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Molecular Therapy

A Cure for Hemophilia: The Promise Becomes a Reality

In the late 1990s, animal studies employing adeno-associated viral (AAV) gene transfer offered the promise that gene therapy could someday be used to treat patients with the bleeding disorder hemophilia. These studies in mice and dogs with hemophilia B (deficiency of coagulation factor IX, FIX) quickly led to a first trial in humans. However, two decades of cycling back and forth between the laboratory and the clinic, including multiple clinical trials, were needed to get it right in patients. At long last, the lead investigators of two clinical trials have reported spectacular successes with AAV gene transfer for both hemophilia A (deficiency of factor VIII, FVIII) and hemophilia B at the World Federation of Hemophilia (WFH) Congress (Orlando, Florida, July 2016).

To treat hemophilia B, Lindsey George (The Children's Hospital of Philadelphia) and her collaborators from Spark Therapeutics (Philadelphia) delivered a version of FIX that has approximately eightfold higher biological activity than that of the wild-type molecule. They administered an engineered AAV capsid and a vector dose of merely 5×10^{11} vector genomes (vg)/kg, and achieved sustained circulating levels of 20–40% of normal coagulation activity in all four treated patients without toxicity. No subsequent bleeding episodes occurred in these patients, and a case of recurring joint bleeding was resolved. Although <50% of normal coagulation activity is still considered mild hemophilia, levels of ~30% should confer a mostly normal life, with a requirement of some additional supplementary FIX concentrate only after severe trauma or for surgery.

In the hemophilia A trial (sponsored by BioMarin Pharmaceutical Inc., San Rafael, CA), six of seven patients treated by John Pasi and colleagues (Royal London Hospital, UK) achieved normal or even superphysiological levels of FVIII and consequently no longer experienced bleeding episodes in the absence of FVIII protein administration. This was achieved at the highest vector dose of 6×10^{13} vg/kg, using an AAV5 vector to deliver a codon-optimized complementary DNA expressing B domain–deleted FVIII. The remarkable outcomes of both trials have exceeded expectations and promise a future, in which—thanks to gene therapy—hemophilia will no longer exist in most adult patients of the developed world.

This is in stark contrast to the modest goal of the initial trials in the 1990s, which was to elevate factor levels to >1% of normal to change the disease phenotype from severe to moderate with the hope of reducing the frequency of bleeding episodes. Both current trials are based on targeting hepatocytes for therapeutic gene expression. An AAV vector with a hepatotropic capsid containing a single-stranded DNA genome was infused intravenously, and gene expression is controlled by a hepatocyte-specific promoter. As hemophilia is X-linked, the patients enrolled in these studies are all male. Hepatocytes are the natural site of FIX expression, whereas FVIII is normally expressed by liver endothelial cells. However, hepatocytes are generally a good target, because they are abundant and efficient in secreting proteins into the blood (which is essential for treatment of hemophilia). Furthermore, optimized transgene expression in hepatocytes tends to induce immune tolerance.

Nonetheless, prior trials with AAV2 and AAV8 serotype vectors were hampered by vector dose–dependent $CD8^+$ T-cell responses to the viral capsid, causing hepatotoxicity and loss of expression. Transient immune suppression was successfully applied to solve this problem in some but not all studies. It appears that this problem can be entirely circumvented by using low vector doses—made possible here through optimization of the vector and incorporation of a more highly active version of FIX. For the hemophilia A trial, the researchers adapted a different strategy. They packaged a codon-optimized FVIII sequence into AAV5, a capsid that has reduced homology to those typically found in humans. Therefore, AAV5 may be less prone to activating memory lymphocyte responses. This may be further supported by a third clinical trial (sponsored by UniQure, Amsterdam, the Netherlands). Wolfgang

Miesbach (University Hospital Frankfurt, Germany) reported average FIX levels of ~5% of normal in five hemophilia B patients who were treated with an AAV5 vector at 5×10^{12} vg/kg. Only one patient showed elevated liver enzyme levels and was treated with steroid drugs. However, even when these results are adjusted with the consideration that this study used the wild-type instead of the enhanced-activity version of FIX, an approximately 10-fold dose disadvantage is apparent compared to the trial at Penn.

This outcome may be due to a lower transduction efficiency of AAV5 in human hepatocytes, as suggested by a recent article in *Molecular Therapy*.[1] Therefore, higher vector doses are required to achieve therapy. Given that FVIII is typically less efficiently expressed than FIX is, Pasi and colleagues used very high doses of 6×10^{13} vg/kg in the hemophilia A trial. Here, very mild elevations of liver enzyme levels were observed, and thus steroid drugs were administered. However, the reason for mild liver toxicity (which may not be immune mediated but still related to the high vector doses) is unclear. Only one patient had previously been treated at a threefold lower dose of 2×10^{13} vg/kg. He achieved substantially lower levels of 2–5% of normal. This discrepancy in the dose response remains to be resolved. An optimal vector dose would result in FVIII levels near normal but not superphysiological, with no or minimal/manageable toxicity. Thus far, the patients discussed here have been followed for up to 8 months. Long-term follow-up will establish whether this cure will last and whether factor levels will be stable. Encouragingly, hepatic AAV gene therapy in hemophilia A and B dogs directed stable levels for well over a decade.

It has been estimated that a lifetime treatment of a patient with severe hemophilia amounts to a cost of US$25–50 million when using recombinant protein products. Gene therapy should be a much more cost-efficient alternative. Moreover, gene therapy may hold the key to treat hemophilia in the third world, where very few could afford these expensive protein drugs. Indeed, the majority of hemophilic patients worldwide currently are untreated. Hemophilia A is the more prevalent form of the disease and thus will require further improvements of the vector technology to be treatable at vector doses that are feasible to produce for large populations. At the same time, additional questions must be addressed for further development of this technology in the developed world as well. Currently utilized AAV capsids have low seroprevalence in the population, but patients with preexisting neutralizing antibodies nonetheless exist. Alternative capsids will need to be developed to enable giving gene therapy to these patients. Assuming that gene therapy in adult patients will be deemed safe, treatment of children may be considered so as to eliminate the disease earlier in life. Loss of episomal AAV vector genomes is a concern in a growing liver, and the risk of antibody (inhibitor) formation against the FVIII or FIX antigen is less clear in young patients with limited exposure. However, cautiously beginning by treating older pediatric patients may be a path to proceed initially. In any case, a cure for hemophilia by gene therapy is no longer merely a promise; it is a reality.

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REFERENCE

1 Vercauteren, K, Hoffman, BE, Zolotukhin, I, Keeler, GD, Xiao, JW, Basner-Tschakarjan, E *et al.* (2016). Superior *in vivo* transduction of human hepatocytes using engineered AAV3 capsid. *Mol Ther* **24**: 1042–1049.