Gene Therapy for Sickle Cell Disease: A Safety/Efficacy Trial

Elizabeth Hexner

A. Introduction

Sickle cell disease (SCD) is an autosomal recessive disease of red blood cells (RBCs). A single amino acid substitution in the ß chain of hemoglobin results in abnormal aggregation of the molecules inside RBCs. The cells become less deformable and are distorted into many shapes including the so-called "sickle cells", subsequently becoming trapped inside blood vessels ("vaso-occlusion"). Clinically a patient with SCD can experience severe debilitating pain, is at increased risk of infection, and despite progress in supportive care lives only into his or her fifth or sixth decade. Some of the most devastating consequences of the disease are stroke and acute chest syndrome, which is the most frequently reported cause of death in patients with SCD.

a. Rationale

120,000 infants are born each year with SCD, and one of every 600 African Americans has the disease. Currently, treatment is largely directed at the relief of symptoms and the prevention of complications of the disease. Allogeneic bone marrow transplant (BMT) has thus far been the only attempt to correct the genetic defect and thereby cure the disease. BMT is an expensive and high-risk procedure that requires a matched donor. Correcting the single gene mutation by gene therapy is promising, potentially curative approach if it can be done safely and effectively.

b. Review of the literature

Progress in gene therapy of the hemoglobinopathies has been slow. Broadly speaking, this has been due to three technical hurdles:

- the difficulties associated with identifying a pure population of stem cells to serve as the proper substrate for gene manipulation. In biological terms, a hematopoietic stem cell maintains an ability to differentiate and to repopulate the host bone marrow, as well as to renew itself. From a technical point of view, stem cells are rare and poorly defined by current methods (they are a subgroup of CD34 positive, CD38 negative marrow cells).
- the requirement of cell division for permanent integration of the transgene into the host genome, leading to the potential differentiation of the stem cell and a subsequent loss of its future ability to self-renew.
- the low levels of expression of the transgene, due to silencing, once integration has taken place.

Considerable progress has been made on all three fronts (reviewed in 1, 2), and within the last few years there have been some encouraging results from stem cell gene therapy trials in humans. One was a report of immune reconstitution of two infants with severe combined immunodeficiency disease (SCID) by transfer of a gene into their stem Cell. [3] Two other studies described the transfer of the multidrug resistance 1 (MDR1) gene into the stem cells of cancer patients undergoing autologous BMT.[4,5] Their purpose was to render the subjects' stem cells more resistant to marrow-toxic chemotherapy. In both of these Phase I trials the vector used was a modified retrovirus (Harvey murine sarcoma virus) containing the MDR1 cDNA. The procedure proved to be safe, and the more recent study using improved techniques for the ex-vivo transduction of stem cells had the highest long-term engraftment of retrovirally transduced stem cells to date: there was expression of MDR at one year in peripheral blood cells.

The pace of research in gene therapy for the hemoglobinopathies has been limited by the complexity of the ß globin gene. It is a large, highly regulated and tissue specific gene. Nonetheless, there are new

promising animal data. A recent report in Nature described the successful introduction of a functional human ß globin gene into the stem cells of mice with ß thalassemia.[6] The investigators modified the transgene so that the transcriptional silencing effect that has commonly limited the utility of ß globin gene therapy appeared to be attenuated. The modification incorporated larger segments of the locus control region (LCR), an important regulatory element in the globin locus, based on studies suggesting that this additional sequence is required to achieve physiologic levels of expression. A lentiviral vector was used: derived from HIV-1, it has the unique ability to infect both dividing and non-dividing cells.

In vitro, when compared to control vectors, there was increased efficiency of transduction as well as an increased level of ß globin expression per cell. Lethally irradiated mice transplanted with transduced marrow cells produced large amounts of human ß globin (up to 13% of the total hemoglobin). The same transplant cells in a mouse model for ß thalassemia intermedia were sufficient to ameliorate the anemia and change the RBC morphology. This offers the rationale for a similar approach in humans.

B. Hypotheses

1. ß globin gene therapy is safe and results in efficient and stable expression of the normal gene product.
2. A transfer of stem cells of patients with SCD modified by this technique can change the phenotype of their disease.

