



PHOTODISC

One of the landmarks in the field of gene therapy has been the initiation of three human clinical trials. It should be noted that all of these trials are in very early stages (phase 1).

As summarized in Table 3, phase I studies are not designed to assess whether the treatment works (*efficacy*) but instead to examine the safety, dosage and timing of a new therapy. Only a few patients (usually those with a severe form of a disease or those who have failed other treatment options), are typically involved in phase I studies. Phase II studies are somewhat larger, and may be combined with phase I trials (referred to as phase I/II trials). Although safety remains the main focus in phase II trials, efficacy is also assessed at this stage. If acceptable results are obtained in phase I and II trials, phase III clinical trials may be initiated. These are the large studies required for approval by the US Food and Drug Administration (FDA). Phase III trials not only continue the examination of safety and efficacy, but also address whether a new treatment is better than the current standard of care with respect to efficacy, side effects or impact on the patient's quality of life. For patients with hemophilia, the current standard of care is considered to be factor replacement therapy.

Gene Therapy Trials and Safety

The gene therapy community was rocked in the fall of 1999 by the unexpected death of a young man enrolled in a gene therapy trial (the trial was not for hemophilia). Jesse Gelsinger had a hereditary disorder that made his liver very fragile. After receiving a high dose of an early-generation adenoviral vector, he developed liver, kidney and lung failure, and, consequently, he passed away. It still is not known whether this was due to his genetic disorder or if it was a freak event. It also isn't clear whether a different vector, such as a gutless adenoviral vector, would have caused such an effect. What is

clear, however, is that gene therapy, like any new form of treatment, involves some risks, and investigators who run gene therapy trials must proceed cautiously and comply fully with Good Clinical Practice.

Safety is a somewhat relative term when treating medical conditions, because some diseases are routinely fatal and the patient may decide that the risk of a new therapy is warranted. Hemophilia is not such a medical ►

exploring gene therapy: CLINICAL TRIALS



► condition, and factor-replacement therapy is an effective, although not always convenient, form of treatment. Therefore, in gene therapy trials conducted in patients with hemophilia, the safety risks must be very low. Preclinical testing should be extensive, dose escalation should be slow and the potential formation of *inhibitors* (antibodies that bind to factor VIII or IX and prevent them from participating in clotting), should be monitored closely.

The issue of inhibitors is of major importance in hemophilia gene therapy trials. There is a significant concern that the body will see the factor VIII or IX molecule made by the transgene as a foreign substance and mount an immune response against it. Because of the different ways in which the body's immune system "sees" proteins made by the body versus those introduced from outside of the body, inhibitors could even occur in gene therapy patients who have never before had problems with inhibitors during factor replacement therapy.

Inhibitors have been detected in animal models with all of the vector systems being studied, and with both factor VIII and IX transgenes. The appearance of inhibitors is highly variable among studies; in some studies, it has been a trivial problem, while in others it has been such a huge problem that it is almost impossible to accurately monitor clotting-factor levels. The factors that influence inhibitor development are poorly understood. The specific vector type, clotting factor, target cell and animal model all appear to affect the incidence of inhibitor formation. Patient characteristics, such as genetic characteristics and what type of prior factor-replacement therapy the patient has received, may also influence the development of inhibitors. To date, there have been no reports of inhibitors in human clinical trials; however, very little information is available on this issue. It is clear that a more complete understanding of what causes inhibitor development, as well as the risk of inhibitor formation in patients receiving gene therapy, is essential if gene therapy is to become a viable option for the treatment of hemophilia.

TABLE 3. Characteristics of the three phases of US clinical trials.

