Gene Therapy: The Promise of a Permanent Cure

Christopher D. Porada, Christopher Stem, Graça Almeida-Porada

Gene therapy offers the possibility of a permanent cure for any of the more than 10,000 human diseases caused by a defect in a single gene. Among these diseases, the hemophiliases represent an ideal target, and studies in both animals and humans have provided evidence that a permanent cure for hemophilia is within reach.

Gene therapy, which involves the transfer of a functional exogenous gene into the appropriate somatic cells of an organism, is a treatment that offers a precise means of treating a number of inborn genetic diseases. Candidate diseases for treatment with gene therapy include the hemophiliases; the hemoglobinopathies, such as sickle-cell disease and ß-thalassemia; lysosomal storage diseases and other diseases of metabolism, such as Gaucher disease, Lesch-Nyhan syndrome, and the mucopolysaccharidoses (including Hurler syndrome); diseases of immune function, such as adenosine deaminase deficiency; and cystic fibrosis.

Although one might assume that the majority of these diseases could be corrected by simply providing an exogenous source of the missing or defective protein, this is not always the case. Even when the required protein can be purified or produced in recombinant form in sufficient quantities to be therapeutically useful, there is still the challenge of providing the missing protein, or replacing the defective protein, in a therapeutic fashion, which may require the delivery of the complex and often fragile protein to the precise subcellular location in which it is normally expressed. In addition, many patients suffering from a given genetic disease have never produced the specific protein in question, so their immune system has never “seen” this protein. Thus, infusion of the purified or recombinant protein could be followed by an immune response in which the cells of the immune system identify as a foreign entity the very protein that could treat the patient’s disease; this immune system response can lead to loss of therapeutic benefit, despite continued protein infusions. Even in the absence of these immunologic hurdles, protein-based treatments can never cure the underlying disease. Rather, they require a lifetime of regularly spaced infusions to keep the disease process at bay. Even after years of treatment, the symptoms will return if the patient misses even a single dose of replacement protein, with potentially life-threatening consequences.

By providing a normal copy of the defective gene to the affected tissues, gene therapy would eliminate the problem of having to deliver the protein to the proper subcellular locale, since the protein would be synthesized within the cell, utilizing the cell’s own translational and posttranslational modification machinery. This would ensure that the protein arrives at the appropriate target site. In addition, although the gene defect is present within every cell of an affected individual, in most cases transcription of a given gene and synthesis of the resultant protein occurs in only selected cells within a limited number of organs. Therefore, only cells that express the product of the gene in question would be affected by the genetic abnormality. This greatly simplifies the task of delivering the defective gene to the patient and achieving therapeutic benefit, since the gene would only need to be delivered to a limited number of sites within the body. Furthermore, if the gene could be specifically targeted to the organs that are most affected by the disorder, the risk of side effects from ectopic expression of the therapeutic gene would be avoided. Gene therapy, if targeted to the appropriate somatic cells, could thus promise permanent correction of the genetic defect following a single treatment. It is this promise that drives the myriad preclinical and clinical gene therapy studies for a wide range of diseases and disorders.

In most preclinical and all clinical gene therapy trials to date, the therapy has been performed on either children or adults, but it bears mention that many of the diseases being considered as candidates for gene therapy can be diagnosed early in gestation, making it feasible to treat the fetus in utero rather than waiting until after birth. Methods for accessing the human fetus are well established and clinically viable, and in utero stem cell–based therapies have been safely performed in the clinic for decades for a number of different diseases [1-3]. Performing gene therapy early in gestation would correct the defect prior to disease onset, allowing the birth of a normal, healthy baby who ideally would require no further treatments.

