

Last year CBER was notified by Dr. Alain Fischer of a leukemia-like illness in a patient in a gene therapy clinical trial he was conducting in France. CBER was again informed by Dr. Fischer of a second similar serious adverse event in this same clinical trial that uses retroviral vector-mediated ex vivo gene transfer into CD34⁺ cells for the treatment of X-linked Severe Combined Immunodeficiency Disease (X-SCID) [1] [2]. Although this clinical trial is not being performed under United States (US) Investigational New Drug (IND) regulations, Dr. Fischer has been very committed to sharing information concerning these events with other regulatory health authorities and the gene therapy community. CBER now seeks the advice of the committee on how to proceed with retroviral vector-mediated gene transfer clinical trials in SCID and other clinical indications in the United States (specific questions for the committee are found on the blue page contained in this notebook).

Brief Review of October, 2002, BRMAC Discussion and CBER Actions

In October 2002, CBER convened an emergency meeting of the BRMAC to discuss the implications of the first serious adverse event reported by Dr. Fischer on US INDs for treatment of SCID that also used retroviral vector-mediated gene therapy [3]. The complete transcripts of that meeting are available at <http://www.fda.gov/ohrms/dockets>, and the Briefing Document and Summary Minutes of the meeting are found in **Appendix 1**. The Committee reached consensus on the following major issues:

- ?? The benefit of gene therapy over existing treatments for children with X-linked SCID who do not have an HLA-identical bone marrow donor is significant, and provides an impetus for allowing retroviral vector-mediated clinical trials in subjects with SCID to proceed. However, the committee recommended that the following two conditions should be met to allow trials to proceed:
 - o Revision of the informed consent document to explain in layman's terms the nature of the leukemia in the clinical trial in France so that subjects and their families understand that the risk of cancer is real, not theoretical.
 - o Develop plans to monitor peripheral blood samples from subjects for the clonality of vector integrants over time.

In response to the BRMAC's recommendations at the October 10, 2002, meeting, CBER issued three letters (see Table 1). The first letter was sent to only the three IND sponsors of retroviral vector-mediated gene transfer clinical trials in subjects with SCID. This letter described the conditions that needed to be met in order for these clinical trials to resume:

1. Revise the informed consent document. The letter comment provided the following suggested language for insertion into the section entitled "Risks associated with the study agent:

Risk of Cancer

When retroviral vectors enter a normal cell in the body, the DNA of the vector inserts itself into the normal DNA in that cells; this process is called integration. Most integration is expected to cause no harm to the cell or to the patient. However, there is a chance that there may be some regions of the normal human DNA where integration of the viral vector's DNA may result in activation of neighboring genes.

For example, if one of these genes were a growth factor, this may cause uncontrolled division of the cell, resulting in a cancer. This type of event has occurred in one animal study in mice where the vector integration site correlates with the occurrence of cancer in these mice.

More recently, the first report of a similar event has been identified in a child who received a retroviral vector in an experimental gene therapy study for X-linked Severe Combined Immunodeficiency (SCID) conducted in France, not under the jurisdiction of the U.S. Food and Drug Administration (FDA). While most of the children who participated in this clinical trial appear to have been cured of their disease, one child developed a leukemia (a form of cancer of the blood) approximately 30 months after receiving the gene therapy treatment. The patient had extensive testing done to determine the cause of the leukemia. A group of experts in this field looked at all the test results, and concluded that the gene therapy caused the leukemia. The child appears to be responding to the treatment of his/her leukemia, but his/her long-term prognosis is unknown at this time. The risk of another cancer, including leukemia, developing in the children already treated in France, or in your child, should you volunteer to have your child entered into this experimental study, is of unknown magnitude, but you need to be aware of this possible risk.

2. Develop plans to monitor subjects for the clonality of the vector integration sites. In the letter we recommended the following:

Please revise your clinical protocol to include analysis of patients' peripheral blood mononuclear cells (PBMCs) for the clonality of vector integration sites.

- a. Patient follow-up should occur at a minimum of every six months for the first five years and then yearly thereafter for the next ten years.
- b. When analysis of a subject's sample reveals a predominant clone or monoclonality, the sample should be subjected to methods that would allow determination of the integration site. If a predominant integration site is observed, a second test should be performed at an interval of no more than three months after the first to see if the clone persists or is transient.
- c. If the clonality is persistent, the resulting sequence should be analyzed against the human genome to determine whether the sequences are associated with any known human cancers.
- d. In all instances where monoclonality is persistent and particularly in instances where there is expansion of the clone, regardless of whether or not the sequence is known to be associated with a known human cancer, the subject should be monitored closely for signs of malignancy, so that treatment, if available, may be initiated early.

