

# Adenosine Deaminase Gene Therapy Protocol Revisited

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The remarkable success of the clinical trial of retroviral-mediated gene transfer in infants with the X-linked form of SCID (XSCID) [1] stands in contrast to the previous studies on the adenosine deaminase (ADA)-deficient form of SCID. The ADA trials have yielded modest and often equivocal results at best, with significant clinical benefit difficult to discern. Studies targeting T cells have shown that ADA gene transduction and expression in a relatively large proportion of circulating T cells can be achieved for a sustained period (over 10 years in the first subject) [2]. However, the subjects have continued to receive ADA enzyme replacement therapy (PEG-ADA), which by itself can detoxify deoxyadenosine metabolites and restore immunity. Therefore, it cannot be objectively determined whether the gene transfer had any useful effect on immune function. Studies targeting hematopoietic stem cells (HSC) from ADA-deficient subjects have shown that ADA gene transduction and engraftment of low numbers of HSC can give rise to gene-containing T cells that have a selective survival advantage compared with noncorrected T cells when PEG-ADA is weaned [3]. Again, it was not possible to demonstrate evidence for significant immune function as a result of the gene-containing cells that were present, as these subjects have also remained on PEG-ADA due to concerns of incomplete immune protection.

When clinical gene transfer studies yield less than salutary results, the failure is usually attributed to the inadequacies of the tools, in this case oncoretroviral vectors. But the failure may lie not in the tools, but rather in the study design. All of the ADA gene transfer studies performed so far have mandated that the subjects be treated with PEG-ADA enzyme replacement therapy, based on ethical considerations to avoid putting them at risk of opportunistic infections by withholding or withdrawing PEG-ADA to test the gene transfer effects. One factor that may have led to the clear-cut benefits to immunity in the XSCID study was the absence of an analogous form of protein replacement therapy that can "rescue" nontransduced T cells and thus blunt the selective advantage that gene-corrected cells are expected to display in SCID subjects.

A few rare "experiments of nature" have shown that ADA-expressing T cells do have a strong selective advantage over ADA-deficient T cells: Isolated cases have been reported of patients with ADA-deficient SCID who show spontaneous improvement in T-cell numbers and function [4,5]. In these subjects, it has been possible to docu-

ment that there had been a spontaneous reversion of a mutant ADA allele, presumably in a single stem or progenitor cell, which then gave rise to a sufficient number of ADA-expressing T cells to restore immunity to a significant extent.

Claude Bordignon and his coworkers have now taken advantage of unique circumstances to carry out ADA gene transfer in the absence of PEG-ADA enzyme replacement therapy in two ADA-deficient SCID patients who lacked suitable histocompatible bone marrow donors and who did not have access to PEG-ADA because of financial constraints [6]. The initial results of this clinical trial were reported in Oral Session at the 43rd Annual Meeting of the American Society of Hematology, in Orlando, Florida, December 10, 2001. These two subjects have undergone gene transfer procedures, similar to those used in the XSCID study by Cavazanna-Calvo *et al.* [1], using *ex vivo* retroviral-mediated transduction of CD34+ cells isolated from their bone marrow in culture medium supplemented with interleukin-3, c-kit ligand, flt-3 ligand, and thrombopoietin on recombinant fibronectin. They are not being treated with PEG-ADA enzyme replacement therapy, so any selective survival advantage of gene-corrected cells should be manifest.

One additional novel aspect in this study was that the two children also received a moderate dose of the chemotherapeutic agent busulfan before the re-infusion of the CD34+ cells. It has been shown that partial cytoreduction—administration of relatively low dosages of total-body irradiation (for example, 100–400 cGy)—can significantly increase the engraftment of transplanted HSC, presumably by killing endogenous HSC, and thereby "making space" for the transplanted HSC. The dosage of busulfan given (4 mg/kg) is less than 25% of the typical dosage used in bone marrow transplantation, in which at least 16 mg/kg of busulfan is usually given in combination with high dosages of other agents, such as cyclophosphamide, with the goal of complete cytoablation. The moderate dosages of busulfan were tolerated well, without severe neutropenia or toxicity. This is the first time that cytoreduction has been used in a study of gene transfer for a genetic disease.

PCR-based analysis of peripheral blood cells shows that the ADA gene is present in cells of multiple lineage, consistent with transduction and engraftment of HSC (although definitive proof of HSC transduction will require retroviral vector proviral integration site analyses). The level of gene-containing peripheral blood

myeloid cells, which do not benefit from the presence of the ADA gene, can be used as an index of the engraftment level of transduced HSC; 7–16% of the myeloid cells contain the inserted ADA gene, which is significantly higher than levels achieved in prior studies that did not apply cytoreduction. These results support the utility of partial cytoreduction to enhance engraftment of gene-modified HSC.

More importantly, the subjects are undergoing immune reconstitution, with the majority of peripheral blood T and B lymphocytes and NK cells containing the vector (75–100%). The numbers and functional activity of T cells increased from the very low state seen in ADA-deficient SCID to protective levels in both subjects over 3–6 months. The subjects were reported to be clinically well without protective isolation.

Therefore, it is possible that the gene transfer tools have been adequate all along, at least in the “best-case scenario” of treating SCID, but that the continued administration of PEG-ADA thwarted the possibility of improvement. As with the XSCID study, the continued evaluation of these patients, literally over years and

decades, will be important to determine the extent and duration of immune reconstitution. Although the tools needed to treat the majority of blood diseases that do not have this profound selective advantage will require further development, this landmark study provides exciting new evidence that gene therapy can be effective, given the correct clinical setting.

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3. Kohn, D. B., *et al.* (1998). T lymphocytes with a normal ADA gene accumulate after transplantation of transduced autologous umbilical cord blood CD34+ cells in ADA-deficient SCID neonates. *Nat. Med.* **4**: 775–780.
4. Hirschhorn, R., *et al.* (1996). Spontaneous *in vivo* reversion to normal of an inherited mutation in a patient with adenosine deaminase deficiency. *Nat. Genet.* **13**: 290–295.
5. Ariga, T., *et al.* (2001). T-cell lines from 2 patients with adenosine deaminase (ADA) deficiency showed the restoration of ADA activity resulted from the reversion of an inherited mutation. *Blood* **97**: 2896–2899.
6. Aiuti, A., *et al.* (2001). Correction of ADA-SCID defect without PEG-ADA therapy by stem/progenitor cell gene therapy combined with a non-myeloablative conditioning. Oral Abstract Session, 43rd Annual Meeting of the American Society of Hematology, Orlando, FL, December 10, 2001.