CHAPTER 38

Gene therapy applications to transfusion medicine

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Introduction

The delivery of genetic material into a patient’s cells as a medical therapy is referred to as gene therapy. The specific manner by which gene therapy is performed varies significantly based on the disease being treated, the amount of genetic material to insert, the target cells, and the route of administration. In the last several years, the number of worldwide clinical trials for cellular therapy, including gene-modified cells, has increased from approximately 151 in 2011 up to 373 in 2014.1 On the other hand, the total number of gene therapy clinical trials registered worldwide progressively increased from just one in 1989 to 120 in 2013.2

Traditionally, transfusion medicine has only been able to provide supportive therapy for patients with inherited hematologic diseases, rather than a curative treatment. For example, chronic transfusion can be used to treat anemia associated with sickle cell disease (SCD) or thalassemia, and the administration of clotting factor concentrates can be used to treat or prevent hemorrhage in patients with hemophilia. However, in the future, novel therapies directed at the genetic basis of these diseases may reduce, or even prevent, the need for such patients to require chronic supportive therapy. The focus of this chapter will be on the possible applications of gene therapy to transfusion medicine.

Gene therapy and transfusion medicine

Gene therapy falls within the purview of transfusion medicine when it requires the infusion of either gene-modified cells or direct administration of vectors that contain the therapeutic genetic material. In addition, transfusion medicine physicians will surely be impacted by gene therapies used to treat diseases that are currently managed with transfusion or factor infusion. For example, hemophilia B, a disease in which patients lack expression of functional clotting factor IX, has been successfully treated with gene therapy in small, early-phase clinical trials.3 Gene therapy also shows promise as a possible treatment for sickle cell anemia and hemophilia A.4,5

Three major considerations for the design of a gene therapy strategy are: which gene to insert, which vector to use, and how to administer the vector. Because hematopoietic stem cells can be mobilized, modified, and reinfused to patients, many hematologic disorders are uniquely suited to gene therapy. At the same time, hematological disorders also pose several unique challenges. This is due to the difficulty of delivering the often large genes required to treat hematologic disease and the risk of malignancy from gene insertion. Yet another challenge is to isolate hematopoietic cells in suitable quantities for transduction–transfection and to support them ex vivo in culture.

Gene selection and targeted insertion

Based on the disease to be treated, the gene that will be inserted can have a variety of attributes. The gene can provide a functional form of a missing or defective gene, it can augment a dysfunctional gene, or it can regulate cell survival. Ideal vectors should be designed to affect specific target cells. In the last few years, the importance of controlling where a gene therapy vector integrates into the genome has become clearer. For example, in the case of gene therapy trials in X-linked immunodeficiency, the retroviral vectors integrated into the genome at sites that activated proto-oncogenes, subsequently causing leukemia in treated patients.6 In addition, a gene therapy trial for hemophilia B was stopped after the viral vector was detected in a subject’s semen, raising concern that a therapeutic gene could be inherited by a subject’s future offspring.7

Gene therapy administration

Gene therapy vectors can be delivered to cells ex vivo and then returned to the patient. Alternatively, nonviral and replication-incompetent viral vectors can be directly administered to the patient for transduction of target cells in vivo. There are several benefits to the ex vivo approach, in particular that transduced cells can be selected prior to reinfusion (see Table 38.1).8 The ex vivo approach also minimizes infection of nontarget cells, significantly decreases the potential risks of exposing the patient to large amounts of viral vector, and allows for the insertion of an additional gene that could be activated to destroy the designed cell, if necessary. On the other hand, direct administration of vectors to the patient overcomes the difficulty of maintaining fully functional cells during ex vivo culture. Because there are risks associated with transgene expression in unintended host cells, it is critical that a vector administered in vivo has target specificity or minimal toxicity if expressed by cell types other than the intended target.
For gene therapy are discussed in the following sections as well. These viral vectors. Nonviral techniques that are under development are the generation of replication-competent viruses and gene immunotherapy. No replication-competent particles are present. Clinical trials, each batch must be tested extensively to ensure that no replication-competent viruses are present. For safety reasons, all viral vectors for gene therapy must be incapable of replication in the human host. For example, replication-incompetent retroviruses (RIRs) are produced by removal of the gag, pol, and env genes. Before viral vectors can be used in clinical trials, each batch must be tested extensively to ensure that no replication-competent particles are present.

