

EDITORIALS



Gene Therapy Fulfilling Its Promise

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From its earliest conception, gene therapy held the promise of correcting inherited diseases by inserting a normal copy of the relevant gene into somatic cells.¹ Common monogenic diseases of blood cells, such as sickle cell disease or β -thalassemia, were originally considered important candidates for gene therapy because they were well understood at the molecular level and because the target cell, the hematopoietic stem cell, is easily accessible and can be explanted, genetically corrected in the laboratory, and then retransplanted.² The advantage of gene therapy over the conventional transplantation of hematopoietic stem cells from compatible donors is that gene therapy is in principle available to all patients and should avert the problems of the immunologic barriers that can lead to graft rejection or graft-versus-host disease. It was soon recognized, however, that the technical challenges of correcting hemoglobin disorders by means of gene therapy were daunting, most likely requiring gene transfer in high numbers of hematopoietic stem cells and high levels of expression of the β -globin gene in erythrocyte precursors.

Thus, in the mid-1980s, several groups turned to a far rarer disorder, severe combined immunodeficiency disease (SCID) due to deficiency of the enzyme adenosine deaminase (ADA), which was considered to be potentially more tractable with the gene-transfer techniques that were then available. It was known from experience with patients who had SCID and an HLA-matched sibling who could be a hematopoietic stem-cell donor that there is a strong selective-amplification effect whereby only a small amount of engrafted marrow can completely restore the immune system.³ Thus, if the ADA gene could be inserted even into only a modest number of hematopoietic stem cells

obtained from a patient with SCID due to ADA deficiency and be expressed in the progeny blood cells produced after retransplantation of the transduced cells, there is a good chance of clinical benefit.

Initial efforts at gene therapy for SCID due to ADA deficiency in the early 1990s did not produce the cures that had been hoped for, probably because of the low numbers of gene-corrected hematopoietic stem cells that were engrafted in the first handful of patients.^{4,5} These pioneer experiments were followed by incremental improvements in the laboratory techniques used to introduce genes into hematopoietic stem cells, and a second generation of clinical trials were begun in the late 1990s, directed at both SCID due to ADA deficiency and the X-linked form of SCID.

Thus, in 2000 and 2002, investigators from France and Italy reported results suggesting that the fulfillment of the promise of gene therapy was at hand. Cavazzana-Calvo et al.⁶ reported immune reconstitution in five infants with X-linked SCID who underwent gene therapy in Paris, and Aiuti et al.⁷ described initial signs of immune reconstitution in two infants with SCID due to ADA deficiency treated in Milan. The gene-transfer methods used in the two studies were similar, but only the patients with SCID due to ADA deficiency were given a chemotherapeutic agent, busulfan, intended to “make space” for the gene-corrected hematopoietic stem cells to enhance their engraftment after reinfusion. Except for the expected transient neutropenia and thrombocytopenia, the clinical effects of the reduced dose of busulfan chemotherapy used in the study were much milder than those of the “full-dose” conditioning typically used for bone marrow transplantation. Since these two studies were published, the encourag-

ing results obtained in patients with X-linked SCID were reproduced in another trial carried out in the United Kingdom.⁸

In this issue of the *Journal*, Aiuti et al.⁹ add to the accomplishments of gene therapy by reporting findings from the extended follow-up of the two previously studied patients with SCID due to ADA deficiency as well as an additional eight patients treated according to the same protocol. Of these 10 patients, 8 have had excellent and persistent immune reconstitution in the absence of enzyme-replacement therapy, as documented with the use of multiple laboratory tests and, most importantly, through their continued clinical well-being without the need for a protective environment to prevent infection. Excellent immune recovery was observed in one child who was almost 6 years of age at the time of gene therapy; although this is quite young by most standards, it is an age when thymic function, essential to provide the niche for T-cell development, has already declined considerably as compared with infancy.

Essentially all the circulating T cells in these patients and most of the B cells and natural killer cells contain the corrective *ADA* gene, whereas the levels of *ADA*-containing granulocytes, monocytes, and bone marrow stem cells or progenitor cells (which are not adversely affected by *ADA* deficiency) are one tenth to one hundredth the levels in the lymphocytes. These findings clearly demonstrate the occurrence of selective amplification of gene-corrected lymphocytes from a small number of gene-corrected hematopoietic stem cells and justify the choice of SCID for developing this approach. Immune reconstitution was unsatisfactory in two patients, one who received the lowest dose of treated bone marrow cells and one with pre-existing autoimmunity, in whom *ADA* enzyme-replacement therapy was subsequently restarted.