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In addition to the clinical advantages of such an approach, key differences between a fetus and an adult make the fetus a more suitable gene therapy recipient. For example, as a result of the active cycling of cells and the continuous expansion that occurs in all of the fetal organs throughout gestation, one can envision that initial transduction of even small numbers of target cells would lead to significant levels of gene-correction by birth. There are also immunologic advantages to performing gene therapy in utero, because exposure to foreign antigens during the period of early immunologic development can result in permanent tolerance if the presence of the antigen is maintained [4]. Over the past several years, we and others have demonstrated that it is possible to take advantage of the unique opportunities presented by the early gestational fetus to achieve significant levels of gene transfer to cells within several major organ systems following a single injection of vector [5-13], while simultaneously inducing immune tolerance to the vector-encoded transgene [14, 15]. Collectively, these findings provide compelling evidence that fetal gene therapy could represent a viable therapeutic option for diseases of multiple organs. Moreover, even if such therapy is not curative, the ability to induce lifelong tolerance would overcome the immune-related hurdles that currently hinder postnatal protein-based treatments. Despite its great promise, however, in utero gene therapy is still in the experimental stages, and carefully designed risk-to-benefit studies will need to be done in appropriate preclinical animal models before a therapy of this type could move into the clinical arena.

The Need for Better Treatments for the Hemophilias

Hemophilia A is caused by a defect in or deficiency of coagulation factor VIII. It is the most common inheritable coagulation deficiency, affecting about 1 in 5,000 males. Hemophilia B is far less common, only occurring in about 1 of every 30,000 male births; it is caused by a deficiency of or defect in coagulation factor IX. Roughly 60% of individuals with hemophilia A or hemophilia B present with the severe form of the disease (meaning they have less than 1% of the normal amount of clotting factor in their blood) [16]; these individuals experience frequent spontaneous hemorrhaging, leading to chronic debilitating arthropathy, hematomas of subcutaneous connective tissue or muscle, and potentially life-threatening internal bleeding. Over time, the collective complications of recurrent hemorrhaging result in chronic pain, absences from school and work, and permanent disability [17]. Current state-of-the-art treatment consists of frequent prophylactic infusions of plasma-derived or recombinant factor VIII or factor IX to maintain hemostasis. Although this treatment has greatly increased life expectancy and quality of life for many patients with hemophilia, this therapeutic approach is still far from ideal, because lifelong infusions are needed and the treatment is extremely expensive ($150,000–$500,000 per year). Even setting these shortcomings aside, factor replacement therapy is not available for approximately 75% of individuals with hemophilia worldwide, placing these patients at great risk of severe, permanent disabilities and life-threatening bleeding [18]. Even for patients with hemophilia A who are fortunate enough to have access to factor VIII and the means to afford prophylactic infusions, there is no guarantee of a life free from treatment complications. Approximately 30% of patients with severe hemophilia A develop inhibitory antibodies to the infused factor VIII protein, which greatly reduces treatment efficacy, increases morbidity and mortality, decreases quality of life, and can ultimately lead to treatment failure [19]. Although inhibitors are far less common in patients with hemophilia B [20], their formation can trigger severe immune responses, which can include anaphylaxis, placing patients in grave danger. Thus, there is a significant need to develop novel hemophilia therapies offering longer-lasting benefit or a permanent cure [21]. Gene therapy offers the promise of being such a treatment.

