Peripheral Blood Stem Cell Collections in the Age of Gene Therapy

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Conflict of Interest

• No Disclosures
Objectives

• Brief History of Gene Therapy
• Vector design
• How are Cells Targeted for Therapy
• Diseases Treated
• Current Trials
• PBSC Mobilization of Select Patient Populations
Gene Therapy

**Direct Delivery**

- Therapeutic gene
  - The therapeutic gene is packaged into a delivery vehicle such as a retrovirus
  - And injected into the patient

**Cell-based Delivery**

- Genetically modified ES cells (can block immune rejection from patient)
  - OR
  - ES cell HLA bank
  - OR
    - SCNT

- ES cells
  - Adult stem cells are isolated and propagated in the laboratory.
  - Adult stem cells
    - The genetically modified cells are reintroduced into the patient.
  - in vitro differentiated stem cell
    - The therapeutic gene is packaged into a delivery vehicle such as a retrovirus and introduced into the cells.
Gene Therapy: Definitions and History

- The FDA defines gene therapy as products “that mediate their effects by transcription and/or translation of transferred genetic material and/or by integrating into the host genome and that are administered as nucleic acids, viruses, or genetically engineered microorganisms”.

- The products may be used to modify cells *in vivo* or transferred to cells *ex vivo* prior to administration to the recipient.

- In 1990, Martin Cline became the first to attempt gene therapy using recombinant DNA. He applied to the UCLA Human Subjects committee for permission to treat patients with β-thalassemia, by altering their hematopoietic stem cells. One patient was treated in Italy and one in Israel. However, he did so without having received permission to perform those studies from the UCLA Institutional Review Board.

- Steven Rosenberg at the NIH aimed at using gene marking techniques to track the movements of tumor-infiltrating blood cells in cancer patients.
Gene Therapy: Definitions and History

• In September 1990 the FDA approved the first gene therapy trial with a therapeutic attempt in humans. Two children with adenosine deaminase deficiency (ADA-SCID, a genetic disease leading to severe immunodeficiency) were treated with cells taken from the blood of these patients and modified ex vivo to express the normal gene for making adenosine deaminase. One patient, Ashanti DeSilva, exhibited a temporary response, whereas the response in the second patient minimal.

• This initial success launched gene therapies which escalated in use with intense interest, until the death of a patient in 1999. This patient with deficiency of ornithine transcarbamylase, a liver enzyme required for the removal of excessive nitrogen from amino acids and proteins. An adenoviral gene delivery vector triggered a hyperactive immune system response and resulted in his death secondary to multiorgan failure.

• This led a considerable pause for the field, especially in the USA
Gene Therapy: Definitions and History

• Many trials were still in progress at the turn of the century and yielded very positive outcomes.

• In early 2000, X-linked severe combined immunodeficiency (SCID-X1) was successfully treated by gene therapy, albeit with subsequent complications associated with insertional mutagenesis.

• Chronic granulomatous disease was another disease that was being tackled using gene therapy in the early 90’s. There was a focus on X linked disorders given the replacement copy number.

• Today there are hundreds of gene therapy trials worldwide. The focus of this review is those involving mobilized peripheral blood stem cells.
Gene Therapy: Vector Technology

• The majority of gene therapy clinical trials to date have involved ‘gene addition’, rather than gene correction.
• Over 60% of trials have been for cancer, and also for a diverse range of conditions including Parkinson’s disease, viral infections, antibody gene expression and gene editing to remove pathogen receptors.
• Viruses of the family retroviridae integrate their genome into host cell DNA as part of their life cycle, and transmitted to daughter cells when the infected cell divides.
• MLV vectors is that they can only transduce dividing cells, however, vectors engineered from HIV can transduce most non-dividing cells. These lentiviral vectors are commonly used in trials.
• AAV (Adeno-Associated Virus) vector genome forms double-stranded DNA episomal circles or concatamers and generally do not integrate
Gene Therapy: 
Vector Technology

- The improved safety of lentiviral vectors compared with LTR-containing MLV (retroviral) vectors in bone marrow gene therapy is because the lentiviral vectors have been engineered to remove any enhancer activity from the LTR, reducing the risk of activation of expression of adjacent genes.
- Insertional mutagenesis is still one of the major concerns with integrating vectors. The main risks arise from vector integration into gene regulatory areas or into transcriptionally active areas, which can result in insertional mutagenesis and oncogenesis.
- When a lentiviral vector was used to treat a patient with β-thalassaemia a different mechanism of cellular gene up-regulation was seen, involving truncation of a cellular mRNA by provision of a splice acceptor in the lentiviral vector.
- Ongoing work in lentiviral vector design aims to eliminate splice donors and acceptors. Continuing improvement towards vectors (gene therapy is based on delivery)
Technique of oncoretroviral gene addition [for X-linked chronic granulomatous disease (CGD)].