Phase I

- Initial study in humans
- Designed to gather data on dosage, timing and safety, *but not efficacy*
- Involves a small number of patients
- Low dose? Dose escalation? Maximum tolerable dose

Phase II

- Continued evaluation of safety
- Initial evaluation of efficacy
- Larger number of patients

Phase III

- Large-scale study to assess efficacy, with continued evaluations of safety
- Designed to determine if the new treatment is better than the current standard of care

Current Clinical Trials

The three pharmaceutical companies that are currently sponsoring hemophilia clinical trials are Avigen, Transkaryotic Therapies and Chiron. The Avigen trial is an *in vivo* delivery trial for patients with hemophilia B, and involves injection of an AAV vector carrying the factor IX transgene directly into muscle. Data for the first patients to receive the lowest dose of this vector have been published and are encouraging.¹⁵ Vector sequences and expression of factor IX could be detected in muscle cells, and no inhibitor antibodies to factor IX were detected. Even at the low doses used, detectable changes in blood clotting were observed. More recently, two of six patients who received the lower vector doses attained factor IX levels between 1% and 2%. Expression levels in the three patients that received the highest vector dose have not yet been reported. Avigen does not plan to continue the intramuscular vector delivery protocol, and will next target the liver through intraarterial delivery. Based on animal studies, liver delivery is expected to yield higher clotting factor levels. ►► TO | 57



FROM 54 | gene therapy

► The other two clinical trials are being conducted in patients with hemophilia A. As discussed previously, the trial being conducted by Transkaryotic Therapies involves the *ex vivo* transfer of naked DNA containing the factor VIII gene into fibroblasts by electroporation. The genetically modified cells are then injected into the patient's abdomen. In some patients, a rise in factor VIII levels was observed. Encouragingly, the patient that received the highest cell dose showed the highest factor VIII expression levels, up to 4% of normal. However, levels were not constant, and factor VIII expression lasted less than a year. Thus, sustained therapy will require periodic administration of the genetically modified cells.

The Chiron trial is testing an *in vivo* gene therapy approach in which large quantities of a retroviral vector containing the factor VIII gene were administered intravenously. Treatment of 13 patients resulted in sporadic increases in factor VIII levels up to approximately 1%.

In all three trials, some patients reported fewer bleeding episodes and less frequent factor usage, suggesting that low level factor expression may be clinically beneficial. However, since phase I trials do not include a placebo group, benefits reported by patients must be interpreted with caution. Finally,

no toxicities or inhibitor development were reported in any of these clinical trials.

Conclusions

Exciting progress has been made in hemophilia gene therapy in the last decade. Multiple vector systems and approaches have been developed for both factor VIII and factor IX, and animal models have greatly improved researchers' abilities to test these systems.

Nevertheless, some challenges do remain. Scientists are actively working to improve available vector systems to achieve high-level, sustained expression of clotting factors. Safety must also be carefully assessed. We must learn more about the potential toxicities of various vector systems. We also need a better understanding of inhibitor generation. Some of this information can only be gained from clinical trials, but these trials must be conducted with as much attention to safety as possible.

Although these challenges are significant, the rapid progress made in hemophilia gene therapy in the last few years suggests that they can—and will—be met. The ultimate “payoff” will hopefully be a more effective and convenient form of treatment for patients with hemophilia. ►

GLOSSARY

Basepair: the smallest unit of information in a double-stranded DNA molecule.

DNA: deoxyribonucleic acid, the chemical substance that makes up genes.

Efficacy: treatment effectiveness.

Electroporation: a laboratory technique that increases the permeability of cells by subjecting them to a strong electric field.

Ex vivo: outside of the body.

Gene therapy: a means of treating human disease by transferring genes into a patient's somatic cells.

Gene: a segment of DNA that contains all of the genetic information required for a particular product, such as a clotting factor.

Genome: the complete set of genes contained in each cell.

Inhibitors: antibodies that bind to and neutralize factor VIII or factor IX, resulting in the failure of factor replacement therapy.

Integrate: to insert into and become one with. When a viral vector integrates into host DNA, it becomes a part of that DNA.

In vivo: inside the body.

Mutation: a change in the information contained in a gene. It can involve one basepair or many basepairs.

Myoblast: an immature muscle cell.

Somatic cells: the non-reproductive cells of the body, such as liver and muscle cells.

Transduction: the process of transferring a gene into a cell.

Transgene: the therapeutic gene carried by the vector.

Vector: the vehicle for transferring genes into a cell.



REFERENCES

Palmer TD, Thompson AR, Miller AD. Production of human factor IX in animals by genetically modified skin fibroblasts: potential therapy for hemophilia B. *Blood* 1989;73:438-45.

