ADVANCES IN HEMATOLOGY

Current Developments in the Management of Hematologic Disorders

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Principles of Gene Therapy for the Hematologist

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H&O What is the definition of gene therapy?

JS The most basic definition of gene therapy is the use of genes to prevent or treat disease. More specifically, it refers to the use of recombinant DNA or messenger RNA, delivered via a vector, to either provide a missing gene or repair a damaged gene.

H&O How is gene therapy carried out?

JS The 2 major strategies for gene therapy are gene transfer and gene editing. With gene transfer, functional genetic material provides compensation for a nonfunctional gene. With gene editing, genetic repair is used to correct the nonfunctional gene.

We have various ways to carry out these strategies. Both gene transfer and gene editing can be carried out either in vivo, in which genetic material is directly injected into the patient's body, or ex vivo, in which we remove the patient's own cells and alter them before returning to them to the patient (Figure). A patient's bone marrow stem cells are often used as the basis for ex vivo gene therapy.

Genes are delivered to the body using vectors, which can be integrating or nonintegrating as well as viral or nonviral. So that adds 4 additional possibilities: viral integrating vectors, nonviral integrating vectors, viral nonintegrating vectors, and nonviral nonintegrating vectors. Any of these possibilities can be performed using either in vivo or ex vivo strategies.

H&O When did clinical trials of gene therapy first begin in hematologic disorders?

JS Physicians first started to inject patients with plasma DNA in an effort to compensate for nonfunctional genes in the early 1970s, which led to the formation of the Recombinant DNA Advisory Committee in 1975. Clinical research on human gene therapy in hematologic disease began in 1990 with the administration of autologous peripheral blood T lymphocytes containing the normal adenosine deaminase deficiency (ADA) gene to a patient with severe combined immune deficiency (SCID) caused by ADA deficiency. The treatment was considered successful because it appeared to improve outcomes in early patients, although that may have been because the treated patients also received protein replacement.

H&O In what hematologic disorders have clinical trials of gene therapy been conducted?

JS Trials have been conducted since the 1990s across various hematologic disorders, including hemophilia A and B, thalassemia, porphyria, severe combined immunodeficiency, Wiskott-Aldrich syndrome, chronic granulomatous disease, aplastic anemia, and hemoglobinopathies.

H&O Where has gene therapy been shown to be effective?

JS Gene therapy has been shown to be effective in both

hemophilia A and B, but to date we consider it as leading to an improvement in symptoms rather than a cure. If a patient has severe hemophilia (<1% of the normal factor IX level), current gene therapy may increase the level of factor IX to greater than 30%. However, the level may not be in the normal range and/or it may drop over time. Most patients receiving factor IX gene therapy experience an improvement to more than 5% of the normal level, which puts them in the mild hemophilia range. This represents a tremendous improvement.

In a trial of severe combined immunodeficiency (SCID) that began in 1999 in France and expanded to the United Kingdom, ex vivo retroviral vector gene therapy led to a dramatic improvement in symptoms.[1-4] In this trial, published by Hacein-Bey-Abina and colleagues in 2002, researchers removed bone marrow cells from 21 patients with SCID, corrected the mutation, and reinfused the cells. All 21 patients had their immunodeficiency corrected, making this a highly successful procedure that significantly improved patient symptoms. Unfortunately, 5 of these patients developed leukemia, which led to death in 1 patient (4 patients were cured of their leukemia). The link to leukemia centered around the tendency of the vector to insert near the *LMO2* oncogene. Although preclinical research had suggested that this was safe, the clinical trial showed the opposite.

> We must accept that there may be risks we simply have not anticipated or identified: the unknown unknowns.

H&O What are the benefits of gene therapy vs other approaches to hematologic disorders?

JS For some disorders, the benefits are still more theoretical than proven, but a major advantage is the possibility of treating a disease for years or decades with a single infusion. The goal here is to allow patients to live as if they do not have the disease, which offers both psychological and physical benefits. In the hemophilia community, Krumb and Hermans have described this goal as living with a "hemophilia-free mind."[5] We are not there yet, because people who receive gene therapy for hemophilia may still have mild hemophilia and/or side effects after gene therapy. However, gene therapy brings us closer to that goal.

