Bone Marrow Transplantation and Alternatives for Adenosine Deaminase Deficiency

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KEYWORDS

• Adenosine deaminase • Severe combined immunodeficiency
• Stem cell gene therapy • Enzyme replacement therapy

Severe combined immunodeficiency (SCID) arises from several different genetic defects. The majority of these abnormalities relate to factors that are specific to the immune system and which lead to lymphoid-specific lineage development abnormalities. In contrast to this is adenosine deaminase (ADA)-deficient SCID, which is thought to comprise approximately 10% to 15% of all cases of SCID.[1] ADA is an enzyme involved in the purine salvage pathway that is required for the recycling of adenosine (Ado) and deoxyadenosine (dAdo) after DNA breakdown, and is expressed in all tissues of the body. The absence of ADA enzyme activity through naturally inherited mutations in the ADA gene leads to the buildup of intracellular and extracellular substrates, all of which have adverse effects on the functions of different cell types. The clinical effects of ADA deficiency are manifest in different organ systems, but most dramatically so in the immune system where it leads to severe lymphopenia with abnormal development of T, B, and natural killer (NK) cells.

ADA deficiency, like other forms of SCID, is invariably fatal in the first year of life and requires early intervention. Hematopoietic stem cell transplantation (HSCT) remains the mainstay of treatment but, unlike for other forms of SCID, 2 other treatment options are available, namely enzyme replacement therapy (ERT) with PEG-ADA and autologous hematopoietic stem cell gene therapy (GT). These different options have coexisted for at least the last decade, and in the case of ERT and HSCT for at least 2 decades. In this article the author reviews the available data on treatment by these

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different options, and offers an overview on when each of the different treatment options should be used.

BIOCHEMICAL BASIS OF ADA-SCID

ADA (EC3.5.4.4) is an enzyme of the purine salvage pathway, expressed at different levels in all tissues of the body, with the highest levels detected in the thymus. ADA catalyzes the conversion of deoxyadenosine (dAdo) and adenosine (Ado) to deoxyinosine and inosine, respectively (**Fig. 1**).[1] In the absence of ADA activity, dAdo accumulates in extracellular compartments and within cells, where it is converted by the enzyme deoxycytidine kinase (dCydK) to deoxyadenosine triphosphate (dATP). The buildup of both dATP and dAdo has deleterious effects on lymphocyte development and function, and is the major cause of the immunologic defects. dATP inhibits ribonucleotide reductase, an enzyme that participates in DNA replication and repair,[2] induces apoptosis in immature thymocytes,[3] and interferes with terminal deoxynucleotidyl transferase (TdT) activity, thus limiting V(D)J recombination and antigen receptor diversity.[4] dAdo accumulation inactivates the enzyme *S*-adenosylhomocysteine hydrolase (SAHH),[5] resulting in inhibition of transmethylation reactions necessary for effective lymphocyte activation. Elevated levels of Ado, acting through cell surface G protein coupled receptors, may contribute to immune dysfunction[6,7] and pulmonary inflammation associated with ADA deficiency.[8] However, the profound

