Molecular Therapy
Editorial

First hemophilia B gene therapy approved: More than two decades in the making

Prevention of bleeds in patients with the severe form of the X-linked bleeding disorder hemophilia B requires frequent intravenous injections of coagulation factor IX (FIX) protein. Gene therapy has the potential to accomplish sustained correction of the bleeding tendency following a single drug administration. In the late 1990s, a body of preclinical work rapidly evolved, demonstrating the ability of adeno-associated viral (AAV) gene transfer to skeletal muscle or liver to generate lasting therapeutic levels of FIX in small and large animal models of the disease. Now, roughly 25 years after the initial demonstration that gene therapy could potentially cure hemophilia B in animals, the first gene therapy drug for hemophilia B has obtained regulatory approval in the United States: Hemgenix (*etranacogene dezaparvovec*). Hemgenix is an AAV5 vector expressing the hyper-active FIX-Padua variant for hepatic gene transfer in adult patients. This CSL Behring gene therapy product (initially developed by UniQure) was approved by the Food and Drug Administration (FDA) based on safety and effectiveness in studies of adult men with severe or moderately severe disease. It is currently the most expensive drug in the world with a price tag of $3.5 million. In clinical trials, FIX activity increased to nearly the normal range 2 years after vector infusion, consistent with decreased incidence of bleeding (annualized bleeding rates, ABR), and approximately 95% of treated patients remained off prophylaxis (i.e., did not require FIX protein infusions to prevent bleeds). The availability of the first FDA-approved gene therapy product for hemophilia B offers a new treatment option for patients afflicted by this bleeding disorder. This is also the first FDA-approved liver-directed gene therapy product, thus having broad implications for the field at large.

Following the initial preclinical proof of concept studies in mouse models, our subsequent observations demonstrated a sustained therapeutic effect in hemophilia B dogs lasting for more than a decade.[1] This and other preclinical studies paved the way toward several early phase I gene therapy trials that were based on either muscle- or liver-directed AAV2 gene delivery in patients with severe hemophilia B. In 1999, the first hemophilia B patient was injected via multiple intramuscular sites with an AAV2 vector expressing FIX. Muscle-targeted AAV gene therapy was safe and resulted in persistent FIX expression in the skeletal muscle that lasted for 10 years but failed to reach the desired efficacy. Further, muscle gene transfer had an increased risk of antibody formation against FIX in animal studies, so that only patients with missense mutations were included in the trial. Patients with other mutations could be included in the more tolerogenic approach of gene transfer to hepatocytes, which also represents the normal site of FIX biosynthesis.[1,2]

Hepatic gene transfer was successful in patients lacking pre-existing humoral immunity to AAV and resulted in FIX expression up to 12% of normal levels. However, FIX levels subsequently declined, which was associated with an unexpected complication due to apparent $CD8^+$ T cell responses against the viral capsid in humans. The significance of these first gene therapy trials for hemophilia B (reflecting a collaboration between The Children's Hospital of Philadelphia, Stanford University, and Avigen) reaches far beyond hemophilia, as they pioneered both muscle-directed and hepatic gene transfer with AAV vectors. A subsequent trial out of St. Jude's Children's Hospital and University College London changed the serotype to AAV8 and depleted the FIX coding sequence of CpG motifs to reduce TLR9 activation. The investigators further added transient immune suppression in cases when an increase in liver enzyme levels was observed. These approaches have since been adapted in many other trials. Sustained expression of ~5% (for at least 8 years) was accomplished and was sufficient to prevent most bleeds.[1,2]