Johann P. Hossle et al. Physiology 2002;17:87-92
Gene therapy using an adenovirus vector

Vector binds to cell membrane

Modified DNA injected into vector

Vector is packaged in vesicle

Vesicle breaks down releasing vector

Vector injects new gene into nucleus

Cell makes protein using new gene
Gene Therapy

Cell Manipulations in the Lab

• Gene therapy entails many prolonged steps in the lab, albeit not as involved as those for CAR-T cells. Much of the time is spent with product validation.

• Additionally, there are many steps that may increase the final number of cells in the dose.

• Many procedures are not in a closed system as one needs to feed cells, centrifuge, or transfer cells.

• There is variability in transduction and vector production.

• Selection of cell populations (e.g. CD34) requires beads or cell sorting.

• There are customized requirements for each type of collection that are exponentially increasing.
Gene Therapy Trials

• Primary Immune Deficiencies constituted the first and majority of trials that involved transduction of HSC’s.

• The first successful gene therapy treated two children without suitable bone marrow donors, who did not have funding for recombinant enzyme therapy.

• In General:
  – HSC’s from the patients were isolated using magnetic beads coated with an antibody to the surface marker CD34.
  – They were cultured for 3-4 days with cytokines and an MLV vector carrying an ADA cDNA, then reinfused.
  – Patient’s have conditioning therapies to “make room” for the infused cells. Previous attempts had largely failed because insufficient numbers of cells were engrafted.
Gene Therapy- PID

- X-linked SCID, accounting for 40%–50% of SCID cases reported world-wide, is caused by mutations in the gamma subunit of the IL2 receptor gene leading to defective expression of the common gamma chain (γc), a subunit shared by a host of cytokine receptors including interleukin (IL)-2, 4, 7, 9, 15 and 21 receptor complexes.
- These cytokines play a vital role in lymphocyte development and function.
- SCID-X1 patients present with profound immunological defects caused by low numbers or complete absence of T, B and NK cells.
- Early allogeneic transplantation from HLA-identical donor has a high success rate, however many patients do not have suitable donors or may be too ill for transplantation.
Gene Therapy- PID

- While the initial trials used a retroviral vector, current trials are using a SIN-LV construct
- SIN-LV contain codon-optimised cDNA under the control of the elongation factor 1/ short (EFS) promoter. This vector entered clinical trials in 2012 in London and Los Angeles.
- The treatment protocol involves the harvest of autologous CD34+ cells from bone marrow (BM) or of mobilized peripheral blood stem cells (PBSCs).
- Cell Dose is important, with reinfusion of higher numbers of gene-corrected progenitor
- Potentially, harvest of mobilized PBSCs through leukapheresis rather than BM harvest allows the collection of larger numbers of CD34+ cells.
- Mobilized PBSC are better targets for viral transduction
- Reduced-intensity conditioning improves engraftment of gene-modified HSCs
Gene Therapy- WAS

• Wiskott–Aldrich syndrome (WAS) is a rare X-linked recessive disease characterized by eczema, thrombocytopenia and immune deficiency. It is also sometimes called the eczema-thrombocytopenia-immunodeficiency syndrome.

• Gene therapy is an attractive approach as there is an expected selective advantage for “rescued” cells.

• Restored gene expression correlated with improvements in platelet counts and corresponding resolution of bleeding, eczema and auto-immunity.