The second letter was sent to a subset of IND sponsors of retroviral vector-mediated gene transfer studies that target stem cells (hematopoietic and other phenotypes), and these sponsors were asked to also develop a plan to monitor

the clonality of the vector integrants (2nd comment of the first letter). The third letter was sent to IND sponsors of all clinical trials using retroviral vector-mediated gene transfer methods. These sponsors were asked to revise their informed consent documents as recommended for the SCID clinical trials.

Table 1. Letters sent to sponsors after October 10, 2002, BRMAC meeting

Category of Retroviral Vector Gene Therapy Clinical Trial	A. Revision to Informed Consent	B. Monitor Clonality	A and B are Required or Recommended
X-SCID	Yes	Yes	Required
Hematopoietic stem cells and other stem cell phenotypes	Yes	Yes	Recommended
All other retroviral vector clinical trials	Yes	No	Recommended

Second Leukemia-Like Illness Reported by Dr. Fischer

Dr. Fischer reported to the FDA on 12/20/02 that patient #5 treated in his gene therapy clinical trial for X-SCID developed a T cell proliferation (188,000/?1 of blast cells in the blood), anemia, and thrombocytopenia. The proliferating T cells have been identified as positive for the alpha/beta T-cell receptor and CD8. The child is reported to be responding to the chemotherapy that is being used to treat the leukemia.

As with patient #4, detailed molecular analyses of the proliferating T cells are being performed in order to understand the mechanism and the cause. There may be up to three subclones with different T cell receptor phenotypes, but these are most likely monoclonal with respect to the retroviral vector integration site. All the cells are positive for gamma-c expression. Sequence analysis has revealed that the vector is again integrated in close proximity to the LMO-2 gene locus, very close to the genetic locus where the retroviral vector was found in the monoclonal T cell expansion of patient #4 (see **Appendix 1** for more information on LMO-2). However, the specific sites of integration are different in patients #4 and #5.

As of January 30, 2003, these are the confirmed data that are currently available on patient #5, with more confirmation and quantification analyses ongoing.

Table 2. Comparison of Patients #4 and #5

Characteristic	Patient #4	Patient #5
Age at Treatment	1 month	3 months

Total Cells Received	27 million	38 million
Gamma c ⁺ cells received	14 million	20 million
Months post-treatment to detection of monoclonal vector integration in peripheral blood cells	30 months	31 months
Months post-treatment to development of leukemia-like disease	36 months	34 months
Family history of childhood cancer	Yes	No
Infectious episodes post-treatment	Yes, varicella zoster infection, 30 months post-treatment (benign course)	Mild pneumonitis, 20 months post-treatment
General Clinical Status Prior to Leukemia-Like Illness	Well, Normal Growth	Well, Normal Growth

CBER’s Actions in Response to the Second Leukemia-Like Illness

In response to the finding that data on patient #4 (subject of October 10, 2002 BRMAC) and now patient #5 indicate a strong likelihood of a causal relationship between the vector integration and the proliferative event, CBER has placed all clinical trials using retroviral vectors to transduce hematopoietic stem cells on clinical hold, pending the recommendations of the February 28, 2003 BRMAC discussion. Sponsors of studies to treat serious and/or life-threatening conditions for which there are no alternative treatments available were advised that they could provide written justification for allowing treatment of such patients to proceed prior to February 28, 2003; FDA would weigh whether the potential benefits outweighed the potential risks for such patients and would consider removing the clinical hold based on those case-by-case assessments. Letters were also sent to all sponsors of retroviral vector-mediated gene therapy clinical trials informing them of the second event in order that they may revise their informed consent documents to reflect the second event with proposed specific language. The proposed language for the informed consent document was identical to that provided previously with the exception of the final paragraph:

More recently, two instances of a similar event have been reported in two children who received a retroviral vector in an experimental gene therapy study for X-linked Severe Combined Immunodeficiency) conducted in France, not under jurisdiction of the U.S. Food and Drug Administration (FDA). While most of the children who participated in this clinical trial appear to have been cured of their disease, one child developed a leukemia (a form of cancer of the blood) approximately 30 months after receiving the gene therapy treatment, and a second child developed a leukemia 34 months after receiving the gene therapy treatment. The first patient had extensive testing done to determine the cause of the leukemia. A group of experts in this field have looked at all the test

results, and concluded that the gene therapy caused the leukemia in the first child. The first child appears to be responding to the treatment of his/her leukemia that he/she has been given, but his/her long-term prognosis is unknown at this time. The clinical status (health status) of the second child is not known to us at this time. A detailed analysis of the cause of the leukemia in the second child is currently underway. Very preliminary studies suggest that the leukemic cells have a common origin, and that the therapeutic gene is inserted near a gene that may be involved in the control of cell growth. The risk of another cancer, including leukemia, developing in the children already treated in France, or in you (or your child – as appropriate to your study), should you volunteer to enroll (or have your child entered into – as appropriate to your study) in this experimental study, is unknown, but you need to be aware of this possible risk.

