Gene therapies for hemophilia hit the mark in clinical trials

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Two recent studies describe clinical successes for single-dose gene therapy in trials for two forms of hemophilia.

Although the first gene therapy proposals are almost 50 years old[1], the past year has been a coming of age for this field. In March, after approval by the European Medicines Agency the previous year, the first patient was treated with a commercial gene therapy to correct an inherited immunodeficiency by modifying blood stem cells *ex vivo*. In August, a genetically modified cell product was approved for the first time by the US Food and Drug Administration (FDA), comprising engineered T cells for cancer immunotherapy. In November, a patient was treated for a genetic deficiency with *in vivo* genome editing for the first time. That same month, remarkable clinical results of gene therapy for spinal muscular atrophy were published, which saved the lives of the infants treated with this approach[2]. In December, the FDA approved for the first time a gene therapy to correct a genetic disease, in this case a rare form of vision loss. This landmark year for gene therapy crescendoed with two publications in the *New England Journal of Medicine*[3,4] describing clinical successes in trials of gene therapy for two forms of hemophilia (**Fig. 1**).

Hemophilia is an inherited X-linked bleeding disorder resulting from deficiencies in blood clotting factors—coagulation factor VIII in hemophilia A and coagulation factor IX in hemophilia B. Prophylactic intravenous infusion of exogenous coagulation factor is the current standard of care for patients with hemophilia[5]. Frequent infusions, up to three times per week, are necessary to reach levels that maintain adequate hemostasis[5], thus significantly affecting patients' quality of life.

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For sustained factor expression from a single treatment, liver-directed gene therapy using recombinant adeno-associated virus (AAV) vectors[6–9] has been tested but was unsuccessful owing to inefficient factor expression, lack of sustained expression and immune responses directed against the AAV capsid proteins that make up the shell of the viral vector. Continued efforts of gene therapy for hemophilia are focused on using single-vector infusions for durable factor expression that provide clinical benefit while minimally activating immune responses.

For hemophilia A gene therapy, the inefficient expression and large size of factor VIII make the use of AAV vectors challenging. To overcome these obstacles, Rangarajan *et al.*[3] developed the vector AAV5-hFVIII-SQ based on a previous construct[8]. This AAV serotype 5 vector contains a codon-optimized expression cassette for a truncated human factor VIII variant with a hybrid liver-specific promoter. Rangarajan *et al.*[3] infused a single intravenous dose of AAV5-hFVIII-SQ into nine men with severe hemophilia A. A dose-dependent increase in factor VIII activity levels was observed with an accompanying marked decrease in the frequency of participant-treated bleeding episodes and cessation of exogenous factor VIII use. At one year after treatment, all seven participants in the high-dose cohort had sustained therapeutic factor VIII levels in their blood. The only serious adverse event observed was progression of preexisting chronic arthropathy in one participant. A mild asymptomatic increase in serum alanine aminotransferase levels, indicative of an immune response to AAV-modified cells, was observed among participants but resolved without major complication, similar to what has been observed in other AAV-based gene therapy trials[6,9].

In contrast to factor VIII levels in hemophilia A, the effective level of factor IX coagulant activity required to eliminate spontaneous bleeding events in patients with hemophilia B remains unknown. Previous AAV-mediated factor IX gene transfer trials have resulted in short-lived or suboptimal factor expression levels[6,9], possibly owing to dose-dependent immune responses to the AAV vector. To improve factor IX activity while minimizing AAV capsid immune responses, George *et al.*[4] intravenously infused a single low dose of a recombinant AAV vector into ten men with hemophilia B. This AAV vector, SPK-9001, uses a liver-specific human promoter to drive expression of codon-optimized factor IX—R338L that has a naturally occurring mutation known to enhance specific activity[10]. After 28 or 78 weeks, high levels of sustained vector-derived factor IX coagulant activity were observed in all participants and there was a significant reduction in annualized bleeding rates and factor use in participants. Importantly, no serious adverse events were observed following treatment with the low dose of vector. In contrast to the study of Rangarajan *et al.*[3] and other AAV-based gene therapy trials[6,9], elevated asymptomatic alanine aminotransferase levels were only observed in two participants.

Long-term safety and durable factor expression are critical for hemophilia gene therapies. As adverse events may not manifest for several years following gene therapy administration, continued monitoring and reporting will be necessary. Importantly, host immune responses in the form of factor VIII and factor IX inhibitor production were not observed during follow-up testing in either study. However, consistent with results in previous studies, AAV-capsid-specific antibodies did develop in all participants, although AAV-capsid-directed cellular immune responses were not detected in participants from the study by Rangarajan *et al.*[3]