A seminal discovery greatly facilitated further development of AAV gene therapy for hemophilia B: a pro-thrombotic, naturally occurring, single amino acid substation was discovered in an Italian family.[3,4] FIX-Padua exhibits an almost 1-log increase in FIX enzymatic activity. While a major health problem for individuals who carry this mutation, this finding has been a blessing for gene therapy. Following our initial demonstration that it yielded superior FIX activity levels after liver-directed gene therapy in preclinical models, one can now achieve the desired coagulation activity in hemophilic patients using much reduced vector doses, thereby simplifying manufacturing and, importantly, reducing risks of cellular immune responses, toxicities, and vector integration. In particular, use of this FIX-Padua variant enabled development of a more effective AAV5 vector that constitutes the basis of *etranacogene dezaparvovec*, raising FIX activity to 37% of normal levels at 24 months after vector infusion, regardless of the presence of pre-existing AAV5 neutralizing antibodies (NAbs) titers up to a certain titer. These elevated FIX levels were associated with a significant decrease of ABR to 54% compared with the 6-month lead-in period on FIX prophylactic replacement therapy. Most importantly, this obviated the need for prophylaxis in 94% (51 out of 54) of treated patients. The most common side effects observed in this pivotal trial were liver enzyme elevations presumably due to inflammatory reactions, mandating the use of transient immune suppression with corticosteroids. Such elevations of liver enzyme levels have been frequently observed in gene therapy trials that depend on the systemic administration of high AAV vector doses, including the pioneering liver-directed gene therapy trials for hemophilia B. This may be due, at least in part, to $CD8^+$ T cell

responses against the viral capsid, though other immune mechanisms may also play a role.

While this AAV5 serotype is not the most efficient vector to transduce human hepatocytes, it has some of the lowest pre-existing immunity in humans. In addition, these antibodies rarely show strong neutralizing activity in humans, which increases the number of candidates to receive the treatment, overcoming one of the critical matters involving eligibility. A similar drug from Pfizer (initially developed by Spark Therapeutics) is currently undergoing further testing in Phase III trials. It utilizes an engineered capsid and also expresses FIX-Padua. Both drugs are given intravenously, taking advantage of liver tropism of the viral capsid and utilizing hepatocyte-specific promoters, albeit the Pfizer drug appears to have a dose advantage. Another difference is the production platform, as the CSL Behring product is manufactured in insect cells using the baculovirus system, while the Pfizer vector is produced in transiently transfected mammalian cells. A baculovirus-produced AAV5 vector (developed by BioMarin) expressing factor VIII in hepatocytes for treatment of hemophilia A, the more common form of the disease, has recently received conditional approval in Europe and is under review for approval in the United States.[5]

One potential limitation of the latest generation AAV-FIX-Padua gene therapy vectors, including the FDA-approved *etranacogene dezaparvovec*, pertains to the substantial inter-patient variability. The underlying mechanisms that account for this variability are poorly understood. Consequently, the therapeutic outcome for each individual treated patient cannot be predicted, and some patients may end up with relatively low FIX levels that still puts them at risk of trauma-induced bleeds and arthropathy. Hopefully, the vector and/or protocol will further evolve to minimize inter-patient variability and increase predictability of gene transfer.

While data on durability of FIX expression are encouraging, it remains to be seen if AAV-based gene therapies represent a lifetime cure or may wane at some point. Vector re-administration is complicated by the persistence of high-titer neutralizing antibodies induced by the AAV injection. Ironically, gene therapy for hemophilia had been pioneered by pediatric institutions, but for now, it is only performed in adult patients. More experience with long-term risks may be prudent before considering pediatric patients. In addition, AAV vector genomes predominantly exist in episomal forms, so that therapy would likely diminish in children with a growing liver. Precise integration into hepatocytes chromosomal DNA using gene editing may potentially overcome this limitation in the future.

Though conventional FIX protein replacement therapy has been improved by products with extended half-life, patients continue to require biweekly injections to prevent bleeds. Consequently, obviating the need for any injections would substantially improve the patient's quality of life. Although the suggested cost of the gene therapy drug may limit its access, the concept of a one-time treatment remains an attractive option for countries in which factor products are not readily available to many patients. Even considering the potential life-long cost-effectiveness benefits, only a suitable payment or alternative reimbursement model will ultimately increase world-wide access to this attractive new treatment option by gene therapy.

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