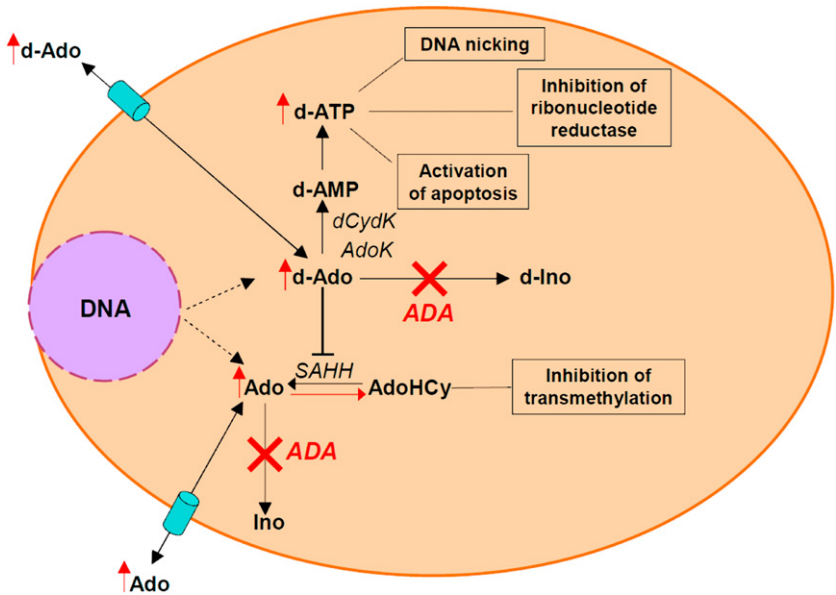

Fig. 1. Pathogenesis of ADA deficiency. The breakdown of DNA leads to recycling of deoxyadenosine and adenosine. In the absence of ADA, deoxyadenosine is converted to deoxyadenosine triphosphate and adenosine is converted to *S*-adenosylhomocysteine, both of whose substrates adversely affect several intracellular mechanisms. ADA, adenosine deaminase; ADCY, adenylyl cyclase; Ado, adenosine; AdoHCy, *S*-adenosylhomocysteine; AdoK, adenosine kinase; d-Ado, 2-deoxyadenosine; d-AMP, deoxyadenosine monophosphate; d-ATP, deoxyadenosine triphosphate; dCydK, deoxycytidine kinase; d-Ino, deoxyinosine; Ino, inosine; SAHH, *S*-adenosylhomocysteine hydrolase.

and relatively selective lymphopenia of ADA deficiency may be best explained by the expression pattern of ADA, which is highest in the thymus as a result of high lymphocyte turnover,[9,10] and also by increased expression of dCydK in lymphocytes, which increases dATP accumulation from dAdo in immune cells more than in other tissues.[11]

The expression of ADA throughout the body does, however, lead to several significant nonimmunologic defects. ADA-SCID patients have been noted to have costochondral abnormalities and skeletal dysplasias,[12] neurologic deficits involving motor function,[13] cognitive and behavioral defects,[14,15] bilateral sensorineural deafness,[16] and hepatic dysfunction.[17] Several of these abnormalities are present despite correction of immunologic abnormalities after bone marrow transplantation,[14] thus highlighting the systemic nature of the disease. Nonimmunologic deficits are also found in ADA-deficient mice, which exhibit hepatocyte degeneration, pulmonary and intestinal defects,[18–20] and neurologic abnormalities,[21] with dATP and dAdo accumulation and SAHH inhibition in affected tissues.

PRINCIPLES FOR THE MANAGEMENT OF ADA DEFICIENCY

The pathogenesis of ADA-SCID arises from the accumulation of toxic metabolites in intra- and extracellular compartments. The nucleosides Ado and dAdo are transported to the plasma once the catalytic activity of dCydK is saturated, whereas dATP is trapped inside the cells. In principle, any form of treatment that aids the clearance of Ado and dAdo from the plasma will shift the dynamic equilibrium of metabolites from within cells and then achieve detoxification, ultimately promoting immune recovery. Thus delivery of sufficient ADA enzyme, whether exogenously (eg, ERT) or packaged within cells (allogeneic stem cell transplant or GT), is the goal for correction of the disease phenotype.

HEMATOPOIETIC CELL TRANSPLANTATION

Hematopoietic cell transplantation (HCT), if available, has been considered the mainstay of treatment for ADA-SCID. HCT is the most readily available treatment for most transplant centers and, if successful, offers permanent correction of the disease. The protocols for the use of HSCT in ADA-SCID and the outcome following these procedures have, however, not been formally reported and with the availability of different treatment options, there remains some uncertainty on when to use transplant and what protocols to use for conditioning. Until very recently, the only data available have come from large follow-up studies on SCID in general where the numbers of ADA-SCID transplants were in the minority. The largest series to be found is a report from the European SCETIDE database, which documents outcome on 475 SCID transplants, 51 of whom were ADA-deficient patients.[22] The 3-year survival data demonstrate an 81% survival for ADA-SCID patients undergoing an HLA-matched donor transplant and 29% survival for those patients undergoing parental mismatched transplants, with no outcome data on the 4 patients undergoing a matched unrelated procedure. In another study of 94 SCID patients transplanted at 2 separate centers, 6 patients were reported and showed 4 matched related donor transplants (3 survivors) and 2 unrelated donor transplants (1 survivor).[23] These data do not effectively represent the use of unrelated donor transplants and importantly do not address several issues, including whether conditioning is necessary for the various different treatment options and also the degree of immune reconstitution and chimerism following the different transplant procedures. There is also a perception from clinicians that ADA-SCID patients are more difficult to transplant than other SCID patients, and that these

patients are more vulnerable to conditioning or infection perhaps as a result of the underlying metabolic defect.