Hemophilia as a Paradigmatic Genetic Disease for Correction by Gene Therapy

Many diseases are being considered as candidates for correction with gene therapy, but several aspects of the basic biology and pathophysiology of hemophilia A and hemophilia B make them ideal targets [21-24]. First, although the liver is thought to be the primary natural site of synthesis of factor VIII and factor IX, neither factor needs to be expressed in either a cell-specific or a tissue-specific fashion to restore hemostasis. As long as the protein is expressed in cells that have ready access to the circulation, the protein can be secreted into the bloodstream and exert its appropriate clotting activity. Moreover, expression of this factor in other tissues of the body exerts no observable deleterious effects. This is in marked contrast to many other genetic diseases, which require that expression of the missing protein be exquisitely controlled, often with respect not only to cell type but also to a specific subcellular locale, in order for the protein to function correctly and to avoid deleterious effects. A second feature of hemophilia A and hemophilia B that sets them apart from many other diseases is that only a small amount of the missing clotting factors is required to achieve a pronounced clinical improvement. Indeed, raising the level of factor VIII or factor IX to even 3%-5% of normal would convert severe hemophilia A or hemophilia B, respectively, to a moderate or mild phenotype. Such a change would be expected to reduce or eliminate episodes of spontaneous bleeding and to greatly improve quality of life. Thus a marked clinical improvement would be anticipated in patients with hemophilia, even with the low levels of transduction that are routinely obtained with many of the current viral-based gene delivery systems. This reasoning prompted the American Society of Gene & Cell Therapy (ASGCT) to include the hemophilias on their list of the 10 diseases that hold the most promise as targets for viable gene therapy.
products within the next 5–7 years (Table 1). This list was part of a “road map” the ASGCT provided to the director of the National Institutes of Health, Francis S. Collins.

To develop and test various gene therapy approaches for treating hemophilia A and hemophilia B, researchers have used several animal models, including dogs with congenital deficiency of factors VIII and IX [25], mouse models obtained by gene targeting and knockout technology [26, 27], and a line of sheep with a form of factor VIII deficiency that accurately mimics the human disease [28]. Marked therapeutic benefit has been obtained using a variety of vector systems in the murine model [29-33]. In dogs, phenotypic correction has been possible but has proved to be far more difficult than in mice [29, 30, 32-39]. In the sheep model, a single infusion of bone marrow stromal cells engineered to express high levels of factor VIII resulted in phenotypic correction and complete reversal of debilitating hemarthroses, but it also triggered the formation of inhibitors of factor VIII [40].

Despite these promising results in animal models, no clinical gene therapy trial has yet shown phenotypic or clinical improvement of hemophilia A in humans. Based on the disappointing results to date, there are currently no active clinical trials of gene therapy for hemophilia A, even though hemophilia A accounts for roughly 80% of all cases of hemophilia. Previous clinical gene therapy trials for hemophilia B were similarly disappointing with respect to clinical benefit [21, 41, 42]. However, a highly successful ongoing trial being conducted jointly by St. Jude Children’s Research Hospital and University College London has recently highlighted the tremendous potential of gene therapy for the treatment of human hemophilia B [43]. In this trial, a single dose of a factor IX–encoding adeno-associated virus–based vector has resulted in expression of therapeutic levels of factor IX that have been sustained for more than 2 years, to date, in 6 adults with severe hemophilia B. The levels of circulating factor IX achieved with this approach, although not high, have enabled 4 of these 6 subjects to completely discontinue routine factor IX prophylaxis. The other 2 patients have not achieved complete independence from factor IX infusions, but this gene therapy–based treatment has allowed them to significantly reduce the frequency with which they need to administer prophylactic infusions [44]. These results represent a leap forward in the treatment and management of hemophilia B and make this an exciting time for the field of gene therapy. We eagerly await news on whether these groups succeed with their plans to adapt this strategy to the treatment of hemophilia A [45]. Even if this adeno-associated virus–based treatment approach does not prove to be fully curative, its ability to mediate long-term expression of factor VIII or factor IX should lessen disease severity, reduce health care expenditures, and dramatically improve the quality of life of patients with hemophilia A or hemophilia B, respectively. NCMJ

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References
11. Porada CD, Park PJ, Tellez J, et al. Male germ-line cells are at risk

### Table 1: Disease Indications Identified by the American Society of Gene & Cell Therapy as Promising Targets for Gene Therapy

<table>
<thead>
<tr>
<th>Disease/Condition</th>
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<td>Leber congenital amaurosis</td>
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<td>Adenosine deaminase severe combined immunodeficiency</td>
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<td>Hemophilia</td>
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<td>X-linked severe combined immunodeficiency</td>
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<td>Parkinson disease</td>
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<td>Age-related macular degeneration</td>
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