• However, reports confirmed the occurrence of leukemia in 4/11 patients due to insertional transactivation of the proto-oncogenes MDS/EVI1 and LMO2 by strong enhancer elements present within the viral LTR (RV)
<table>
<thead>
<tr>
<th>Disease</th>
<th>Vector</th>
<th>Promoter</th>
<th>Conditioning</th>
<th>Stem Cell Source</th>
<th>Centre</th>
<th>Recruiting Since</th>
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<td>X-SCID</td>
<td>SIN-γRV</td>
<td>EFS</td>
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<td>BM</td>
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<td>PBSCs</td>
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<tr>
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<td>EFS</td>
<td></td>
<td>Busulfan 4 mg/kg</td>
<td>BM/ PBSCs</td>
<td>Los Angeles, Bethesda</td>
<td>2013</td>
<td>16</td>
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<td>SIN-LV</td>
<td>WAS</td>
<td>RIC busulfan/fluorouracil&lt;sup&gt;b&lt;/sup&gt;</td>
<td>BM/ PBSCs</td>
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<td>RIC busulfan/fluorouracil&lt;sup&gt;b&lt;/sup&gt;</td>
<td>BM/ PBSCs</td>
<td>Boston, London, Paris</td>
<td>2011</td>
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<td>PBSCs</td>
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<sup>a</sup>Busulfan 6 mg/kg and fludarabine<sup>b</sup>.

Booth et al Trends in Molecular Medicine 2015
Gene Therapy
PBSC transduction
Gene Therapy
PBSC mobilization

• Gene therapy requires a relatively large number of CD34 cells
• Many factors influence HSC mobilization and methods to maximize collection of progenitor cell aphereresis have been studied extensively in transplant.
• Variables shown to significantly affect mobilization in healthy allogeneic HPC aphereresis donors include:
  – donor age, sex, race, and total G-CSF dose administered.
• Red blood cell microcytosis, including iron deficiency is associated with impaired cell yields independent of preapheresis CD34+ cell counts (i.e. not associated with poor mobilization).
• Exposure to myelotoxic drugs (chemotherapy) is associated with poor collections
Gene Therapy
PBSC mobilization

• PID patients suffer from chronic infections or inflammation can result in persistent leukocytosis associated with increased endogenous G-CSF levels. This may result in tachyphylaxis and poor G-CSF responses.

• Collection yields may be improved if patients undergo mobilization when their active infections are controlled, however there are no biomarkers to assess for poor mobilizers.

• Studies correlating inflammatory cytokines or markers (e.g. CRP) have pointed to the utility of a low platelet count or a high ESR in identifying poor mobilizers.
Gene Therapy- CGD

- Chronic granulomatous disorder is a diverse group of hereditary diseases in which neutrophils have difficulty forming the reactive oxygen species (e.g. superoxide radicals due to defective phagocyte NADPH oxidase). This prevents immune cells from killing certain ingested pathogens.
- Gene therapy has been attempted for 20 years and a trial is now open.
- CGD remains one of the most difficult targets for gene therapy for the following reasons:
  - Expression of the wild-type gene does not provide any survival advantage to the transduced stem and progenitor cells. Myeloablative conditioning may be necessary.
  - Cell Dose is important!!
  - Circulating neutrophils have a life-span of few days which means a large number of long-term repopulating HSCs need to be corrected (there is no accumulation of corrected cells).
  - Chronic infections lead to an inflammatory environment in the bone marrow and could exert a negative effect on the successful engraftment of the transduced HSC
Gene Therapy
CGD- Mobilization

CGD and SCID populations are characterized by significantly less robust CD34+ HPC mobilization than healthy controls.
Gene Therapy- SS

- Allo-transplant is curative for both SCD but has a high rate of fatal complications such as graft rejection and graft failure. Furthermore, the frequency of identifying a related or matched unrelated donor is low.

- Gene Therapy is an attractive therapy for these patients however one needs to obtain cells to transduce. GCSF administration has been associated with complications in SCD.

- In patients with SCD, complications may arise from the propensity for vaso-occlusion and the existence of a chronic inflammation associated with leukocytosis (complicating G-CSF administration), increased serum levels of C-reactive protein and cell activation, and hypercoagulability.
The milestones of ex vivo gene therapy research and development for hemoglobin disorders. LG001, HGB204, HGB205, and HGB206 clinical studies are conducted with beta globin lentiviral vectors.
Gene Therapy- SS

• G-CSF is relatively contraindicated in patients with sickle cell disease, there are reports of mobilizing sickle cell patients after “prophylactic” exchange transfusion followed by G-CSF.

• In one report, two patients were mobilized in order to store their marrow as a backup prior to undergoing allogeneic stem cell transplantation. The patients were heavily transfused and their percentage of HbS was <40%.

• Both did experience some pain during the collection procedure but they were able to complete it.