For these reasons, a multicenter retrospective analysis on outcome of transplant for ADA-SCID was initiated, involving European and North American centers. Interim analyses have been presented at European Group for Blood and Marrow Transplantation meetings and were summarized in a recent review article.[24] The major outcomes reported were that matched sibling (n = 31) and matched donor transplants (n = 8) were highly successful, with 3-year survival figures of 87% and 88%, respectively. Matched unrelated donor transplant (n = 11) survival figures were 67%, but much poorer figures were obtained for mismatched related donor transplants (n = 30) (from haploidentical parental donors) where survival was only 43%. The survival following mismatched unrelated transplants was similarly only 29% (n = 7). The reasons for the divergent survival figures are difficult to dissect out, but the major determining factors appear to be HLA disparity and the use of conditioning. The availability of a fully matched related donor does allow the infusion of the donor graft without the need for any prior cytoreductive conditioning regime. The majority of transplants in the matched sibling and matched family donor setting were undertaken without any conditioning and therefore avoided the chemotherapy-related toxicities to which these patients are anecdotally vulnerable. For other donor options for which the outcome figures are less good, the use of conditioning may have played a major factor. It may be argued that given the minimal residual immunity present in ADA-SCID patients, it is possible to perform all transplants without the use of conditioning even in the unrelated and haploidentical setting. However, the data available do not support this. Of 4 unrelated donor transplants performed without conditioning, 2 did not engraft effectively. Data are also available for ADA-SCID haploidentical transplants performed without conditioning.[25] Of 19 procedures performed at a single North American institution, 14 survived (73%) the procedure but only 7 patients were successfully engrafted (disease-free survival 37%) with the remainder rejecting the unconditioned transplant. Together, these data suggest that unconditioned transplants can only be successfully undertaken with fully matched sibling and family donors, but there is a need for cytoreductive conditioning for all other donor types.

Other determining factors were also examined. The perceived susceptibility of ADA-SCID to conditioning has led to the suggestion that patients may benefit from reduced intensity procedures but interestingly, there was no difference between patients undergoing reduced intensity conditioning (n = 8, survival = 50%) and those undergoing fully myeloablative conditioning (n = 38, survival = 50%). The use of PEG-ADA prior to transplant has been argued as both a positive and negative risk factor. Some have argued that its use before transplant may stabilize children and therefore improve transplant outcome. In contrast, others argue that its use may blunt the survival advantage to ADA-containing donor cells. In this study at least, neither argument prevails as no difference in survival was found between those who received PEG-ADA before transplant and those who did not.

One important outcome noted was the quality of immune recovery. The data available suggest that the majority of patients who survive transplant normalize absolute lymphocyte and T-cell counts. Impressively, 92% of patients were able to discontinue immunoglobulin replacement therapy, including 24 of 26 patients undergoing unconditioned sibling donor procedures, suggesting that immune recovery is relatively complete in the majority of surviving patients. Similarly, patients are well detoxified after transplant with marked reduction of dATP levels to a mean of approximately 100 mmol/L which, although not normal, represents approximately 1 log reduction from levels at diagnosis.

No data were available on the degree of donor chimerism. The author's own experience suggests that following unconditioned procedures, the majority of patients only show donor T-cell engraftment, which may be complete or mixed. However, there is little donor cell engraftment in myeloid or B-cell lineages. Other data from animal experiments suggest that in murine neonatal transplants in ADA-deficient mice, engraftment of ADA-replete cells in nonlymphoid organs such as liver or in myeloid cells can lead to cross-correction and development of endogenous T cells.[26] The inference from these different observations is that what is important is not necessarily engraftment of donor cells in any specific lineage but, as alluded to earlier, the delivery of sufficient ADA enzyme in an engrafting cellular source that allows effective systemic detoxification, thereby promoting T- and B-cell development.

An important question for this disease has been the effect of transplantation on the other manifestations of ADA-SCID. Follow-up of transplanted patients demonstrates that despite effective immune recovery and stable detoxification, the majority of patients show cognitive deficits, with a mean IQ score 2 standard deviations below the population normal range, and also show hyperactivity and attention deficit patterns of behavioral disorders.[15] Patients also show a high incidence of audiological problems, with a typical pattern of high-frequency sensorineural hearing loss. For both types of defect, it was not possible to make any correlation with the type of transplant or the conditioning regime, or the degree of chimerism, metabolic detoxification, or immune recovery. The only variable to influence outcome was the level of dATP at the time of diagnosis, which showed a negative correlation with the IQ level, suggesting that greater metabolic derangement at diagnosis led to poorer IQ.[14] This result would suggest that a certain amount of damage may be present at birth that is irreversible, despite immune and metabolic reconstitution, which may relate in part to in utero damage to the developing fetus and may argue for earlier therapeutic intervention.

These data provide some useful guidance on the use of transplant following different donor options. The results from matched sibling donor and matched related donors are extremely good and similar to those seen for other forms of SCID. The ability to undertake these procedures without chemotherapy and thereby avoid long- and short-term chemotherapy-related toxicities makes this a very attractive option, as does the documented quality of cellular and humoral immune reconstitution. By contrast, mismatched transplants especially from parental donors are poorly tolerated and have poor survival figures. These transplants are also unsuccessful without any conditioning, with a high rate of rejection, and therefore it would be advisable to avoid such procedures altogether given that other treatment options such as ERT and GT are available. Matched unrelated donor (MUD) transplants were still poorly represented in this series, with only 11 fully matched procedures undertaken and an overall survival rate of 67%; it is difficult to make any firm recommendation on the basis of such small numbers (see also the later general discussion alongside the use of other treatment options). The ability to avoid nonimmunologic complications is not influenced by the type of transplant or the degree of conditioning, and it is presently not possible to recommend any specific measures.