C. Methods

a. Subjects

For this Phase I type study, the object is to select ten subjects who have already had debilitating clinical events related to SCD. The inclusion/exclusion criteria would be identical to those used for the allogeneic BMT trial decribed above (see table 1) '[7] with 2 exceptions: subjects would be adults (over the age of 18), and, as an autologous system, an HLA-identical donor would not be required.

TABLE 1. CRiteria for Eligibility
for Transplantation in Children
with Sickle Cell Disease.

i. Criteria for inclusion

- Sickle cell disease (sickle cell anemia, sickle cell-hemoglobin C disease, or sickle cell-, ß -thalasswmia)
- Age less than 16 years
- HLA-identical related donor
- One or more of the followinK,
- Stroke or central nervous system event lasting longer *than* 24 hours
- Acute chest syndrome with recurrent hospitalizations or previous exchange transfusions
- Recurrent vaso-occlusive pain (≥ 2 episodes per year for several years) or recurrent priapism
- Impaired neuropsychological function and abnormal cerebral MW scan
- Stage I or II sickle lung disease
- Sickle nephropathy (moderate or severe protainuria or a glomerular filtration rate 30 to 50% of the predicted normal value)
- Bilateral proliferative retinopathy and major visual impairmant in at least one eye
- Osteonacrosis of multiple joints
- Red -cell alloimmunization (≥ 2 antibodies) during longterm transfusion therapy

ii. Criteria for exclusion

- Age greater than 15 years
- Lack of availability of H-LA-identical donor*
- One or more of the following.
- Karnofsky or Lansky functional performance score < 70†
- Acute hepatitis or evidence of moderate or severe portal fibrosis or cirrhosis on biopsy
- Severe renal impairment (glomerular filtration rate, < 30% of the predicted normal value)
- Severe residual functional neurologic impairment (other than hemiplegia alone)
- Stage III or IV sickle lung disease
- Demonstrated lack of compliance with medical care
- Seropositivity for the e human immunodeficiancy virus

*Patients with RLA-matched related donors with the sickle-cell trait were not excluded,
†The Lansky performance score is a measure of functional status in children.

b. Mobilization and isolation of stem cells

Peripheral blood progenitor cells will be mobilized using standard protocols. CD34+ cells will be isolated by immunomagnetic selection.

c. Retroviral vector, transgene, and transduction protocol

Vector: The protocol will use the AM12M1 vector identical to that used in the MDR1 protocols (Genetix Pharmaceuticals, Cambridge, MA) [4,5]. It is derived from the Harvey murine sarcoma virus and packaged by the AM12 amphotrophic packaging cell line. This protocol has been approved by the FDA and the NIH Recombinant DNA Advisory Committee. The vector will be produced at the National Gene Vector Laboratory (Indianapolis, Indiana).

Transgene: The transgene, TNS9, will incorporate the longer segments of the LCR of ß globin[8].

Transduction protocol: The protocol will reflect recent advances in the efficiency of retrovirus-mediated gene transfer. This includes an optimized cytokine combination for the ex-vivo maintenance of the CD34+ stem cells. Culture plates are coated with fibronectin fragments optimized to co-localize retroviral particles to target cells.'," Specifically, cells are maintained in media (IMEM, BioWhittaker) with 10% fetal calf serum containing 100 ng/ml of granulocyte colony stimulating factor, megakaryocyte growth and development factor and stem cell factor (G-CSF, MGDF, SCF) for 48 hours. They are plated onto dishes treated with the fibronectin fragment CH-296 and exposed to vector for 4 hours, collected and incubated again in the cytokineenriched media overnight, then re-exposed to vector (4h) reincubated in media (8-16h) then frozen (as described in ref. 4).

d. Safety assessment

Before cells are transduced, vector supernatants to be used will be assayed for replication competent retrovirus. [4] Additionally, PCR for viral envelope will be done on all samples before transplantation and periodically post-transplantation.

e. Assessment of transduction efficiency

Transduced cells will be screened for percentage of transgenecontaining colonies by plating in methylcellulose-containing medium. Colonies (erythroid burst-forming units and mixed colonies) will be screened by PCR. Provided enough sample is available, a stem cell specific assay using the NOD/SCID mouse model will also be used so that correlative data can be gathered.

f. Minimal ablation and reinfusion

Patients will be prepared using a non-myeloablative radiation regimen (100-300 cGy)[11]. As in the allogeneic BMT protocol,[7] all patients will be transfused and/or exchanged to achieve a starting fraction of hemoglobin S of less than 30%. The transduced cells will be brought frozen to the bedside, thawed, and reinfused through a peripheral intravenous catheter.