**Vector selection**

The ideal vector should have no toxicity, lead to minimal inflammation, and have a large gene carrying capacity. In addition, vectors should be able to target specific cell types and genetic integration sites within the host. Other factors to consider are whether gene expression needs to be inducible and if lifelong expression is necessary. Unfortunately, a single vector that achieves all of these goals is not yet available. However, based on the above needs, a suitable vector can often be selected. Many research laboratories are developing strategies to optimize current vectors to meet the criteria for an ideal vector. Three of the most well-established viral vector systems for clinical trials are retroviruses, adenoviruses, and adeno-associated viruses. (Note that the use of lentiviral vectors, which are a subtype of retroviruses, is covered in Chapter 41, “Adoptive Immunotherapy.”) Table 38.2 summarizes the salient features of these viral vectors. Nonviral techniques that are under development for gene therapy are discussed in the following sections as well.

**Virus inactivation**

For safety reasons, all viral vectors for gene therapy must be incapable of replication in the human host. For example, replication-incompetent retroviruses (RIRs) are produced by removal of the gag, pol, and env genes. Before viral vectors can be used in clinical trials, each batch must be tested extensively to ensure that no replication-competent particles are present.

**Risks**

The major risks associated with viral vector–based gene therapy are the generation of replication-competent viruses and gene integration that could lead to the activation of oncogenes or loss of function of tumor suppressor genes.

**Replication-competent viruses**

Replication-competent viruses develop either by recombination of the constituent parts of the vector system with endogenous viral sequences in the vector packaging cell lines or by activation of endogenous proviral sequences. The significant risks of retroviral reactivation for gene therapy were elucidated when primate studies were initially performed. In these studies, CD34-selected primate marrow cells were infected with replication-incompetent virus from packaging cell lines. There were, however, some replication-competent viruses that had not been detected by existing assays. Upon infusion, three of eight primates subsequently developed lymphomas containing the active, rearranged retrovirus. As mentioned, investigators are optimizing safety by creating replication-incompetent vectors, and minimizing the regions of homology between the vectors and packaging cell lines. Currently, clinical trials require stringent testing to guarantee that the retroviral vector to be used in humans is entirely replication incompetent.

**Genotoxicity (insertional mutagenesis)**

Genotoxicity occurs when genetic material is inserted in proximity to a proto-oncogene or disrupts expression of a tumor suppressor gene. The presence of the strong viral promoter near a proto-oncogene can lead to transcriptional activation of the gene. This has led to the development of malignancy in patients enrolled in a clinical trial for X-linked immunodeficiency. Unfortunately, no strategies are currently available for site-directed insertion of the genetic payload. In addition, insertion of a transgene into a portion of the genomic DNA that is essential to cell survival can lead to disruption of critical cell processes, resulting in cell death.

**Nonviral gene therapy vectors**

Nonviral vectors for gene therapy are also undergoing intensive investigation. Unlike viral vectors, nonviral methods do not need to overcome the extensive immune mechanisms that inhibit transduction in vivo. There is also no risk of replication-competent forms of the vector. These methods include DNA microinjection or chemical transfection of episomal or other exogenous DNA.