Finally, sponsors of inactive clinical trials using hematopoietic stem cells transduced with a retroviral vector received a letter to inform them of the second event and to advise them of actions they must take should they want to reactivate their IND. In all cases, CBER has requested that sponsors of retroviral vector-mediated clinical trials develop plans to monitor for clonality of vector integration sites in a manner similar to the letters sent previously (see above, and see Table 3).

Table 3. Letters sent to IND sponsors after Dr. Fischer’s report about second leukemic event

Category of Retroviral Vector Gene Therapy Clinical Trial	A. Revision to Informed Consent	B. Monitor Clonality	A and B are Required or Recommended
Active hematopoietic stem cells	Yes	Yes	Required
Inactive hematopoietic stem cells	Yes	Yes	Required to resume trial
All other retroviral vector clinical trials	Yes	Yes	Recommended

Potential Mechanisms of Tumorigenesis by Retroviral Vector-Mediated Gene Therapy

At the October 10, 2002, meeting of the BRMAC, two scientific presentations addressed the issue of whether retroviral vectors may be directly involved in tumorigenesis. Dr. Linda Wolff presented an abundance of historical data on wildtype retrovirus infection in animal models that has clearly demonstrated that enhancer elements present in the retroviral long terminal repeat regions are capable of activating distal cellular promoters at distances as long as 200 kilobase pairs away, resulting in dysregulated gene expression (preleukemic phase). Alternatively, virus integration itself, in the absence of enhancer

activation, may also disrupt key regulatory elements of a gene, also resulting in aberrant gene expression. During the preleukemic phase, the cells proliferate and additional oncogenic events occur that produce the malignant transformation and rapid expansion of cells. These events are so well-accepted as critical steps in tumorigenesis, that Neil Copeland and his colleagues at the National Cancer Institute are mapping retroviral integration sites in virus-induced tumors in mouse models in order to identify novel cancer genes (the database can be found at <http://genome2.ncifcrf.gov/RTCGD>). For example, analysis of retroviral integration sites in one mouse model revealed 152 candidate cancer genes in the mouse genome [4].

While experience with wildtype retroviruses in animal models had clearly demonstrated that retroviral integration is an important mechanism of tumorigenesis, it was also assumed that the high frequency of genomic integrations resulting from a high viremic load during virus replication was critical to the development of tumors in these models. The advent of retroviral vectors that do not replicate, and therefore, should have reduced frequencies of genomic integration, were assumed to have a correspondingly decreased risk of tumorigenesis. Hence, the use of retroviral vectors was deemed safe for clinical use. In general, this appears to be the case. However, Dr. Christopher Baum presented data from a murine model of retroviral vector-mediated gene transfer to CD34⁺ hematopoietic stem cells, where 6/10 secondary recipients developed acute myeloid leukemia with long latency [5]. The retroviral vector was integrated within the first exon and caused activated expression of a known proto-oncogene, Evi-1. However, the authors hypothesize and show indirect evidence for the concept that the transgene used in the retroviral vector, a truncated form of the receptor for nerve growth factor, dLNGFR, may also be involved in the tumorigenesis of the AML observed in these animals [5] [6].

The observations from Li et al, suggest that retroviral vector-mediated gene therapy has the potential to play a "two-hit" role in tumorigenesis, both by vector insertion and as a consequence of aberrant transgene expression. This raises the possibility that over-expression of gamma-c may play a collaborating role in the leukemogenic events. Although tumors were not reported in preclinical studies performed in mice or dogs using retroviral vector-mediated gene transfer to over-express gamma-c [7] [8] [9] [10], none of these studies maintained the animals longer than 6 months before necropsy. If long latencies are required for the leukemogenic events to reveal themselves, the potential for tumors might have been missed in the previous studies. The time to development of leukemia-like disease in patients 4 and 5, as well as the lack of leukemia in primary mouse recipients in the report by Li, et al, suggest that longer-term studies would be necessary to identify whether over-expression of gamma-c may be tumorigenic. Data from transgenic mice that over express cytokines that use heterodimeric receptors composed, in part, of gamma-c may shed some light on this issue. Development of leukemias in IL-15 transgenic mice [11] and thymic lymphomas in IL-9 transgenic mice [12], where over-expression of these cytokines results in

constitutive activation of the STAT pathway via gamma-c, suggests that over-expression of gamma-c could play a role in tumorigenesis.