• In another report, 3 of 5 patients had pain during collection but without correlation to percentage of HbS. Two patients with mild VOC and pain had low HbS levels of 14% and 25%, whereas other patients with higher HbS levels had no complications.
Gene Therapy- SS

• There is no known cutoff for percentage of HbS that can assure a safe mobilization with G-CSF.

• Potentially, areas for improvement can include
  – reducing a patient’s HbS to less than 10-20% followed by mobilization and collection
  – using hydroxyurea to time the egress of progenitors
  – The use of plerixafor for collection

• Conventional bone marrow harvest requires general anesthesia, and is also associated with complications, including large volumes of BM to obtain sufficient cells for transduction.

• Two gene therapy trials are open and plerixafor is being used for mobilization.
Hydroxurea withdrawal in SCD patients results in an increase in circulating progenitors
Gene Therapy
β-Thalassemia

- Trials of gene therapy in β-thalassemia have demonstrated transfusion independence in a single adult subject 3 years following transplantation with HSPC modified by a lentiviral vector expressing adult β-globin under the control of the endogenous β-locus control region cloned into the vector.
- A single vector for SCD and Thal of a β-globin gene with an altered sequence to promote dimerization
- The integration of the transgene within the third intron of the high mobility group AT-hook 2 (HMGA2) gene led to increased expression of the transgenic β-globin sequences and accounted for most of the therapeutic β-globin expression
- Cells with this integration pattern represented 2-8% of the blood cells at 28 months, yet were sufficient to prevent transfusion
Gene Therapy
β-Thalassemia-mobilization

• Challenges in this disease for mobilization:
  – Adult thalassemics present with advanced organ damage due to accumulated iron and have a decreased BM stem cell reservoir, due to the BM suppression. In addition, a great proportion of adult patients have undergone splenectomy, which contributes to leukocytosis and alters mobilization.

• One-month HU-pretreatment prevented hyperleukocytosis and allowed successful CD34+ cell collections when an optimal washout period was maintained

• Plerixafor resulted in rapid and effective mobilization in both splenectomized and non-splenectomized patients and was well-tolerated.

• For gene therapy of thalassemia, G-CSF or Plerixafor has proven useful and can be used as mobilization agents
Gene Therapy
β-Thalassemia

