The human coagulation system has evolved over the past 450 million years to function through a complex coordinated interaction involving the vessel wall, circulating cells, and a protein cascade of procoagulants that are regulated by a series of anticoagulant mediators. In normal hemostatic responses, these various components combine to generate a protective fibrin clot, limiting blood loss from the circulation. In pathological states, deficiencies, dysfunction, or aberrant regulation of these hemostatic elements result in either bleeding or thrombotic phenotypes. These pathologies are the consequence of either rare genetic traits or, more often, a complex interaction of genetic and acquired influences.

Abstract: Molecular genetic details of the human coagulation system were among the first successes of the genetic revolution in the 1980s. This information led to new molecular diagnostic strategies for inherited disorders of hemostasis and the development of recombinant clotting factors for the treatment of the common inherited bleeding disorders. A longer term goal of this knowledge has been the establishment of gene transfer to provide continuing access to missing or defective hemostatic proteins. Because of the relative infrequency of inherited coagulation factor disorders and the availability of safe and effective alternative means of management, the application of gene therapy for these conditions has been slow to realize clinical application. Nevertheless, the tools for effective and safe gene transfer are now much improved, and we have started to see examples of clinical gene therapy successes. Leading the way has been the use of aden-associated virus–based strategies for factor IX gene transfer in hemophilia B. Several small phase 1/2 clinical studies using this approach have shown prolonged expression of therapeutically beneficial levels of factor IX. Nevertheless, before the application of gene therapy for coagulation disorders becomes widespread, several obstacles need to be overcome. Immunologic responses to the vector and transgenic protein need to be mitigated, and production strategies for clinical grade vectors require enhancements. There is little doubt that with the development of more efficient and facile strategies for genome editing and the application of other nucleic acid–based approaches to influence the coagulation system, the future of genetic therapies for hemostasis is bright. (Circ Res. 2016;118:1443-1452. DOI: 10.1161/CIRCRESAHA.115.307015.)

Key Words: bleeding ■ coagulation ■ factor IX ■ factor VIII ■ gene therapy ■ gene transfer ■ hemophilia
initial vasoconstrictive response to vascular damage is crucial for the early phase of hemostasis (eg, reduced blood flow, platelet margination, and unfolding of von Willebrand factor), specific therapies aimed at this early element of hemostasis are not routinely used. In contrast, cellular therapies to enhance the contributions of platelets (adhesion and aggregation) and red cells (contributing to platelet margination and fibrin clot strength) to the generation of an optimal hemostatic response are frequently applied to the treatment of bleeding. Currently, most therapies used for coagulation disorder management are aimed at replacing, enhancing, or inhibiting components of the procoagulant protein cascade and the corresponding anticoagulant and fibrinolytic response.

### Procoagulant Treatment Approaches

Interventions aimed at preventing or treating bleeding can involve either single factor strategies or combination replacement therapy. The more common inherited bleeding disorders, hemophilia A (factor VIII [FVIII] deficiency) and B (factor IX [FIX] deficiency), von Willebrand disease, and factor XI deficiency, can be treated with single factor replacement therapies through the infusion of protein concentrates derived from either plasma or recombinant DNA technology. In contrast, most acquired bleeding disorders, such as vitamin K deficiency, liver disease, and consumptive coagulopathies, are managed with the infusion of either defined multifactor concentrates (eg, prothrombin complex concentrates, factors II, VII, IX, and X), cryoprecipitate, von Willebrand factor, factors VIII, XIII, and fibrinogen or plasma. The efficacy of these various treatment regimens can be monitored through clinical assessment and by the performance of either global tests of hemostasis, such as the prothrombin, partial thromboplastin, and thrombin times, or by quantification of single factor levels as in the replacement of FVIII and FIX in the hemophilias.

### Gene Therapy Concepts and Historical Context

The concept of using genetic approaches to treat and potentially cure disease originated in the early 1980s with the cloning of the first human genes. The first, approved, and successful gene therapy study in a human was conducted in 2 patients with inherited adenosine deaminase deficiency in 1990. Since then, the field of gene therapy has had a chequered history, but in 2012, the European Medicines Agency approved for the first time a gene therapy drug Glybera, a gene therapy treatment for inherited lipoprotein lipase deficiency. Now in 2015, there is realistic optimism that this therapeutic modality can offer significant opportunities for long-term benefits in a range of disorders.