What are the implications of these events for patient safety in retroviral vector-mediated gene transfer clinical trials?

The majority of the children born with X-SCID in the gene therapy clinical protocol in France were reported to have both laboratory and clinical evidence of immune function, comparable to, and in some cases, better than standard treatment options for this clinical condition following treatment [1] [2]. The occurrence of a vector-related leukemia-like illness in two of eleven children treated in this clinical trial dictates that the regulatory and scientific communities scrutinize the use of retroviral vectors in all clinical trials, even though similar events have not been reported to date. Given the clinical success seen in the French trial, it is critical to study the science underlying retroviral vectors to determine whether retroviral vector-mediated gene therapy can be applied with an increased margin of safety. The following are some modifications to consider. As noted in the detailed scientific discussion of each of these modifications found in **Appendix 2**, some of these modifications are more theoretical possibilities for the future (noted here in italics), rather than practical solutions for the present (noted here in bold). However, to be thorough, we have presented both for your consideration.

?? Dosing Paradigm

- o **Reduced dose of vector**
- o **Reduced dose of cells**
- o **Dose based on total number of vector integrations**

?? Additional preclinical studies to assess carcinogenic potential

- o **Perform carcinogenicity testing at an earlier stage of clinical development (typically by phase 3)**
- o **Traditional assays are 2-year bioassays, therefore, alternative models may need to be considered if prior to phase 1:**
 - ?? **Transgenic models carrying oncogenes**
 - ?? **Knockout models of tumor suppressor genes**
- o **Perform both sets of evaluations**

?? Cell Target or Culture Conditions

- o *Identify true hematopoietic stem cells in order to minimize the number of cells required for transduction.*
- o *Modify transduction protocols to reduce transcriptional activation of genes*

- *Study of gene expression changes by gene and protein microarray in cells post gamma-c gene transfer*

?? Vector Modifications

- *Insulator sequences to block enhancer activation*
- **Deletion of retroviral enhancer elements within the LTR**
- *Targeted vector integration*
- *Identify cells with vector integration into known tumorigenic sites*

APPENDIX 1

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 (γ_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 β_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.

Question for the Committee

Are there additional data or measures that clinical investigators need to provide before future and present clinical trials in SCID patients should proceed in the US? Please consider in your discussion each of the following:

- a) Consideration of risk/benefit of gene therapy vs. alternative therapies;
- b) Revisions to informed consent documents;
- c) Alterations to the cell dose administered;
- d) Alterations to the vector dose administered;
- e) Mapping of vector insertion sites on all clinical lots of cells prior to release for clinical use;
- f) Alterations in vector design (i.e., SIN vectors)



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Center for Biologics Evaluation and Research
Biological Response Modifiers Advisory Committee

SUMMARY MINUTES
Meeting #33, October 10, 2002
Hilton Hotel, Gaithersburg, MD

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*BRMAC #34, Topic III
Briefing Document*

The summary minutes for the October 10, 2002 meeting of the Biological Response Modifiers Advisory Committee were approved on December 2, 2002.

I certify that I attended the October 10, 2002 meeting of the Biological Response Modifiers Advisory Committee and that this report accurately reflects what transpired.

Gail Dapolito, Executive Secretary

Daniel R. Salomon, M.D., Chair

**FDA BIOLOGICAL RESPONSE MODIFIERS ADVISORY
COMMITTEE
SUMMARY MINUTES
MEETING #33, October 10, 2002**

The Biological Response Modifiers Advisory Committee (BRMAC) met on October 10, 2002 at the Hilton Hotel, Gaithersburg, MD. In open session, the committee discussed safety issues recently identified related to retrovirus vectors in gene therapies for the treatment of patients with severe combined immunodeficiency and receive updates. The committee also received updates of CBER research programs in the Laboratories of Molecular Tumor Biology and Gene Regulation. The committee met in closed session to discuss individual research programs in the Center for Biologics Evaluation and Research.

Daniel Salomon, M.D., Chair, called the meeting to order and introduced the members, consultants, guests and guest speakers. The executive secretary read the conflict of interest statement into the public record. This statement identified members and consultants of the committee with an appearance of a conflict of interest, who were issued waivers to participate. Copies of the waivers are available from the FDA Freedom of Information Office.

The FDA provided a brief introduction to 1) an adverse event recently reported in a retroviral gene therapy trial in France for the treatment of children with X-linked severe combined immunodeficiency (X-SCID), 2) similar trials in the U.S. and 3) specific questions posed by the FDA for committee discussion.

