Pros and Cons of Gene Therapy in ß-Thalassemia
**β-Thalassemia: established and potential new therapeutic approaches**

• Many β-thalassemic patients are treated with blood transfusion and iron chelation. However, the complexity of this disorder and new findings suggest that this conventional approach could be improved upon.

• Jak2 inhibitors and hepcidin agonists might soon be utilized to limit ineffective erythropoiesis, EMH, splenomegaly, limit iron absorption and improve the transfusional therapy.

• The only definitive cure is bone marrow transplantation. But this approach presents severe limitations such as finding a compatible bone marrow donor and avoiding GVHD.

• Gene transfer offers an alternative approach to bone marrow transplant since utilizing autologous hematopoietic stem cells avoids the limitations of finding a compatible donor and prevents GVHD.
Gene therapy of β-thalassemia

• Gene transfer using a viral vector (lentiviral vector)

• Conditioning of the patient (complete or partial)

• Preclinical and clinical data

• Potential pitfalls
  • Poor chimerism (genetically modified vs. untransduced cells)
  • Genotoxicity

• Potential solutions
Gene Therapy Schematic Approach

- **Hematopoietic stem cells**
- **Reinfusion**
- **Transduction**
- **Vector carrying the therapeutic gene**
Therapeutic Levels of Hb in Mice Affected by β-Thalassemia

Therapeutic haemoglobin synthesis in β-thalassaemic mice expressing lentivirus-encoded human β-globin


Nature | Volume 406 | 6 July 2000 | www.nature.com

Plenary paper

A novel murine model of Cooley anemia and its rescue by lentiviral-mediated human β-globin gene transfer

Stefano Rivella, Chad May, Amy Chadburn, Isabelle Riviere, and Michel Sadoulm

Blood, 15 April 2003 • Volume 101, Number 8
Correction of Sickle Cell Disease

Correction of Sickle Cell Disease in Transgenic Mouse Models by Gene Therapy

Robert Pawliuk,1,2 Karen A. Westerman,1,2 Mary E. Fabry,3 Emmanuel Payen,4 Robert Tighe,1,2 Eric E. Bouhassira,3 Seetharama A. Acharya,3 James Ellis,5 Irving M. London,1,6 Connie J. Eaves,7 R. Keith Humphries,7 Yves Beuzard,4 Ronald L. Nagel,3 Philippe Leboulch,1,2,4,8*

14 DECEMBER 2001 VOL 294 SCIENCE www.sciencemag.org

Amelioration of murine β-thalassemia through drug selection of hematopoietic stem cells transduced with a lentiviral vector encoding both γ-globin and the MGMT drug-resistance gene

BLOOD, 4 JUNE 2009 • VOLUME 113, NUMBER 23

Huiwen Zhao,1 Tamara I. Pestina,1 Md Nasimuzzaman,1 Perdeep Mehta,2 Philip W. Hargrove,1 and Derek A. Persons1

Improved Human β-globin Expression from Self-inactivating Lentiviral Vectors Carrying the Chicken Hypersensitive Site-4 (cHS4) Insulator Element

Molecular Therapy vol. 15 no. 10, 1863–1871 oct. 2007

Paritha I Arumugam¹, Jessica Scholes¹, Natalya Perelman¹, Ping Xia¹, Jiing-Kuan Yee² and Punam Malik¹,³,⁴

Correction of β-thalassemia major by gene transfer in haematopoietic progenitors of pediatric patients

2010 EMBO Molecular Medicine

Emanuela Anna Roselli², Riccardo Mezzadra², Marta Claudia Fritto², Giuletta Munaggi², Erika Biral², Fulvio Maullio²,³, Fabrizio Mastropietro², Antonio Amato³, Giovanni Torron², Chiara Refalgh², Maria Domenica Cappellini², Marco Andreani³, Guido Lucarelli³, Maria Grazia Roncarolo¹,²,³, Sarah Marktel², Giuliana Ferraro¹,²,³
β-Thalassemia Treatment Succeeds, With a Caveat

Conversion to Transfusion Independence

Last RBC transfusion 16 months ago

Phlebotomy (200 ml)

New blood. A year after gene therapy, a β-thalassemia patient no longer needed blood transfusions.
Potential problems

Poor chimerism
(genetically modified vs. untransduced cells)
The challenge of obtaining therapeutic levels of genetically modified hematopoietic stem cells in beta-thalassemia patients

Genotoxicity
The possibility that the chromosomal random integration of the vector can lead to insertional mutagenesis
It is expected that the selection will be more effective as the vector will be able to express high levels of the beta-globin gene at single copy, irrespective of the chromosomal site of integration.
Gene Therapy Clinical Trial for SCID-X1 Halted Due to Insertional Mutagenesis

LMO2 gene

+ Oncoretroviral Vector Encoding the T and NK cells γc-Cytokine Receptor Subunit

Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1
Potential solutions:

- Insulators
- Suicide gene
- Targeted integration
Ankyrin-ST9W Lentiviral Vector

- **CMV-5' LTR**
- **WPRE**
- **β-globin gene**
- **Silent mutations**
- **3' SIN-LTR + Ankyrin sequence**

- They might prevent “genome toxicity” or insertional mutagenesis
- Insulators are elements thought to reduce expression interference and favor chromatin opening
A Pre-Clinical Approach

To establish a simple method to analyze, after gene transfer, a large number of thalassemic erythroid cells derived from patients with different mutations
Transduction of human erythroid precursors isolated from peripheral blood

Exapansio In vitro (Phase 1)

Erythroid Differentiation (Phase 2)

Ficoll separation

β-globin gene

Locus Control Region

LTR  RRE  e+  p  HS2  HS3  HS4  Ankyrin-sinLTR

SD  SA

840 bp  1308 bp  1069 bp

1 Kb
Conclusions

• Gene transfer might offer an alternative approach to bone marrow transplant since utilizing autologous hematopoietic stem cells avoids the limitations of finding a compatible donor and prevents GVHD.

• Preclinical studies in mice are extremely promising.

• However, some concerns are still present regarding the possibility that these vectors might trigger the activity of oncogenes.

• It is unclear what is the level of chimerism that need to be achieved.

• Additional preclinical studies using patient cells might be able to address these questions.