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Gene Therapy for Immunodeficiency Due to Adenosine Deaminase Deficiency

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ABSTRACT

BACKGROUND

We investigated the long-term outcome of gene therapy for severe combined immunodeficiency (SCID) due to the lack of adenosine deaminase (ADA), a fatal disorder of purine metabolism and immunodeficiency.

METHODS

We infused autologous CD34+ bone marrow cells transduced with a retroviral vector containing the ADA gene into 10 children with SCID due to ADA deficiency who lacked an HLA-identical sibling donor, after nonmyeloablative conditioning with busulfan. Enzyme-replacement therapy was not given after infusion of the cells.

RESULTS

All patients are alive after a median follow-up of 4.0 years (range, 1.8 to 8.0). Transduced hematopoietic stem cells have stably engrafted and differentiated into myeloid cells containing ADA (mean range at 1 year in bone marrow lineages, 3.5 to 8.9%) and lymphoid cells (mean range in peripheral blood, 52.4 to 88.0%). Eight patients do not require enzyme-replacement therapy, their blood cells continue to express ADA, and they have no signs of defective detoxification of purine metabolites. Nine patients had immune reconstitution with increases in T-cell counts (median count at 3 years, 1.07×10^9 per liter) and normalization of T-cell function. In the five patients in whom intravenous immune globulin replacement was discontinued, antigen-specific antibody responses were elicited after exposure to vaccines or viral antigens. Effective protection against infections and improvement in physical development made a normal lifestyle possible. Serious adverse events included prolonged neutropenia (in two patients), hypertension (in one), central-venous-catheter-related infections (in two), Epstein-Barr virus reactivation (in one), and autoimmune hepatitis (in one).

CONCLUSIONS

Gene therapy, combined with reduced-intensity conditioning, is a safe and effective treatment for SCID in patients with ADA deficiency. (ClinicalTrials.gov numbers, NCT00598481 and NCT00599781.)

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ADENOSINE DEAMINASE (ADA) DEFICIENCY is a fatal autosomal recessive form of severe combined immunodeficiency (SCID), of which failure to thrive, impaired immune responses, and recurrent infections are characteristics.^{1,2} Toxic levels of purine metabolites (adenosine and adenine deoxyribonucleotides) due to the deficiency of ADA can cause hepatic, skeletal, neurologic, and behavioral alterations^{1,3,4} and sensorineural deafness.⁵ A hematopoietic stem-cell transplant from an HLA-identical sibling, the treatment of choice, is available for only a minority of patients⁶⁻⁸; the use of alternative donors is associated with a high risk of death or lack of engraftment.^{1,6} Administration of polyethylene glycol-modified bovine ADA (PEG-ADA) corrects the metabolic alterations and improves the clinical condition of patients^{2,9} but often fails to sustain correction of the immunodeficiency^{10,11}; its use is limited by neutralizing antibodies against the bovine enzyme, autoimmunity, and the high cost of lifelong therapy.¹

Gene therapy is effective in patients with X-linked SCID,¹² but its use has been hampered by the development of T-cell leukemia due to insertional mutagenesis caused by the retroviral vector.^{13,14} Pilot trials have shown the safety and feasibility of gene therapy in patients with SCID due to ADA deficiency,¹⁵⁻¹⁷ but all patients required maintenance with PEG-ADA, and the ADA-transduced stem cells were unable to reconstitute the recipient's immune system. We previously described two patients with ADA deficiency in whom nonmyeloablative conditioning allowed for substantial correction of the metabolic and immune defects 1 year after gene therapy.¹⁸ Here, we describe the long-term outcome of these two children and results in eight additional patients who were treated with nonmyeloablative conditioning followed by infusion of autologous CD34+ cells from bone marrow that had been transduced with a viral vector carrying the ADA gene.

METHODS

PATIENTS

Patients were enrolled from July 2000 through September 2006 in one of three phase 1-2 clinical protocols: one approved by the Hadassah University Hospital Ethics Committee and Israeli National Regulatory Authorities, and two approved by the San Raffaele Scientific Institute's Ethics Committee and the Italian National Regulatory Au-

thorities (Table 1). Children with SCID due to ADA deficiency who lacked a healthy HLA-identical sibling were eligible for enrollment. In addition, patients who had been treated with PEG-ADA for at least 6 months were eligible in case of inefficacy, defined by immunologic measurements or as intolerance, allergic reaction, or autoimmunity.

The Italian Telethon Foundation received, from the European Medicines Agency, an orphan-drug designation for ADA vector-transduced CD34+ cells (EMEA/OD/053/05). The parents of all patients provided written informed consent for experimental treatment. The two Italian clinical trials are registered in the cell and gene-therapy database of the Italian Istituto Superiore di Sanità, which has required patient-by-patient authorization as of the end of October 2002.

GENE THERAPY

Before gene therapy, a central venous catheter was implanted, and bone marrow specimens were obtained and cryopreserved for possible later use. On day 4 before gene therapy, autologous bone marrow specimens were again harvested under general anesthesia; mononuclear cells were isolated by means of density gradients, and CD34+ cells were purified with the use of immunomagnetic beads (CliniMACS, Miltenyi). CD34+ cells were stimulated with cytokines (fms-related tyrosine kinase 3 ligand, KIT ligand, thrombopoietin, and interleukin-3) and transduced with the retroviral vector (GIADAL) based on the Moloney murine leukemia virus carrying the human ADA gene.¹⁸ Supernatant production, cell isolation, and transduction were performed at MolMed according to current Good Manufacturing Practices. Nonmyeloablative conditioning involving the intravenous (or oral, in Patient 2) administration of 2 mg per kilogram per day of busulfan (Busilvex, Pierre Fabre) was performed on days 3 and 2 before gene therapy. Gene therapy consisted of the infusion of CD34+ marrow cells that had been transduced with the ADA-containing vector.¹⁸