ENZYME REPLACEMENT THERAPY

Enzyme replacement therapy (ERT) with PEG-ADA (Adagen, Enzon Inc; obtained outside USA through Orphan Europe) for the treatment of ADA deficiency has been available for almost 20 years and has been designated an orphan drug. The use of PEG-ADA provides another treatment modality for ADA-SCID, but unlike HSCT or

GT it is not a curative therapy but requires regular intramuscular administration. Nevertheless, the effectiveness of PEG-ADA in correcting metabolic and immunologic parameters and, more importantly, in promoting clinical well-being in patients makes it an important option in the care of patients.

PEG-ADA is a compound in which the bovine form of ADA is covalently conjugated to polyethylene glycol (PEG). Pegylation confers several therapeutically beneficial properties to ADA through alteration of its physical and chemical properties, mainly due to an increase in molecular weight.[27,28] The circulating life of the compound is prolonged from minutes to days as clearance from the circulation is inhibited. Pegylation also reduces the immunogenicity of a protein, which again helps to extend its circulating life.[27]

PEG-ADA is administered by once- or twice-weekly intramuscular injections, and results in high levels of ADA activity in the plasma. Of importance is that PEG-ADA cannot cross the cellular membrane and therefore exerts its effect through extracellular ADA activity. High ADA activity in the plasma eliminates Ado and dAdo and, because of the equilibrium maintained between the intra- and extracellular compartments, this results in the movement of Ado and dAdo into the extracellular compartment where further deamination occurs. By drawing adenosine from the cell, this then reduces intracellular accumulation of dATP and its consequent toxic effects.

Since the first patients were treated, the dosing regime has evolved and it is now advised that children should start at a dose of 60 U/kg/wk with biweekly injections until metabolic correction is established (between 1 and 3 months). Once patients show clinical improvement and biochemical stabilization, they can be maintained on a dose of 30 U/kg/wk in a single weekly injection.[29,30] It is important when monitoring patients to assess their immune function as well as metabolic parameters, and this should be taken into account when considering altering the dose of PEG-ADA. Erythrocyte dATP levels can also be measured and used to guide treatment. Initial trough plasma ADA levels (before injection) should be maintained at 50 to 150 μmol/h/ml (normal range <0.4 μmol/h/ml), which equates to approximately 4 to 10 times the normal erythrocyte ADA activity and is required for initial rapid detoxification. Once a maintenance dose of 30 U/kg/wk is established, trough plasma ADA levels can be maintained at 25 to 60 μmol/h/ml. Erythrocyte deoxyadenosine nucleotide levels decrease significantly, and are maintained at levels below those observed after HSCT and SAHH activity is normalized. It is recommended that trough plasma ADA activity levels are monitored every 1 to 2 weeks during the first 2 to 3 months of treatment, twice a month until 9 months of treatment, and then monthly until 18 to 24 months on PEG-ADA. Once patients are established on an effective maintenance dose then plasma ADA levels can be measured every 2 to 4 months unless there is a change in clinical status.

IMMUNE RECONSTITUTION ON PEG-ADA

Immune reconstitution following treatment with PEG-ADA has not as yet been reported in a formal manner, and the data cited here are taken from retrospective and single-center studies. The impression from these different studies is that immune recovery is variable, the reasons for which may be associated with the underlying clinical condition of the child, the age at which treatment is started, and the level of residual thymic activity at the time of PEG-ADA initiation. Up to 20% of patients receiving therapy appear to show no response.[1,30] In the majority of cases, however, full immune recovery is seen in the short term but is followed by waning T-cell numbers.[31] In terms of humoral immunity, continued immunoglobulin replacement is

required in up to 50% of those treated[25] with long term PEG-ADA. What is not clearly documented, however, since most attention has been directed toward immune recovery, is that following PEG-ADA initiation, there is rapid detoxification of dATP levels and this is temporally associated with an increased clinical well-being in patients who start to feed and gain weight. Over time, despite patients having suboptimal immune function, similarly there is well-maintained clinical well-being, with freedom from infection and good growth parameters.