**Gene editing**

Various gene-editing approaches such as transcription activator–like effector nucleases (TALENs), zinc finger nucleases (ZFNs), and clustered regularly interspaced short palindromic repeats (CRISPRs) have been described. All three of these technologies

### Table 38.1 Advantages and disadvantages of different modes of vector administration

<table>
<thead>
<tr>
<th>Manner of Vector Administration</th>
<th>Ex Vivo</th>
<th>In Vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Possible advantages</td>
<td>Ability to select transduced cells prior to reinfusion</td>
<td>No need to maintain ex vivo fully functional cells that can engraft long-term after administration to the patient</td>
</tr>
<tr>
<td></td>
<td>Minimizes infection of nontarget cells</td>
<td></td>
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<tr>
<td></td>
<td>Exposes patient to smaller dose of viral vector</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ability to insert a suicide gene to inactivate a therapeutic gene</td>
<td></td>
</tr>
<tr>
<td>Possible disadvantages</td>
<td>Can be technically difficult to maintain and transduce fully functional cells ex vivo</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Exposes patient to larger dose of viral vector</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vector may unforeseen effects on unintended target cells</td>
<td></td>
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<tr>
<td></td>
<td>Immune system may target the vector for destruction</td>
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</table>

### Table 38.2 Common viruses that have been modified for gene therapy research

<table>
<thead>
<tr>
<th>Family/Subfamily and Characteristics</th>
<th>Specific Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retroviruses (single-stranded RNA, with maximal insert size of approx. 8 kb)</td>
<td>Murine leukemia virus</td>
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<tr>
<td>Oncoviruses</td>
<td>Spleen necrosis virus</td>
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<tr>
<td></td>
<td>Rous sarcoma virus</td>
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<tr>
<td></td>
<td>Avian leukosis virus</td>
</tr>
<tr>
<td>Lentiviruses</td>
<td>Human immunodeficiency virus, type 1</td>
</tr>
<tr>
<td></td>
<td>Human immunodeficiency virus, type 2</td>
</tr>
<tr>
<td>Spumaviruses</td>
<td>Foamy virus</td>
</tr>
<tr>
<td>Adenoviruses (double-stranded DNA, with maximal insert size of approx. 8 kb for first generation; up to 37 kb for new generation)</td>
<td>Adenovirus, type 5</td>
</tr>
<tr>
<td>Adeno-associated virus (single-stranded DNA with maximal insert size of approx. 5 kb)</td>
<td>Adeno-associated virus, type 2</td>
</tr>
</tbody>
</table>
can be used to disrupt, add, or correct genes in animal cells. Unlike gene therapy vectors, which insert genetic information into a patient’s genome, TALENs, ZFNs, and CRISPRs actually correct mutations in the patient’s DNA.

Studies that have assessed the possible application of gene-editing techniques to hemoglobinopathies are discussed in the “Transfusion-Medicine Related Gene Therapy Trials” section, below. Notably, gene-editing approaches have also shown promise for other conditions. For example, a trial of HIV patients treated with autologous T cells that were engineered to have dysfunctional CCR5 genes by a ZFN was recently reported. Although one of the 12 patients in the trial had a transfusion reaction to the infusion of T cells, the study found that infusion of these engineered cells appeared to be safe. Long-term monitoring of study participants is needed to confirm the safety of this approach.

**Transfusion-medicine related gene therapy trials**

**Hemophilia B**

Hemophilia B is an X-linked genetic disease that results in bleeding due to reduced or absent activity of factor IX, a serine protease synthesized in the liver. Patients with mild hemophilia B have between 6% and 49% of normal factor IX activity, and may never be diagnosed unless they undergo a major trauma or surgery. Moderate hemophilia B is classified as factor IX activity between 1% and 5% of normal. Patients with moderate hemophilia B may have spontaneous bleeding or prolonged or major bleeding after injuries. Severe hemophilia B is diagnosed when <1% of normal factor IX is detectable in the blood and can result in debilitating, spontaneous bleeding episodes. At present, bleeding prevention in hemophilia B is accomplished via administration of source plasma–derived or, ideally, recombinant factor IX concentrates. Many patients with severe disease require frequent infusions of factor IX to prevent spontaneous bleeding episodes.

