# Beta-thalassemia Gene Therapy

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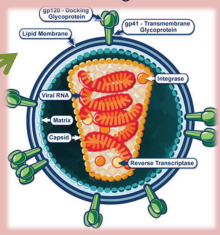
## Introduction

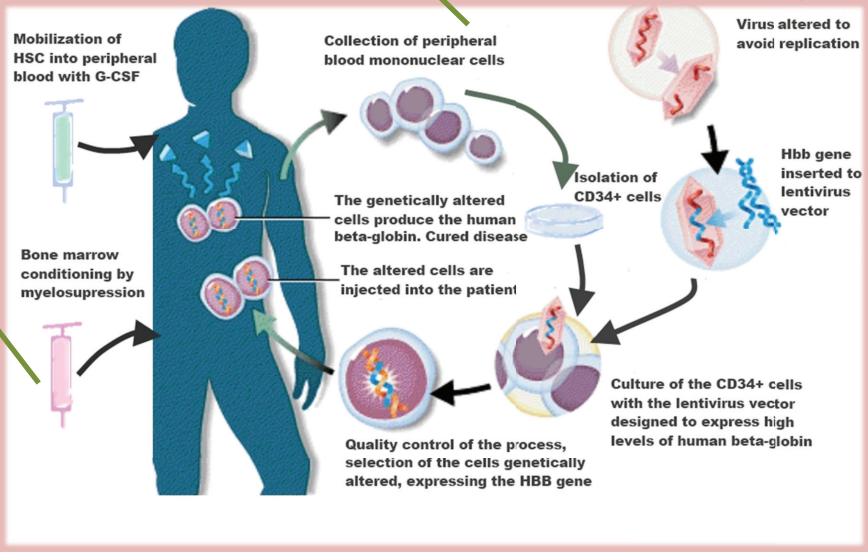
Beta-thalassemia is one of the most common autosomic monogenic recessive diseases worldwide. Individuals suffering from the most severe forms of the disease suffer from life-threatening anemia, hepatosplenomegaly and many more. As they do not produce enough **hemoglobin**, they need blood transfusions frequently. However, blood transfusions provoke an iron overload with several worrying side effects. Bone marrow transplant is the only lifelong cure option, but this treatment is not available for patients who don't have an **HLA** matched donor; Gene Therapy offers a new treatment alternative, making **autologous cell transplant** possible.

## Materials and methods

Being Beta-thalassemia a **monogenic** disease, is easy to imagine why it was the target of some of the first Gene Therapy studies. The approach it offers consists in an autologous bone marrow transplant, as the image shows, performed after the patient cells are modified ex-vivo with a viral vector.

The target cells are hematopoietic stem cells (**HSC**) for their capacity of **self-renewal** and giving rise to different mature lineages. Nevertheless, this characteristics make the **integration** of the vector essential to ensure the therapeutic gene is in all progeny.

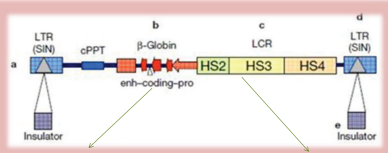



Pretreatment is necessary to achieve **therapeutic levels** of beta-globin. As corrected cells do not experience a selective pressure, the effect of the therapy risks to become diluted. For this reason, ablative **myelosuppression** is administered to the patients in order to extinguish the disease burden and open the bone marrow niche are being studied.

**Lentivirus** vectors are the ones used to carry the ***HBB*** gene to the target cells. They integrate into the hosts genome of both dividing and non-dividing cells; that is particularly important when targeting HSC, as they are quiescent. Retroviral vectors do not achieve efficiencies as high as lentiviral vectors due to their inability to transduce non-dividing cells. In addition, their integration pattern is more dangerous than lentivirus pattern.

The lentiviral vector used is a self inactivating vector (**SIN**). Deletion of **enhancers** in the viral LTRs allows for tighter regulation of the transgene and reduces the potential for activating close **proto-oncogenes**.




The vector is flanked by chromatin **insulators**, in order to further improve vector expression and reduce the chance of activating surrounding cellular genes.

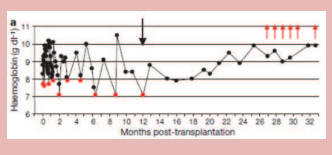
The coding cassette is optimized for expression and size, and it is included in antisense direction relative to vector transcription.

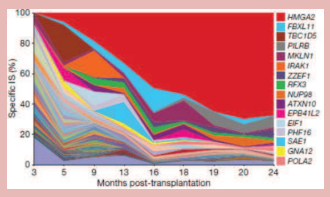
High level globin expression requires from a regulatory element upstream of the beta globin locus (**LCR**), as the LCR is too large, only core derivatives of have been introduced.

## Results

The first relevant clinical trial was initiated in 2007 in France. It was directed towards the use of a **beta-globin lentiviral vector** for the treatment of individuals, the vector encoded the beta-globin mutated at amino acid 87, which enabled them to differentiate endogenous from vector produced hemoglobin. The vector was flanked with a truncated version of an insulator, the **cHS4**.

The autologous cells were harvested from bone marrow and patients were conditioned with full myeloablation. A patient who followed the treatment, dependent on monthly transfusions, become **transfusion independent** for over a year.




Most of the therapeutic benefits resulted from a **dominant clone** in which the integrated vector caused the activation of the proto-oncogen ***HMGA2***. Nevertheless, The overexpression of *HMGA2* is correlated with benign tumour phenotypes.

## Future prospectives

•More Clinical trials are being made or will start in a foreseeable future, with similar characteristics to the approach explained. The treatments are based in diverse beta-globin lentiviral vectors which differ in the gene used, insulators and LCR elements.

•Induced pluripotent stem cells (**IPS**), pave the way for effective gene therapy application in humans. They could be **screened** for safe integration sites or **corrected** by gene targeting, and they expand in vitro and differentiate into transplantable HSC. In addition, they provide the opportunity of performing **gene correction** instead of gen addition, so there is no possibility of insertional mutagenesis. However, nowadays, it is necessary to improve systems for reprogramming adult cells and redifferentiation.

## Conclusions

•Successful preclinical studies, safety increase of vectors due to insulators, specific promoters and SIN vectors, and the amount of data available support the initiation of several clinical trials.

•The optimal vector design, pretreatment for achieving engraftment, and other factors are still unknown; thus, further studies should be addressed to solve them.

•IPS cells, and other new approaches help improving gene therapy and make it a real option.

•The great potential for beta-thalassemia gene therapy is still to be reached, rationally designed clinical trials could help to achieve it .