Guest experts provided presentations to the committee on:

- ~~1/~~ a retroviral gene transfer trial in France to treat children with XSCID and the subsequent detection and confirmation of T cell expansion in one patient related to the therapy
- ~~2/~~ alternative therapies, including bone marrow transplant for patients with SCID
- ~~3/~~ historical overview of insertional mutagenesis and cancer
- ~~4/~~ mouse model of insertional mutagenesis and examples of myeloid leukemia following retroviral gene transfer in a murine model
- ~~5/~~ the role of the LMO2 gene/gene product in hematopoiesis and leukemia

The chair then commenced the open public hearing. The committee heard comments from the audience representing

the views of families of X-SCID and other gene transfer patients and from advocacy groups including the Stop ALD Foundation, Citizens for Responsible Care in Research and the Council for Responsible Genetics. The committee also heard a presentation on self-inactivating LTRs from a representative of Genetics Pharmaceuticals.

Following the open public hearing, the committee began deliberations of questions posed by the FDA related to the safety of current U.S. retroviral gene transfer trials of patients with X-SCID.

Based on the committee comments that followed each of the preceding expert presentations, the Chair charged the committee to consider, in their discussion of the following question, 1) the safety, feasibility and appropriateness of proceeding with gene therapy trials in patients with different forms of SCID, 2) increased efforts for early diagnosis and 3) methods to make gene therapy safer.

The committee began deliberations on the following multipart question:

Are there additional data or measures that clinical investigators need to provide before future and present clinical trials in SCID patients should proceed in the US? Please consider in your discussion each of the following as they pertain to X-SCID and other forms, such as ADA-SCID:

a) Consideration of risk/benefit of gene therapy vs. alternative therapies

The committee reached consensus on the following:

1. The T cell clonal expansion (leukemia-like disease) seen in one of eleven X-SCID patients treated with an ex vivo gene therapy was likely caused by an insertional mutagenesis effect of the retroviral vector used in the gene therapy.
2. X-SCID patients with HLA identical donors, should be excluded from current X-SCID gene transfer trials because of the relatively high clinical success of intervention by HLA identical bone marrow transplantation (i.e. up to 90% survival if transplant is done in the newborn period).

3. In comparison, it was noted that for those children with only haploidentical bone marrow transplants that the benefits are not as great (i.e. 50-75% survival, the potential of requiring life-long IV Ig therapy, increased infection risks and uncertain quality of life). Thus, relative to haploidentical stem cell transplantation it is reasonable to consider gene therapy as an alternative.
4. Retroviral gene transfer trials in the U.S. should proceed only with careful consideration of inclusion/exclusion criteria that will in the best judgment of investigators, reviewers and institutional review boards provide sufficient levels of benefit over risk relative to alternative medical therapies. Moreover, informed consent documents should appropriately reflect the new information from the French X-SCID study on the potential of insertional mutagenesis with retroviral vectors

The committee also offered several viewpoints in the discussion of the appropriate patient population for X-SCID gene transfer trials:

1. Gene transfer trials as salvage therapy:
 - limiting gene therapy to X-SCID patients who first fail haploidentical transplantation could deny many patients the opportunity of gene therapy and is not advisable
 - the patients in the Fischer trial were not transplanted prior to the gene transfer, thus, it is possible that the excellent results are in part due to this selection. Therefore, it is important to consider the possibility that this particular gene therapy might not be as good an approach if used as a “salvage” therapy for X-SCID patients that have failed transplants.
2. Patient’s families should have “an array” of choices with a best effort at accounting for risks and benefits vs. an either/or situation.
3. Risks of secondary cancer are not limited to gene transfer therapies – accepted cancer treatments (i.e. radiation or chemotherapeutics) often carry an increased risk of secondary cancer.

b) Revisions to informed consent documents

The committee agreed on the following:

1. **It is important for investigators to inform all patients presently enrolled in or candidates for retroviral gene therapy trials, that there was an adverse event in a retroviral gene therapy trial and this was due to insertional mutagenesis. Informed consent forms should include strong, non-equivocal language about the retroviral insertion.**
 - **all retroviral vector clinical trials should have revisions in informed consent documents to reflect this event**
 - **ideally, all the revised consent documents should use consensus language clearly describing the event and its implications as a risk element**
2. **There is a need for final implementation of a comprehensive database (managed by NIH and FDA) to follow gene therapy patients and allow for dissemination of this information.**
3. **Informed consent documents should:**
 - **include consensus language that is complete and accurate**
 - **be potent and direct; written in common language**
 - **include full disclosure of positive and negative outcomes**
 - **not include mitigating factors such as multiple hits or the number of patients treated**
 - **emphasize unknowns (ex. role of family pedigree) but include information saying the gene therapy caused leukemia in a gene therapy for X-SCID.**

c) Alterations to the cell dose administered

The committee discussed the theoretical potential of reducing the risk of an insertional mutagenic event by altering the number of CD34⁺ cells that are exposed to the vector, thereby reducing the number of virus hits that could lead to an insertional mutagenic event but still maintain engraftment. The committee discussed the current standard of = 2x10⁶ CD34⁺ cell/kilogram for engraftment as well as

alternate therapies using cord blood that maintain engraftment using 1×10^5 CD34⁺/kilogram.