LABORATORY STUDIES

Blood and marrow samples were obtained from patients with SCID due to ADA deficiency, and blood samples were obtained from healthy children and adults as controls, with approval from the San Raffaele Scientific Institute's Ethics Committee and the Hadassah University Hospital Ethics Committee, according to standard ethical procedures. The Supplementary Appendix (available with

Table 1. Characteristics and Treatment of the Study Patients.*

Patient No.	Clinical Study	Sex	Age at Onset	Amino Acid Mutation	Previous Treatment	Age at Gene Therapy	Infused CD34+ Cells	Transduced CFU	Copies of Vector
			mo			yr	per kg of body weight	%	no./cell
1	Hadassah	F	1	H17P (homozygous)	None	0.6	8,600,000	25.0	2.20
2	SR-I	F	2	L107P, R211H	Haplo-BMT	2.4	900,000†	19.2	ND
3	SR-I	M	2	G74V, R282Q	Haplo-BMT	1.0	5,400,000	50.6	0.85
4	SR-II	F	5	R282Q (homozygous)	Haplo-BMT, PEG-ADA (for 2 mo)	1.9	3,800,000	12.6	ND
5	SR-II	F	2	G216R, E319fsX3	PEG-ADA (for 1.2 yr)	1.6	9,600,000	39.8	1.89
6	SR-II	M	1	R211H (homozygous)	PEG-ADA (for 5.3 yr)	5.6	9,500,000	26.0	1.05
7	SR-II	M	1	G216R, S291L	PEG-ADA (for 1.1 yr)	1.5	9,000,000	16.7	0.83
8	SR-II	F	1	H15D (homozygous)	PEG-ADA (for 2.7 yr)	2.8	10,600,000	29.5	0.12
9	SR-II	M	5	Exon 5, splice-donor site +2	PEG-ADA (for 0.8 yr)	1.4	13,600,000	44.6	0.57
10	SR-II	F	3	G216R (homozygous)	Haplo-BMT, PEG-ADA (for 1 yr)	1.8	10,700,000	21.5	0.35

* The patients were consecutively enrolled in one of three clinical studies with the same protocol at Hadassah University Hospital (Hadassah) or the San Raffaele Scientific Institute (SR-I or SR-II). BMT denotes bone marrow transplantation, CFU colony-forming units, ND not determined, PEG-ADA polyethylene glycol–modified bovine adenosine deaminase, and X a stop codon.

† Patient 2 received a booster of 2,200,000 transduced CD34+ cells per kilogram (with an average of 37.9% transduced CFU) 31 months after the first infusion, without conditioning.

the full text of this article at NEJM.org) describes measurements of cell subgroups, frequencies of transduced cells, results of flow cytometry, in vitro T-cell responses, antibodies generated after immunization,¹⁹ and ADA activity in cell lysates.²⁰

SAFETY

Adverse events were recorded and reported according to Good Clinical Practice, and were updated as of August 31, 2008. Patients were monitored through clinical examination, imaging, and hematologic, immunologic, biochemical, and molecular tests, which included testing for replication-competent retrovirus.

STATISTICAL ANALYSIS

Mean or median values are reported, as appropriate. Clinical follow-up (including safety) data were updated as of August 31, 2008; analyses regarding molecular, biochemical, and immunologic variables were performed on data as of November 2007. Comparisons between values at various time points and between values for different variables at the same time point were performed by means of the Wilcoxon signed-rank test for paired data (two-tailed tests). The rates of infection (events per person-month of observation)

and days of hospitalization were evaluated before gene therapy (from birth) and after therapy (from 4 months after gene therapy onward, to exclude an initial period of procedure-related hospitalization). The degree of correlation was expressed by means of Pearson's correlation coefficient. The data for Patient 2 were evaluated for efficacy until PEG-ADA was introduced at 4.5 years after gene therapy, whereas the data for Patient 8 were evaluated for safety only because PEG-ADA was reintroduced 0.4 year after gene therapy.

RESULTS

Ten patients with SCID due to ADA deficiency who had early-onset manifestations (median, 2 months of age) underwent ADA gene therapy at a median age of 1.7 years (range, 0.6 to 5.6) (Table 1). This disorder was diagnosed at birth in one patient, a bone marrow transplant from a mismatched related donor had failed in four patients, and six patients had received PEG-ADA for more than 6 months, with an inadequate response. PEG-ADA was discontinued 3 weeks before gene therapy, to favor the growth of ADA-transduced cells. Patients underwent nonmyeloablative preconditioning with busulfan (total dose, 4 mg per kilogram of body

