ERYTHROPOIESIS, HEMOGLOBIN EXPRESSION AND SWITCHING.

THE HUMAN δ-GLOBIN GENE AS A THERAPEUTIC TOOL FOR β-HEMOGLOBINOPATHIES. POST GWAS TARGET VALIDATION AND EVALUATION OF MOLECULES IN PRECLINICAL MODELS.

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Objectives and specific aims: β-thalassemia (β-thal) and Sickle Cell Disease (SCD) affect countless people worldwide. The only definitive treatment is bone marrow transplantation which has many limits. Gene therapy and gene editing approaches remain largely experimental. Alternative approaches and new targets need to be evaluated. Our project objective is to evaluate a possible therapeutic approach based on the enhancement of HbA2 (α2δ2).

Background/Rationale: we focused our attention on the δ-globin, the non-α component of HbA2. Although expressed at a low level, HbA2 is fully functional.¹ The therapeutic potential of HbA2 in β-thal and SCD (β-hemoglobinopathies) has been validated in vivo by our lab.²³ In a recent GWAS study CCND3, was found associated with increased HbA2 levels.⁴ Ccnd3 null mice are viable and fertile.⁵ We have preliminary results showing a robust increase of δ-globin expression (and γ to a minor extent) in vivo in a transgenic mouse model on a Ccnd3 null background. These observations suggest that there could be a viable pathway to alter the cell cycle during terminal erythroid differentiation, as in Ccnd3 KO mice, without pathological consequences. Supporting the rational we have recently shown, as proof of principle, that HbA2 can be increased by molecules.⁶ A small molecule inhibitor of Cyclin D3 dependent-kinase is available and additional molecules have shown activity in an ex vivo assay. These data are the starting point for our study aimed at increasing HbA2 levels as a therapy for β-thalassemia and Sickle Cell Disease (SCD). The use of molecules would allow the treatment of β-hemoglobinopathies more universally available.

Objectives and methods: the project objective is to evaluate a possible approach to the treatment of β-hemoglobinopathies based on the enhancement of HbA2 expression. We aim to evaluate if the Ccnd3 deprivation, in vivo, would produce an increase of HbA2 (and HbF) sufficient to cure or ameliorate β-thalassemia in a humanized mouse model, containing the full human β-globin cluster, and in human erythroid cell culture. In human erythroid cells, we will design a genome-editing approach using the CRISPR/Cas9 technology. The effect of CCND3 deprivation will be evaluated in human erythroid cells from healthy donors as well as β-thalassemic and SCD patients. If successful our results would demonstrate that it is possible to improve β-thalassemia (and possibly SCD) through...
a non-lethal modulation of cell cycle in vivo and in vitro. Moreover our results could validate an additional target (CCND3) for genome editing approaches. In parallel we will also evaluate, in vivo, the effect of cell cycle modulators. Modulators will include a validated Cyclin D3 inhibitor as well as other active molecules previously selected by screening in a mouse fetal liver derived erythroid cell line (Telethon RG 14065). We will evaluate the increase in HbA2 expression and the possible improvement of the pathology in a humanized β-thalassemic mice model.

Anticipated output: the results of the proposed project could contribute to the development of new therapeutic strategies and to the validation of a new target for the treatment of β-hemoglobinopathies.

References:
3) Porcu S1, et al. Delta-Globin Gene Expression Improves Sickle Cell Disease In A Humanized Mice Model. (Submitted)

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