Highlights in Gene Therapy and Alternative Therapies in Nonmalignant Hematology From the 2016 American Society of Hematology Meeting

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Autologous Gene Therapy Feasible for Use in Severe Hemoglobinopathies

An experimental autologous gene therapy called LentiGlobin is feasible for use in patients with severe hemoglobinopathies, according to interim results from 2 phase 1/2 trials. The HGB-205 trial included 1 patient with severe sickle cell disease (SCD) and 4 patients with transfusion-dependent ß-thalassemia (TDT); the HGB-206 trial included 7 patients with severe SCD.

All patients underwent bone marrow harvest (for SCD) or mobilization and apheresis (for TDT) to obtain autologous CD34-positive cells. These cells were transduced with the BB305 lentiviral vector, which encodes a β-globin gene that confers antisickling properties. After myeloablative conditioning with busulfan, patients received a single infusion of the transduced cells.

The HGB-205 trial included adolescents and adults with severe SCD or TDT. The patient with SCD was 13 years old, and the 4 patients with TDT ranged in age from 16 to 19 years. In a poster, Dr Marina Cavazzana of the *Imagine* Institute, Hôpital Universitaire Necker-Enfants Malades - APHP in Paris, France, presented results from a median follow-up of 20.8 months after gene therapy.

Engraftment was successful in all participants, with a median time to neutrophil engraftment of 17 days. Vector copy number in the peripheral blood generally remained consistent from month 3, with a range of 0.2 to 3.4 copies per diploid genome. No grade 3 or 4 adverse events related to gene therapy occurred, and no evidence of clonal dominance was observed.

After 18 months of follow-up, the patient with SCD had no clinical symptoms or complications of SCD—even though transfusions had been discontinued 3 months after gene therapy. Compared with baseline values, the unconjugated bilirubin level dropped from 50 to 11 µmol/L, lactate dehydrogenase dropped from 626 to 287 U/L, and the reticulocyte count dropped from 238 × 10^9 to 132 × 10^9/L.

After 12 to 36 months of follow-up, the 4 patients with TDT exhibited ongoing transfusion independence and sustained production of therapeutic hemoglobin (HbA^{T87Q}). Two patients had stable total hemoglobin levels above 10 g/dL, and 1 patient no longer required iron chelation therapy. The fourth patient had been transfusion-free for 9 months.

The HGB-206 trial included adults with a history of symptomatic SCD, adequate organ function and performance status, and no previous hematopoietic stem cell transplant or gene therapy. Dr Julie Kanter of the Medical University of South Carolina in Charleston presented data from 7 study participants (median age, 26 years; range, 18-42 years), 4 of whom had been followed for at least 6 months after treatment. Engraftment was successful in all patients, with a median time to neutrophil engraftment of 22 days (range, 17-29 days). After a median follow-up of 7.1 months, no grade 3 or 4 adverse events related to gene therapy had occurred, and no evidence of clonal dominance or replication-competent lentivirus was found. Grade 3 adverse events related to bone marrow harvest occurred in 5 participants; these included pain, anemia, and vaso-occlusive crisis, all of which resolved with standard treatment.

Dr Kanter and colleagues found that therapeutic hemoglobin (HbA^{T87Q}) was expressed in all participants; the median level at 3 months was 0.4 g/dL. In the 2 participants with the longest follow-up (9 months), the HbA^{T87Q} levels were 0.31 and 1.2 g/dL. Of the 4 participants with at least 6 months of follow-up, 3 had fewer vaso-occlusive crises after treatment than before the study began, although this effect may have been related to the brief follow-up, the effects of busulfan, or transfusions of red blood cells after treatment.

The researchers noted that the median BB305 vector copy number dropped from 0.6 copies per diploid genome in the gene product to .08 copies per diploid genome in the peripheral blood, which would explain the relatively low expression of HbA^{T87Q} in HGB-206 compared with HGB-205. Dr Kanter explained that changes have been made to the manufacturing and treatment protocol to increase HbA^{T87Q} production. HGB-206 is still enrolling patients, with a target enrollment of 29 patients.