The committee reasoned that alterations of the cell dose to a level below that known to result in inefficient engraftment may pose a greater risk to the subject than the risk of insertional mutagenesis. Therefore, they did not recommend alterations to the cell dose from current standards of treatment. The committee encouraged further research on how to improve the purification techniques of hematopoietic stem cells and any other strategies, that might allow for lower target cell doses or reduce the risk of insertional mutagenesis.

d) Alterations in vector dose administered

The committee received information that current vector doses reach approximately one copy per cell. The committee agreed no change was recommended to the current vector dose.

e) Mapping of vector insertion sites on all clinical lots of cell prior to release for clinical use

The committee agreed that lot release mapping of vector insertion sites was not scientifically or technically feasible and is not recommended.

In a further discussion of safety modifications to existing SCID protocols, the committee strongly recommended monitoring for proviral integration and clonal (monoclonal, oligoclonal, polyclonal) outgrowth of subjects samples after engraftment. The committee stated assays are currently available to monitor proviral integration and should be included in all X-SCID retroviral vector gene transfer protocols at defined time intervals (ex. every 3-6 months). It was noted that once a monoclonal integrant is identified that the genomic sequence at the site of vector integration should be determined and compared to existing genomic databases. The committee expressed that knowledge of the insertion site may, in some cases, inform clinical treatment or earlier intervention.

There was consensus by the committee that monitoring programs be developed and included in all retroviral gene transfer trials. However, the committee also stated flexibility should be allowed in the development of monitoring plans and sponsors have the opportunity to justify if monitoring for integration and clonal outgrowth are not necessary.

f) Alterations in vector design (i.e. SIN vectors)

The committee agreed while this is a very important research question, they do not recommend changes to current vector design. The committee did suggest several areas of interest that could be important in the future, such as developing a vector “suicide system” and refinements in the enhancer element of the LTR.

This completed the committee discussion of safety issues related to retroviral gene therapies for the treatment of patients with severe combined immunodeficiency. The committee reconvened after a short break and heard updates on CBER research programs in the Laboratories of Molecular Tumor Biology and Gene Regulation. Following the research updates the open session of the meeting was adjourned.

For more detailed information concerning the open session presentations and committee discussion summarized above, please refer to the meeting transcripts available on the FDA website at <http://www.fda.gov/ohrms/dockets>.

Please submit all external requests to the FDA Freedom of Information Office.

Appendix 2: Consideration of Modifications to Increase the Safety Margin of Retroviral Vectors

1. Dosing Paradigm

Currently, retroviral vector-mediated gene therapy clinical protocols using ex vivo modified cells include a dosing regimen based on the number of cells (for example, a typical dose for CD34⁺ cells is 10⁶ cells/kg). With changes in the transduction protocols combined with increasingly more efficient vectors, the total number of vector integration events will likely increase. For example, Woods, et al, carefully analyzed the vector integration frequency in NOD/SCID repopulating cells transduced with a lentiviral vector, and found that the average vector copy number per vector-expressing cell (GFP⁺) was 5.6, with a range of observed integrants from 3.7 up to 11.7 per GFP⁺ cell [1]. One approach to modulate this effect would be to reduce the dose of vector used for transduction.

Alternatively, the total number of vector integration sites could be modulated by reducing the cell dose, or to dose based on the total number of vector integrations. Of note, patients #4 and #5 received a total of 14 and 20 million CD34/gamma-c positive cells, corresponding to 2-4-fold higher doses of gamma-c positive cells than the previous three patients received [2].

2. *Additional preclinical studies to qualify retroviral vector and transduction systems for carcinogenic potential*

The evaluation of pharmaceuticals for carcinogenic potential is generally required and completed prior to license approval (Guidelines for Industry: "The Need for Long-term Rodent Carcinogenicity Studies of Pharmaceuticals", ICH S1A; and "S1B Testing for Carcinogenicity of Pharmaceuticals", both available at <http://www.ich.org/ich5s.html#Carcino>). As per the recommendations of the International Conference on Harmonisation, this assessment is usually not needed prior to large-scale clinical trials unless there is a special cause for concern, such as a special patient population (i.e., pediatric), or disconcerting preclinical/clinical data have surfaced (Guidance for Industry: "M3 Nonclinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals", available at <http://www.ich.org/ich5s.html#Multi>).