Table 2. Clinical Outcomes of the Study Patients.*

Patient No.	Clinical History before Gene Therapy	Years of Follow-up	Relevant Infections after Gene Therapy	Serious Adverse Events after Gene Therapy	PEG-ADA	Clinical Condition after Gene Therapy
1	Recurrent respiratory infection, failure to thrive	8.0	None	None	No	Well
2	Chronic diarrhea, recurrent respiratory infection, scabies, failure to thrive	7.5	Skin molluscum, urinary infection	None	Initiated 4.5 yr after gene therapy	Well
3	Recurrent respiratory infection, dermatitis, failure to thrive, eating disorder	6.3	None	Prolonged neutropenia and thrombocytopenia	No	Well
4	Recurrent respiratory infection, oral infection with <i>Candida albicans</i> , skin BCG and bacterial infections, chronic diarrhea, failure to thrive	5.9	Varicella	None	No	Well
5	Recurrent respiratory infection, aseptic meningitis, chronic diarrhea, failure to thrive	4.4	Varicella	None	No	Well
6	CMV lung infection, EBV infection, recurrent respiratory infection, hearing deficit, failure to thrive	3.8	CVC-related infection, EBV reactivation, varicella	None	No	Well
7	Facial dysmorphism, eating disorder, staphylococcal infection, oral and genital infection with <i>C. albicans</i> , failure to thrive	2.8	None	Autoimmune hepatitis	No	Well, but with eating disorder
8	Developmental delay, recurrent respiratory infection, autoimmune hemolytic anemia, macrophage activation syndrome, hearing deficit, failure to thrive	2.5	Recurrent respiratory infection, urinary infection	Hypertension, prolonged neutropenia, autoimmune thrombocytopenia	Restarted 0.4 yr after gene therapy	Mild symptoms
9	<i>Pneumocystis jiroveci</i> pneumonia	1.9	Gastroenteritis	None	No	Well
10	Postvaccinal BCG infection, recurrent respiratory infection, developmental delay, neurosensory deafness, genital ambiguity, congenital adrenal insufficiency, hypothyroidism, failure to thrive	1.8	CVC-related infection (two)	None	No	Well, but with developmental delay

* Serious adverse events were those other than serious infections during the follow-up period after gene therapy. BCG denotes bacille Calmette–Guérin, CMV cytomegalovirus, CVC central venous catheter, EBV Epstein–Barr virus, and PEG-ADA polyethylene glycol–modified bovine adenosine deaminase.

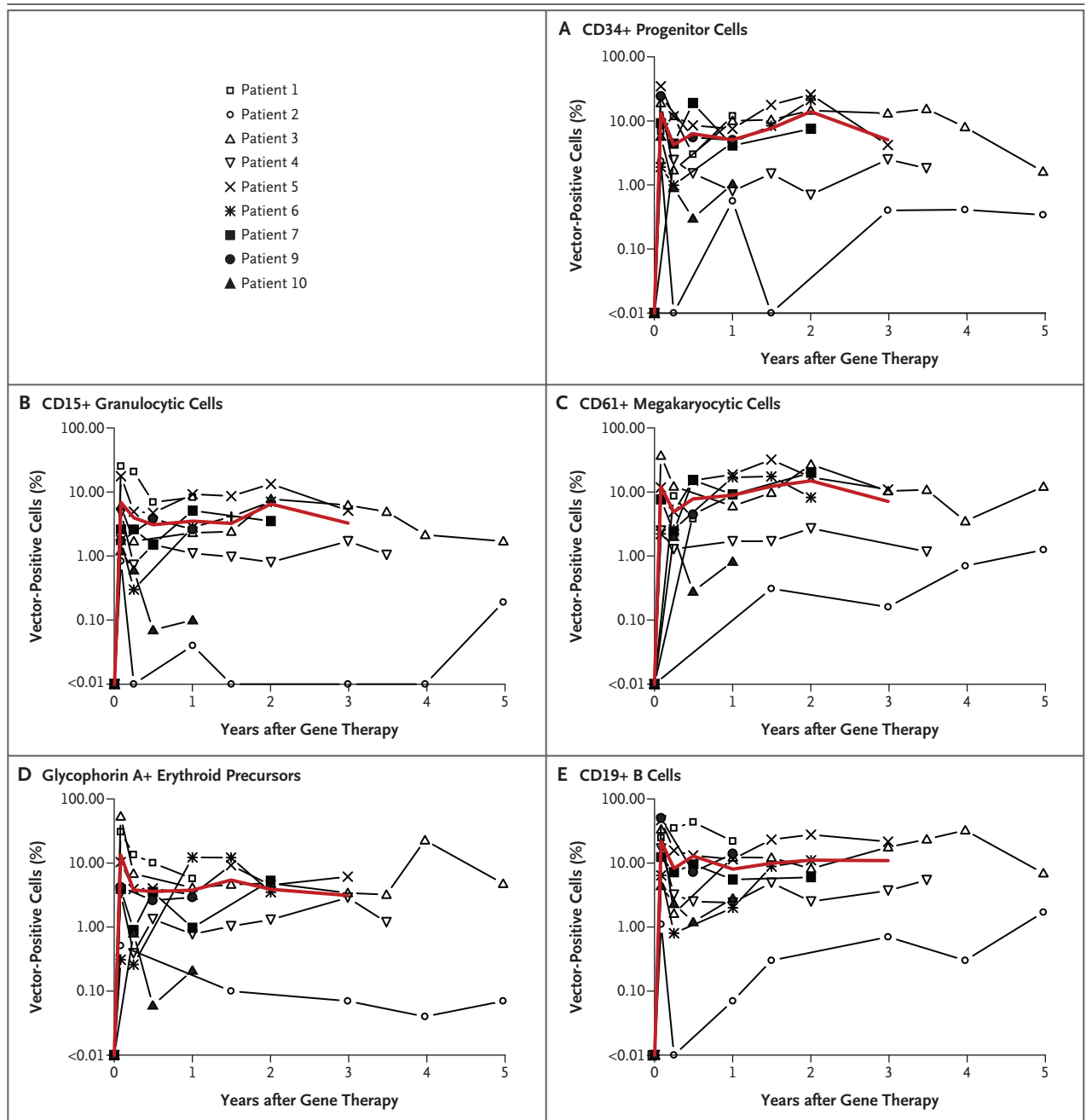


Figure 1. Persistence of ADA-Transduced Cells in Bone Marrow.