Ribeil JAM, Hacein-Bey-Abina S, Payen E, et al. Update from the Hgb-205 phase 1/2 clinical study of LentiGlobin gene therapy: sustained clinical benefit in severe hemoglobinopathies [ASH abstract 2311]. *Blood*. 2016;128(22)(suppl).

Kanter J, Walters MC, Hsieh MM, et al. Interim results from a phase 1/2 clinical study of LentiGlobin gene therapy for severe sickle cell disease [ASH abstract 1176]. *Blood*. 2016;128(22)(suppl).

Commentary: These studies indicate that gene therapy is rapidly advancing as a potential alternative treatment modality for common hemoglobinopathies that are associated with significant clinical complications. Although gene therapy may not become the treatment of choice for all patients with SCD or TDT, these reports suggest that the phenotype of severe disease can be modified safely and effectively in selected individuals. These data should reassure patients of the short-term (and hopefully long-term) safety of gene therapy, and encourage them to participate in further clinical trials.

Only time will tell if these early promising successes in gene therapy will lead to meaningful improvements in quality of life and overall survival. Now is the time for hematologists, health economists, ethicists, and patients to determine which types of hemoglobinopathies should be eligible for gene therapy. Gene therapy is expected to become increasingly successful and pervasive in the treatment armamentarium.

Gene Therapy Boosts Factor IX Activity in Hemophilia B

An investigational gene therapy product called SPK-9001 effectively boosts levels of factor IX in patients with hemophilia B, according to a small study. Patients who received the treatment, which employs a liver-directed recombinant adeno-associated viral vector, were able to discontinue their factor IX infusions entirely or nearly entirely.

Dr Lindsey A. George of the Children's Hospital of Philadelphia in Philadelphia, Pennsylvania, and colleagues administered a single dose of SPK-9001 to 9 men (aged 18-52 years) with moderate to severe hemophilia B. Participants had a baseline factor IX:C level of no more than 2%, a Spark100 neutralizing antibody (NAb) titer of less than 1:5, no history of inhibitory antibodies, and no evidence of liver fibrosis more advanced than stage 2. They were either being treated prophylactically for bleeding events or were experiencing arthropathy or 4 or more bleeding events per year.

After 12 to 52 weeks of follow-up, the mean steady-state factor IX activity was 28.3% (±10.0%) of normal, and the number of bleeding events was significantly reduced from baseline. Grade 1 transaminase toxicity occurred in 1 patient, but there were no unexpected adverse events related to the agent or the procedure. One patient received an infusion of factor IX in response to a suspected ankle bleed; this was the only instance of a patient experiencing a bleeding event or requiring factor IX. No patients developed inhibitory antibodies. Two patients received corticosteroids to treat immune responses to the treatment.

Dr George said that the study patients' need for factor IX was reduced 99% compared with their need during the 52 weeks before gene therapy, which represents a cost savings of more than $2 million over the cumulative follow-up of 238 weeks.

The low dose of gene therapy used in this study (5×10^{11} vg/kg), which was chosen to minimize immune responses requiring the use of corticosteroids, did not reduce sustained factor IX expression compared with previous studies. It also produced the highest levels of sustained factor IX activity following gene transfer.

SPK-9001 has received breakthrough therapy designation from the US Food and Drug Administration.

George LA, Sullivan SK, Giermasz A, et al. Spk-9001: adeno-associated virus mediated gene transfer for hemophilia B achieves sustained mean factor IX activity levels of >30% without immunosuppression [ASH abstract 3]. *Blood*. 2016;128(22)(suppl).

Commentary: This abstract was one of several presented at the meeting that highlighted the recent successes of gene therapy in modulating the bleeding phenotype in individuals with hemophilia B. Similar successes are also emerging for hemophilia A. These findings suggest that eventually, these hemophilias can be "cured."

Now that gene therapy approaches to disease treatment are emerging, hematologists are poised to become the gene therapists of the future. Hemophilias have been ideal test cases for the development of gene therapy because hematologists have been successful at characterizing the genetic defects (genotypes) and quantitating the phenotypes in these diseases. They also have developed a network of comprehensive care centers to monitor the effectiveness of various treatment modalities, along with their economic impact.