Immune recovery is evidenced initially by an increase in B-cell numbers within a few weeks of starting treatment, and is followed by an increase in T-cell count that may take several months to occur. Symptoms of immune dysregulation such as hemolytic anemia and immune thrombocytopenia can be seen during this period, and may be related to dysregulated cellular and humoral recovery. Recovery of T cells following initiation of PEG-ADA is variable. Again a formal description of T-cell recovery in large numbers of patients is not available but the author's practice has seen 2 major patterns of T-cell recovery. There are patients who show T-cell recovery with regeneration of CD4 and CD8 populations and the development of some naïve T-cell precursors. Over time, these individuals run levels of T cells that are below the normal range for age and are significantly T-lymphopenic. The other pattern seen and which appears to be most common is patients who show T-cell recovery but this being composed almost entirely of memory T cells. In these individuals, despite maintaining high levels of ADA activity and effective detoxification, very little CD4 T-cell and especially naïve CD4 T-cell output is seen. It is most probable that in these patients there has been, either in utero or in the early neonatal stage, significant damage to the thymus as a result of high levels of circulating toxic metabolites. The capacity for these individuals to generate naïve T cells through ERT appears to be severely limited, and such individuals are protected by the development of more mature T-cell populations.

Data on long-term outcome in patients with ADA deficiency treated with PEG-ADA has been published by several groups.[25,32,33] Chan and colleagues[33] published a retrospective review of 9 patients treated in North America with follow-up data from 1995 to 2002. The patients were mostly typical ADA-SCID patients with early presentation (one late-onset patient) who had received PEG-ADA for 5 to 15 years. Their clinical course over this time was good, with little in the way of recurrent or severe infections, although any immune reconstitution associated with treatment declined over time. The majority of children also received other forms of treatment including HSCT and GT. Absolute lymphocyte counts (ALC) remained below the normal range despite improving from baseline. ALC peaked between 1 and 3 years on PEG-ADA at 250 to 1480 cells/mm^3, but fell after 5 to 12 years of therapy to 12 to 500 cells/mm^3. Proliferative responses normalized in some patients after an average of 4 months of treatment before declining over time. Immunoglobulin production was difficult to assess as patients received supplemental immunoglobulin. Again it was postulated that limited thymic reserve or age-related decline in thymic function may have given rise to the decrease in T-cell numbers over time.

Malacarne and colleagues[32] explored thymic output and immune reconstitution in 5 patients (ages 5–9 years) with ADA-SCID treated with PEG-ADA for 5 to 8 years. B-cell and T-cell numbers again increased 5 to 14 months following initiation of treatment, but remained low in comparison with normal controls. Patient responses to phytohemagglutinin (mitogen) stimulation increased but were variable even within the same patient. Four of the 5 patients developed specific antibodies after immunization with tetanus and showed an increase in serum immunoglobulin levels. Normal T-cell development and thymic output were measured through T-cell receptor excision circles (TRECS), and these proved to be consistently low compared with healthy

age-matched controls, again suggesting a compromise in thymic function. B-cell repertoire was examined and found to be restricted, but it is not clear if this relates to an intrinsic B-cell problem or is secondary to defects in the T-cell compartment. Patients remained clinically well on long-term therapy with PEG-ADA.

A European survey of patients receiving PEG-ADA therapy was undertaken in 2005 and preliminary data were published in 2007.[25] Data gathered from 42 patients based in several European centers showed that PEG-ADA was started in the first 6 months of life in over half of the patients (n = 27). Two-thirds of patients received PEG-ADA only and the remainder progressed to HSCT or GT. Four reported deaths were infection related (cytomegalovirus viremia, respiratory syncytial virus infection, encephalitis, and pneumonitis) and were unlikely to be related to PEG-ADA. As previously reported, immune recovery was variable, with T-lymphocyte numbers below the normal range after 1 year of treatment (mean CD3+ count: 460 cells/mm^3). Immunoglobulin levels improved to the extent that 40% of patients received immunoglobulin replacement after 1 year. Overall survival in this cohort was 85% for children who received PEG-ADA alone (n = 26). Survival for those who went on to receive HSCT was 70% and 100% for those progressing to GT.

At present the long-term consequences of PEG-ADA therapy are unknown, but it is clear that immune recovery especially in the T-cell compartment is below normal levels and in some patients runs at levels that are of concern with regard to opportunistic infection. The reasons for this have not been determined, but reduced thymic function either at the onset of therapy or over the course of therapy may play an important role. The majority of patients have remained clinically well without experiencing major infective problems, but several case reports suggest that in certain patients ongoing low T-cell numbers has led to significant problems. One boy developed Hodgkin lymphoma after 13 years of treatment,[31] another developed Burkitt lymphoma again after 13 years of treatment,[34] and another child developed Epstein-Barr virus–positive malignant brain lymphoma after 10 years of treatment.[35] It is likely that such lymphoproliferative disease arises due to reduced immune surveillance. Further data gathering is necessary to determine overall outcome of patients on long term therapy.