D. Analysis

a. is expression achievable and safe?

Detecting the transgene: Bone marrow aspirates will be assayed by PCR of genOMiC DNA and RT-PCR of mRNA at 4 weeks, 12 weeks, 6 months and one year. The primers at the genomic level will detect differences in the sizes of intronic sequence (the transgene's is partially deleted) and at the level of MRNA, primers will discern the $ß^a$ mutation from $ß^A$

Safety: Because this study involves the manipulation of stem cells, overall hematopoietic function (bone marrow and peripheral) will be periodically assessed.

b. Can it change the disease phenotype?

This would be assayed using hemoglobin electrophoresis of peripheral RBCs to determine the relative amounts of hemoglobin S versus hemoglobin A. For SCD patients who have had clinical events (i.e. stroke or acute chest) that require exchange transfusions, a reduction of hemoglobin S to less than 30% is sufficient to improve outcomes.[12] It was recently shown that the same threshold is effective when used as primary prevention for patients who have not had their first neurologic event but who have an abnormal screening test. 13 It is therefore reasonable to assume that even a modest expression of hemoglobin A could change clinical outcomes for SCD patients.

Other secondary outcomes that would be assessed would be hematocrit, reticulocyte count, and an examination of RBC morphology.

E. References:

[1] Persons, D.A. and Nienhuis, A.W. Gene therapy for the hemoglobin Disorders: Past, present and future. PNAS 97, 5022-5024 (2000).
[2] Dick J.E. Gene therapy turns the corner. *Nature Medicine* 6, 624-626 (2000).
[3] Cavazzana-Calvo, M. et al. Gene Therapy of Severe Combined Immunodeficiency (SCID)-X1 Disease. *Science* 288, 669-671 (2000).
[4] Abonour, R et al. Efficient retrovirus-mediated transfer of the multidrug resistance 1 gene into autologous long-term repopulating hematopoietic stem cells. *Nature Medicine* 6, 652-658 (2000).
[5] Hesdorffer, C. et al. Phase I Trial of Retroviral-mediated Transfer of the Human MDR1 Gene as Marrow Chemoprotection in Patients undergoing High-Dose Chemotherapy and AutologouS SteM-Cell Transplantation. *Journal of Clinical oncology* 16, 165-172 (1998).
[6] May, C. et al. Therapeutic haemoglobin synthesis in P-thalassaemic mice expressing lentivirus-encoded human P-globin. *Nature* 406, 82-86 (2000).
[7] Walters, M.C. et al. Bone Marrow Transplantation for Sickle Cell Disease. New *England Journal of Medicine* 35, 369-376 (1996).
[8] May, C. et al. *Nature* 406, 82-86 (2000).
[9] Hanenberg, H. et al. Colocalization of retrovirus and target *cells* on specific fibronectin fragments increases genetic transduction of mammalian cells. *Nature Medicine* 2, 876-882 (1996).
[10] Kiem, H.P. et al. Improved gene transfer into baboon marrow repopulating cells using recombinant human fibronectin fragment CH-296 in combination with interleukin-6, stem cell factor, FLT-3 ligand, and megakaryocyte growth and development factor. *Blood* 92, 1878-1886 (1998).
[11] Amado R.G. and Chen I.M. Lentiviral vectors - the Promise of Gene Therapy Within Reach? *Science* 285, 674-676 (1999).
" Storb R. Hematopoietic stem cell transplantation in nonmalignant diseases. *Journal of Rheumatology - Supplement*. 48:30-5, May. 972-942 (1997).
[12] Adams R.J. et al. Prevention of first stroke by transfusions in children with sickle cell anemia and abnormal results on transcranial Doppler ultrasonography. New *England Journal* of Medicine 339, 5-11 (1998).
[13] Emre, U. et al. Effect of transfusion in acute chest syndrome of sickle cell disease. *Journal* of *Pediatrics 1*27,1 901-904 (1995).