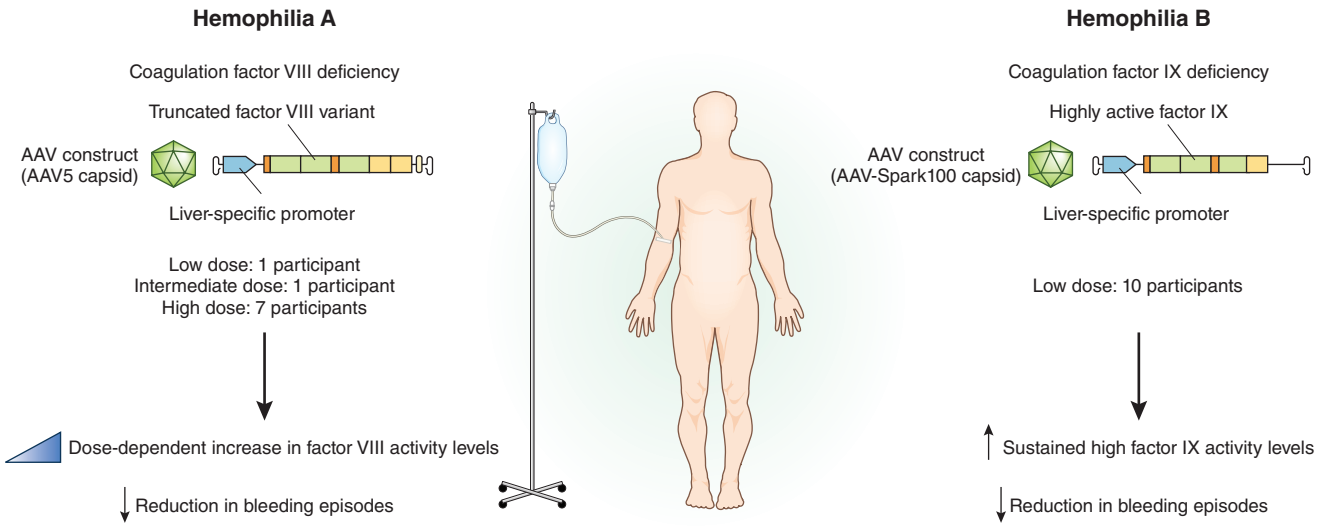

Figure 1 Gene therapy strategies for hemophilia. Rangarajan *et al.*[3] were able to treat hemophilia A with a single high dose of a truncated factor VIII variant. George *et al.*[4] treated hemophilia B with a single dose of a highly active variant of factor IX. There was a significant reduction in the number of bleeding episodes in both groups.

and glucocorticoids were used to safely and successfully treat the AAV-capsid-directed cellular immune responses observed in two participants from the study by George *et al.*[4]. The low frequencies of these immune responses and the ability to control them with steroid treatment are very promising for both these therapies and other AAV-based gene therapies. Nonetheless, results at later time points after treatment will be critical to understand whether expression of the coagulation factors from the AAV vectors is maintained and protected from silencing, degradation or loss via cellular turnover. This will be critical because AAV-directed antibodies would likely prevent successful readministration with the same vector. Likewise, a significant fraction of the patient population has already been exposed to these viral vectors and thus harbors preexisting antibodies. Future improvements to these therapies may incorporate vectors engineered to circumvent preexisting host immunity[11]. Additionally, targeted integration of transgenes encoded by AAV vectors into the genome via gene editing[12], as a strategy to ensure sustained expression following a single vector dose, has recently entered the clinic for hemophilia B and other enzyme deficiencies.

The safety and efficacy demonstrated in these studies are not just promising for the treatment of hemophilia but also contribute to the positive outlook for the gene therapy field in general. This is particularly true in light of the other recent tremendous gene therapy successes, including AAV-based treatments in the eye and central nervous system[2]. Given the recent success and growing interest from both academic and commercial sectors, there is no reason to expect the pace of advances to slow in the near future.

COMPETING FINANCIAL INTERESTS
The authors declare competing financial interests: details are available in the online version of the paper.

1. Sinsheimer, R.L. *Am. Sci.* **57**, 134–142 (1969).
2. Mendell, J.R. *et al. N. Engl. J. Med.* **377**, 1713–1722 (2017).
3. Rangarajan, S. *et al. N. Engl. J. Med.* **377**, 2519–2530 (2017).
4. George, L.A. *et al. N. Engl. J. Med.* **377**, 2215–2227 (2017).
5. Srivastava, A. *et al. Haemophilia* **19**, e1–e47 (2013).
6. Manno, C.S. *et al. Nat. Med.* **12**, 342–347 (2006).
7. Jiang, H. *et al. Blood* **108**, 107–115 (2006).
8. McIntosh, J. *et al. Blood* **121**, 3335–3344 (2013).
9. Nathwani, A.C. *et al. N. Engl. J. Med.* **371**, 1994–2004 (2014).
10. Simioni, P. *et al. N. Engl. J. Med.* **361**, 1671–1675 (2009).
11. Maheshri, N., Koerber, J.T., Kaspar, B.K. & Schaffer, D.V. *Nat. Biotechnol.* **24**, 198–204 (2006).
12. Sharma, R. *et al. Blood* **126**, 1777–1784 (2015).