• Trial of plerixafor in β thalassemia patients
• The primary end point of the study was the collection of at least $6 \times 10^6$ CD34 cells/kg in two or fewer apheresis collection (column limitations)
• This end point was achieved by 65% of the evaluable subjects who received plerixafor alone (11/17) in 1.83 days required time. Of the remaining 6 patients (35%) who failed to reach the study’s target cell dose, only 2/17 (12%) were identified as truly poor mobilizers yielding $2.5 \times 10^6$ CD34+ cells/kg by two aphereses (P13, P17).
• Myeloablation before the infusion of gene-corrected HSCs facilitates the establishment of near complete vector-carrying cell chimerism; however, a non-myeloablative conditioning should be considered to reduce peritransplant risks of bone marrow aplasia or in the case of graft failure.
• This approach has been successfully applied in the case of inherited immunodeficiencies, but it presents a challenge when the genetically corrected stem cells lack a selective advantage such as in thalassemia or sickle cell disease.
<table>
<thead>
<tr>
<th></th>
<th>Plerixafor only—all patients</th>
<th>Preceding single-agent mobilization (plerixafor n = 3, G-CSF n = 1) of remobilized patients</th>
<th>Plerixafor + G-CSF—remobilized patients</th>
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<tbody>
<tr>
<td>Patient number</td>
<td>17 (10 SPL, 7 non-SPL)</td>
<td>4 (3 SPL, 1 non-SPL)</td>
<td>4 (3 SPL, 1 non-SPL)</td>
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<tr>
<td>Blood CD34⁺ cells during apheresis/µl</td>
<td>53 ± 37.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.4 ± 12.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>115.5 ± 13.3&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Splenectomized 65.4 ± 44&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>21.6 ± 14.4&lt;sup&gt;e&lt;/sup&gt;</td>
<td>118.0 ± 15.1&lt;sup&gt;d,e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Nonsplenectomized 37.2 ± 19.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>17</td>
<td></td>
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<tr>
<td>Total CD34⁺ cell yield, ×10⁶/kg</td>
<td>6.3 ± 2.2</td>
<td>2.5 ± 1.4&lt;sup&gt;f&lt;/sup&gt;</td>
<td>8.9 ± 2.9&lt;sup&gt;f&lt;/sup&gt;</td>
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<td></td>
<td>Splenectomized 6.7 ± 2.4</td>
<td>2.5 ± 1.5&lt;sup&gt;g&lt;/sup&gt;</td>
<td>7.6 ± 1.6&lt;sup&gt;g&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Nonsplenectomized 5.6 ± 1.8</td>
<td>2.5</td>
<td>12.7</td>
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<tr>
<td>Days of aphereses</td>
<td>1.88 ± 0.33&lt;sup&gt;h&lt;/sup&gt;</td>
<td>2 ± 0</td>
<td>1.00 ± 0.0&lt;sup&gt;h&lt;/sup&gt;</td>
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<tr>
<td>CD34⁺ cell yield per apheresis, ×10⁶/kg</td>
<td>3.6 ± 2.0&lt;sup&gt;i&lt;/sup&gt;</td>
<td>1.2 ± 0.7&lt;sup&gt;j&lt;/sup&gt;</td>
<td>8.9 ± 2.9&lt;sup&gt;ij&lt;/sup&gt;</td>
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<tr>
<td>Total CFCs, ×10⁶/kg</td>
<td>379 ± 189&lt;sup&gt;k&lt;/sup&gt;</td>
<td>n/a</td>
<td>737 ± 134&lt;sup&gt;k&lt;/sup&gt;</td>
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<tr>
<td>CFU-GM</td>
<td>257 ± 112&lt;sup&gt;l&lt;/sup&gt;</td>
<td>n/a</td>
<td>496 ± 66&lt;sup&gt;l&lt;/sup&gt;</td>
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<td>Failure to reach 6×10⁶ CD34⁺ cells/kg</td>
<td>6/17 (35%)</td>
<td>4/4 (100%)</td>
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<tr>
<td>Failure to yield &gt;2.5×10⁶ CD34⁺ cells/kg</td>
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<td>3/4 (75%)</td>
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<td>Max WBCs, ×10³/µl</td>
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<td>41.2 ± 28.1</td>
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<td>Splenectomized 38.9 ± 13&lt;sup&gt;n&lt;/sup&gt;</td>
<td>Plerixafor: 28 ± 17&lt;sup&gt;h&lt;/sup&gt;/G-CSF: 88</td>
<td>82.2 ± 19.7&lt;sup&gt;mn&lt;/sup&gt;</td>
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<td>Nonsplenectomized 20.6 ± 2.9</td>
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<td>Platelet counts, ×10³/µl</td>
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<td>Baseline</td>
<td>458 ± 191&lt;sup&gt;o&lt;/sup&gt;</td>
<td>440 ± 139&lt;sup&gt;p&lt;/sup&gt;</td>
<td>455 ± 151&lt;sup&gt;q&lt;/sup&gt;</td>
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<td>After leukapheresis</td>
<td>224 ± 109&lt;sup&gt;o&lt;/sup&gt;</td>
<td>176 ± 51.2&lt;sup&gt;p&lt;/sup&gt;</td>
<td>169 ± 23.6&lt;sup&gt;q&lt;/sup&gt;</td>
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<td>Spleen volume, cm³</td>
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<tr>
<td>After mobilization</td>
<td>703 ± 302.4</td>
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<td>% Splenic enlargement over baseline(max)</td>
<td>10.74 ± 13.9</td>
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SPL, splenectomized; non-SPL, non-splenectomized. BFU-E, erythroid burst-forming unit; CFU-GM, colony-forming unit-granulocyte, macrophage.
Plerixafor alone resulted in an earlier, shorter lived response that when in combination with G-CSF
Thalassemia patients mobilize slower than normal donors.
Gene Therapy - Cell Dose

• For X-SCID gene therapy- vector copy number correlated functional immune reconstitution and clinically relevant efficacy was achieved only with high VCN
• The requirement for a high VCN does raise concern with the increased risk of insertional mutagenesis.
• When fewer cells are transduced, there is an added risk of oligoclonal expansion, especially with lymphocytes.
• HSC transduction at lower VCN leads to partial T and B cell recovery with and associated with the production of autoreactive T cells similar to those seen in patients with hypomorphomic RAG-SCID
• Future push to treat patients with even higher cell numbers
Gene Therapy - Future work

• Genomic safe harbours (GSHs) are regions of the human genome where newly integrated transgenes can be expressed stably without adverse effects on the cell - prevent integration mutagenesis.