H&O Can you discuss more of the clinical trials using gene therapy in hematologic disorders?

JS Regarding hemophilia, multiple preclinical and clinical trials in the 1990s, 2000s, and 2010s used lentiviral, adenoviral, and adeno-associated viral (AAV) vectors for gene therapy. The first use of AAV in muscle occurred in 1999, involving a patient with severe factor IX deficiency. In a study published by Roth and colleagues in the *New England Journal of Medicine* in 2001, 6 patients with hemophilia A were given modified fibroblasts.[6] After transduction, little to no factor VIII expression was observed, but in 4 of the patients, factor VIII levels were measurable only in plasma. No patients developed long-term complications or inhibitors to factor VIII.

In a study supported by GenStar Therapeutics, an adenoviral vector was administered intravenously to express full-length factor VIII in a patient with severe hemophilia A, leading to transient expression of factor VIII. The patient developed liver toxicity, high inflammatory markers, disseminated intravascular disease, and other infusion-related adverse events, but fortunately the vector did not cause a fatal event.

In a 2000 publication by Kay and colleagues, expression of factor IX in a patient with severe hemophilia B was approximately 1.5% after AAV vector infusion.[7] That was followed by a 2006 trial by Manno and colleagues that also used an AAV for factor IX, in which a patient with severe hemophilia B developed 3% expression of factor IX and another patient developed 12% expression.[8] These early trials established safety and provided the incremental steps to further our understanding of gene therapy for hemophilia, but had limited efficacy.

In 2011, a dose-escalation trial was published in the *New England Journal of Medicine* in which 6 patients with severe hemophilia B received a single dose of an AAV expressing a factor IX transgene.[9] All patients expressed factor IX after treatment, which ranged from 2% to 11% of normal levels. The same group published 3.2-year follow-up data in the same journal in 2014 on 6 patients (2 of the original patients plus 4 more) who received high-dose treatment.[10] The results showed a consistent increase in the factor IX level to a mean of 5.1%, which resulted in a reduction of more than 90% in both bleeding episodes and the use of prophylactic factor IX concentrate. We expect to see further follow-up in these patients to learn whether they continued to express factor IX.

Finally, the results of the phase 3 HOPE-B trial led to the 2022 US Food and Drug Administration approval

