Hemophilia B Gene Therapy in Humans Shows Promise

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Study Hypothesis

The initial hope that gene therapies might treat many diseases has not been largely realized, in part, owing to limitations of delivery mechanisms, adverse effects of therapies, and failure to produce results superior to standards of care. Hemophilia B, a known X-linked deficiency of the factor IX gene (FIX), results from low FIX levels and leads to increased mortality and substantial morbidity, including crippling chronic joint disease, requiring frequent and expensive prophylactic or acute protein therapy. This study hypothesized that the use of a single injection of a modified adeno-associated virus (AAV) vector expressing FIX could establish long-term therapeutically relevant levels of this blood protein in men affected with hemophilia B.

How Was the Hypothesis Tested?

AAV vectors allow for the delivery of transgenes and may have superior profiles to other targeting strategies, particularly, owing to muted immune responses, though it can still be challenging to obtain therapeutic doses without triggering host immune responses. The authors previously designed a FIX AAV vector (scAAV2/8-LP1-hFIXco) with several features, including a liver-specific promoter, designed self-complementary in a tail-to-tail dimer, to increase transcriptional activation and transduction efficiency, and codon optimization for sequences frequently found among highly expressed eukaryotic genes. Additionally, the AAV2 vector was pseudotyped with an AAV8-capsid to blunt immune responses, since AAV8 has lower seroprevalence in humans than AAV2. Administration of this vector in primates demonstrated sustained FIX expression at presumed therapeutic levels, with enhanced expression in hepatocytes, an important target for FIX expression, and a favorable safety profile in mice and primates. In the current study, 6 men with severe hemophilia B were enrolled (FIX activity, <1% of normal values) in a combined Phase 1 and 2 trial (ClinicalTrials.gov number NCT00979238) and received a single peripheral vein infusion of the vector expressing the FIX transgene (scAAV2/8-LP1-hFIXco). There were 2 participants enrolled in each of 3 dose categories: low (2×10^{11} vg/kg), intermediate (6×10^{11} vg/kg), and high (2×10^{12} vg/kg). Initial administration of vector did not include coadministration of immunosuppressive therapy. Patients were followed for 6 to 16 months. Plasma FIX activity, anticapsid and anti-FIX antibody levels, vector shedding and cellular immune responses, vital signs, and laboratory chemistries were collected frequently. Efficacy was defined as persistence of active FIX at 3% or greater of normal levels.

Principal Findings

Two patients with low-dose treatment achieved about 1% to 2% of normal FIX activity, patients with the intermediate dose (3× higher) averaged about 2.5% to 3% of normal FIX activity, and patients with the high dose (10× higher than low dose) achieved about 7% of normal activity. Measurable expression levels of FIX were observed in all patients for more than 6 months, indicating ongoing endogenous synthesis, and FIX protein therapy was eliminated (2 patients with high-dose treatment and 1 patient with intermediate-dose treatment), other than in response to acute injuries, or substantially reduced (3 other patients).

Between 49 to 58 days postinfusion, Participant 5 in the high-dose group experienced elevated aminotransferase levels, deemed a Grade III serious adverse event. After ruling out other potential explanations for the liver injury, an immunologic response to the scAAV2/8-LP1-hFIXco treatment was suspected, and the patient received prednisolone, starting on day 58. Liver enzyme levels returned to normal ranges, prednisolone was tapered, expression of FIX persisted at...
about a 3% level, and the patient remained free of spontane-
ous hemorrhage more than 6 months.

Immunologic results in all patients indicated no responses
to the FIX transgene product, but a primary immune response
to AAV8 was observed. AAV8 capsid-specific T cells in
peripheral blood mononuclear cells were significantly elev-
ated among those in the intermediate- and high-dose groups.
Major (Participant 5) and minor (Participant 6) elevations in
liver enzyme levels among the high-dose group suggest the
AAV8 response may have represented immune-mediated
destruction of transduced hepatocytes. Both patients received
prednisolone, liver enzymes returned to normal, and capsid-
specific T cells were no longer detected, whereas FIX
expression remained over subsequent months, suggesting that
transduced hepatocytes may have cleared the AAV8 capsid
peptide through proteolytic degradation and escaped T cell
clearance.

**Implications**

This study represents a major gene therapy advance as the
first to achieve long-term blood protein expression at thera-
apeutically relevant levels. Substantial cost reductions were
already achieved with decreased prophylactic therapy. Fur-
ther studies in larger samples are needed to understand
whether the approach is safe, appropriate dosing, and if there
are important contributing factors to therapeutic course, such
as prior exposure to AAV and individual variation in antigen
processing and presentation. If the approach proves safe and
efficacious, it could lead to a major shift in hemophilia B
treatment, including in developing nations where prophylaxis
is often not feasible, and may indicate a promising strategic
path for the treatment of additional genetic disorders.

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**Disclosures**

None.
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