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A COMBINED GENE THERAPY STRATEGY FOR β-THALASSEMIA TREATMENT

A research project proposal

Universitat Autònoma de Barcelona

Background

β-thalassemia disease is a highly prevalent hereditary type of anemia caused by the absence or reduction in β-globin chain synthesis, one of the subunits of adult hemoglobin (HbA), mainly due to very heterogeneous mutations in the β-globin gene. The basis of β-thalassemia lies in the fact that HbA cannot be formed because of this lack. Free α-globin chains then accumulate in red blood cells (RBC) and their precursors, causing their apoptosis, peripheral hemolysis, and ineffective erythropoiesis. The result of both events is chronic-hemolytic anemia, which leads to extramedullary hematopoiesis, iron overload, and many other adverse events such as organ systems impairment (Figure 1).

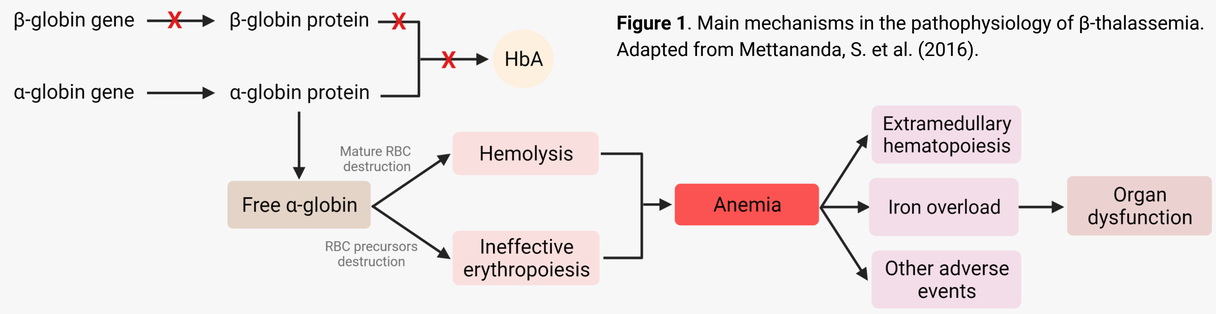

Figure 1. Main mechanisms in the pathophysiology of β-thalassemia. Adapted from Mettananda, S. et al. (2016).

There are several ways to treat β-thalassemia. However, these therapies still have some drawbacks (Table 1). What is proposed here is to combine the addition of the β-globin gene and the induction of γ-globin expression. The latter can take place by repressing BCL11A, a transcription factor that has been validated as an essential element in γ-globin silencing. This way, an improvement in current therapies can be achieved as it may give less likelihood of manifesting problems associated with NTDT.

Table 1. Current treatments of β-thalassemia and their main disadvantages.

Treatment	Description	Main drawbacks
RBC transfusion	It makes survival possible. Patients classify as transfusion-dependent (TDT) if there is an inability to survive without it, or non-transfusion-dependent (NTDT).	The disorder is not cured. Iron overload can be increased, so an additional iron chelation therapy is necessary. Life expectancy and lifestyle are affected.
Allogeneic HSC transplantation	Hematopoietic stem cells are transplanted from a suitable donor. It is the only available cure with clinical use.	Lack of suitable HLA-matching donors. Risks of immune-mediated rejection, graft-versus-host disease (GVHD), graft failure.
Gene therapy	Definitive correction of genetically defective erythroid precursor cells of the own patient. There are two main strategies: 1) Virus-mediated insertion of the β-globin gene 2) Reactivation of γ-globin expression	They do not definitively cure the patients but turn them into transfusion independents. The unbalanced production of globin chains does not completely disappear, so patients must still be controlled for extramedullary erythropoiesis and iron overload.

Hypothesis

The simultaneous addition of the β-globin gene and inhibition of BCL11A in β-thalassemic HSCs significantly reduces α/non-α globin chain imbalance in erythroid cells. Consequently, this also leads to an improvement in the symptomatology of the disease comparing to previous successful gene therapies.