The effects of PEG-ADA on the metabolic disturbances and immune reconstitution seen in ADA deficiency have been well described, but its effect on systemic manifestations of disease in not clear. In several major centers, children with ADA deficiency proceed to HSCT when an appropriate donor becomes available and PEG-ADA treatment is discontinued, and therefore long-term systemic outcome data are lacking. In those patients who have remained on long-term ERT, there are no formal data on the outcome of systemic pathology. The cognitive and behavioral abnormalities and sensorineural deafness described in ADA-deficient patients are certainly not affected by metabolic correction and persist after transplantation; these are now well documented, but no such data exist for patients on PEG-ADA. This lack of data perhaps is because no individual center has a sufficiently large cohort on long-term ERT to perform such a study and because such studies, especially on behavioral function, are difficult to perform across several different countries and continents.

The development of specific IgG antibody to bovine peptide epitopes of PEG-ADA has been reported by several groups, and often coincides with an improvement in humoral immunity.[25,30,36,37] There are no reports of antibody formation to PEG itself; it can be detected by enzyme-linked immunosorbent assay in up to 80% of patients on long-term PEG-ADA therapy but is clinically insignificant in most. Neutralizing antibodies to PEG-ADA that directly inhibit catalytic activity and accelerate clearance from plasma have been identified in 9 patients.[36,37] Seven of these patients were able to

either continue ERT after dosage adjustment, or they underwent successful HSCT. However, loss of efficacy contributed to death in 4 patients, including 3 who also developed refractory hemolytic anemia.

The development and use of PEG-ADA has provided an important alternative option for treatment of patients with ADA deficiency. The rapid metabolic detoxification afforded by high-level enzyme replacement allows clinical stabilization of patients and provides longer-term treatment options when no suitable donor is available. The long-term immune recovery on PEG-ADA appears to be suboptimal, although clinical well-being is maintained in the majority of patients. However, PEG-ADA is not readily available in all countries, which together with its high cost may limit its applicability to all patients.

GENE THERAPY FOR ADA-DEFICIENT SCID

ADA-SCID has long been seen as an attractive target for hematopoietic cell GT. Indeed the very first clinical trials of GT for any genetic disease were performed on patients with ADA-SCID in the early 1990s. The reasons for its emergence as an ideal target disorder for GT include (1) its monogenic nature; (2) its role as a ''housekeeping'' enzyme with an uncomplicated pattern of gene regulation; (3) the fact that experience with HSCT demonstrates that the most severe manifestations of the disease can be corrected using ADA-replete hematopoietic cells; and (4) the poor outcomes in mismatched donor transplants. The advantages of GT over transplantation, which were stated then and which still hold true today, are that modification of autologous cells avoids the complications of graft-versus-host disease and because of the perceived survival advantage to ADA-replete cells, GT can be undertaken with little or no conditioning.

Several studies were conducted in the early 1990s, all of which used different forms of conventional gammaretroviral vectors encoding the ADA gene under the transcriptional control of the viral LTR (long terminal repeat).[38–41] The cellular target for the different studies varied from autologous peripheral blood lymphocytes (PBLs), to a combination of bone marrow and PBLs, to selected CD34+ stem cells from bone marrow or umbilical cord blood. In all these studies, no conditioning was given and all patients received PEG-ADA while undergoing GT. The major findings from these studies were that gene transfer into progenitor cell populations over extended time periods could be achieved and that there was little or no toxicity to patients as a result of gene transfer. However, no demonstrable improvement in immune function was seen as a result of the GT procedure.

It was not until early 2000s that more effective protocols were designed that eventually resulted in successful correction of the clinical and immunologic phenotype.[42–44] The major changes to the protocols were the withdrawal of PEG-ADA prior to the return of gene-transduced cells, thereby restoring a potential survival advantage to ADA-replete cells, and the use of mild nonmyeloablative conditioning to allow the long-term engraftment of a greater number of gene-modified cells at the outset. Trials in Milan and London, and a joint study between the Children's Hospital Los Angeles (CHLA) and the National Institutes of Health (NIH) treated a total of more than 20 patients. Each group used a different gammaretroviral vector and all transduced autologous selected CD34+ stem cells. The Milan and the United States studies used intravenous busulfan conditioning at a dose of approximately 4 mg/kg although the dosing regimes have varied between the 2 studies, and the London group used melphalan, 140 mg/kg, based on their experience of this chemotherapy regimen in their allogeneic transplant program.

The combined results from these 3 studies are very encouraging, and are summarized in **Table 1**. The majority of patients treated in these studies had previously shown an inadequate response to ERT or had failed an allogeneic transplant. In all patients, PEG-ADA was not given after the return of gene-modified cells. Following GT the majority of patients (67%) showed improved recovery of T-cell numbers compared with pre-GT levels. In these patients, it has not been necessary to restart ERT and several patients have remained off ERT for longer than 5 years, with the longest follow-up being approximately 8 years. Most impressively, all patients to date have survived the procedure and there have been no significant adverse events.