The coagulation genes were among the first to be characterized in the 1980s, with major contributions derived from Earl Davie’s laboratory at the University of Washington (Figure 1A). These discoveries confirmed that the coagulation proteins were derived from genes which encoded modular domains that were shared between related families of coagulation factors (eg, the vitamin K-dependent factors and factors V and VIII). Soon after the cloning of the FVIII and FIX genes, translational application of this knowledge was initiated through the introduction of molecular diagnostics, the development of recombinant clotting factors for replacement therapy, and the initial preclinical trials of gene therapy.

The hemophilias are an ideal model genetic disease for the application of gene therapy. They result from recessive mutations in 2 well-characterized genes with minimal influence from other genetic modifiers. Furthermore, it was well recognized from the natural history of moderately severe and mild hemophilia (clotting factor levels between 1% and 40%) that small increments in plasma clotting factor levels would result in significant benefits in reducing the risk of bleeding. Thus began the 30-year quest to convert what seems to be a simple therapeutic goal, to deliver a normal gene copy to compensate for the existing mutant gene.

### Spectrum of Gene Therapy Initiatives for Coagulation Disorders

It is clear, for the reasons detailed earlier, that the hemophilias have been leading candidates for the application of gene therapy since the dawn of molecular medicine. However, advances in the development of gene therapy strategies for other pro- or anticoagulant deficiencies has been modest, a fact that relates to a combination of factors, including the infrequent incidence of monogenic inherited bleeding and thrombotic disorders, the existence of safe and effective alternative therapies, and in some instances, inadequate biological knowledge to effectively design gene therapy strategies.

Aside from FVIII and FIX gene therapy, the only other procoagulant targets that have been explored to any extent in preclinical studies have been von Willebrand factor and factor VIIa. The former target might find a place for the treatment of the rare type 3 form of von Willebrand disease (incidence 1 per million), but even in this population, with no detectable circulating von Willebrand factor, spontaneous bleeding rates can often be surprisingly low. The limited activated factor VII gene therapy studies have been focused predominantly on the potential for developing a sustainable, long-term strategy for bypassing the presence of FVIII antibodies (FVIII inhibitors) in hemophilia A.

Gene therapy for anticoagulant purposes has been similarly underdeveloped. Again, this relates to the availability of safe and effective alternative treatments and to the fact that monogenic traits directly responsible for initiating thrombosis are rare. Finally, in contrast to the hemophilias, the levels of transgenic protein expression required to produce a phenotypic benefit are likely to be beyond the capability of current gene transfer technologies.

One thrombotic disorder where gene therapy has produced promising preclinical results is the inherited form of
The therapeutic goal for coagulation factor gene therapy is the delivery of a normal gene copy to produce therapeutically effective levels of the protein that is either deficient or dysfunctional as a result of a germ line mutation (Figure 1B). Several strategies for genome editing, using technologies such as zinc finger nucleases and transcription activator-like endonucleases, have the potential for in situ mutation correction. 

Figure 1. Gene therapy as a treatment for coagulation factor disorders. A. Gene therapy is the ultimate goal for treatment of inherited coagulation disorders. Characterization of the clotting factor genes/proteins in the 1980s allowed for the molecular diagnosis of inherited bleeding and thrombophilic disorders. Subsequently, recombinant protein technologies provided the first clotting factor–specific replacement therapies. Current strategies for improving treatment of inherited coagulation disorders include improving the pharmacokinetics of recombinant protein therapies and increasing the safety and efficacy of gene transfer protocols. B. Potential strategies for gene therapy for coagulation factor deficiencies include viral and nonviral in vivo, methods of delivery of a normal gene copy, ex vivo modification of host cells to induce normal gene expression, and in situ mutation correction. CRISPR-Cas9, clustered regularly interspaced short palindromic repeats/clustered regularly interspaced short palindromic repeat–associated 9; iPS, induced pluripotent stem cells; and TALENS, transcription activator-like effector nucleases.