Of course, gene therapy may not be the best choice for all individuals with hemophilia. The emergence of clotting factor replacement products with an extended half-life and the implementation of prophylaxis treatment regimens have minimized the need for a gene therapy cure, particularly for those with hemophilia B. At the same time, gene therapy may be very useful in subsets of patients with hemophilia A or B who are at elevated risk of developing inhibitory antibodies (based on such factors as family history, the presence of large gene deletions, and the presence of nonsense mutations) or whose genotype manifests as a very short plasma circulating time for their specific replacement therapy.

As advances in hemophilia continue, we must remember that less than 10% of affected individuals globally receive standard of care treatment. This disparity raises ethical considerations—who should be eligible for very expensive treatments? We also need to answer many questions regarding the long-term effects of gene therapy, which may include oncogenesis, hepatic toxicity, and vector-induced immunogenicity. How should we proceed if the effectiveness of gene therapy dissipates? Can levels of coagulation factor activity be modulated in vivo to prevent development of hypercoagulability in the long term? These and other questions remain to be answered.

RNA Interference Agent Lowers Antithrombin Levels in Hemophilia

Fitusiran appears to be an effective agent for lowering antithrombin (AT) levels in patients with hemophilia A or B who have inhibitory antibodies, according to a phase 1 study. Fitusiran is an experimental agent that harnesses RNA interference.

The study, which was presented as a poster by Dr K. John Pasi of Barts Health NHS Trust in London, United Kingdom, enrolled 16 patients aged 21 to 65 years with severe hemophilia A or B and inhibitory antibodies. The patients received a monthly dose of subcutaneous fitusiran; 6 patients (average age, 32 years) received a 50-mg dose and 10 patients (average age, 37 years) received an 80-mg dose.

After follow-up ranging from 43 to 147 days, the mean AT level was reduced by approximately 80%. More than half the patients (56%) had no bleeds, and more than two-thirds (69%) had no spontaneous bleeds during follow-up. The 80-mg dose of fitusiran appeared to be more effective at preventing bleeds, with a 70% rate of no bleeds and a 90% rate of no spontaneous bleeds. The median annualized rate of bleeding was 0% for all patients, compared with 31% in the period before the study.

All adverse events were mild or moderate and included injection site reactions and cough. Alanine aminotransferase increases to more than 3 times the upper limit of normal, all of which were asymptomatic and reversible, developed in 3 patients. D-dimer increases occurred in some patients, but none were clinically significant. There were no thromboembolic events, and no patients developed antibodies to fitusiran or discontinued it use owing to adverse events. All bleeding events were successfully managed with bypassing agents.

Seven patients who completed phase 1 of the study have transitioned to a phase 2 open-label extension, and they have continued to tolerate the agent well after up to 7 months of continuous dosing.

Pasi KJ, Georgiev P, Tim Mant, et al. Fitusiran, an investigational RNAi therapeutic targeting antithrombin for the treatment of hemophilia: updated results from a phase 1 and phase 1/2 extension study in patients with inhibitors [ASH abstract 1397]. *Blood*. 2016;128(22)(suppl).

Also see: Ragni MV, Georgiev P, Mant T, et al. Fitusiran, an investigational RNAi therapeutic targeting antithrombin for the treatment of hemophilia: updated results from a phase 1 and phase 1/2 extension study in patients without inhibitors [ASH abstract 2572]. *Blood*. 2016;128(22)(suppl).

Commentary: The genetic inheritance of AT deficiency is known to produce hypercoagulability, and case reports of patients with severe hemophilia A support the finding that coinheritance of AT deficiency results in a very mild bleeding phenotype.

