE), anemia, splenomegaly, iron-overload, bone fragility and osteoporosis, whose management still calls for improvement. We recently identified Transferrin receptor 2 (TFR2) as a brake of erythropoiesis, which balances red blood cells production to iron availability. TFR2 is mainly expressed in the liver, where it regulates systemic iron homeostasis, and in the erythroid compartment, where it is a partner of erythropoietin receptor (EPOR). We proved that *Tfr2* deletion in the bone marrow enhances EPOR signaling and erythropoiesis both in wild-type mice (1) and in a model of transfusion-independent β-thalassemia (*Hbb*$^{th3/+}$) (2), paving the way toward the design of a novel potential therapeutic approach for β-thalassemia based on TFR2 targeting. In this context, RNA degradation technologies as small-interfering RNAs (siRNAs) represent an innovative and promising opportunity, rapidly transferable to the clinics. However, achieving effective downregulation of the target of interest within erythroid cells is a challenging task, because of poor cellular uptake. To overcome this issue, we aim at assessing the efficacy of *Tfr2*-siRNAs conjugated to specific groups for organ-targeted delivery.

Recent evidence proves that *Tfr2-ko* mice are characterized by increased bone mass because of an augmented bone turnover (3). We will then verify whether *Tfr2* inactivation might also correct bone abnormalities in β-thalassemia mice. The potential involvement of fibroblast growth factor 23 (FGF23), a recently identified mediator of the erythropoiesis-bone relationship, will be also evaluated.

Overall, these studies have the potential of validating a TFR2-targeted therapeutic approach for the concomitant correction of erythropoietic, iron and bone abnormalities of β-thalassemia.

In detail the project includes:
- the treatment of wild-type and *Hbb*$^{th3/+}$ thalassemic mice with functionalized *Tfr2*-siRNAs for the characterization of their effectiveness in stimulating erythropoiesis *in vivo*;
- the evaluation of the bone phenotype of *Hbb*$^{th3/+}$ mice with germ-line *Tfr2* inactivation and/or treated with *Tfr2*-siRNAs;
- the evaluation of whether TFR2 affects FGF23 production by erythroid cell, exploiting *TFR2*-null cell lines and primary cells;

- the assessment of the *in vivo* effect of a FGF23 blocking peptide in *Hbb*$^{th3/+}$ mice with germ-line *Tfr2* inactivation, to verify whether TFR2 modulates erythropoiesis and/or bone homeostasis through FGF23 production.

**Skills to be acquired by the student:**

- *In vitro* and *ex vivo* culture of cell lines and primary cells
- RNA extraction and real-time PCR; hematological analysis and iron quantification; Western-Blot and FACS analysis
- Management, genotyping and manipulation of mouse models; bone marrow transplantation procedure

**References** (max. 3)

1. Nai A, et al. *Blood*. 2015;125(7):1170-9
2. Artuso I, et al. *Blood*. 2018;132(21):2286-97
3. Rauner M, et al. Nat Metab. 2019;1(1):111-124