Transgene Delivery Approaches

The first critical hurdle to overcome in any gene transfer protocol is the efficient delivery of the therapeutic transgene to somatic cells of the patient (Figure 2A). This goal can be achieved by either in vivo or ex vivo approaches, and there are advantages and weaknesses to both strategies. Ex vivo transgene delivery requires the isolation of a recipient cell population that can be readily transduced and has the potential for prolonged survival after delivery back into the recipient. The advantages of this approach are that efficient rates and
cell-type specificity of transgene delivery can be achieved (Figure 2B) and that the immunologic complications of in vivo vector administration are avoided. However, ex vivo strategies are inherently complex and resource-intensive, and re-establishment of the genetically modified cells may require some form of conditioning to ensure engraftment. In contrast, in vivo gene transfer is straightforward to perform, but ensuring efficient transgene delivery to specific cell types, without inciting immune reactions to the delivery vehicle, is challenging.

A single human clinical trial for ex vivo delivery of a FVIII transgene has been performed for hemophilia A. This study, involving 12 patients, used autologous fibroblasts that were electroporated ex vivo with a FVIII transgene, selected for optimal FVIII expression, expanded, and subsequently injected into the omental membrane. There were no adverse events associated with these studies, but also no evidence of sustained FVIII transgene expression, although several patients showed evidence of transient (2–3 days) minimal increments of plasma FVIII levels (2%–6%). Other preclinical studies of ex vivo transgene delivery for hemophilia have involved hematopoietic stem cells, mesenchymal stem cells, and endothelial progenitors. Results from the hematopoietic stem cell studies have been especially promising and may advance to a phase II/III clinical trial in the near future, but the endothelial progenitor investigations have been complicated by the generation of immune responses to the transgene product.

One particular ex vivo strategy for ectopic hemophilia gene therapy deserves further commentary. The generation of FVIII transgenes that are delivered to and expressed exclusively in megakaryocytes and platelets has shown promise in promoting an effective hemostatic response in standard animal models of hemophilia A and in the presence of FVIII inhibitory antibodies. Whether this strategy can be sufficiently optimized in terms of transgene delivery and protein production without the requirement of prohibitive conditioning regimens remains to be established. One potential approach to circumvent the requirement for bone marrow conditioning is the use of intraosseous vector infusion to mediate in vivo hematopoietic cell transduction. Using a lentiviral-based strategy with a platelet-specific regulatory sequence, long-term persistence of intra-platelet FVIII has been demonstrated at sufficient levels to mediate hemostatic protection in the presence of anti-FVIII antibodies.

Modes of Transgene Delivery

The goal of transgene delivery is to introduce the therapeutic gene construct as efficiently and safely to the recipient, usually to selected types of somatic cells. Importantly, avoidance of delivery to germ cells is a requirement for all clinical studies because the safety of germ line modification remains unresolved.

There are essentially 2 modes of transgene delivery: the application of viral vectors or nonviral vector-mediated strategies. Some of the latter approaches have been used for in vitro molecular biology studies since the 1970s, but in general, physicochemical protocols are relatively inefficient delivery approaches for achieving long-term persistence of transgenes. Electroporation has also been applied in some ex vivo protocols but again lacks sufficient efficiency for routine clinical translation. Finally, localized application of hydrodynamic gene transfer protocols has been proposed as a further approach to deliver expression plasmids to specific organs or regions of organs. However, the inevitable, albeit transient, vascular and local tissue damage caused by this mode of delivery initiates a strong innate immune response, thus increasing the likelihood of a secondary adaptive response to the neoantigenic transgene protein.

In marked contrast to the limitations of the various nonviral modes of transgene delivery, viral vectors use
properties that have evolved over millions of years to facilitate the efficient entry of viruses into cells. The generation of viral vectors involves the modification of the viral genome to eliminate the possibility of viral replication and to provide sufficient space for insertion of the therapeutic transgene in a viral particle that can still be efficiently packaged. Subsequent to the generation of the modified vector genome, the vector particles are produced in cell culture, providing packaging and structural proteins from cotransfected helper plasmids.

Gene therapy clinical trials for hemophilia have used 3 types of viral vector (Figure 3A). One patient received an adenoviral-mediated treatment for hemophilia A; there has been one trial of retroviral gene transfer of FVIII, and the remaining studies have used AAV vectors to deliver FIX transgenes (Table).

In contrast to AAV vectors that are discussed later, adenoviral vectors are not appropriate for the delivery of coagulation transgenes, in large part because of the significant proinflammatory innate immune response that their capsid proteins incite. This response can be limited to the development of fever, thrombocytopenia, and the elevation of liver transaminases, but can, under certain circumstances, advance to a fatal systemic inflammatory state such as that which killed Jesse Gelsinger during a trial of ornithine transcarbamylase gene therapy in 1999. Unless this obstacle can be eliminated (which may simultaneously reduce the transduction efficacy of the vector), adenoviral vectors will not find a place in clinical coagulation factor gene therapy.

