

**BIOLOGICALS RESPONSE MODIFIERS ADVISORY COMMITTEE  
MEETING #33, OCTOBER 10, 2002  
Retroviral Gene Therapies for the Treatment of Patients with  
Severe Combined Immunodeficiency – Safety Issues**

CBER is convening this meeting in response to the recent notification of an adverse event in a clinical trial in France that uses retroviral vector-mediated gene therapy in children with X-linked Severe Combined Immunodeficiency. In particular, we are seeking the advice of the committee on how to proceed with similar clinical trials in the US (Question for the Committee is found on the last page of this document).

**Severe Combined Immunodeficiency (SCID)**

Severe Combined Immunodeficiency (SCID) is a group of inherited disorders that all share a defect in T cell differentiation giving rise to deficiencies in immune cell function (5). Current therapeutic options include bone marrow transplantation. In those cases where a HLA-identical donor (meaning that the donor marrow is a perfect match for the recipient) is used, survival is 100%, as reported in a long-term study of infants with SCID (3). Survival is reduced to 78% in those children who receive HLA-haploidentical donor marrow (the donor marrow is 50% identical to the recipient) (3). Although bone marrow transplantation seems to result in normal T cell function, most children who receive the HLA-haploidentical marrow still have abnormal B cell function, resulting in the need to treat with intravenous immune globulin in over 60% of the cases (3). In contrast, a study in neonates comparing data on bone marrow transplantation performed in 21 SCID infants who received the transplants in the neonatal period found that the survival rate was 95%, even in those cases where the transplant was from a haploidentical donor (9).

The genetic lesions underlying many of the clinical forms of SCID have been elucidated (5). One type of SCID caused by a genetic defect in the gene encoding adenosine deaminase (ADA) can be successfully treated in 90% of the patients by weekly administration of PEG-ADA (ADA coupled to polyethylene glycol) (5). Defects in the gene encoding the common gamma chain ( $\gamma_c$ ) have also been shown to cause X-linked SCID. Other genetic defects resulting in SCID include mutations in the gene encoding Jak-3, interleukin-7 receptor alpha chain, Rag-1 and Rag-2, or CD45 (reviewed in (5)). The inheritance pattern is either X-linked or autosomal recessive for all these known genetic mutations. The facts that SCID is caused by a genetic defect and that the genetic defect underlying the disease is known, in most cases, make SCID an attractive target for gene therapy approaches, whereby one could potentially correct the genetic defect by providing a normal copy of the gene.

Initial clinical trials using a gene transfer approach were performed in children with SCID-ADA by treating their T cells with a retroviral vector encoding the ADA protein. While T cells carrying the retroviral vector sequences have been detected long-term, the levels have been very low, and the continued use of PEG-ADA rendered the studies difficult to interpret with regard to clinical benefit of the gene transfer (2) (1). Several subsequent studies have been performed in children with SCID-ADA using retroviral vectors to deliver the ADA gene to hematopoietic stem cells (reviewed in (6)). Again, patients were maintained on PEG-ADA and the levels of T cells carrying the retroviral vector sequences were maintained for years after treatment, but always at low levels. The success of the gene transfer itself was again difficult to assess because of the concomitant administration of PEG-ADA.

More recently, gene therapy clinical studies have been initiated in children with X-SCID, and for the first time, retroviral vectors have been used to treat hematopoietic stem cells has resulted in not only laboratory evidence for gene transfer, but also laboratory and clinical evidence of immune function suggesting there may be clinical benefit (4) (7). Evidence of successful engraftment was reported in 4/5 infants treated with CD34+ hematopoietic stem cells that were exposed to a retroviral vector encoding  $\gamma_c$ . In addition, longer-term follow-up data on these four patients, varying from 1.6 to 2.5 years at the time of the report, indicated almost normal numbers of T cells and natural killer (NK) cells as well as normal responses to antigen proliferation in vitro or after immunization. In addition, unlike those patients who receive haploidentical bone marrow transplants, the levels of antibody production were sufficient to obviate the need for intravenous immunoglobulin administration. Importantly, the children who were treated in this study were showing evidence of normal growth and ability to lead normal lifestyles (7).

## **Retrovirus Vectors**

Retrovirus vectors most commonly used in clinical trials of gene therapy are based on a murine gammaretrovirus. The vector sequences are deleted compared to the wildtype virus so that cells exposed to retrovirus vectors express only the therapeutic gene product, but do not make new viral particles. This is a critical safety feature of all retroviral vectors used in clinical trials of gene transfer. However, because the parental murine gammaretrovirus can, under some circumstances, cause tumors in mice via insertion of retroviral DNA into the host cell genome, retroviral vectors have always been perceived to carry the potential risk of tumorigenesis. While most integration events of the vector DNA are not expected to cause harm to the cell or to the patient, there is an unknown (but thought to be low) risk that in some cases the integration event may result in activation of neighboring genes which could result in uncontrolled

cell division or a tumor (an event called "insertional mutagenesis"). Since tumorigenesis is thought to be a multi-step phenomenon, it would be likely that an additional event would be required before a vector insertion at a given locus would necessarily result in tumor formation. In all cases, the potential risk of tumorigenesis from a retroviral vector has been included in informed consent documents used in retroviral vector-based clinical trials in the US.