Severe hemophilia B is an attractive target for gene therapy because even a small increase in endogenous factor IX activity could reduce dependence on factor IX infusions and prevent spontaneous bleeding episodes. In the late 1990s, researchers using various animal models of hemophilia B found that a gene therapy technique using an adeno-associated virus (AAV) vector could be used to induce sustained circulation of factor IX. Shortly thereafter, a human trial established that administration of an AAV vector to human subjects was safe. A subsequent study of seven patients with severe hemophilia B showed that treatment with a recombinant AAV vector (rAAV-hAAT-F.IX) led to only transient elevations in circulating factor IX. Because the reduction in factor IX activity was associated with an increase in transaminases, the study authors concluded that it was immune-mediated destruction of transduced hepatocytes expressing the AAV capsid may have occurred. In contrast to animal models, humans are frequently infected by AAV during childhood. This may explain why animals were capable of a sustained response to AAV-mediated gene therapy while humans generated a secondary immune response that led to the destruction of transduced hepatocytes. However, a more recent trial of 10 patients with severe hemophilia B treated with a different AAV vector (scAAV2/8-LP1-hFIXco) demonstrated a sustained, dose-dependent increase in factor IX activity. After a median of 3.2 years of follow-up, these patients have required fewer factor IX infusions, have had fewer bleeding episodes, and have not had significant treatment-related toxicity. The authors of the study estimate that, in addition to the benefits to the patients, the gene therapy treatment has saved $2.5 million in factor IX injections to these 10 patients.

There are three clinical trials (all phase 1 or phase 1/2) of AAV vector–mediated gene therapy for hemophilia B registered with ClinicalTrials.gov. All three trials (NCT01620801, and NCT01687608) are recruiting patients. Projected primary completion dates for these trials range from June 2015 to November 2019. The results of these trials will likely determine the fate of AAV vector–mediated gene therapy as a treatment for hemophilia B.

**Hemophilia A**

Hemophilia A is caused by a deficiency in factor VIII, which is the only clotting factor synthesized by endothelium (rather than the liver). Similar to hemophilia B, patients with hemophilia A are stratified into mild (6–49% of normal factor VIII activity), moderate (1–5% of normal factor VIII activity), and severe (<1% of normal factor VIII activity) categories based on their level of circulating factor VIII. Also similar to factor IX deficiencies, even a small increase in factor VIII activity would be expected to protect patients from bleeding episodes. Therefore, hemophilia A is another appealing target for gene therapy.

However, unlike factor IX, factor VIII is difficult to efficiently express in vitro. Attempts to induce cultured cells to produce factor VIII can result in the production of misfolded factor VIII protein, which can, in turn, activate the unfolded protein response and trigger apoptosis of the cells. Secondly, the gene that encodes factor VIII is significantly larger than that of factor IX, and, in the absence of complex laboratory manipulations, is too large to fit into the AAV vector. Finally, patients with hemophilia A are more likely to develop circulating inhibitors to factor VIII, making long-term expression of the transduced gene product difficult. For these reasons, gene therapy for hemophilia A lags behind gene therapy for hemophilia B. At least one group has published plans for a clinical trial of an AAV vector–mediated approach to hemophilia A therapy as early as 2015. At present, no gene therapy trials for hemophilia A are registered with ClinicalTrials.gov.

Attempts to use other vectors for the treatment of hemophilia have shown promise, but advancement beyond the preclinical stage has been elusive. Over a decade ago, Roth et al. showed that skin-derived fibroblasts could be transfected with the factor VIII gene without the use of a viral vector. When these fibroblasts were injected into the omentum of patients with hemophilia A, some patients experienced less bleeding and had increased factor VIII activity levels. Later studies demonstrated that infusion of a retroviral vector containing the factor VIII gene with a deleted β-domain resulted in a temporary increase of circulating factor VIII in more than half of participants. More recently, Du et al. reported that long-term bleeding prophylaxis can be accomplished by inducing factor VIII expression in blood cells, including platelets, using a lentiviral vector to modify gene expression in hematopoietic progenitor cells in a canine model of hemophilia A. Gene therapy to induce platelet-specific expression of factor VIII could one day be used to treat human hemophilia A in patients with factor VIII antibodies (inhibitors). Hopefully, these preclinical and stage I trials will continue to show promise.