The single clinical trial using an FVIII retrovirus in hemophilia A involved the systemic delivery of a classical γ-retroviral vector that would require cellular replication for successful transduction. None of the 12 patients entered in this phase 1 study developed complications, but only low level (~4%) and transient levels of FVIII were documented, indicating either inadequate transduction and transgene expression.

Although γ-retroviral vectors are no longer being pursued for hemophilia therapy, the development of lentiviral vectors, capable of transducing both dividing and nondividing cells, has continued. Lentiviral vectors can be used as the means for transgene delivery either in ex vivo strategies for modifying stem cell populations or via systemic in vivo delivery. Both strategies have been used successfully in animal models of hemophilia, and some form of lentiviral-based treatment may well enter the clinic in the future. Nevertheless, in 2015, the clearly favored vector system for clinical coagulation gene therapy was AAV.

**AAV-Mediated Gene Therapy for Hemophilia**

The development of AAV-based protocols for gene transfer of FIX has been one of the highlights of clinical gene therapy advances in the past 5 years. AAVs are small infectious agents (≈20 nm) that belong to the family of paroviruses. In humans, infection with AAV is asymptomatic. AAV has a small single-stranded DNA genome of ≈4.7 kb, with 2 open reading frames that encode capsid and other structural proteins and proteins facilitating integration of the wild-type virus into a specific region on chromosome 19. In the construction of AAV vectors, both these coding regions are deleted, allowing the insertion of transgenes of ≤5 kb without adversely influencing the packaging efficiency of vector particles. This packaging limitation has not affected the generation of FIX transgenes, in which the coding sequence spans ≈1.3 kb. In contrast, the FVIII cDNA occupies ≈8.5 kb, and even after deletion of the nonessential B domain–encoding sequence, the cDNA is still ≤5 kb. Thus, promoter/enhancer elements for AAV FVIII constructs have to be compact, and even then, a reduction in vector packaging efficiency is a significant concern (Figure 3B).

To date, human clinical trials of skeletal muscle and liver-directed AAV FIX gene therapy have been conducted. In each instance, strong tissue-specific promoter/enhancer

---

**Table 3. Viral vectors for in vivo gene transfer.**

<table>
<thead>
<tr>
<th>Viral Vector</th>
<th>Characteristics</th>
<th>Advantages</th>
<th>Limitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>36 Kb dsDNA</td>
<td>Large genome</td>
<td>Easy to produce high titer</td>
</tr>
<tr>
<td>Retrovirus (lentivirus)</td>
<td>8 Kb ssRNA</td>
<td>Larger genome</td>
<td>High infection efficiency</td>
</tr>
<tr>
<td>Adeno-associated virus (AAV)</td>
<td>4.7 Kb ssDNA</td>
<td>Low immunogenicity</td>
<td>Infects many cell types</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>AAV Vector</th>
<th>Codon Optimization/ cDNA contraction</th>
<th>Transgene cDNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rep Cap</td>
<td>~5 kb ss DNA</td>
<td>4.7 kb ss DNA</td>
</tr>
</tbody>
</table>

**5’ Regulatory Elements**
- Constitutive
- Regulated
  - Cell-specific
  - Developmental-specific
  - Drug-inducible

**3’ Regulatory Elements**
- Enhancer
- Poly A tail

---

**Figure 3.** Viral vectors for in vivo gene transfer. A. Gene therapy clinical trials for hemophilia have used adenovirus (1 patient), γ-retrovirus (12 patients), and adeno-associated virus (AAV; 31 patients). B. Adenovirus-associated inflammation and inadequate transduction and transgene expression by γ-retrovirus have discontinued their use in coagulation factor gene therapy. One challenge of AAV-mediated gene therapy is the limited packaging size of the recombinant vector genome. Strategies for overcoming this limitation include cDNA contraction and the use of compact promoter/enhancer elements. ITR indicates inverted terminal repeat.
elements have been used, and the vectors have been delivered by either intramuscular injection or portal or peripheral vein injection for the skeletal muscle and liver studies, respectively. Tissue tropism of the AAV vectors is influenced by the differential efficacy for transduction mediated by serotype-specific capsids.56 Thus, AAV8 possesses strong hepatotropism and can be delivered efficiently to the liver via peripheral vein infusion without the risk of significant transduction of other tissue types.