The results by Aiuti et al. present a key difference from those of the two gene-therapy trials of X-linked SCID. Although 18 of 20 treated infants with X-linked SCID are alive and well with restored immunity, a T-cell lymphoproliferative syndrome developed within 2 to 5 years after the procedure in 5 children; 1 of these children died as a consequence of complications of the syndrome, despite therapy. Investigations have implicated insertional oncogenesis in the pathogenesis of the leukemia-like illness, in which the insertion of the corrective retroviral vector may activate expression of cellular proto-oncogenes near the integration site.¹⁰

The sharp dichotomy between the absence of this complication in the patients with SCID due to *ADA* deficiency and its occurrence in 25% of the patients with X-linked SCID is important to understand if we are to retain the therapeutic efficacy of gene therapy while minimizing its risks. The time to immune recovery in the patients with SCID due to *ADA* deficiency is markedly slower (6 to 12 months) than the rapid development (over 3 to 6 months) of T cells in the patients with X-linked SCID receiving gene therapy, which may reflect important biologic differences between the corrected hematopoietic stem cells in X-linked SCID and SCID due to *ADA* deficiency.

The gene responsible for X-linked SCID encodes the common γ (γ c) chain, a component of the receptor for multiple cytokines involved in lymphocyte development and function. The γ c protein provides a proliferation signal that may cooperate with the concomitantly deregulated expression of a proto-oncogene in proximity to the gene-transfer vector-integration site, favoring the establishment of malignant cells. On the contrary, *ADA* expression merely provides protection against apoptosis in *ADA*-deficient cells, which is expected to place less selective pressure on the survival of *ADA*-deficient hematopoietic stem cells containing vector integrations that might have caused oncogene activation.

Despite the widely publicized adverse events in the X-linked SCID trials, it is vital to dispassionately compare gene-therapy results with those of the current standard of care. Transplantation of parental or unrelated allogeneic hematopoietic stem cells in the approximately 80% of infants with SCID who lack an HLA-matched sibling donor has success rates of 50 to 85%, with a considerable number of patients dying from a host of complications. Certainly, the outcomes of gene therapy for SCID reported in recent trials are at least as good as, and arguably better than, the results reported for allogeneic transplantation, justifying further study of this procedure that, in the case of SCID due to *ADA* deficiency, has already received orphan-drug status by the European Medicines Agency.

The prospects for continuing advancement of gene therapy to wider applications remain strong. Ongoing and upcoming clinical trials will use safer designs of retroviral vectors, newer types of vectors for viral gene delivery, and emerging methods for direct in situ gene repair (Fig. 1). These