The proportions of vector-positive cells (on a log₁₀ scale) for each of the 9 patients evaluated for efficacy and on average (red line) are shown for several cell lineages from bone marrow specimens: CD34+ progenitor cells (Panel A), CD15+ granulocytic cells (Panel B), CD61+ megakaryocytic cells (Panel C), glycophorin A+ erythroid precursors (Panel D), and CD19+ B cells (Panel E). The data were averaged for as many patients (as long as there were at least three) as were undergoing follow-up at each time point. The long-term persistence of ADA-transduced cells results in efficient detoxification.

weight) followed by the infusion of CD34+ marrow cells that had been transduced with the ADA-containing vector (mean dose, 8.2×10^6 CD34+ cells per kilogram, with an average of 28.6%

transduced colony-forming units) (Table 1). In seven patients, absolute neutrophil counts were less than 0.5×10^9 per liter for more than 1 day after receipt of busulfan, and the duration of

neutropenia was related to the area under the concentration–time curve of busulfan ($r=0.65$) (Table 1 and Fig. 1 in the Supplementary Appendix). In two patients, neutropenia lasted over 30 days (Table 2); Patient 3 had neutropenia and thrombocytopenia and received platelet transfusions and infusion of the autologous marrow held in reserve (1.4×10^6 CD34+ cells per kilogram) at day 30 after gene therapy. The neutropenia in Patient 8 resolved after administration of granulocyte colony-stimulating factor. Patients were discharged after a median period of hospitalization of 42 days (range, 34 to 110), and follow-up continued according to the protocol and the guidelines of the Italian regulatory agency. Of the 10 patients, 8 did not require PEG-ADA during the follow-up period (Table 2). None of the patients received an allogeneic transplant after gene therapy.

SAFETY

There were no adverse events that could be attributed to the ADA-transduced cells. A mild and transient increase in liver enzyme levels was detected in four patients within 2 to 3 weeks after treatment. At present, data from a median duration of follow-up of 4.0 years are available for the 10 patients, and no events suggestive of leukemic transformation have been seen. Moreover, no abnormal expansion or clonal outgrowth was detected in immunologic and molecular studies (Fig. 2A in the Supplementary Appendix).²¹ Serious adverse events included two cases of prolonged neutropenia, one of hypertension, three of central-venous-catheter–related infection, one case of Epstein–Barr virus reactivation, which resolved after preemptive therapy with one dose of anti-CD20 monoclonal antibody, and one case of autoimmune hepatitis (Table 2). Patient 8, who had recurrent autoimmune hemolytic anemia and the macrophage activation syndrome and had received corticosteroids for 2 years before gene therapy, had three episodes of autoimmune thrombocytopenia, requiring long-term corticosteroid administration, reintroduction of PEG-ADA approximately 5 months after gene therapy, and treatment with anti-CD20.