**Hemoglobinopathies**

Hemoglobinopathies such as SCD and thalassemia are some of the most common single-gene defects worldwide. SCD affects
approximately 275,000 and β-thalassemia affects approximately 56,000 newborns every year. The current treatment for these diseases is primarily supportive therapy, which includes chronic transfusion for the management of anemia. However, this approach comes with several consequences, including alloimmunization, iron overload, and splenomegaly. While hematopoietic stem cell transplantation is an option as a definitive therapy, very few patients have suitable matched sibling donors, and there is a significant chance of adverse outcomes, including graft-versus-host disease and graft failure. Alternative therapies that lead to decreased reliance on transfusion and other supportive therapies would likely be beneficial to patient outcomes.

Much like hemophilia, the hemoglobinopathies are attractive targets for gene therapy. However, these diseases have been more difficult to treat with this approach as they require large genetic sequences with stable, long-term expression to be effective. In addition, they require a high level of gene expression for correction, which is difficult to achieve with traditional vectors. As of this writing, no approved gene therapies are currently available for the treatment of SCD. Mouse models of SCD have demonstrated that lentiviral vectors carrying an antisickling globin are able to persistently express the globin gene at levels high enough for symptomatic correction. In addition, approaches that use the γ-globin gene with a β-globin promoter, to ensure expression in adults, have been promising in mouse models, and an SCD gene therapy trial recently began enrolling patients.

Clinical trials for the treatment of β-thalassemia with gene therapy have progressed more quickly. Several trials have enrolled patients in phase 1/2 trials in several centers across the United States, but long-term patient outcomes have yet to be reported. A single patient with β°/β°-thalassemia, without an HLA-matched hematopoietic stem cell donor, has been reported to have achieved transfusion independence three years after lentiviral β-globin gene transfer.

Other novel approaches to hemoglobinopathies use a gene-editing technique. Although these are not yet available in clinical trials, early studies using induced pluripotent stem cells have shown that TALENs and CRISPRs can be used to correct mutations causing β-thalassemia and CRISPRs and ZFNs can be used to correct the SCD mutation. Similarly, TALENs have been reported to correct mutations causing α-thalassemia major in induced pluripotent stem cells. With the promising results so far, it is hoped that gene therapies for the hemoglobinopathies will continue to progress quickly through clinical trials.

Wiscott-Aldrich syndrome (WAS)
WAS is a rare, X-linked immune deficiency caused by mutations in the WAS gene. Patients classically present with thrombocytopenia (with small platelets), susceptibility to infections, and eczema. Affected patients also have an increased risk for the development of autoimmune diseases and cancer. Patients are typically treated with a stem cell transplant, if an HLA-matched donor is available. Patients with WAS may come to the attention of the transfusion service as a result of bleeding in the context of thrombocytopenia.

A phase I/II clinical trial of three pediatric patients with WAS, who lacked an HLA-matched donor or were otherwise ineligible for stem cell transplant, was recently reported. In this trial, autologous CD34+ cells were transduced with a normal WAS gene ex vivo using a lentiviral vector. When the engineered hematopoietic stem cells were infused, they engrafted successfully. The patients went on to demonstrate improvements in hemostasis and immunity. Encouragingly, a similar approach using a lentiviral vector to engineer hematopoietic stem cells for treatment of children with metachromatic leukodystrophy also showed success without apparent evidence of genotoxicity. Although long-term safety monitoring is needed, the results of these trials are promising for patients without HLA-matched stem cell donors suffering from WAS or other diseases.

Summary
Gene therapy continues to offer much promise for the treatment of genetic and acquired diseases. Transfusion medicine laboratories are currently involved in clinical gene therapy trials and, in the future, if these therapeutic modalities are approved, will likely oversee what may be “routine” administration of gene therapy vectors and vector-infected cells.

Disclaimer
The authors have disclosed no conflicts of interest.

Key references
A full reference list for this chapter is available at: http://www.wiley.com/go/simon/transfusion