More detailed analysis of the data from these studies shows that T-cell recovery, although sufficient to protect individuals from infection, is at the lower end of the normal range. However, analysis of thymic function by TREC analysis or by surface phenotyping suggests that thymopoiesis is established in treated individuals and that T cells undergo thymic education before entering the peripheral circulation, suggesting that prethymic progenitors have been successfully transduced. These data are in keeping with the demonstration of a polyclonal T-cell repertoire and normal response to mitogenic and antigenic stimulation. Humoral function is less well reconstituted, although 5 of 10 children in the Milan study were able to discontinue immunoglobulin replacement therapy and showed vaccine-specific antibody responses.[44] The ability of patients to remain off ERT also demonstrates effective metabolic detoxification, and there is an impressive decrease in dATP levels. In some patients an increase in erythrocyte ADA levels was seen, indicating successful transduction of erythroid progenitors that were now capable of giving rise to ADA-replete red cells. Analysis of transgene levels showed that the majority of T and NK cells were gene marked, but also that there were lower but significant levels of marking in B cells and in the myeloid lineage.

GT in ADA-SCID has been highly successful, but these studies have run in parallel to studies of GT for SCID-X1[45,46] and X-linked chronic granulomatous disease (CGD).[47] In both latter studies, the success of GT has also been associated with gammaretroviral-mediated leukemogenesis.[47–49] Insertion of the gammaretroviral vector into the target cell chromosome is not a random process (as was thought at the initiation of these studies), and such vectors have a propensity to insert in and around the transcription start site of active genes. In 5 of 20 patients in studies of SCID-X1 and in 2 of 4 patients treated with GT for X-CGD, insertion of the vector into protooncogenes led to aberrant gene transcription of these genes and the proliferation of specific clones that eventually underwent leukemic transformation. Detailed analysis suggests that the powerful enhancer elements of the viral LTR were able to interact with nearby promoters, thereby activating aberrant protooncogene transcription. These

Table 1
Summary of clinical trials of GT for ADA deficiency

Center	No. of Patients	Follow-Up (Years)	Off Enzyme	Survival	DFS
Milan	10	1.8–8.0	8/10	100%	80%
London	6	1.0–6.0	3/6	100%	50%
CHLA-NIH	6	0.5–3	3/6	100%	50%
UCLA-NIH	3	0.1–0.5	3/3	100%	n.e.
Total	25	0.1–8.0	17/25	100%	67%

Abbreviations: DFS, alive without bone marrow transplantation or PEG-ADA restart; n.e., not evaluable; UCLA, Unicersity of California, Los Angeles.

observations have naturally led to the analysis of retroviral integration sites in patients undergoing GT for ADA-SCID, and in keeping with findings from the other studies, vector integrations into or around protooncogenes have also been identified in ADA patients.[50,51] However, despite these similar integration profiles and similar duration of follow-up, no evidence of clonal dominance or proliferation has been observed in ADA-SCID patients. The reasons for these differing outcomes are difficult to explain, especially because the vector constructs and LTR sequences have in some cases been very similar and may indicate that GT for ADA-SCID has a more favorable risk profile, although extended follow-up is necessary before such statements can made more definitively. The observations do demand, however, that safety monitoring should be continued to be strictly implemented over the long term in all patients, according to guidelines of regulatory agencies. Further, the potential risks associated with the use of gammaretroviral vectors have led several groups to investigate the use self-inactivating vectors, such as lentiviral vectors,[52,53] which may in time improve the safety of GT for ADA-SCID.

DISCUSSION

The data available from the 3 different therapeutic options have led to guidance on how to treat patients when faced with different donor availabilities. The initial stages for care of a child with ADA-SCID would be as for any child with SCID with treatment of active infection and stabilization of the clinical state, including optimal nutrition. In some cases and especially in infants with very poor nutrition or respiratory compromise, it may be advisable to start PEG-ADA to stabilize the child. Respiratory distress in an ADA-SCID child may in many cases be as a result of metabolic derangement rather than infection, and therefore detoxification with ERT may be acutely beneficial. As for other SCID forms, an urgent search for a donor should be undertaken.

If a fully matched sibling or family donor is available, the evidence currently available suggests that a transplant should be undertaken without any conditioning. The success rates associated with such procedures and the effective long-term reconstitution in both T- and B-cell compartments does not at present warrant the use of alternative therapies. Some have argued that the use of a conditioning regime may improve stem cell engraftment and promote an improved quality of immune reconstitution long term. However, the current data do not show any waning of immune function even more than 10 years after unconditioned HSCT, and the majority of patients have remained free of immunoglobulin therapy. Further strong evidence to the contrary would be required before this recommendation is changed. If PEG-ADA has been started, it is unlikely that there will have been significant immune recovery (which usually takes 3–6 months) before a matched sibling donor (MSD)/matched family donor (MFD) is found, in which case PEG-ADA can be stopped at the time of the unconditioned procedure.