Dai Y, Roman M, Naviaux RK, Verma IM. Gene therapy via primary myoblasts: long-term expression of factor IX protein following transplantation in vivo. *Proc Natl Acad Sci USA* 1992;89:10892-5.

Bosch A, McCray PB Jr, Chang SM, et al. Proliferation induced by keratinocyte growth factor enhances in vivo retroviral-mediated gene transfer to mouse hepatocytes. *J Clin Invest* 1996;98:2683-7.

VandenDriessche T, Vanslembrouck V, Goovaerts I, et al. Long-term expression of human coagulation factor VIII and correction of hemophilia A after in vivo retroviral gene transfer in factor VIII-deficient mice. *Proc Natl Acad Sci USA* 1999;96:10379-84.

Greengard JS, Jolly DJ. Animal testing of retroviral-mediated gene

therapy for factor VIII deficiency. *Thromb Haemost* 1999;82:555-61.

Connelly S, Andrews JA, Gallo AM et al. Sustained phenotypic correction of murine hemophilia A by in vivo gene therapy. *Blood* 1998;91:3273-3281.

Connelly S, Mount J, Mauser A, et al. Complete short-term correction of canine hemophilia A by in vivo gene therapy. *Blood* 1996;88:3846-53.

Gallo-Penn AM, Shirley PS, Andrews JA et al. Systemic delivery of an adenoviral vector encoding canine factor VIII results in short-term phenotypic correction, inhibitor development and biphasic liver toxicity in hemophilia A dogs. *Blood* 2001; 97: in press.

Connelly S, Kaleko M. Unpublished results.

Morrall N, O'Neal W, Rice K, et al. Administration of helper-dependent adenoviral vectors and sequential delivery of different vector serotype for long-term liver-directed gene transfer in baboons. *Proc Natl Acad Sci USA* 1999;96:12816-21.

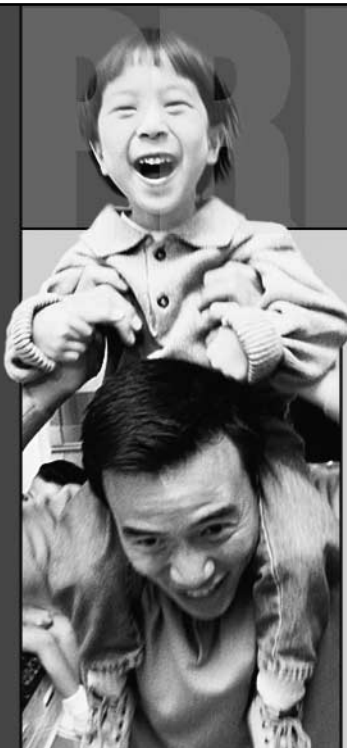
Chao H, Mao L, Bruce AT, Walsh CE. Sustained expression of human factor VIII in mice using a parvovirus-based vector. *Blood* 2000;95:1594-9.

Snyder RO, Miao C, Meuse L, et al. Correction of hemophilia B in canine and murine models using recombinant adeno-associated viral vectors. *Nat Med* 1999;5:64-70.

Herzog RW, Yang EY, Couto LB, et al. Long-term correction of canine hemophilia B by gene transfer of blood coagulation factor IX mediated by adeno-associated viral vector. *Nat Med* 1999;5:56-63.

Kafri T, Blomer U, Peterson DA, Gage FH, Verma IM. Sustained expression of genes delivered directly into liver and muscle by lentiviral vectors. *Nat Genet* 1997;17:314-7.

Kay MA, Manno CS, Ragni MN, et al. Evidence for gene transfer and expression of factor IX in haemophilia B patients treated with an AAV vector. *Nat Genet* 2000;24:257-61.



Do the 5:

- 1 Get an annual comprehensive checkup at a hemophilia treatment center.
- 2 Get vaccinated—Hepatitis A and B are preventable.
- 3 Treat bleeds early and adequately.
- 4 Exercise to protect your joints.
- 5 Get tested regularly for blood-borne infections.

NHF NATIONAL PREVENTION PROGRAM

Key steps today for giant strides tomorrow.

Collaborating with the CDC, chapters, associations, HTCs, and the community to prevent or reduce the complications of hemophilia.