In this case, where the potential for cancer induction is suspected in patients, the performance of preclinical studies to evaluate the *in vivo* carcinogenic potential of the various contributory components of the final clinical product (i.e., retroviral vector backbone, transgene, and target cell combination) should perhaps be considered at an earlier stage of the clinical development program.

The carcinogenic potential of a pharmaceutical product has been traditionally assessed in the rodent lifetime (i.e., 2-year) bioassay [3]. If required prior to initiation of Phase I trials, the long duration of these studies would potentially hinder the forward progression of some gene transfer products in a timely manner.

Therefore, it may be necessary to explore the feasibility of alternative in vivo models to address this issue. The repertoire includes the use of genetically engineered animals expressing a transgene and/or having one or both alleles of a gene knocked out [3, 4] [5] [6]. Some of the better-characterized genetically modified models include, but are not limited to the following:

- ?? Tg.AC mouse model – tissue specific oncogene model; homozygous for a mutant *v-Ha-ras* transgene, providing a reporter phenotype (skin papillomas) in response to genotoxic or nongenotoxic carcinogens, including tumor promoters [7].
- ?? p53+/- homozygous knockout mouse model – heterozygous for a wild-type Trp53 tumor suppressor gene and a null allele that is not transcribed or translated [8] [9]; appears to be especially sensitive to mutagenic carcinogens.
- ?? *rasH2* mouse model – hemizygous, carrying multiple copies (about 3-5) of the human prototype *c-Ha-ras* oncogene integrated in a tandem array into chromosome #15; elevated levels of transgene detected in tumors; appears sensitive to genotoxic and some nongenotoxic carcinogens[10].

Many other alternative animal models are cited in the scientific literature and include, but are not limited to, the neonatal mouse [11], *Xpa*-/- knockout mouse, *Xpa*-/- / p53+/- knockout mouse [9], and models subject to premature aging or having telomere dysfunction [12] [13].

The use of an alternative model is generally not intended to serve as a stand-alone assessment of potential carcinogenicity. For example, many transgenic models have mutations in only one pathway that might be relevant to the human cancer process for a particular product. Also a specific gene defect may affect tumor development and tumor type, making extrapolation to the human outcome difficult. In addition, the strain and genetic background of the animal model can alter tumor type, location, and incidence [14]. The route of administration can also influence the study results, depending on the model used. The data collected for these short-term (generally about 6-9 months in duration) studies may not be as biologically informative as the information gathered in the traditional, longer bioassays. Multiple organ effects, interactions over time, animal age, and other factors may individually or collectively contribute to the overall assessment of carcinogenic potential. Depending on their characteristics and applicability, several models may need to be used in

order to provide the optimal testing strategy for a particular product or product component.

Animal models of disease have been used with increasing frequency in the development of biopharmaceuticals to evaluate both the activity and the toxicity of the product in a potentially more appropriate testing system. As they may also be important in the determination of host resistance to cancer, the use of disease models may also contribute to the understanding of the overall mechanism of action of any toxicity associated with the clinical product or product component.

In addition, due to the intended chronic nature of therapy with these products, the possibility for the performance of long-term toxicology studies in animals in parallel with early phase clinical trials should not be overlooked. Such a study would, by its design, help to reveal any preneoplastic/neoplastic changes that may occur in the animals. Information about the onset, incidence, and tumor type could be gathered as well. Additional, important data that could be obtained in such a study include local/systemic toxicities over time, the persistence of the transgene over time, and the immune response to the vector/transgene (and how this response may affect tumor development).

All *in vivo* studies performed need to consider some common, basic elements in the study designs in order to better assess the overall safety profile for the clinical product. Study designs should: 1) consider the historical background data available for the animal model used, 2) the use of appropriate positive and negative controls, 3) the animal's strain and age, 4) the inclusion of several dose levels of the product, 5) the dosing route, 6) the dosing schedule, 7) include appropriate in-life testing parameters, and 8) include histopathology, molecular toxicology, and immunohistochemical evaluation.

The current consensus of both industry and the regulators is that the alternative models should not be used as the sole means to investigate the potential carcinogenicity of a pharmaceutical, but instead should be employed in conjunction with the standard rodent bioassays, as well as with the long-term toxicity study, in order to provide the most comprehensive safety profile for a particular product. Such a series of studies is both time-consuming and costly. Potentially the concept of platform studies could be applied by various sponsors/institutions in order to achieve a common goal - to determine the *in vivo* carcinogenic potential of the final product and product components – the retroviral vector backbone, transgene, and target cell combination (Pilaro, AM, "Platform studies for AAV vectors in gene therapy" presented at the FDA/NIH Workshop on Nonclinical Toxicology Study Design Issues for Development of AAV-based Gene Therapies, Bethesda, MD, 5/2/99, copies of slides presented found in Appendix 3.