If a matched MSD/MFD is unavailable, a search for an unrelated donor should be undertaken, during which time PEG-ADA should be started to promote improved nutrition and clinical well-being. If no matched donor is available, the transplant choice may be for a mismatched unrelated donor or a parental haploidentical donor transplant. The clear message from the currently available data is that these transplants are poorly tolerated and have a high degree of mortality in the conditioned setting as well as a high rate of rejection if performed without conditioning. For these reasons, it is advisable that these procedures are only undertaken if there is no access to ERT, perhaps for economic reasons, or if there is no possibility of enrolling the child into

a trial of GT. Cord blood donations may tolerate a higher degree of HLA disparity, but there is currently insufficient evidence to inform any strong recommendation.

Therapeutic decision making is most problematic when faced with the choices of continuing ERT, undertaking a fully matched unrelated donor transplant, or enrolling the patient into a trial of GT. The continued administration of PEG-ADA represents the easiest and, in the short term, the safest choice. It is clear that patients benefit with clinical well-being, freedom from infection, and improved nutrition. However, the long-term data are fairly conclusive in showing that, over time, immune recovery is suboptimal and many children run very low T-cell numbers. Emerging reports documenting opportunistic infection in such individuals suggest that this option may not be beneficial long term, although it is difficult to specify the exact time period. The other concern is that the observed decrease in thymic function may ultimately limit the efficacy of a definitive procedure such as GT or allogeneic transplant. If physicians or parents are not keen to enter a definitive procedure initially and wish to continue ERT, a prudent approach may be to monitor patients carefully and at the first signs of waning T-cell or thymic function, consider the options of HSCT or GT. A further reason to discontinue ERT and offer HCT or GT would be the development of autoimmune cytopenias or neutralizing antibody that is refractory to immune modulation.

The choice between HSCT or GT as a definitive procedure is again difficult. Simply put, the choice may between the short-term risks associated with MUD HSCT against the potential for long-term side effects associated with GT. MUD HSCT has a 67% survival outcome given the present data, but this involves only 11 individuals. These figures may change significantly even if only a handful of further transplants are undertaken. It is clear, however, that outcome following these procedures is very good, with almost complete T- and B-cell reconstitution, and presently no evidence of long-term side effects. By contrast, GT has an excellent survival outcome and to date more than 20 children in 3 trials have survived the procedure without any significant side effects. The drawbacks of GT are twofold. First, there is the potential for insertional mutagenesis using the current vector technologies, although importantly the follow-up does not show any evidence of clonal proliferation in treated individuals. The other issue is that of immune recovery; at present 67% have been able to discontinue ERT on a long-term basis and approximately 50% have been able to discontinue Ig replacement. Thus the choice will eventually be determined by several factors, including the availability of the 2 modalities at treatment centers and, probably most importantly, the parental and physician attitude toward the risks and benefits associated with the 2 different treatment options.

This field is a rapidly evolving one. Advances in both HSCT- and GT-related technologies are likely to be implemented in the next few years and may greatly improve the safety profiles of these treatments. In HSCT, the use of newer chemotherapy agents such as treosulfan and the use of novel antibody-based conditioning agents[54] may reduce the toxicities associated with HSCT. Self-inactivating lentiviral vectors have already shown an improved safety profile in in vitro and in vivo models of stem cell transformation,[55,56] and if such safety improvements are demonstrated in clinical studies, this would represent a significant advance. Lentiviral vectors also show improved transduction of hematopoietic stem cells and preservation of stem cell capacity, which in turn may allow for improved ADA gene expression and immune recovery. Thus, the balance between these 2 modalities will need to be reviewed on a regular basis as results from the anticipated improvements emerge.

Further issues surround the best treatment for the nonimmunologic manifestations of ADA-SCID, and most importantly the cognitive and neurologic defects. At present, data are available only from those patients who have undergone HSCT, which highlights the

incidence of mild to severe problems in this cohort. No data are available from patients undergoing ERT or GT. It may be hypothesized that better systemic detoxification may result in an amelioration of nonimmunologic abnormalities, and it will be important to formally study these patients. Such analysis is being planned. At present, however, these considerations cannot guide any specific treatment choice.

ADA-SCID is one of the most severe forms of SCID as a result of both the immunologic and metabolic abnormalities, and is a challenging disorder to treat. The availability of 3 different therapeutic options makes management decisions more difficult, but ultimately this does present choices that are not available for other SCID patients. Some strong recommendations can already be made based on current data and, as further developments in transplant and GT occur, it is likely that safer and more effective therapies will become available.

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