Fate of AAV Vectors

Transduction by AAV vectors involves an interaction with a range of serotype-specific cell surface receptors.56–58 The vector particles are taken into endocytic vesicles, where, in the acidic milieu of the endosome, the capsid is removed and the nucleic acid released for transport to the nucleus.59 In efforts to accelerate this process, showing that long-term expression of the FIX transgene was achievable.43,62,63 Latterly, hemophilia gene therapy trials have targeted the liver for transgene delivery using either portal vein infusion or, more recently, peripheral vein injection of hepatotropic AAV8 vectors.

The FIX transgene constructs that have been generated have used small and potent tissue-specific enhancer/promoter elements, and one study has used a codon optimized FIX cDNA.45 In the most recent FIX clinical trial, the FIX transgene also contains a gain-of-function missense variation (Arg338Leu–FIX Padua64,65) that increases the specific coagulant activity of the transgenic protein by ≈7-fold.

For native single-stranded AAV transgenes to mediate mRNA expression, a second complementary DNA strand has to be synthesized. In efforts to accelerate this process, some investigators have constructed self-complementary AAV transgenes that mediate earlier expression after delivery.57

Vector doses in the clinical trials have ranged between 2×10^{11} and 5×10^{12} vector genomes/kg.

After transgene delivery, FIX plasma levels start to increase after 2 to 3 weeks and have, until the most recent study using the FIX Padua transgene, resulted in FIX levels of 2% to 10% in the short term and persistent levels of 1% to 6%.43,45,46 Preliminary results from the FIX Padua study, in patients receiving one of the higher vector doses, have shown transient levels of FIX >50% that have subsequently fallen to levels similar to the other studies that have been reported.66 In the context of hemophilia, these relatively low levels of FIX have had significant benefits in terms of

<table>
<thead>
<tr>
<th>Vehicle</th>
<th>Transfer Method</th>
<th>Gene</th>
<th>Patients Treated</th>
<th>Best Outcome</th>
<th>Complications</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gamma-retrovirus transduction of autologous fibroblasts</td>
<td>Ex vivo</td>
<td>F9</td>
<td>2</td>
<td>Transient expression (2%)</td>
<td>No adverse effects reported</td>
<td>Xinfang et al42</td>
</tr>
<tr>
<td>Electroporation of autologous fibroblasts (BDD-FVIII)</td>
<td>Ex vivo</td>
<td>F8</td>
<td>12</td>
<td>Transient expression (4%)</td>
<td>No adverse effects reported</td>
<td>Roth et al42</td>
</tr>
<tr>
<td>Gamma-retrovirus (BDD-FVIII–IV)</td>
<td>In vitro</td>
<td>F8</td>
<td>12</td>
<td>Transient expression (4%)</td>
<td>No adverse effects reported</td>
<td>Powell et al43</td>
</tr>
<tr>
<td>“Gutless” adenovirus</td>
<td>In vitro</td>
<td>F8</td>
<td>1</td>
<td>Transient expression (1%–3%)</td>
<td>Transaminitis, thrombocytopenia</td>
<td>No published reports42</td>
</tr>
<tr>
<td>ssAAV2/2–Intramuscular</td>
<td>In vitro</td>
<td>F9</td>
<td>8</td>
<td>Transient expression (&lt;2%)</td>
<td>No adverse effects reported</td>
<td>Manno et al44</td>
</tr>
<tr>
<td>ssAAV2/2–hepatic artery infusion</td>
<td>In vitro</td>
<td>F9</td>
<td>7</td>
<td>Transient expression (10%) over 8 wk</td>
<td>Transient transaminitis</td>
<td>Manno et al44</td>
</tr>
<tr>
<td>scAAV2/8: (FIXco)–IV</td>
<td>In vitro</td>
<td>F9</td>
<td>10</td>
<td>Persistent expression (1%–6%) ≤3 y</td>
<td>Transient transaminitis</td>
<td>Nathwani et al45,46</td>
</tr>
<tr>
<td>scAAV2/8: (FIX Padua)–IV</td>
<td>In vitro</td>
<td>F9</td>
<td>6</td>
<td>Variable expression (0.5%–25%) ≤8 mo</td>
<td>No adverse effects reported</td>
<td>Monahan et al47</td>
</tr>
</tbody>
</table>

BDD indicates B-domain deleted; FIXco, codon optimized FIX; sc, self-complementary; and ss, single strand.

