

## UPDATE ON RETROVIRAL VECTOR-MEDIATED TUMORIGENESIS IN GENE TRANSFER CLINICAL TRIALS

### Brief Update

In late 2002, Dr. Alain Fischer notified CBER of the detection of leukemia in two subjects in a retroviral vector-mediated gene transfer clinical trial he was conducting in France for the treatment of X-linked Severe Combined Immunodeficiency Disorder (X-SCID). In response to these events, CBER convened two meetings of its Biological Response Modifiers Advisory Committee to discuss the impact of the findings in the clinical trial in France on similar US clinical trials (October, 2002 and February, 2003). Experts concluded that the finding of retroviral vector sequences integrated in or near the *Lmo-2* gene combined with the transcriptional activation of *Lmo-2*, provides strong evidence for a causal relationship between administration of the retroviral vector-modified cells and the development of leukemia. We have attached the briefing documents and corresponding summaries for each meeting (**Tab 3, 4**).

In recent weeks, CBER learned of three additional events that have prompted us to seek additional advice from an Advisory Committee to discuss the impact and interpretation of these events in the context of the clinical use of retroviral vectors for gene therapy in SCID, as well as in other clinical indications:

- 1) The first child reported by Dr. Alain Fischer to have developed leukemia following successful reconstitution of his immune system following gene therapy died from a relapse of his leukemia (following bone marrow transplantation).
- 2) A third child in the same clinical trial in France has developed "uncontrolled T lymphocyte proliferation" (investigations in progress, press release, **Tab 5**).
- 3) Dr. Cynthia Dunbar from the NHLBI has reported to us the death of a monkey 6 years after administration of CD34<sup>+</sup> cells transduced by a retroviral vector containing a marker gene (GFP) and a drug resistant marker [1]. At the time of autopsy, the monkey is reported to have had widespread myeloid sarcoma, with myeloperoxidase positive tumor cells infiltrating many organs (liver, kidney, skin, choroids plexus), despite the absence of blastic cells in the peripheral blood. Subsequent molecular analysis is reported to have indicated presence of retroviral vector sequences in the tumor cells. Additional studies are ongoing to determine whether the vector insertion site may have contributed to the development of tumorigenesis in this animal. The results of these studies will be presented to the committee.

### CBER Actions

In response to these recent events, CBER has placed a clinical hold on INDs that use retroviral vector-mediated gene transfer to treat Severe Combined Immunodeficiency Disease, without regard to the genetic basis for the

immunodeficiency. The clinical investigators were asked to update their informed consent processes and notify their Institutional Review Boards (IRBs).

In addition, all sponsors of clinical trials that use retroviral vector-mediated gene transfer for any clinical indication have received a letter informing them of the recent events. In addition, we have also transmitted this information to the IRBs overseeing the safety of subjects in these clinical trials.

## **Retroviral Vector-Mediated Gene Transfer Clinical Trials in SCID**

In response to each report of a Serious Adverse Event (SAE) in the clinical trial in France for X-SCID, CBER placed on clinical hold retroviral vector-mediated gene therapy clinical trials under US IND in all SCID indications. We would like the Committee to consider whether there is sufficient cumulative evidence to suggest that the events in France indicate a higher risk when using retroviral vector-mediated gene transfer for X-linked SCID than other genetic forms of SCID, and at what point do the risks to the study subjects outweigh the potential benefits (see questions #1 and 2).

### **1. X-SCID**

To date, the X-SCID gene therapy clinical trial conducted by Dr. Fischer is the only retroviral vector-mediated gene therapy clinical trial where development of leukemia related to the gene therapy has been reported. Several characteristics were common to the first two children who developed leukemia, and hence, identified as potential risk factors:

- a) They were the youngest children treated (1 and 3 months);
- b) They received the highest cell dose (14 and 20 million gamma-c-positive cells, respectively) [2].
- c) The retroviral vector was integrated into or near the *Lmo-2* locus [2].

The new case of "uncontrolled T lymphocyte proliferation" was reported in a subject who was 9 months of age at the time of product administration and received a lower cell dose than the first two subjects who developed leukemia. Therefore, the first two potential risk factors are not present in this third child. Molecular analyses to determine the site of vector integration are underway; therefore, it is unknown whether the third potential risk factor is present. However, the time course from treatment to detection of lymphoproliferation is similar in all cases: lymphoproliferation was detected in the first two cases at 34 and 36 months post-transplantation of the retroviral vector-modified cells and in this third child, it was detected at 32 months.

What is unique about this trial? A comparative summary of the methods used for cell isolation, culture, transduction, and retroviral vectors used and a summary of key clinical aspects of all ongoing or proposed clinical trials for X-SCID using retroviral vector-mediated gene transfer are found in **Tab 6 (Tables 1 and 2)**. Analysis of this information leads to identification of the following characteristics unique to Dr. Fischer's trial:

- a) Use of fetal calf serum as a medium additive;
- b) Higher concentration of IL-3 and use of PEG-MGDF instead of TPO, in the culture medium;
- c) Shorter prestimulation time;
- d) Use of an amphotropic envelope;
- e) Longer follow-up of subjects;

Development of leukemia in subjects in trials other than Dr. Fischer's may indicate that the first four characteristics are not critical variables. On the other hand, if during follow-up beyond three years, no subjects in other trials develop leukemia, then one or more of these characteristics may be relevant.