Objectives

1) Design a lentiviral vector that includes the β-globin gene and an shRNA targeting BCL11A.
2) Prove if the vector increases the total Hb content and reduces free α-globin in RBC from β-thalassemia patients comparing to previous successful therapies.
3) Test if the above results can be obtained *in vivo* in a mouse model of β-thalassemia and if treatment with the new vector ameliorates the symptomatology of the disease.

Methods

LENTIVIRAL VECTOR DESIGN AND PRODUCTION

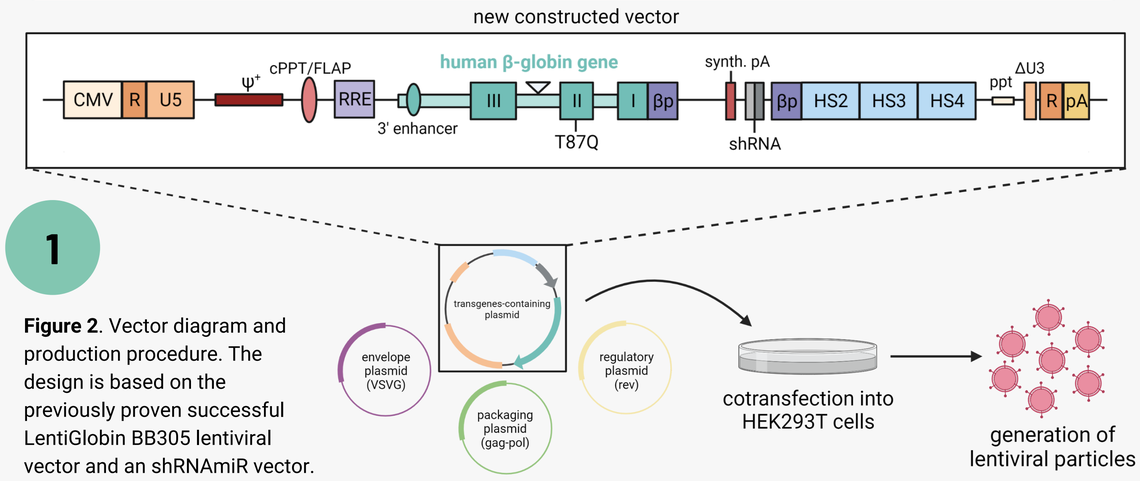

Figure 2. Vector diagram and production procedure. The design is based on the previously proven successful LentiGlobin BB305 lentiviral vector and an shRNAmiR vector.

