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Assessment of Novel γ-Globin Lentiviral Vectors for β-Thalassemia Gene Therapy

β-Thalassemia syndromes represent a group of hereditary, monogenic, blood disorders, characterized by reduction or absence of β-chain synthesis (1). As a result, α-chain molecules are produced in excess and precipitate in red blood precursors, leading to impaired erythrocyte maturation, mechanical damage and apoptosis (2).

Current therapies for β-thalassemia include mainly blood transfusions with iron chelation, hydroxyrea treatment for HbF induction and allogeneic HSC transplantation of HLA-matched donors (3). The latter leads to therapy, however it is restricted by the limited donor availability and the need for long-term immunosuppression to prevent or delay GVHD. Therefore, a molecular approach, such as gene therapy that overcomes most of the aforementioned limitations and exploits the beneficial effects that originate from the re-infusion of the corrected cells, seems quite promising.

As lentivuruses (LVs) can cross the nuclear membrane of interphasic cells, they are able to infect both dividing and non-dividing cells. They can therefore be employed for therapeutic transgene delivery in genetically modifying tissues, such as the hematopoietic system. The significant breakthrough in the field of globin gene therapy using LVs, occurred with the construction of an HIV-based vector combining the β-globin gene along with its regulatory elements, which led to amelioration of the disease in β-thalassemic mice (4,5). Following this approach, several groups working on hemoglobinopathies, employed self-inactivating (SIN) β-globin LVs in their studies, leading to correction of murine β-thalassemia (6), as well as to the first successful clinical trial of β-thalassemia gene therapy in France, in 2007 (7).

The observation that compound thalassemic patients with HPFH syndrome typically have less anemia, milder clinical symptoms and are often transfusion-independent drew the attention towards constructing γ-globin vectors (8,9,10). We have generated a novel insulated SIN γ-globin LV, designated as GGHI (11), which managed to produce efficient amounts of HbF *in vitro*, restoring hemopoiesis and thus resulting in phenotypic correction in erythroid cultures of $CD34^+$ cells isolated from peripheral blood or bone marrow of thalassemic patients (11). Current projects in our laboratory involve incorporation of novel regulatory elements in GGHI vector that will both enhance transgene expression and stability and provide high tropism for targeted human $CD34^+$ cell transduction.

My project involves assessment of these LVs, both *in vitro* in human $CD34^+$ cells from normal and thalassemic individuals and *in vivo* in mouse models. In the former case, human $CD34^+$ cells are isolated form peripheral blood or bone marrow, purity is confirmed using human anti-CD45 and anti-CD34 Abs and are then being transduced with the therapeutic LVs. Following a 24h-transduction, cells are washed and resuspended in medium supplemented with rhSCF, rhFlt-3L, hTPO, rhEPO, rhIL-3 and rhIL-6. Under these conditions, $CD34^+$ cells will commit to erythroid lineage. Vector-induced HbF increase is assessed in cells expressing erythroid-specific markers, such as anti-CD71 and anti-CD235a, 21 days post transduction. At the same time, and in order to assess the reduction in apotosis in thalassemic cells as a result of HbF increase by our therapeutic vector, or the extent of cell death that may indicate LV toxicity, Annexin-V staining is employed. In certain cases and in order to assess the $CD34^+$ cell tropism of certain LVs when in a heterogeneous population, such as mononuclear cells, the percentages of the transduced “non-target” cell populations, i.e lymphocytes, dendritic and NK cells are determined using flow cytometry and anti-CD3, anti-CD11c and anti-CD56 Abs respectively.

Lastly, in order to assess the *in vivo* therapeutic effect of both GGHI and the novel LVs, experiments will be carried out in thalassemic and NSG mice. With regards to the thalassemic mouse model, total bone marrow cells will be transduced with the different LVs in the presence of mIL-1a, mTPO, mSCF and mIL-3 before transplanted to thalassemic mice. Restoration of thalassemic phenotype *in vivo* will be determined by assessment of hematological parameters, as well as expression of markers, such as HbF (combined with isotype control IgG1) and Ter119. Xenografts will be also performed and thalasssemic human $CD34^+$ cells, transduced with our novel LVs, will be transplanted in NSG mice, which support human erythropoiesis. Engraftment and chimerism will be assessed by staining of mouse bone marrow cells with human and mouse anti-CD45 Ab respectively.

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