Uncover the world of gene therapy as an answer for β-thalassemia

Laura Franco Vallès, Grau en Genètica, Universitat Autonoma de Barcelona.

Universitat Autònoma de Barcelona

Introduction, purpose and objectives.

β - thalassemia is a monogenic blood disorder that is characterized by reduced or absence of β - globin production, resulting in anemia. Current therapies include blood transfusion combined with iron chelation, and the stimulation of fetal hemoglobin. BM and cord blood transplantation, although are the only curative treatments, are restricted by the matched donor limitation.

Different gene therapy strategies have been evolving in the last years with the aim to develop an efficient and effective curative treatment for this disease. The success of gene therapy lies on the desing of efficient globin vectors, like lentiviruses, that can effectively and stably transduce HSCs and on the improvement of the iPSC reprogramming techniques, aiming to achieve higher yield of genetically modified HSCs.

Since gene therapy development is growing so fast, the purpose of this research paper is going to be an easy explanation of β – thalassemia and its gene therapy, that will facilitate the patients and its families to become familiar with the mechanismis of the two most promising treatements.

In this poster is shown the information used to do the booklet destinated to the patients and their families.

β - thalassemia is a blood disorder that produces the reduction or the abcense of the production of hemoglobin A, which comprises the 98% of adult human hemoglobin. Hemoglobin is composed by four chains: 2 α globin chains and 2 β globin chains.

This reduction or absence in the production occurs when the gene that controls the production of β-globin (*HBB*) is defective. The accumulation of α chains that cannot match the β chains and form hemoglobin molecules leads to a premature death of red blood cells. This fact causes a lack in circulating red blood cells, resulting in anemia and limiting tissue oxygenation.

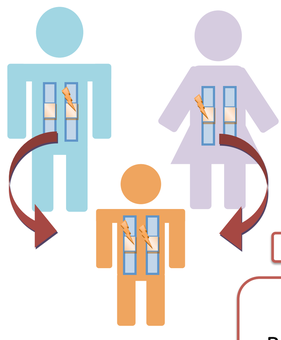
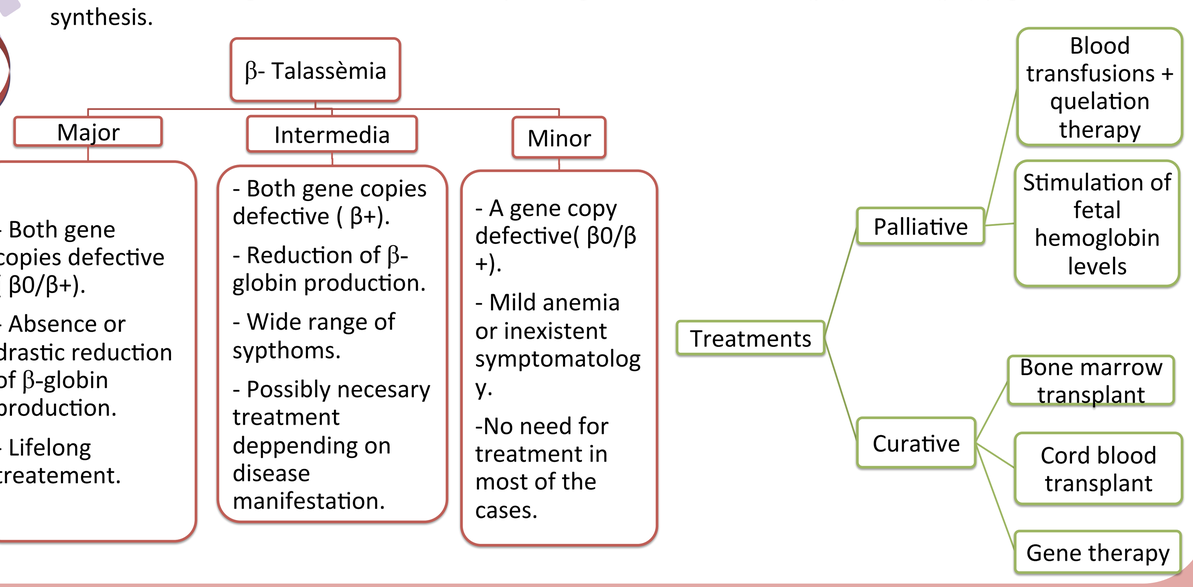


Figure 1: The pattern of inheritance: autosomal recessive. (Rare dominant pattern of inheritance)

β - thalassemia is caused by more than 200 mutations, mostly associated with one or limited number of nucleotides. The vast majority are point mutations within the gene or its immediate flanking and can alter the transcription, the RNA processing and the mRNA translation. These mutations are classified according to whether the corresponding allele results in no (β0) or low (β+) β-globin chain synthesis.



Gene therapy: modify the genetic material using the insertion of a functional gene into a living cell to achieve a therapeutic effect.