IN VITRO EXPERIMENTS WITH β-THALASSEMIC HSCs

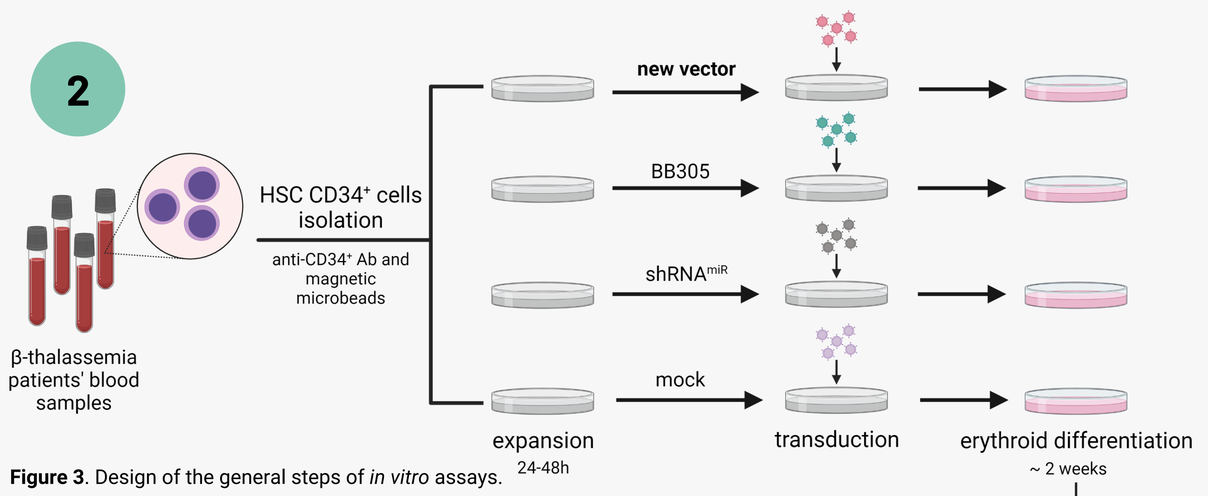

Figure 3. Design of the general steps of *in vitro* assays.

IN VIVO EXPERIMENTS WITH A MOUSE MODEL FOR β-THALASSEMIA

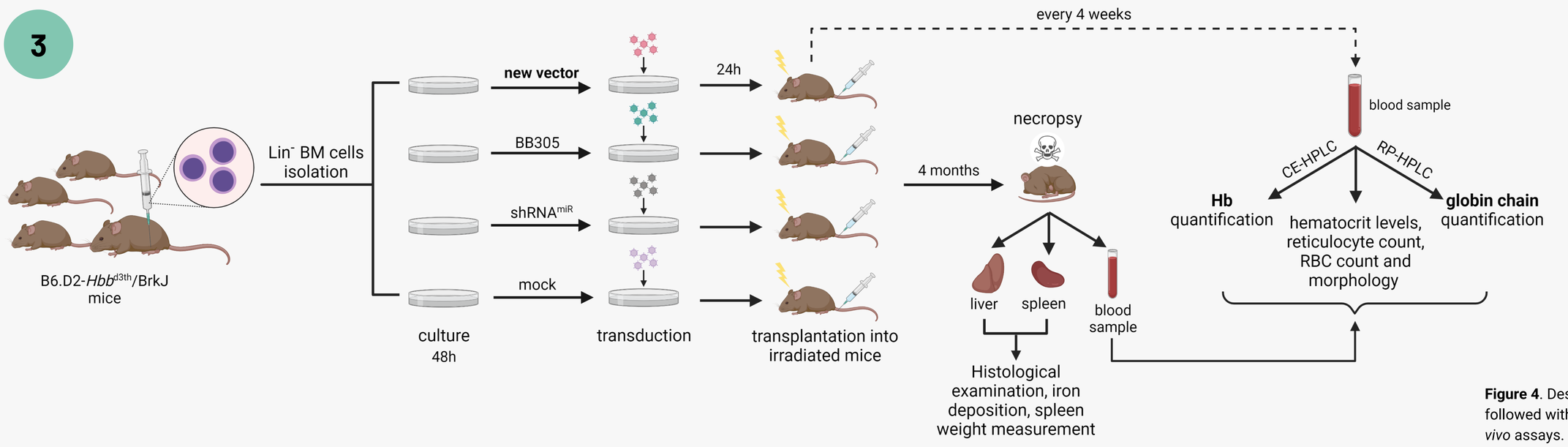

Figure 4. Design of the general procedure followed with β-thalassemic mice in the *in vivo* assays.

Expected results

***In vitro* results:** It is expected to see β- and γ-globin chains, HbA, and fetal Hb synthesis, in addition to the highest total Hb concentration, in erythroid cells from β-thalassemia patients treated with the new vector. Furthermore, a peak seen in untreated cells corresponding to α-globin chain aggregates should disappear in the study group of cells.
***In vivo* results:** Regarding globin chains and Hb concentration, it is expected to see the same pattern of results as *in vitro* studies, considering that, in this case, mouse embryonic globin is produced instead of γ-globin. Moreover, liver and spleen histology in treated mice should show average weight, normal tissue characteristics, and few iron depositions, indicating a reduction in anemia, extramedullary erythropoiesis, and splenomegaly.

Relevant references:

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