Recently, these assumed risks were demonstrated to be real when scientists reported that acute myeloid leukemia developed in mice receiving hematopoietic stem cells transduced with a retroviral vector (8). In all cases the leukemic cells had the same site of insertion of the retroviral vector, causing inappropriate expression of the gene at the insertion site (Evi1). However, it was postulated that in addition to the dysregulated expression of Evi1 that additional factors, such as the transgene used in the retroviral vector and the target cell population, likely contributed to the occurrence of leukemia (8).

The long-recognized risks of tumorigenesis from retroviral vectors were initially addressed by FDA/CBER initially nearly 10 years ago when a letter was issued to all sponsors of gene therapy clinical trials using retroviral vectors requesting life-long follow-up of all subjects who participated in these clinical trials. The policy was later published (10/18/2000) in a guidance document: Guidance for Industry: Supplemental Guidance on Testing for Replication Competent Retrovirus in Retroviral Vector Based Gene Therapy Products and During Follow-up of Patients in Clinical Trials Using Retroviral Vectors (available at <http://www.fda.gov/cber/genetherapy/gtpubs.htm>). The guidance document recommends that all subjects should be followed life-long on an annual basis. In addition, the topic of long-term follow-up was also discussed at several previous meetings of the FDA Biologicals Response Modifiers Advisory Committee (November, 2000; April, 2001; and October, 2001 – transcripts are available at <http://www.fda.gov/cber/advisory/brm/brmmain.htm>).

### **Adverse Event in Retroviral Vector Gene Therapy Clinical Trial in X-SCID in France**

One child in the gene therapy clinical trial in X-SCID children in France (4) (7) has had a serious adverse event related to the retroviral vector gene therapy. Although the clinical trial is not under US IND, the clinical investigator has been very cooperative and has shared many of the data with CBER. The child was treated three years ago and had positive clinical and laboratory evidence of immune function. He had a mild lymphocytosis in April, 2002, preceding a varicella zoster virus (VZV) infection (chicken pox). He was able to clear his infection, but maintained a somewhat elevated, but stable, T cell count, until August, 2002, when the T cells began to increase an additional 10-fold and the child presented with hepatosplenomegaly. At that point he was treated with

steroids and vincristine, to reduce his T cell counts, and subsequently also received Daunorubicine. His T cell counts have been reduced to 500, and the patient is in good condition.

The expanded T cells are gamma delta T cells, and are monoclonal with respect to both the form of the T cell receptor expressed and the site of retroviral vector insertion into the genome. Using a PCR-based method, the investigators have shown that the retroviral vector has inserted into the first intron of the LMO-2 gene on chromosome 11. There is over-expression of LMO-2 in these cells, suggesting that the vector insertion may have caused dysregulation of the LMO-2 gene expression. LMO-2 (the second member of the LIM-only family of genes) is normally expressed during early stages of hematopoietic differentiation and its expression appears to be critical for development of lymphoid and myeloid cell lineages (reviewed in (10)). In addition, the chromosomal translocation t(11;14)(p13;q11) in T-ALL (acute lymphocytic leukemia) results in joining of the T cell receptor D or J segments to the LMO-2 locus. This translocation is thought to be the result of aberrant RAG-mediated V(D)J recombination, highlighting the multi-step nature of the leukemogenic process (10).

It is important to consider that there are likely several factors that may have played a role in the T cell expansion in this patient. The retroviral vector insertion and activation of LMO-2 may have been a necessary step in these events, but the insertion alone may not have been sufficient. Additional factors that should be considered are the role of the VZV infection in stimulating T cell proliferation and possible genetic predisposition, since there are two childhood cancers in the family, including a cancer in the patient's sister.

### **CBER's Actions**

Upon notification of the adverse event in the gene therapy clinical trial in France, FDA/CBER reviewed the currently active gene therapy clinical protocols under IND in the US. We identified three clinical trials that were most similar to the one ongoing in France in terms of the clinical indication, target cell, retroviral vector, and route of administration. While the serious adverse event in France was being evaluated, we placed each of the INDs in SCID subjects using retroviral vector-mediated ex vivo transduction of CD34+ hematopoietic stem cells on clinical hold, pending further analyses of this event. In addition, we notified sponsors of similar clinical trials that are in active or inactive status (i.e., no longer actively treating patients) of this event and requested that they contact their patients' families to discuss the event and its implications. We now seek the advice of the committee and its experts to determine what future regulatory actions should be taken.