β – thalassemia a perfect candidate:

-Monogenic disease
-Severe disease
- Current treatments limitated.

Lentivirus:
- Retrovirus
- Dividing non dividing cells
- 3 main genes
-Regulatory genes

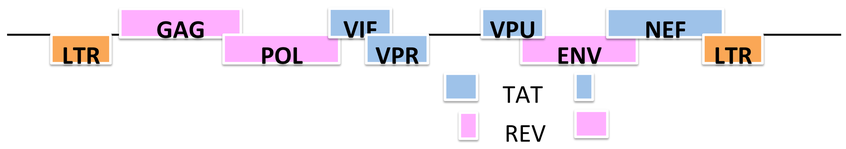


Figure 2: HIV genome. LTR; Unstranslated promoter region. Gag; matrix, capsid, nucleocapsid and P6 protein. Pol; protease, integrase and reverse transcriptase. Vif; infectivity for cell-free transmission. Vpr; transactivator, entry into non-dividing cells. Vpu; virus export facilitator. Tat; transcriptional activator. Rev; regulator of structural gene expression. Env; envelope glycoproteins. Nef; CD4 downregulation, unknown *in vivo* phatogenetic function.

Lentiviral vector
- Problems: RCL and insertional toxicity
- SIN vector
-Third generation packaging system
- Still infectingividing non dividing cells

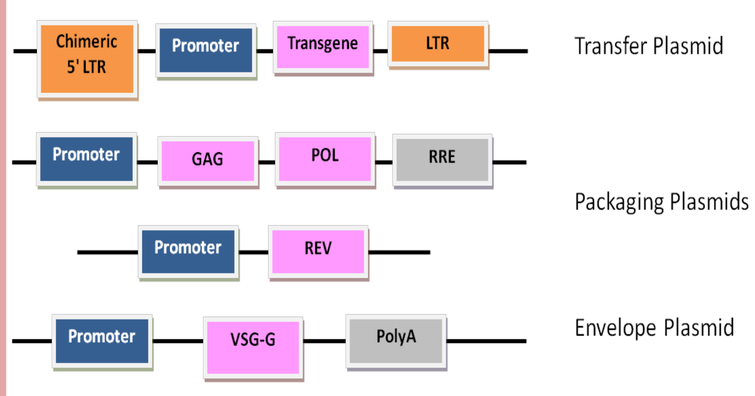


Fig.3: Third generation packaging method using a SIN vector. The packaging plasmid contains the *gag* and *pol* genes under the influence of a promoter. The rev plasmid expresses *rev* cDNA. The transfer vector plasmid contains HIV-1 cis-acting sequences and an expression cassette for the transgene. It is the only portion transferred to the target cells and does not contain wild-type copies of the HIV LTR. The 5' LTR is chimeric, with the RSV enhancer and promoter replacing the U3 region to rescue transcriptional dependence on *tat*. The 3' LTR has an almost completely deleted U3 region. The envelope plasmid encodes a heterologous envelope to pseudotype the vector, here shown coding for vesicular stomatitis virus (VSV)-G.

