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. 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. 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. Therefore, 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. 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. reported immune reconstitution in five infants with X-linked SCID who underwent gene therapy in Paris, and Aiuti et al. 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 infusion. 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 (γc) chain, a component of the receptor for multiple cytokines involved in lymphocyte development and function. The γc 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
Self-inactivating vectors to eliminate strong LTR enhancers

Cellular promoters for more physiologic regulation of gene expression

Chromatin insulators and other boundary elements to block transactivation

Viral vectors with better gene-transfer and integration-site choices

Retroviral vector

5' LTR

Promoter

Therapeutic gene

3' LTR

Integration

Cellular gene

Retroviral vector

Murine γ-retroviruses

Lentiviruses, foamy viruses, and nonmurine retroviruses

Hematopoietic stem cell

Direct in situ gene repair to obviate random gene insertion

Homologous recombination

Non–chemotherapy-based methods to “make space”

Monoclonal antibodies

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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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