Results of AAV-Mediated Gene Therapy for Hemophilia

To date, ≈31 hemophilia B patients have been treated in 5 AAV gene therapy trials (Table). A clinical study using AAV to deliver FVIII has just enrolled its first patient. In the hemophilia B trials, initial studies involved intramuscular injection of the vector, showing that long-term expression of the FIX transgene was achievable.43,62,63 Latterly, hemophilia gene therapy trials have targeted the liver for transgene delivery using either portal vein infusion or, more recently, peripheral vein injection of hepatotropic AAV8 vectors.

In the most recent FIX clinical trial, the FIX transgene also contains a gain-of-function missense variation (Arg338Leu–FIX Padua64,65) that increases the specific coagulant activity of the transgenic protein by ≈7-fold.45

For native single-stranded AAV transgenes to mediate mRNA expression, a second complementary DNA strand has to be synthesized. In efforts to accelerate this process, some investigators have constructed self-complementary AAV transgenes that mediate earlier expression after delivery.57

Vector doses in the clinical trials have ranged between 2×10^{11} and 5×10^{12} vector genomes/kg.

After transgene delivery, FIX plasma levels start to increase after 2 to 3 weeks and have, until the most recent study using the FIX Padua transgene, resulted in FIX levels of 2% to 10% in the short term and persistent levels of 1% to 6%.43,45,46 Preliminary results from the FIX Padua study, in patients receiving one of the higher vector doses, have shown transient levels of FIX >50% that have subsequently fallen to levels similar to the other studies that have been reported.66 In the context of hemophilia, these relatively low levels of FIX have had significant benefits in terms of
Reducing bleeding frequency, with patients being able to stop their routine FIX protein prophylactic infusions. In the study with the longest follow up of 10 hemophilia B patients, therapeutically effective levels of FIX between 1% and 5% have been maintained for a median of 3.2 years after single peripheral vein vector infusions.46 Follow-up of patients in all of these trials is ongoing.

Challenges to Gene Therapy for Hemophilia

The first obvious fact is that progress with FIX gene therapy has been significantly advanced compared with FVIII. This is because of a combination of limitations presented by FVIII. First, the FVIII cDNA, even with the B domain–encoding region deleted, is ≈4.5 kb, and thus, generation of FVIII transgene constructs that can be efficiently packaged in AAV particles is difficult. Second, achieving high-level expression of FVIII has been notoriously problematic, for reasons that have been only partially explained. Finally, the immunogenic potential of FVIII is significantly greater than that of FIX, and preclinical studies of FVIII gene transfer have frequently been complicated by the development of an anti-FVIII immune response.32,67 Of course, FIX gene therapy is included within a larger group of coagulation disorders that include hemophilia A (FVIII deficiency) and hemophilia B (FIX deficiency). Studies of FIX delivery have not been complicated by the development of an anti-FIX immune response, and in the latest clinical trials, plasma levels of transgenic FIX have been adequate to produce a significant long-term clinical benefit. Nevertheless, the predictability and consistency of these FIX levels are still not well understood.

The most significant problem that has arisen in the recent AAV clinical trials has been the development, in ≈25% of patients, of an anti-AAV capsid immune response that has resulted in transduced hepatocyte cytolysis.36,70,71 This complication is the result of CD8+ cytotoxic T cell responses to AAV capsid peptides being presented on the surface of transduced hepatocytes (Figure 4A). The timing of the subsequent transaminitis that develops has varied from as early as 3 weeks to as late as 10 weeks post vector delivery, probably reflecting either a secondary recall response or a later primary immune response to the AAV capsid. With the aim of minimizing this immunologic problem, patients with evidence of preexisting anti-AAV immunity, as demonstrated by the presence of anti-AAV antibodies, are currently excluded from participation in these clinical trials. This affects different numbers of patients for the different AAV serotypes but ranges from ≈30% of the population with antibodies to AAV8 to ≈60% of the population with preexisting anti-AAV2 immunity.72 Unfortunately, even with the generation of novel AAV capsids, the issue of cross-reactivity of the immune response may still be an obstacle to avoiding the subsequent cellular immune attack.73