3. Cell Target or Culture Conditions

Hematopoietic stem cells (HSCs) are a desirable target population for gene therapy due to their self-renewal and differentiation properties. CD34 has served as a marker for isolation of HSCs used in therapeutic clinical trials, but data suggest that some stem cell populations express low or undetectable levels of CD34 [15] [16] [17]. Additionally, data from some of these studies indicate that the CD34⁻ cell population is more primitive than CD34⁺ cells [16] [17]. In theory, if the most primitive HSC population was used in gene transfer clinical trials, fewer cells would be required to be transduced, thereby, reducing the exposure to vector integration sites in the genome.

An additional factor that may influence whether integration of a retroviral vector may result in tumor formation is whether genetic loci with tumorigenic potential are transcriptionally active at the time of transduction. Both gammaretroviruses and lentiviruses have been shown to preferentially integrate into sites of transcriptionally active genes [18] [19]. Therefore, if one could identify vector transduction conditions favorable to efficient vector transduction in the absence of gene transcription activation, this may be another potential strategy to increase the safety margin of retroviral vectors. Another area of investigation that could be pursued would be to examine transduced cells using gene and protein microarrays in order to determine the gene expression profile. As a result of gamma-c chain gene transfer, activation of LMO-2 or other involved gene(s) might be detected prior to cell infusion.

4. Vector Modifications

If the major mechanism operative in the development of leukemia-like illnesses in the two children in Dr. Fischer's clinical trial of X-SCID gene therapy is due to retroviral enhancer-mediated activation and dysregulation of cellular gene(s) after vector insertion, then one should consider whether alterations in the vector design could decrease this risk to subjects.

One such vector design element under consideration is the use of insulator elements. Insulators are sequences of DNA that have been identified at the boundaries of genetic elements. Two functions have been identified with these sequences: 1) protection against position effects within the genome (i.e., silencing); 2) the ability to block enhancers – preventing inappropriate gene activation [20]. Investigators have included insulators in their retroviral vectors in order to protect against silencing of the vector genome [21] [22], with variable results, depending upon the particular configuration of the insulator in the retroviral vector. While it's been postulated that addition of insulators may prevent the distal activation of cellular promoters by enhancer sequences present in the retroviral LTR, this has not been experimentally proven.

A second strategy under investigation is to delete the enhancer elements within the retroviral LTR. Although this approach has been successfully achieved in lentiviral vectors [23] [24], introduction of enhancer deletions in gammaretroviral vectors has generally resulted in vectors with lower titers [25, 26]. One exception is an interesting variation of the enhancer deletion reported by Hwang et al, where the retroviral enhancer and promoter were replaced by the bacterial tetracycline operator. When expressed in packaging cells expressing the tetracycline trans-activator, LTR-derived RNA is expressed allowing for production of relatively high titer retroviral vector (approximately 10^5 CFU/ml). In contrast, target cells where the tetracycline trans-activator is not present will not allow for LTR-derived expression [27]. In those cases where retroviral enhancers are deleted, vectors would still need to carry an exogenous enhancer to control transgene expression, which may still have the ability to inappropriately activate cellular promoters.

One way to avoid inappropriate gene expression would be to use only tissue-specific and gene-specific promoters. For example, the minimum region of the gamma-c promoter required for hematopoietic-specific gene expression has been identified [28], and use of this type of element would avoid the constitutive expression resulting from exogenous strong promoters typically used.

Another approach to avoid vector insertion-mediated effects would be to develop a mechanism whereby integration into the genome would be targeted to regions where vector insertion would not have deleterious consequences. Unfortunately, only limited progress has been made in the development of site-specific integrating retroviral vectors. For example, in vitro studies of fusion proteins between retroviral integrase and DNA-binding proteins have resulted in site-specific integration [29] [30]. Endogenous genetic elements called retrotransposons have been shown to integrate into the genome in a site-specific manner [31] [32], suggesting that incorporation of these elements into retroviral vectors may provide a site-specific delivery system. However, this idea has not been tested empirically. The best example to date of a site-specific integrating vector is with adeno-associated virus, (AAV), where AAV vectors engineered to contain certain cis-acting sequences from wildtype AAV results in site-specific integration [33] [34]. Alternatively, although currently not feasible, it would be desirable to consider whether methods could be developed that would allow one to select against cells with vector integrated into sites of known tumorigenic potential (for example, LMO-2).

APPENDIX 3 - REFERENCES

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