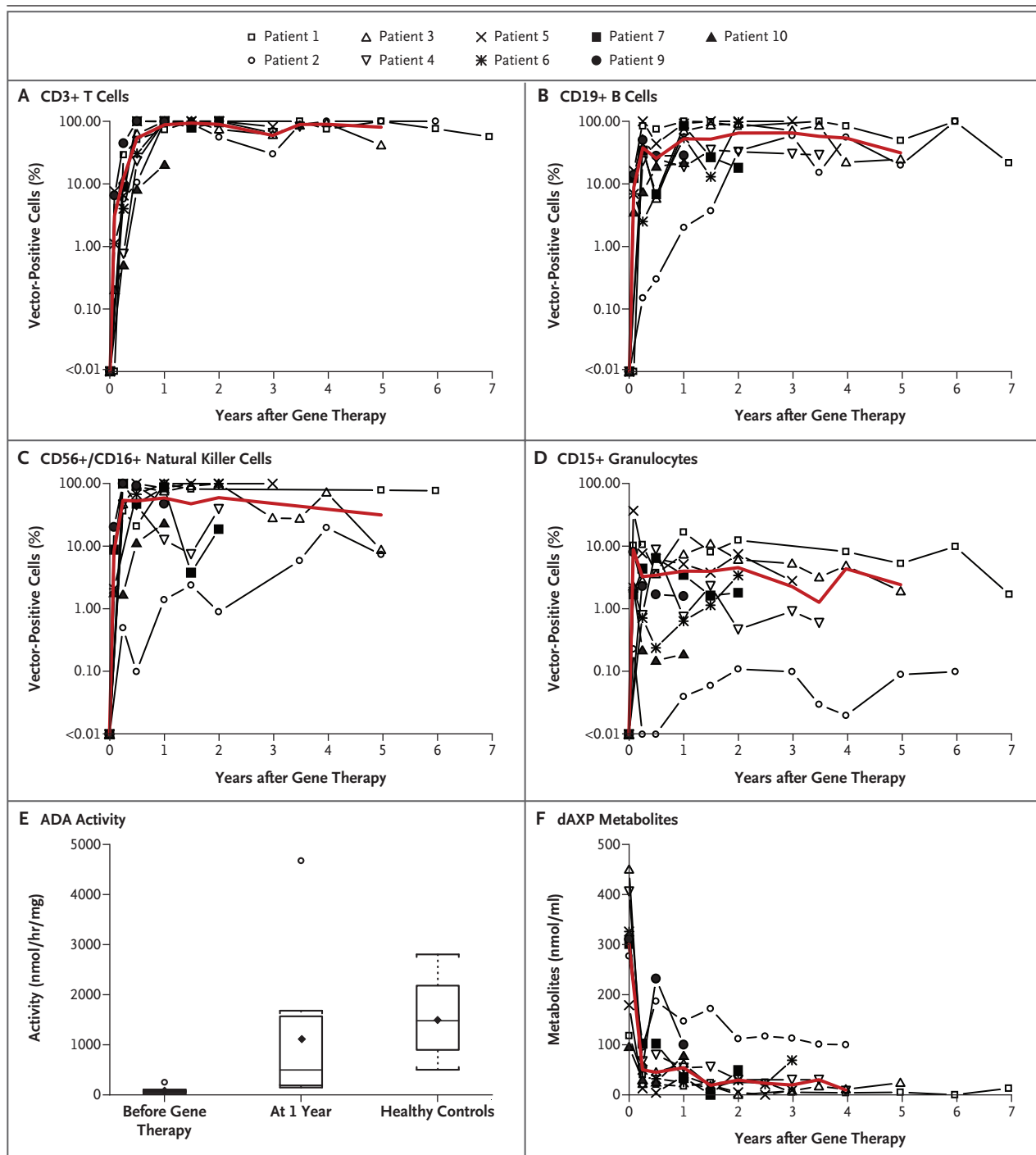
ENGRAFTMENT OF VECTOR IN MULTIPLE CELL LINEAGES

ADA-transduced CD34+ cells and their progeny were found in purified marrow (Fig. 1) and blood (Fig. 2) specimens. One year after gene therapy, the mean proportion of bone marrow cells carry-

Figure 2 (facing page). Persistence of ADA-Transduced Cells, ADA Activity, and Purine Metabolites in Peripheral Blood.

The proportions of vector-positive cells (on a \log_{10} scale) for each of the 9 patients evaluated for efficacy and on average (red line) are shown for several cell lineages from peripheral-blood specimens: CD3+ T cells (Panel A), CD19+ B cells (Panel B), CD56+/CD16+ natural killer cells (Panel C), and CD15+ granulocytes (Panel D). The long-term persistence of ADA-transduced cells results in efficient detoxification. The data were averaged for as many patients (as long as there were at least three) as were undergoing follow-up at each time point. Panel E shows ADA activity in blood mononuclear cells in nine patients before and at 1 year after gene therapy, as compared with levels in 17 healthy controls. In the box-and-whisker plots, the box contains the data points that fall between the first and third quartiles, the horizontal line indicates the median, the diamond indicates the mean, and the brackets delineate 1.5 times the interquartile range. Panel F shows the systemic breakdown of toxic purine metabolites (deoxyadenosine nucleotides [dAXP]) in red cells before gene therapy (the levels at diagnosis for patients who received polyethylene glycol–modified bovine ADA [PEG-ADA]) and during the follow-up period. Data are shown for each of the nine patients included in efficacy analyses; the median is also shown (red line). Data for Patient 2 are shown through 4 years, after which PEG-ADA was reintroduced. For comparison, the average value of 2'-deoxyadenosine-5'-triphosphate is 103 nmol per milliliter after standard bone marrow transplantation²² and less than 3 nmol per milliliter in healthy controls.

ing the retroviral vector was 5.1% of CD34+ cells, 3.5% of granulocytic cells (CD15+), 8.9% of megakaryocytic cells (CD61+), 3.8% of erythroid cells (glycophorin A+), and 8.0% of B cells (CD19+) (Fig. 1). In peripheral-blood specimens at 1 year, the mean frequencies of transduced T cells, B cells, and natural killer cells were 88.0%, 52.4%, and 59.2%, respectively ($P=0.004$ for each comparison with granulocytes) (Fig. 2A through 2D). In the B-cell lineage, the proportion of vector-positive B cells was significantly higher in the blood than in the bone marrow ($P=0.004$). The frequency of vector-positive CD34+ cells at 1 year after gene therapy correlated with the proportion of transduced colony-forming units ($r=0.60$) and vector copy number in CD34+ cells ($r=0.75$). Moreover, the patients who received a dose of more than 8×10^6 of CD34+ cells per kilogram (Table 1) or had neutropenia for more than 15 days (Table 1 in the Supplementary Appendix), as compared with the remaining patients, had higher mean percentages of transduced CD34+ marrow cells (6.3% vs. 0.7%) and CD15+ marrow cells



(4.3% vs. 0.6%) at 1 year. ADA-transduced cells persisted in all hematopoietic lineages, including mature granulocytes, through the last evaluation (Fig. 1 and 2).

ADA EXPRESSION AND PURINE METABOLISM

The presence of ADA was documented through detection of its enzymatic activity in blood mono-

nuclear cells (Fig. 2E), marrow mononuclear cells (data not shown), T cells,²³ and red cells and was confirmed through flow cytometry of T and B cells, and monocytes (Fig. 2B in the Supplementary Appendix). The median ADA activity in blood mononuclear cells and red cells was significantly higher at 1 year than at baseline (mononuclear cells, 497 vs. 65 nmol per hour per milligram; red

cells, 0.35 vs. 0.06 μmol per hour per milliliter; $P=0.004$ for both tests), which corresponded to 33.6% and 1.9% of median levels in controls, respectively (Fig. 2E and data not shown). The ADA activity in white cells and red cells resulted in a significant reduction of toxic levels of purine metabolites (deoxyadenosine nucleotides) in red cells at 1 year as compared with levels at diagnosis in the same patients ($P=0.004$) (Fig. 2F). With the exception of Patient 2, deoxyadenosine nucleotide levels remained low throughout the follow-up period in all patients who were not receiving PEG-ADA.

IMMUNE RECONSTITUTION

After administration of the ADA vector, there was a progressive increase in T-cell counts, which reached maximum levels at 1 to 3 years (Fig. 3A). In children who had been treated with PEG-ADA, the increase occurred after a transient reduction following the discontinuation of enzyme-replacement therapy (Fig. 3A). As compared with values before gene therapy, the median CD3+ T-cell count was 0.51×10^9 per liter at 1 year of follow-up and 1.07×10^9 per liter at 3 years ($P=0.004$ and $P=0.03$, respectively) (Fig. 3B). Median levels of CD4+ T cells and CD8+ T cells 3 years after gene therapy were 0.52×10^9 per liter and 0.47×10^9 per liter, respectively ($P=0.03$ for both comparisons with baseline values).