With the development of a cytotoxic T cell response to AAV capsid peptides, transduced liver cells are killed and the transgene is lost, thus prevention or rapid intervention of this immune attack is essential to achieve long-term transgene expression. To date, mitigation and eventual extinction of the immune response has been possible with transient corticosteroid administration started after early recognition of liver transaminase increases.45 Although the use of prophylactic immunosuppressive therapy for this complication has been discussed repeatedly, this strategy has not yet been used. Finally, in addition to the cell-mediated immune response to the AAV capsid, a potent humoral response is consistently documented after AAV vector administration that prevents effective repeat AAV delivery. This obstacle to vector readministration might be circumvented by strategies such as infusing capsid decoys to divert the antibody blockade.74

Figure 4. Complications of gene therapy. A. Complications of gene therapy include innate and adaptive immune responses, insertional mutagenesis, and failure of transgene expression. B. Approximately 25% of patients treated with adeno-associated virus (AAV)-mediated gene therapy develop an anti-AAV capsid cell–mediated immune response. Presentation of AAV capsid protein to cognate CD8+ T-cells results in cytolysis of transgene-expressing cells. MHC indicates major histocompatibility complex.
Future Considerations for the Application of Gene Therapy for Coagulation Disorders

After a 30-year period of in vitro experimentation and preclinical assessment, the field of gene therapy is beginning to show robust evidence of clinical benefit in a range of genetic disorders. The recent success of gene transfer for hemophilia B highlights the potential of this therapeutic modality for management of coagulation pathologies. As further evidence of the promise of gene therapy initiatives, the partnership in hemophilia gene therapy trials by the biopharmaceutical industry has increased dramatically in the past 2 years. Nevertheless, before gene therapy can be extended to widespread clinical utility, several critical hurdles need to be overcome. First, is the development of gene therapy vectors that can be produced in large scale with high and reproducible quality. Evidence that the AAV vectors used in a recent FIX clinical trial contained only \( \approx 1\% \) of transgene containing particles illustrates the need for improved and more efficient vector production protocols. With current clinical trials being limited to study populations of 5 to 10 patients, there is a long way to go before the widespread application of gene transfer can be envisaged.

Next is the issue of immune obstacles to successful gene transfer. With levels of preexisting immunity to current AAV-based vectors ranging from 30% to 60%, many otherwise eligible patients are excluded from this form of treatment. Whether AAV capsid modifications or the use of novel AAV serotypes will circumvent this obstacle remains to be seen. Similarly, the use of other vector types, such as lentiviral constructs, would substantially reduce this problem. Aside from the problems posed by immune responses to the vector, immune reactions to the novel transgenic protein may also complicate some applications of gene transfer, particularly when the transgenic protein presents peptide sequences that are novel to the recipient.

Although immediate adverse effects of gene transfer using AAV and lentiviral vectors have been negligible, the long-term outcome of gene therapy will require formal monitoring, particularly for genotoxicity outcomes. Studies performed in small and, more critically, large animal models with greater longevity have not shown any evidence of an enhanced incidence of chronic pathologies (most importantly, no evidence of increased cancer development). Nevertheless, these observations will need to be strengthened by formal long-term, multi-year surveillance in human gene therapy recipients. Finally, the efficacy of gene therapy in contrast to currently available or next-generation infused factor replacement therapies will need to be evaluated in randomized clinical trials.

The promise of genetic therapies for improved management of coagulation disorders is now beginning to be realized. Although gene replacement strategies are the most prominent of these approaches, the application of inhibitory oligonucleotides and small inhibitory RNA molecules to alter nucleotides and small inhibitory RNA molecules to alter the hemostatic balance has demonstrated how other nucleic acid–based strategies have shown considerable potential in recent clinical trials. With enhanced access to genome editing technologies, this momentum toward translational benefits is likely to continue.

Sources of Funding

D. Lillicrap is the recipient of a Canada Research Chair in Molecular Hemostasis. L.L. Swystun is the recipient of a CIHR fellowship.

Disclosures

None.

References


Downloaded from http://circres.ahajournals.org/ by guest on November 26, 2017


individual with severe hemophilia B. Mol Ther. 2006;14:452–455. doi: 10.1016/j.mther.2006.05.004.


Gene Therapy for Coagulation Disorders
Laura L. Swystun and David Lillicrap

Circ Res. 2016;118:1443-1452
doi: 10.1161/CIRCRESAHA.115.307015
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2016 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/118/9/1443

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/