Could the gamma-c transgene protein product play a role in the pathogenesis of retroviral vector-associated leukemia in X-SCID? Leukemia has not been reported in other retroviral vector-mediated gene therapy clinical trials in X-SCID suggesting that the gamma-c transgene may not play a role in these events. However, results from a preclinical study in mice indicate that the gamma-c transgene may play a cooperative role in the tumorigenic process.

Dr. Copeland of the NCI's Center for Cancer Research has been using replicating retroviruses to "tag" retroviral integration sites identified in murine hematopoietic tumors (Mouse Retroviral Tagged Cancer Gene Database, <http://RTCGD.ncifcrf.gov>). A retrospective analysis of this database identified two leukemias associated with integrations at *Lmo2*, two leukemias associated with integration at *Il2rg* (coding region for gamma-c) and one leukemia with integrations at both sites [3]. While the level of *Lmo2* gene transcription was approximately 15-fold higher than other murine T cell leukemias, the *Il2rg* transcriptional levels were not altered. However, the authors note that statistical analysis reveals that the chance occurrence of a leukemia with insertions at both of these gene loci is approximately 3/2,000,000, suggestive of a "co-selection", rather than a random event. Indeed, they have conducted previous studies indicating that the identification of "co-selected" genes often correlates with a cooperative effect in inducing leukemia [4].

The observation that the mouse tumor has activation of *Lmo2* gene transcription in the absence of any obvious dysregulation of *Il2rg* is analogous to what had been observed in the first two patients where *Lmo2* was transcriptionally activated without evidence of dysregulation of cell activation pathways due to gamma-c expression [2]. One interpretation may be that neither gamma-c expression nor oncogene activation alone is sufficient to induce tumors, but rather both events are prerequisites to initiating this process of tumorigenesis. Perhaps the normal function of gamma-c as a component of several cytokine receptors, resulting in induced cell proliferation upon activation by cytokines, may be necessary to potentiate the effect of a particular vector integration event. In addition, the more than 30-month time period between product administration and detection of leukemia indicates other events are required beyond the initial vector integration and gamma-c expression.

## 2. ADA-SCID

ADA-SCID is an inherited disorder caused by a defect in the gene encoding adenosine deaminase (ADA). Absence of ADA activity leads to toxic levels of ADA substrates, and this cytotoxic effect causes severe combined immunodeficiency that is usually diagnosed during infancy. However, in some individuals the mutation in the ADA gene results in partial ADA activity, and a milder form of the disease, so that these individuals may not be diagnosed until later in childhood or adulthood [5]. Survival after HLA-identical sibling bone marrow transplant is nearly 100%, but survival after haploidentical transplantation varies from approximately 50-75%, depending upon the transplant center (see slides presented by Dr. Rebecca Buckley of Duke University, found in **Tab 7**; for a detailed review, see also [6]). Patients with ADA-SCID have another treatment alternative, enzyme replacement with pegylated-ADA (PEG-ADA). A 1995 review of patients with ADA-SCID treated with PEG-ADA or who received a haploidentical bone marrow transplant indicated comparable rates of mortality and morbidity, supporting the use of PEG-ADA as first line treatment in patients who are too ill to undergo a haploidentical transplant [7, 8]. However, it is important to note that neither therapy provides complete immune reconstitution. For that reason, gene therapy has been investigated for over 10 years for its potential as an improved alternative.

At this time, there is one active IND in the US for administration of retroviral vector-modified hematopoietic stem cells as a potential treatment of ADA-SCID, sponsored by Dr. Don Kohn of The Saban Research Institute of Childrens Hospital Los Angeles. In addition, Dr. Claudio Bordignon at the San Raffaele Telethon Institute for Gene Therapy in Milan, Italy, is conducting an ADA-SCID clinical trial using retroviral vectors. In **Tab 6**, **Table 3** provides a comparison of these two trials with respect to the methods used for cell isolation, culture and retroviral transduction, while **Table 4** provides a summary of some key clinical aspects. In 2002, Dr. Bordignon reported evidence of engraftment and immune cell function in two subjects administered retroviral vector-modified cells, including a reduction in the toxicity normally associated with lack of ADA activity [9]. Several characteristics distinguish the gene therapy clinical trials for ADA-SCID from those for X-SCID:

- a) As shown in Table 4, some subjects in both Dr. Bordignon's and Dr. Kohn's trials have been followed for over three years without evidence of vector-associated leukemia, although the total number of subjects is low (n=6, combined).
- b) The number of transduced cells administered in Drs. Bordignon's and Dr. Kohn's trials is lower compared to Dr. Fischer's X-SCID trial.
- c) The function of the protein product of the ADA transgene is to detoxify the cells, rather than to induce proliferation (as with gamma-c).

In considering the lack of leukemia in ADA-SCID gene therapy clinical trials to date, combined with the difference in disease etiology between ADA-SCID and X-SCID, we request that the committee comment on the possible differences in the risks of retroviral vector-mediated insertional tumorigenesis in these two indications (see question #3).

**REFERENCES** (Bolded references provided in **Tab 8**)

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