According to the most recent follow-up data available for patients not receiving PEG-ADA, T-cell counts were above the lower limits of normal²⁴ in five patients (Table 3). T-cell counts did not increase in Patient 2, despite treatment with PEG-ADA (Fig. 3A). An increase of more than one \log_{10} unit in the median numbers of circulating naive CD4+CD45RA+ cells and T-cell-receptor excision circles (a marker of recent emigrants from the thymus) in circulating T cells ($P=0.06$ and $P=0.03$, respectively, for the comparison for 3 years vs. baseline) (Fig. 3 in the Supplementary Appendix) indicated restoration of thymic activity. Remarkably, thymic activity was restored after gene therapy in Patient 6, the oldest subject, who had no detectable T-cell-receptor excision circle at 5.5 years of age while receiving PEG-ADA.

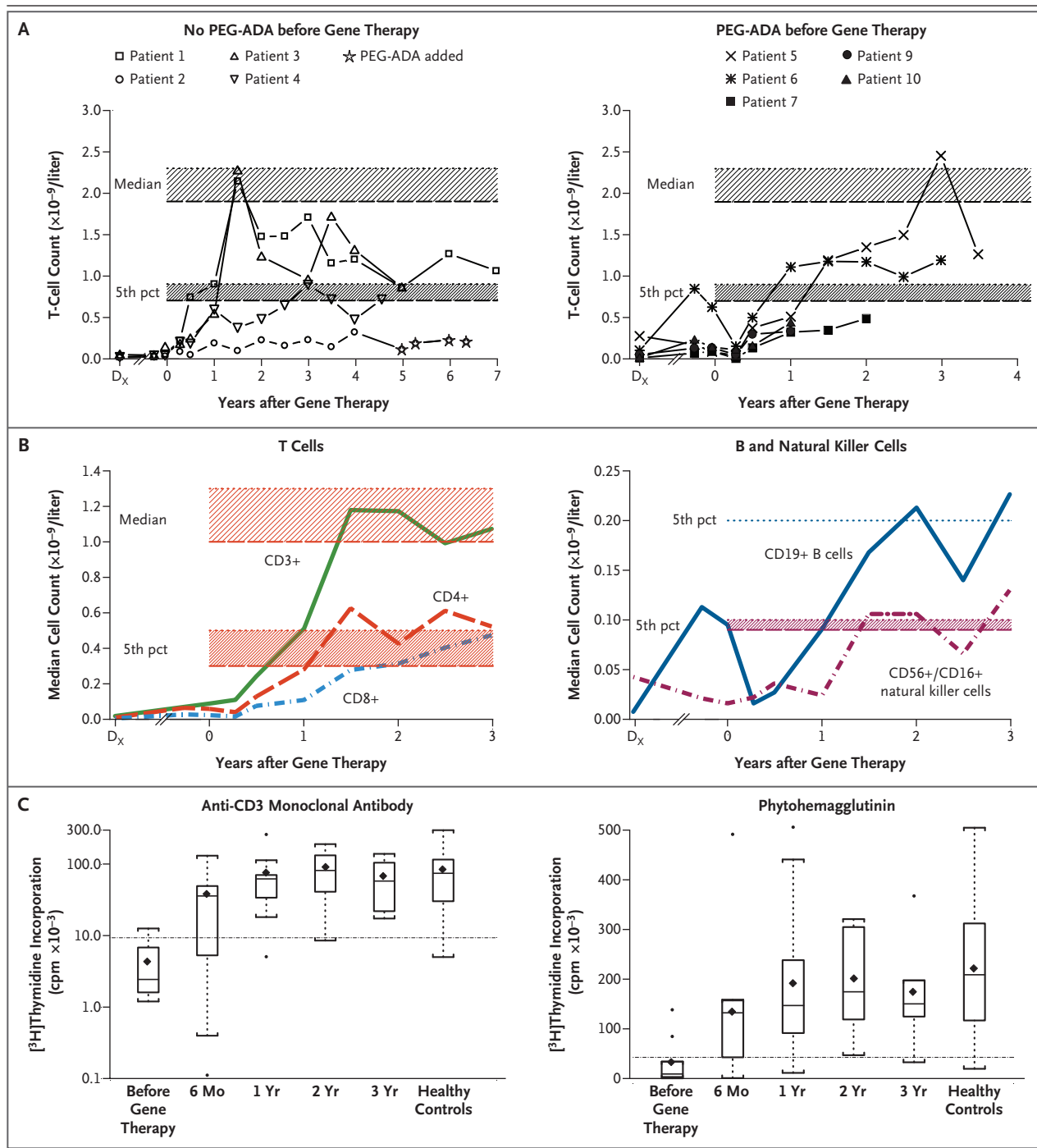
Levels of natural killer cells were significantly increased at 3 years as compared with baseline ($P=0.03$) (Fig. 3B) and displayed normal cytotoxic activity against K562 cells. Proliferative responses of T cells against mitogens (anti-CD3 monoclonal antibody and phytohemagglutinin) were

Figure 3 (facing page). Immune Reconstitution after Gene Therapy.