These observations suggest that reducing AT activity in patients with severe hemophilia by interfering with its synthesis in the hepatocyte may lead to reduced bleeding and reduced use of replacement clotting factor concentrates. The remarkable results of the phase 1 and 2 trials that employ small interfering messenger RNA (mRNA) technology to reduce circulating AT activity in vivo provide a proof of principle for this approach. No excessive thrombogenicity has been noted, although these patients have yet to experience intrinsically hypercoagulable states such as septicemia, extensive surgeries, and cancer. The availability of AT concentrates may be able to mitigate these risks, but more studies are necessary to determine the long-term safety of this therapy. This research is also teaching us more about the role of AT as a naturally occurring serine protease modulator of coagulation in vivo, because the hemophilia A cohort has not yet developed any thrombophilic complications despite levels of AT that are traditionally associated with the development of venous thromboembolism. We look forward to seeing results from the phase 3 trials that soon will be initiated.

Givosiran Shows Clinical Activity in Acute Hepatic Porphyria

Interim results from a randomized phase 1 trial demonstrate that the investigational RNA interference agent givosiran (ALN-AS1) can reduce porphyria attacks and hemin use in patients with acute hepatic porphyria. Givosiran reduces the production of aminolevulinic acid (ALA) and porphobilinogen (PBG), which are responsible for attacks, by targeting ALA synthase 1 (ALAS1) in the liver.

Dr Eliane Sardh of the Karolinska University Hospital in Stockholm, Sweden, presented the results of the 3-part study on a poster. The first 2 study parts were conducted in asymptomatic patients with acute intermittent porphyria (AIP), who received either a single ascending dose of givosiran (n=20) or multiple ascending doses of givosiran (n=8). As previously reported, parts 1 and 2 of

the study found that givosiran was generally well tolerated and led to mean reductions of 95% in urinary PBG and of 86% in urinary ALA.

In the third part of the study, 16 patients with AIP with recurrent attacks are receiving multiple doses of givosiran over a 6-month treatment period. Unblinded data from cohort 1 (n=4; 2.5 mg/kg given once quarterly) showed a mean reduction of 74% in the annualized rate of attacks, a mean reduction of 75% in the annualized number of hemin doses, and a 10.5-time longer maximum attack-free interval (mean increase in length, approximately 82 days) compared with the run-in period. Aggregated blinded data from cohort 2 (n=4; 2.5 mg/kg given monthly) provided additional evidence of clinical activity, with a 50% mean reduction in hemin doses. No drug-related serious adverse events or discontinuations caused by adverse events occurred. One fatal case of acute pancreatitis complicated by a pulmonary embolism occurred that was unlikely to be related to the study drug. Further results from the third part of the phase 1 study will be presented in 2017. Following the treatment phase, all patients are eligible to receive givosiran in an open-label extension study.

A phase 3 study with givosiran is planned for late 2017.

Sardh E, Harper P, Al-Tawil N, et al. Interim data from a randomized, placebo controlled, phase 1 study of ALN-AS1, an investigational RNAi therapeutic for the treatment of acute hepatic porphyria [ASH abstract 2318]. *Blood*. 2016;128(22)(suppl).

Commentary: The porphyrias have always been a challenge to diagnose and treat. In most cases, the consequences of the disease ultimately bring the patient to the attention of the hematologist. Givosiran is an investigational mRNA interference therapeutic that targets ALAS1 for the treatment of acute hepatic porphyrias. It works very much like fitusiran does for hemophilia (described earlier). The phase 1 results for porphyria show the promise and power of small interfering mRNA technology to target the synthesis of key proteins, which can modulate the phenotypes of many other hematologic diseases. Because hematologists will be the primary purveyors of such therapy, more hematologists need to become refamiliarized with the basic pathology of these rare diseases.

Also of Note

Park SH, Lee CM, Deshmukh H, Bao G. Therapeutic Crispr/Cas9 genome editing for treating sickle cell disease [ASH abstract 4703]. *Blood*. 2016;128(22)(suppl).

Adair JE, Becker PS, Chandrasekaran D, et al. Gene therapy for Fanconi anemia in Seattle: clinical experience and next steps [ASH abstract 3510]. *Blood*. 2016;128(22)(suppl).

Johnson BA, Chauhan AK, Staber JM. Long-term expression of von Willebrand factor via piggyBac-mediated gene transfer [ASH abstract 3511]. *Blood*. 2016;128(22)(suppl).

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