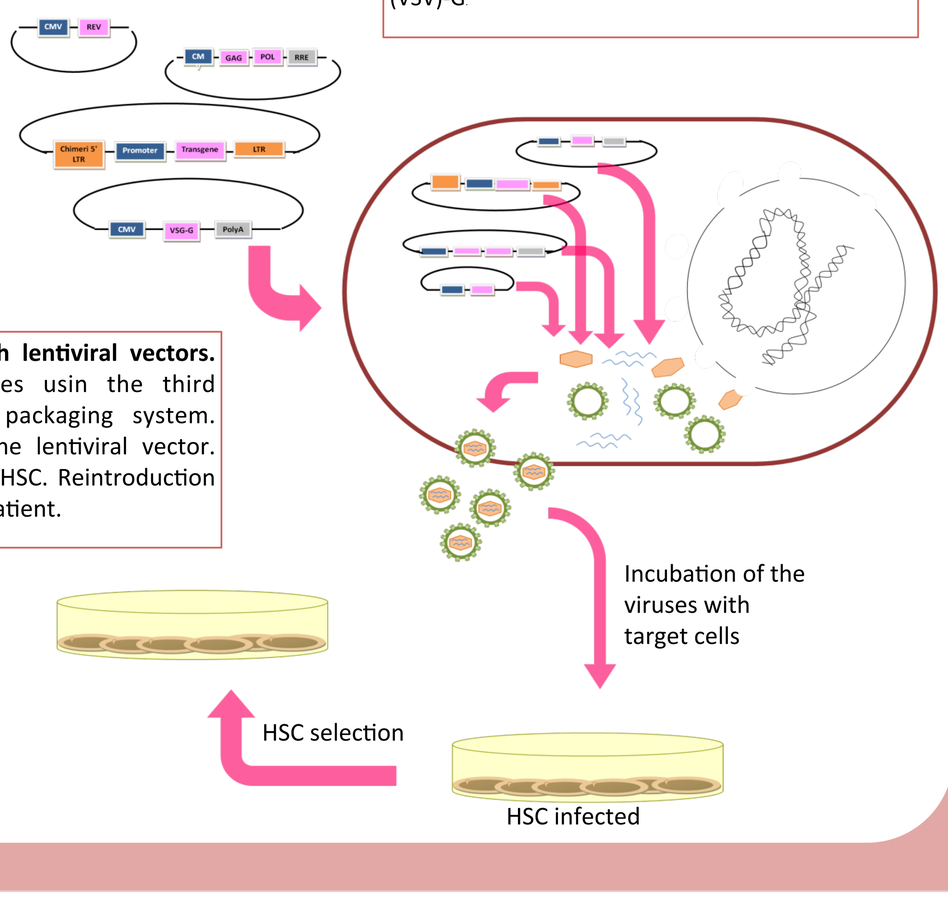
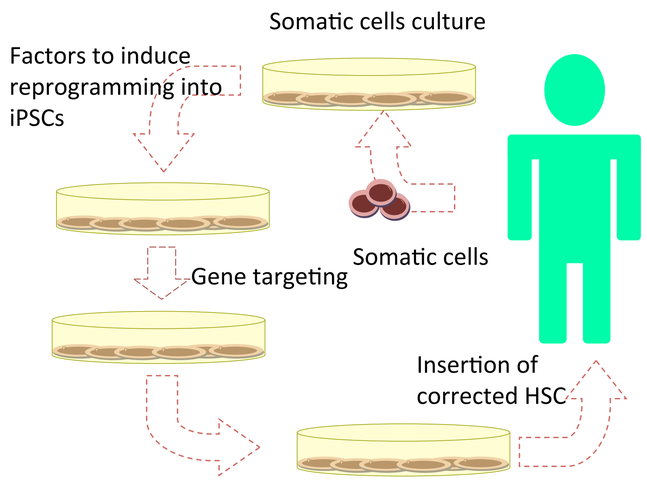


Fig.4: Gene therapy with lentiviral vectors. Generation of the viruses usin the third generation of lentivirus packaging system. Incubation of HSC with the lentiviral vector. Selection of the corrected HSC. Reintroduction of corrected HSC into the patient.

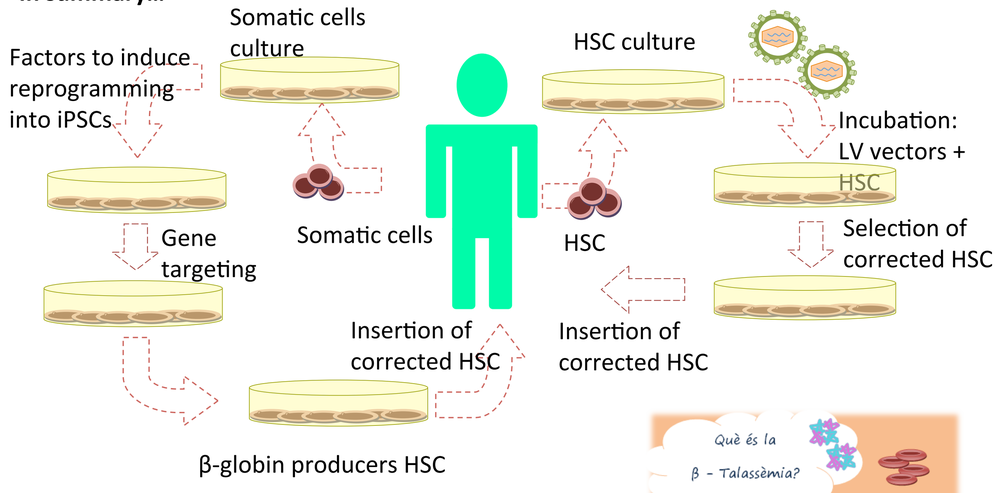
iPSCS

-Induced pluripotent stem cells from mainly somatic cells
-Genes and surface proteins expressed almost identical to the ones expressed in embryonic stem cells
-Induction by genes such as Oct3/4, Sox2,Klf4, c-Myc or Nanog.
-Correction by gene targeting
-Generation of every type of cell in human body

Fig.5: iPSCS gene therapy. Extraction of somatic cells from the patient. Reprogramming this cells into iPSCs using specific factors and genes mentioned before.
Gene targeting, an homologous recombination technique, to insert the functional β-globin gene. Differentiation of this cells into HSC. Reintroduction of the corrected HSC into the patient



In summary...



Conclusions

A lot of techniques, mechanisms and approaches are evolving really fast. Lots of them can be used as treatment for different severe disease so we should make an effort updating the patients with all the information that they may need in a way that can be understood with no problems.