Panel A shows T-cell counts for individual study patients not receiving polyethylene glycol–modified bovine adenosine deaminase (PEG-ADA) before gene therapy (Patients 1 through 4, left side) and for patients receiving PEG-ADA (for >6 months) before gene therapy (Patients 5, 6, 7, 9, and 10; right side). The data are shown from the time of diagnosis (D_x). PEG-ADA supplementation began during the follow-up period in Patient 2, as indicated in Panel A. Panel B (left side) shows the median cell counts for CD3+ T cells, CD4+ T cells, and CD8+ T cells after gene therapy. Reference values for T cells (Panel A) and CD4+ T cells (Panel B) are also shown: the top shaded areas represent the median values for healthy controls 2 to 5 years of age (dotted upper boundaries) and 5 to 10 years of age (dashed lower boundaries), and the bottom shaded areas, the 5th percentiles (pct) for those age classes, respectively.²⁴ For additional comparisons in Panel B, the reference values for CD8+ T cells in healthy controls for both age classes are 0.8×10^9 per liter for the median and 0.3×10^9 per liter for the 5th percentile. Panel B (right side) also shows the median cell counts for CD19+ B cells and CD56+/CD16+ natural killer cells at various time points. The 5th percentile for healthy controls 2 to 5 years of age and 5 to 10 years is shown for B cells (blue broken line; same value for both age classes) and natural killer cells (purple broken upper line, value for 2 to 5 years of age; purple broken lower line, value for 5 to 10 years of age). The median values for healthy controls in the two age classes are 0.8×10^9 to 0.5×10^9 per liter for B cells and 0.4×10^9 to 0.3×10^9 per liter for natural killer cells. Panel C shows data for the in vitro proliferative responses to anti-CD3 monoclonal antibody (on a \log_{10} scale, left side) and to phytohemagglutinin (on a linear scale, right side). The data are expressed as counts per minute (cpm) in ADA-deficient patients (for nine study patients before gene therapy and 6 months and 1 year afterward, for seven patients 2 years afterward, and for five patients 3 years afterward) and in 114 healthy controls. For the box-and-whisker plots, the box contains the data points that fall between the first and third quartiles, the horizontal line indicates the median, the diamond indicates the mean, and the brackets delineate 1.5 times the interquartile range (with data outside this range shown as individual points). The dashed horizontal line represents the 5th percentile for healthy controls (children and adults).

normal by 6 to 12 months of follow-up in all patients and remained normal during the follow-up period (Fig. 3C). Proliferative responses to alloantigens, *Candida albicans*, and tetanus toxoid were also observed in most patients (Table 3).

The T-cell-receptor repertoire was polyclonal, as ascertained by means of flow cytometry (Fig. 2A in the Supplementary Appendix). In Patients 1 through 4, a mean (\pm SD) of $92 \pm 8\%$ of the T-cell-



receptor $V\beta$ families after gene therapy were polyclonal, according to spectratyping analyses. In three patients previously given PEG-ADA, the proportion of $V\beta$ T-cell receptors displaying a polyclonal profile increased from $18 \pm 10\%$ to $79 \pm 9\%$ after gene therapy.

B-cell counts increased progressively after gene therapy (Fig. 3B), and as of the most recent fol-

low-up visit, the counts were normal in four patients (Table 3). The proportion of CD27+ memory B cells was similar in patients who underwent gene therapy and age-matched controls, with a polyclonal immunoglobulin-gene rearrangement in both groups (data not shown). Serum levels of IgA and IgM reached normal values in the majority of patients (Table 3), and serum IgG levels

Table 3. Long-Term Immune Reconstitution after Gene Therapy.*

Variable	Patients with Normal Value no./total no.
Cell count	
CD3+ T cells	5/9
CD4+ T cells	4/9
Natural killer cells	3/9
B cells	4/9
In vitro proliferative responses	
PHA mitogen	9/9
Anti-CD3 mitogen	9/9
<i>Candida albicans</i>	7/9
Alloantigens	8/9
TT	5/5
Serum immunoglobulins	
IgG	5/9
IgM	7/9
IgA	5/9
Antibodies to specific antigens	
Vaccine including TT, DT, BPT, and Hib	5/5
Pneumococcus (IgM)	4/5
MMR vaccine or other viral antigens†	5/5

* Results are from the most recent time point at which the patient was not receiving polyethylene glycol–modified bovine adenosine deaminase (PEG-ADA). Data for Patient 8 are not included here; this patient was evaluated for safety only because PEG-ADA was reintroduced 0.4 year after gene therapy. Some results are listed only for the five patients whose serum IgG levels were within the normal range after discontinuation of intravenous immune globulin supplementation. The normal values of cell counts, serum immunoglobulin levels, and in vitro proliferative responses are those reported for age-matched subjects (Eibl et al.,¹⁹ Comans-Bitter et al.,²⁴ and laboratory controls). BPT denotes *Bordetella pertussis* toxin; DT diphtheria toxin; Hib *Haemophilus influenzae* type b; MMR measles, mumps, and rubella; PHA phytohemagglutinin; and TT tetanus toxoid.

† Viral antigens consisted of antibodies against varicella, Epstein–Barr virus, or cytomegalovirus.

were within the normal range in five patients after discontinuation of intravenous immune globulin supplementation. In these five patients, antibodies against toxoid, conjugated or bacterial polysaccharide antigens, or measles, and rubella were detectable after immunization with these antigens (Table 3).

CLINICAL OUTCOME

All 10 patients are alive. The nine patients who could be evaluated for efficacy (all but Patient 8) are well, with the duration of follow-up ranging from 1.8 to 8.0 years (Table 2). Patients 1 through

6 go to school regularly or, if of preschool age, have normal social relationships with other children and parents. All but two patients (Patients 1 and 9) were below the reference, the 5th percentile, in height and weight before gene therapy; at 1 year after gene therapy, their median weight increased from the 1.7th to the 13.6th percentile and the median height increased from the 3.4th to the 10.9th percentile (Fig. 4 in the Supplementary Appendix).