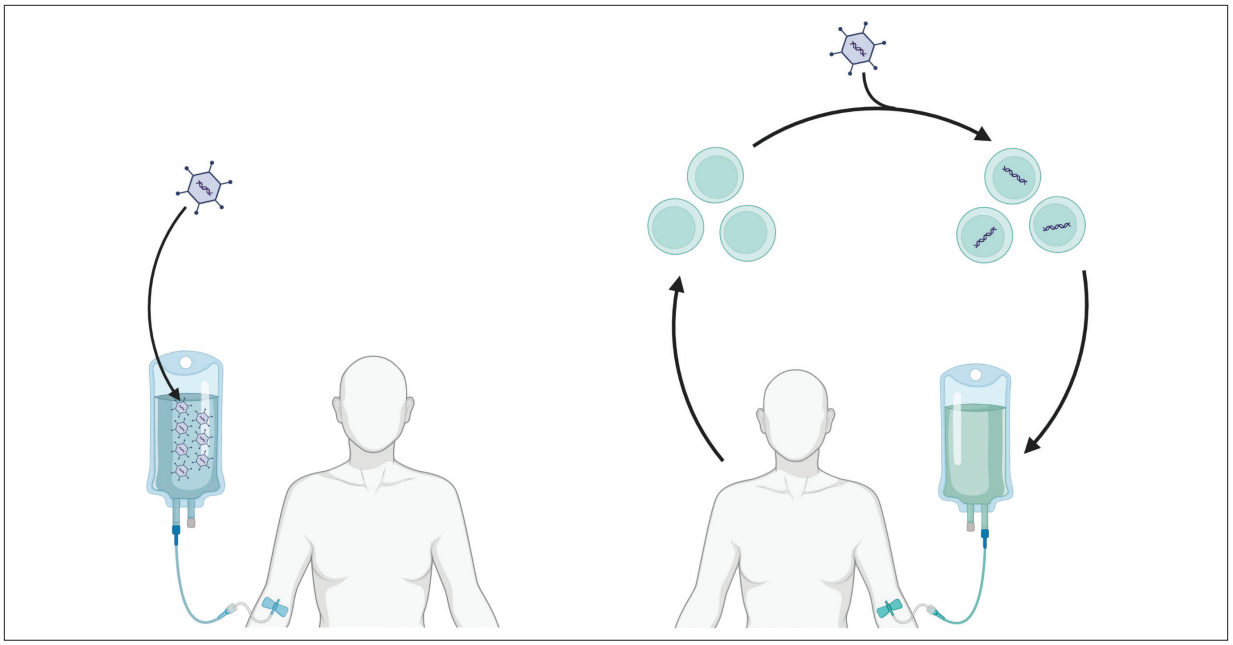

Figure. In vivo (left) vs ex vivo (right) methods of gene transfer and gene editing. Image created with BioRender.com.

of the gene therapy etranacogene dezaparvovec, also known as AMT-061 (Hemgenix, uniQure/CSL Behring), in patients with moderate to severe hemophilia B.[11] The HOPE-B trial enrolled 54 men with moderate to severe hemophilia B, making it the largest trial of an experimental gene therapy for this condition. At 1 year after the infusion, patients achieved a mean of 41.5% of normal factor IX activity levels. In addition, a single dose of AMT-061 reduced the annualized rate of both total and spontaneous bleeds requiring treatment by 80% or more.

The next agent expected to receive approved is valoctocogene roxaparvovec for treatment of hemophilia A, based on the results of the phase 3 BMN 270-301 trial from BioMarin. Valoctocogene roxaparvovec is an AAV5-based gene therapy vector designed to address factor VIII deficiency. In the trial, which was published by Ozelo and colleagues in the *New England Journal of Medicine* in 2022, valoctocogene roxaparvovec treatment provided endogenous factor VIII production and significantly reduced bleeding episodes and the use of factor VIII concentrate in 134 participants with severe hemophilia A.[12]

H&O What are the risks associated with gene therapy?

JS Clinicians and patients should know that treatment may not lead to the results they want. A gene therapy product to increase factor VIII may lead to 5% expression, or it may lead to more than 225% expression. A potential risk is sexual transmission of the product, and therefore we discuss with patients the importance of abstinence and/or the use of barrier contraceptive methods for at least one year. Also, because these agents target the liver, patients need to understand that their liver health could be affected. Finally, we must accept that there may be risks we simply have not anticipated or identified: the unknown unknowns (as our community has frequently heard Dr Glenn Pierce say).

It should be noted that although rare, gene therapy has resulted in death. In 1999, the first person to die in a gene therapy trial was 18-year-old Jesse Gelsinger, who had mild ornithine transcarbamylase deficiency and was enrolled in a trial that was meant for patients with severe disease even though he was relatively healthy. After this event, hesitation and ethical questions arose regarding gene therapy. Although no deaths have occurred in patients with hematologic disorders during or after clinical trials using AAV, deaths have occurred after receiving AAV-mediated gene therapy to treat patients with other diseases.[13,14]

H&O What new technologies are being used in gene therapy?

JS I expect to see alternatives to AAVs, such as nanoparticles, nucleic acids, and other nonviral vectors. The use of nonviral vectors could be advantageous because viruses tend to activate the immune system, which can affect the health of the liver and other organs. Nanoparticles can

be specifically designed to target a particular cell type, so there may be fewer off-target effects.

An exciting gene therapy approach is the use of zinc-finger nucleases (ZFNs), transcription activator-like effector nuclease (TALENs), or clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9) for gene editing. The use of CRISPR/Cas9 has been studied in preclinical models of sickle cell disease and β-thalassemia, and some success has been reported in early clinical trials.[15]

Disclosures

Dr Staber has served on the advisory boards for Sanofi, Novo Nordisk, and Takeda.

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