• Most recently, a bacterial clustered regularly interspaced short palindromic repeats (CRISPR) RNA together with a CRISPR-associated (Cas) protein has been used to target and mutate a mammalian cell locus.

• Has gene therapy been unseated for gene correction?
Potential Factors Leading to Improved Safety and Efficacy of Haematopoietic Stem Cell (HSC) Gene Therapy. Developments in three main areas have the potential to improve both the safety and the efficacy of HSC gene therapy. Greater accessibility to tr...
Gene Therapy - Mobilization Summary

- GCS-F mobilization of CD34+ cells from the marrow into the blood in gene therapy candidates is the best method to generate large number of cells in the periphery for collection.

- Underlying disease complicated the timing and the number of cells that are mobilized.

- Factors such as infection or inflammation alter mobilization; treatment of underlying disease and timing may be able to ameliorate this response.

- Attention to reversible RBC microcytosis (i.e. iron deficiency anemia).

- Measurement of PB CD34 on D4 and addition of plerixafor if not >40 for collections of at least 5 e6 cells/kg.

- Timing of G-CSF dose with attention to the relatively short half of the drug.

- Vascular access - low threshold for a device rather than peripheral access.

- **Communication** with the team - early and often.
Reservations Concerning Gene Therapy

The attention recently given the prospects of gene therapy requires a realistic appraisal of the potential as well as a sober consideration of the liabilities of this therapeutic approach.

There is no doubt that the development of techniques for transfer of genes and chromosomes in laboratory studies of mammalian cells will provide a powerful research tool toward the achievement of both normal and abnormal cellular processes and will ultimately provide a rationale for the treatment of many human diseases. Gene therapy, however, involves direct application of this technology to individuals suffering from genetic disease. Possibilities under discussion include: introduction of DNA of or from chromosomes directly by somatic cell fusion; transfer of genetic material from a host to another by virus-like particles containing DNA of the host cell, infection with active or inactive virus containing genes that can determine some particular biochemical function; or infection with a viral nucleic acid to which some cellular gene has been coupled.

Although the number of newborns suffering from disorders that can be described as genetic is very large, only a small fraction of these disorders would even in principle be amenable to intervention by any of these techniques. Neither genetically dominant disorders, nor multifactorial traits, nor disorders resulting from extra chromosomes can be alleviated. The major remaining class is that of the recessive "inborn errors of metabolism." These occur with a collective frequency of about 1 per 1000 individuals and include, conservatively, between 100 and 1000 different disorders. Gene therapy would be likely to involve the isolation of somatic cells from a diseased individual, the alteration of their genetic endowment in vitro, and their replacement in the individual.

For example, it seems unlikely that sickle-cell anemia would be relieved if a few percent of the blood-forming cells were replaced by cells capable of producing normal hemoglobin, or that the consequences of phenylketonuria would be relieved by the presence of a few somatic cells capable of converting phenylalanine to tyrosine. On the whole, it does not seem probable that more than a small fraction of the inborn errors could be helped by these techniques, and, when new somatic cells in the understanding of the immune response, these disorders will probably be treated more easily and effectively by tissue transplantation or some sort of enzyme therapy.

Furthermore, there are certainly hazards, both known and unknown, that accompany the presently conceived strategies. Many of the procedures are likely to be mutagenic, and we can only guess how many dominant effects, visible only in the whole individual, might appear? Most of the viruses under consideration as vectors are tumor-producing. Even the fractionated virus-like particles containing cell DNA are certain to include some particles containing viral DNA. Damaging alterations of regulatory processes and even uncontrolled tumor-like growth could easily be the consequences of introducing additional chromosomes or a host of viral genes.

The premises offered by the proponents of gene therapy largely ignore its limitations and hazards. To mislead the public in this regard risks another period of disappointment and reaction. We are still primarily in a descriptive phase in our understanding of human genetics, with little, if any, idea of how to intervene safely at any level. Let us not do to ourselves what we have done to our environment. Let us seek public support for research toward a better understanding of normal and abnormal human biology, rather than promise quick glamorous cures.—MAURICE S. FOX, Massachusetts Institute of Technology, and JOHN W. LITTLEFIELD, Harvard Medical School.