Gene Therapy for Beta Thalassemia

From the Bench to the Bedside

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Beta-Thalassemia

- Recessively inherited anemias that result from different mutations in the \( \beta \)-globin gene cluster
- The effect of mutations is reduced (\( \beta^+ \)) or absent (\( \beta^0 \)) \( \beta \)-globin, and therefore, reduced or absent adult hemoglobin (Hb A)
- The relatively excess \( \alpha \)-globin precipitates in erythroid precursors, resulting in membrane damage, apoptosis and ineffective erythropoiesis
- The standard of care for thalassemia major is life-long blood transfusions with iron chelation
- The curative therapy for \( \beta \)-thalassemia major is an allogeneic hematopoietic cell transplant (HCT)
Life Expectancy, Therapeutic Options and Causes of Death in Thalassemia

- Life expectancy is in 40-50 years in developed countries.
- HCT from a sibling, if performed early, is curative
- Regular transfusions and daily chelation is the mainstay of therapy in those with no match
- Most deaths occur in children in developing countries
- Iron overload is the major cause of death
Sickle Cell Anemia (SCA)

- A point mutation in $\beta$-globin gene – $\beta^S$-globin; HbS
- More than 100 million people afflicted worldwide
- 115,000 Americans with SCD.
- 2,000 affected infants born in the United States every year
- Similar numbers born everyday in Africa
- >$1.1$ billion in healthcare costs/yr in the US alone

Graham R. Serjeant. 1985. Sickle Cell Disease (modified)
Life Expectancy and Causes of Death in Sickle Cell Anemia

- Chest syndrome
- Pulm hypertension
- Sudden Death
- Stroke
- Vaso-occlusion - acute multi-organ failure.
- Renal Failure
- Peri-operative
- Iron overload
- Infections

Platt, NEJM, 94
Hematopoietic Stem Cell Transplant (HCT) for $\beta$-Thalassemia
1. Optimal medical therapy is the key for a successful HCT
2. High risk patients had high TRM with standard Bu-Cy
3. Reducing conditioning regimen intensity results in graft rejection
4. Adult patients had a high TRM

Courtesy: Angelucci, et al, ASH 2010
Rationale For Gene Transfer into Autologous HCT

- HCT for SCA/thalassemia have resulted in cures

- HCT are limited by:
  - Availability of matched donors – available only in 10-15%
  - Potentially serious immunological side effects (graft versus host disease and graft rejection)
  - Myeloablative chemotherapy conditioning

- Insertion of a normal $\beta$- or $\gamma$- globin gene into autologous HSC
  - A single gene defect; potential of a one-time permanent correction
  - Not be limited by the availability of matched donors
  - May not need myeloablative chemotherapy conditioning
An HIV-1 Based Self-inactivating Lentivirus Vector

GbG

αγ Globin exons

CMV - GAG - RRE - cPPT - β UTR - 3’ enh - * - β promoter - LCR - HS2 - HS3 - HS4 - ΔU3 - 3’ LTR
Myeloablative Transplant using Humanized Berkeley Sickle Mice
Correction of Organ Pathology and Improved Survival

Marrow
Kidney
Liver
Spleen

Mock
Gb^bG

Survival (%)
Weeks #

Gb^bG (n=8)
Mock (n=6)
Human β Thalassemia Major Bone Marrow CD34+

Gene Transfer
IL6/SCF/Flt3-L/Tpo

In Vitro Model of Human Erythropoiesis

Splenectomy (d–5) 4Gy

β2m NOD-SCID
Expansion of CD34+ cells in Erythroid Cultures

Fold expansion

Days in culture

- NBM
- NBM-K6I
- TBM
- TBM-BGI

p<0.001
p<0.002
HPLC analysis of $\beta$-Globin in Erythroid Cultures

Normal  
(100% $\beta$-like globins)

Thalassemia Major  
(7% of all $\beta$-like globins)

Thalassemia Major + BGI  
75% of all $\beta$-like globins  
2-2.5 vector copies/cell
Correction of Ineffective Erythropoiesis in Hu $\beta^0$ Thal

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Effective human erythropoiesis is observed in vivo in NOD-SCID β2Mnull mice 3-4mo post gene transfer.

Normal Bone Marrow CD34+ cells

Thal Bone Marrow CD34+ cells

Thal Bone Marrow CD34+ cells with BGI

Human Hemoglobin A
Gene Transfer of the γ-Globin Vector Corrects Beta Thalassemia

Thalassemia Mouse Model

Hb (gm/dL)

Mock          GbG
8.2           12.5

Gene Transfer Diagram:
GbG
5’LTR SD GAG RRE cPP1 SA

β UTR 3’enh

β promoter

LCR

HS2 HS3 HS4

Aγ Globin
Safety Features of the Lentivirus Vector

Self-inactivating design

Expression of $\gamma$-globin from this vector is restricted to the erythroid lineage

Erythroid transcriptional machinery is lost during terminal differentiation

Safety studies on primary murine progenitors show nearly 200 fold lower genotoxicity as compared to the conventional retroviral vectors
A Phase I/II Pilot Studies of Gene Transfer for Sickle Cell Disease and Beta Thalassemia
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<th>Condition</th>
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<td>Sadelain et al.</td>
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<td>Leboulch et al.</td>
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Conclusions

- Therapeutic efficacy of $\beta/\gamma$ globin gene transfer in HSC has been adequately demonstrated, resulting in the correction of mouse/human models of thalassemia & SCA

- The design of lineage- and differentiation stage-restricted expression via lentivirus vectors represents a major step in reducing the risk of trans-activating cellular oncogenes

- Clinical trials in hemoglobinopathies have begun in France, and are at the horizon at three centers in the US, making HSC gene transfer a potential therapeutic reality