Stem Cell Gene Therapy for Hemoglobinopathies

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The Case for the Hemoglobinopathies

- Sickle cell disease first described 100 years ago in a student from the West Indies (Herrick, Arch Intern Med. 1910; 5, 517)
  - First disease for which molecular defect identified (Pauling et al, Science, 1945; 110, 543)
  - Abnormal Hb polymerization upon deoxygenation
    - Severe anemia, frequent pain, end organ damage, shortened lifespan
- Thalassemias result from reduced or absent production of one of the globins
  - Severe anemia, frequent transfusions, iron overload, end organ damage, shortened lifespan without adequate chelation
- Ideal for bone marrow stem cell based approach

Herrick, Arch Intern Med. 1910; 5, 517
Hematopoietic stem cells as vehicles for therapeutic gene delivery

**Allogeneic stem cell transplantation**
- Transplantation using stem cells from a normal donor
  - Bone marrow stem cells from a matched brother or sister carrying the normal gene

**Autologous stem cell gene transfer**
- Transplantation using the patient’s own modified stem cells
  - Bone marrow stem cells exposed to viral vectors carrying a normal or therapeutic gene

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**Hematopoietic stem cells as vehicles for therapeutic gene delivery**

**Autologous stem cell gene transfer**

- **Murine**
  - High gene transfer rates easily achieved in vivo
- **Early human clinical**
  - Equally high gene transfer rates estimated by in vitro assays
    - In vivo levels of <1/100,000 cells
    - Too low to expect clinical benefit
- **Predictive human HSC assays needed**
  - Nonhuman primate competitive repopulation model developed
Rhesus competitive repopulation model

Steady state bone marrow comparable to G-CSF or G-CSF/SCF mobilized peripheral blood as stem cell source
(Stem Cells, 2004)

Neo not toxic to differentiation
(Human Gene Therapy, 1999)

Immune reaction not limiting
(Human Gene Therapy, 2001)

Optimal cytokine support
(Blood, 1998)

Clinically feasible methods
(Molecular Therapy, 2000)

100 cGy TBI sufficient in mice
(Human Gene Therapy, 2001)

Low level engraftment in rhesus
(Molecular Therapy, 2001)

Low-dose busulfan promising
(Experimental Hematology, 2006)

Clinical success feasible in simple disorders?
Rhesus competitive repopulation model

Steady state bone marrow comparable to G-CSF or G-CSF/SCF mobilized peripheral blood as stem cell source (Stem Cells, 2004)

Neo not toxic to differentiation (Human Gene Therapy, 1999)

Immune reaction not limiting (Human Gene Therapy, 2001)

Retroviral globin vectors including LCR elements unstable and prone to rearrangement

Optimal cytokine support (Blood, 1998)

Clinically feasible methods (Molecular Therapy, 2000)

True stem cell transduction (Blood, 2000)

100 cGy TBI sufficient in mice (Human Gene Therapy, 2001)

Low level engraftment in rhesus (Molecular Therapy, 2001)

Low-dose busulfan promising alternative

letters to nature

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

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NATURE | VOL 406 | 6 JULY 2000 | www.nature.com
Preclinical testing needed to improve the odds of successful clinical application
Nonhuman primate model for therapeutic β-globin gene transfer

- Modified vector developed to facilitate analysis and improve transduction rate in nonhuman primates
- Vector produced at preclinical scale
  Both SIV and HIV (with alternate cyclophilin binding domain) backbone compared
- Developed human β-globin specific detection assays
- Optimized lentiviral transduction procedures
- Initiated in vivo non-human primate studies

In vivo expression of human β-globin at day 30 after transplantation

Confirmed by RNAsc protection assay
32% and 13% of BM and PB cells positive, respectively, by genomic Southern blotting
Clonogenic bone marrow progenitors positive for vector at 54%
In vivo expression of human β-globin at extended follow up in both animals


In vivo persistence of genetically modified cells limited by poor HSC transduction

In vivo persistence of genetically modified rhesus cells limited by species specific block to HSC transduction
Production of chimeric vectors to overcome restriction from TRIM5-alpha and APOBEC3G

**HIV1-Gag/Pol + SIV-CA plasmid**

![Diagram of HIV1-Gag/Pol + SIV-CA plasmid]

**HIV1- Rev/Tat + SIV-Vif plasmid**

![Diagram of HIV1- Rev/Tat + SIV-Vif plasmid]

Dose escalation of chimeric vectors on human and rhesus cell lines

![Graphs showing dose escalation of chimeric vectors on human and rhesus cell lines]

The HIV1 vector with sCA (γHIV) allowed efficient transduction of human and rhesus blood cell lines. Addition of sVif reduced transduction efficiency for the human blood cell line.
Competitive repopulation assay to compare \(\chi\text{HIV}\) with HIV1 vectors

Transduction (MOI=50) Single 24 hr

Chi-HIV-GFP vector mixture

HIV1-YFP vector Transplantation

G-CSF/SCF mobilized PBSCH

Rhesus CD34+ cells

Rhesus CD34+ cells

G-CSF/SCF mobilized PBSCH

Rhesus macaques

Rhesus macaques

Total Body Irradiation (2x5Gy)

Total Body Irradiation (2x5Gy)

The \(\chi\text{HIV}\) vector achieves superior expression rates in vivo

\[\text{Granulocytes} \quad \text{Lymphocytes} \quad \text{Red Blood Cells} \quad \text{Platelets}\]


animal 1
The $\chi$HIV vector achieves multilineage expression in vivo

In vivo GFP among red blood cells:
Preclinical testing of potentially therapeutic vectors feasible
Tissue specificity decreases viral titers

- MSCV-LTR U3 promoter
- Erythroid specific globin expression cassette
- Globin expression cassette with cHS4 insulator

LETTERS

Transfusion independence and HMG2 activation after gene therapy of human β-thalassaemia

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In vivo expression of human β-globin results in transfusion independence at 1 year

- Hb rises to 10 g/dl
  - Permitting phlebotomy
- HPLC demonstrates vector derived globin
  - Concomitant rise in HbF noted
- Vector derived Hb rises over time
  - Over 3 g/dl contribution

Dominance of clone bearing integration in HMGA2 over time

- Integration site analysis performed
  - Rise in contribution by clone with integration at HMGA2 seen over time
- Percentage of modified blood cells derived from HMGA2 increases over time
Clonal dominance of clone bearing integration in HMGA2 over time

- Expression of HMGA2
  - Increased by 10,000 fold
- Expression plateaued at 15 mo
- Predominant mRNA species sequenced
  - Truncated by alternative splicing with the cHS4

Remaining Barriers:

- The difficulty in achieving high gene transfer rates and expression levels while minimizing the risk of insertional mutagenesis
  - Need for tissue specific globin lentiviral vectors
- Poor predictive value of rodent models for human HSC behavior and need for a suitable large animal disease model
- Need for clinical phenotype which reliably predicts poor short term outcome and a patient subpopulation appropriate for consideration on experimental therapeutic trials
- Lack of infrastructure required to perform clinical gene transfer trials at most medical centers
- Difficulty in securing funding for incremental work required to move from murine to human studies.
“Incremental”

- Definition (from Merriam-Webster): of, relating to, being, or occurring in especially small increments

“How many more years, how many more vectors, how much more money will we need to see results?”

“Consider alternative approaches…”

Innovative is the enemy of incremental, and can be at odds with translation.

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