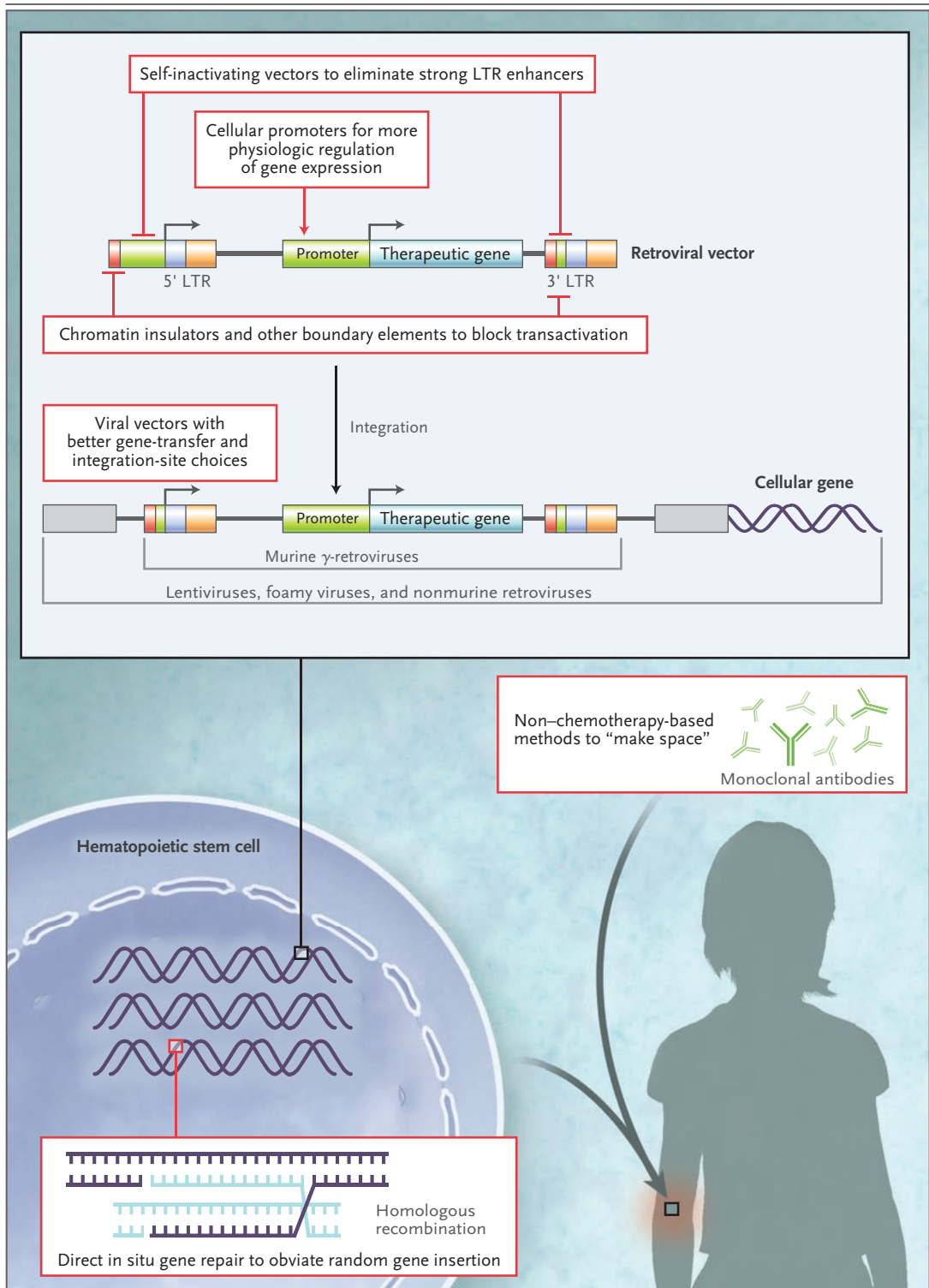


Figure 1 (facing page). Increasing the Safety and Efficacy of Gene Therapy through the Use of Hematopoietic Stem Cells.

Long-lasting gene correction of hematopoietic stem cells requires persistence of the corrective gene for it to be passed on to all the progeny blood cells. At least six methods that ensure such persistence in ways that are safer for patients, yet still retain the efficacy seen in the clinical trials of gene therapy for severe combined immunodeficiency disease (SCID), are under study. The first method is the use of self-inactivating vectors to eliminate strong long terminal repeat (LTR) enhancers. The LTR sequences at the ends of retroviral-vector sequences possess strong enhancer activity that may play a key role in the transactivation of adjacent cellular proto-oncogenes. Vector designs that lead to “self-inactivation” of these LTR enhancer sequences have significantly reduced transactivation activity.¹¹ The second method is the use of cellular promoters for increased physiologic regulation of gene expression. The use of the promoters of cellular genes, rather than the strong viral promoters often used in vectors, to drive expression of the therapeutic gene may reduce the risks of activating adjacent cellular genes.¹² The third method is the use of chromatin insulators and other genomic boundary elements to block transactivation. DNA sequences present throughout the genome act to block interactions between adjacent transcriptional units, and these boundary elements (including insulators and matrix-attachment regions) may prevent integrated vectors from transactivating adjacent cellular genes.¹³ The fourth method is the use of gene-transfer vectors that make better gene-transfer and integration-site choices. The gene-transfer vectors used in most studies to date, derived from murine γ -retroviruses, have a high predisposition toward integrating near the 5' ends of cellular genes, close to the transcriptional control elements of the cellular gene. Newer vectors being developed from lentiviruses, foamy viruses, and nonmurine retroviruses (such as avian sarcoma and leukosis virus) tend to integrate across broader regions of the genome, which may decrease their potential to transactivate cellular genes.¹⁴ The fifth method is the use of direct in situ gene repair to obviate random gene insertion. Methods to perform gene repair by means of efficient homologous recombination are being developed, aided by sequence-specific endonucleases that facilitate the process. Gene correction, rather than gene addition, would not lead to random insertion of transgene sequences.¹⁵ The sixth method is the use of non-chemotherapy-based approaches to favor safer engraftment of gene-corrected stem cells. In the study by Aiuti et al.,⁹ a chemotherapeutic agent, busulfan, was administered to patients to “make space” in their bone marrow for the gene-treated stem cells to engraft. Methods that use less-toxic agents, such as monoclonal antibodies that bind to and deplete stem cells, may be able to facilitate stem-cell engraftment with fewer potential short- and long-term side effects.¹⁶

approaches to the treatment of hemoglobinopathies, hemophilia, muscular dystrophy, congenital retinopathies, neurodegenerative disorders, and other genetic diseases may further fulfill the promise that gene therapy made two decades ago.

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