The rate of severe infections, expressed as the number of events per 10 person-months of observation, decreased from 0.93 before gene therapy to 0.13 after gene therapy. The median number of hospitalization days dropped from 45 before gene therapy to 2 after therapy. Three of the six infections were due to catheter-related bacteremia (Table 2); no life-threatening opportunistic infections have occurred. Most patients had abnormalities in neuropsychomotor development at onset that improved during the follow-up period. Three patients had hearing deficits before gene therapy that persisted afterward.

DISCUSSION

We found that treatment of SCID due to ADA deficiency by means of nonmyeloablative chemotherapy followed by an infusion of autologous hematopoietic stem cells that had been transduced with a retroviral vector bearing the ADA gene is not associated with adverse events during a median follow-up period of 4.0 years. The treatment supplies the patient with hematopoietic stem cells that pass a functional ADA gene to all their progeny. Of the 10 patients with SCID due to ADA deficiency who were treated in this manner, there was restoration of immune function and protection against severe infection in 9. The sustained expression of ADA in multiple hematopoietic-cell lineages allowed for the detoxification of purine metabolites and improvement in the patients' physical development.

The mortality rates in ADA-deficient patients who receive transplants from unrelated and haplo-identical donors after cyto-reduction are 37% and 70%, respectively.^{6,25} Transplantation without conditioning, involving marrow from a parent, is successful for most types of SCID,^{7,26} but only half of ADA-deficient patients with SCID have sustained donor engraftment. Our protocol, in contrast, affords excellent survival without serious complications, such as graft-versus-host dis-

ease.^{1,6,8} In addition, gene therapy is suitable for older children with SCID due to ADA deficiency, who have a higher risk of failure and complications after transplantation.^{6,26} Patients from whom a small number of bone marrow cells were harvested or who have preexisting chromosomal alterations in the marrow²⁷ may not be candidates for gene therapy.

Enzyme-replacement therapy is effective in most patients with ADA deficiency² but often fails to sustain lymphocyte counts and T-cell function.^{10,11,25} We found that ADA gene therapy improves immune function in patients who had insufficient immune reconstitution during PEG-ADA therapy. Taken together, these results indicate that the intracellular expression of ADA after gene transfer is superior to extracellular detoxification by PEG-ADA in permitting the maturation and survival of functional lymphocytes.

The use of nonmyeloablative conditioning^{18,28} and withdrawal of PEG-ADA were crucial factors in the successful outcome of our trial. Earlier gene-therapy trials for SCID due to ADA deficiency,¹⁵⁻¹⁷ which did not include conditioning regimens, were hampered by limited engraftment and immune reconstitution. Our data show that nonmyeloablative conditioning allows for the engraftment of transduced stem cells. Conditioning with busulfan was also used (at a dose of 8 mg per kilogram) in a gene-therapy protocol for treating chronic granulomatous disease,²⁹ resulting in the engraftment of 10 to 15% of transduced granulocytes. Another gene-therapy trial for SCID due to ADA deficiency used melphalan for conditioning and achieved metabolic and T-cell reconstitution, but only 0.1% of granulocytes carried the ADA gene.²² In our trial, the number of infused CD34+ cells and the efficiency of *in vitro* gene transfer were also critical.

Previous gene-therapy studies with mature lymphocytes³⁰ or hematopoietic stem cells^{17,18,22} indicated that enzyme-replacement therapy inhibited the outgrowth of ADA-transduced cells. Our study supports the notion that a toxic environment caused by high levels of purine metabolites at the time of stem-cell engraftment is advantageous in supporting the differential expansion of gene-corrected cells, especially in lymphoid lineages.

Gene therapy restored normal immune function in five patients and resulted in significant improvement in lymphocyte counts and functions

in the other five patients, leading to protection from infectious complications. The reconstitution of lymphocyte levels was considerably slower in comparison to recovery after a bone marrow transplant including T cells from an HLA-identical donor. This difference is most likely due to the time required for the differentiation of T cells from purified, vector-containing stem cells. We believe that early intervention with gene therapy in patients with SCID due to ADA deficiency can reduce the risk of thymic involution and that optimization of the conditioning procedure will improve engraftment and immune recovery.

Gene therapy has been shown to benefit patients with X-linked SCID or chronic granulomatous disease, but the results were seriously limited by the development of leukemic proliferation (in 5 of 19 patients with X-linked SCID^{31,32}) and clonal expansion of myeloid cells (in 2 patients with chronic granulomatous disease²⁹). These complications were associated with retroviral-vector insertions near cellular proto-oncogenes. Our long-term follow-up and the experience in other trials of patients with SCID due to ADA deficiency^{17,22,33,34} did not reveal such complications. This is consistent with the polyclonal pattern of vector integration and T-cell repertoire, and the lack of *in vivo* skewing for potentially dangerous insertions.²¹ (See the Supplementary Appendix for further discussion of this complication of gene therapy in SCID.)

In conclusion, gene therapy with nonmyeloablative conditioning is an option to be considered for all patients with SCID due to ADA deficiency who lack an HLA-identical sibling donor. Our study suggests that gene therapy in combination with appropriate conditioning regimens could be successfully extended to the treatment of other congenital diseases involving the hematopoietic system.

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Dr. Bordignon reports being the chief of the board and chief executive officer (CEO) of MolMed, a drug company authorized to produce and release gene-therapy-based medicinal products for human use. MolMed manufactured the vector and engineered cells under Good Manufacturing Practices as a service to Telethon. Dr. Bordignon left the clinical study when he became CEO of MolMed in 2006. No other potential conflict of interest relevant to this article was reported.

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