

BRMAC briefing document for Day 2 November 17, 2000

Follow-up of Subjects in Gene Transfer Clinical Trials

Session III. Issues in long term follow up of clinical trial participants

Background

The purpose of this session is to obtain advice on what types of gene transfer clinical trials need long-term follow-up, what type of long-term follow-up should be performed, and how best the data should be captured to facilitate analysis by FDA staff.

Clinical exposure to gene transfer vectors that integrate into the genome differs from most other forms of medical treatment. Traditional pharmacologic agents have finite half-times in vivo, typically limited by the pharmacokinetics of the agent. In contrast, integrating gene transfer vectors may expose recipients to the expressed gene product and the integrated vector sequences indefinitely. Long-term survival of exogenously introduced genetic sequences in the human genome may pose risks that do not become apparent until years later. For example, the long-term expression of a transgene may be a desired outcome, but has the potential of inducing unwanted immune responses leading to autoimmune disease. Another hidden long-term consequence could result from the interaction of the introduced genetic sequences with other elements in the cellular genome causing abnormal gene expression (for example, activation or suppression of cellular genes that may be detrimental or potentially tumorigenic in the cell target).

Safety evaluations are a critical part of drug development. In clinical trials, the timing of these safety assessments varies depending on the nature of the intervention and the duration of the study. Typically extensive safety evaluations are performed on all study subjects at specific time periods while they are receiving study medication and for a defined period of time off-treatment, e.g., 30 days from the last dose. The purpose is to identify acute and mid-term toxicities possibly related to the intervention. In order to detect latent or long-term effects possibly related to an intervention, clinical follow up is performed for considerably longer periods of time, such as life-long or out to the next generation.

Long-term follow up is generally much less intensive than during the immediate study period, and practical and ethical constraints may limit the completeness and accuracy of this type of data collection. Multiple factors may impede the success of long-term follow-up, e.g: 1) study participants move and may be lost to follow up, 2) participants may refuse to return for follow up testing, 3) patient tracking requires substantial resources and tracking, 4) companies and or investigators may lose interest in the product and be reluctant to devote effort and resources years or decades later 5) companies that funded the initial studies may go out of business, without contingency plans for who will be responsible for continuing long term follow up, 6) academic investigators who initiate

a clinical trial may leave the institution or lose their funding and lose the ability to follow patients. Given the difficulties and the resource issues, long term follow up efforts are most likely to be successful if focussed on obtaining the most important data. Thus, it is important to carefully consider when it is necessary to request long-term follow up, and what types of data would be most critical to collect.

This issue was recently driven home as we reviewed responses to an FDA letter to all gene therapy sponsors in which the agency asked for information about trial monitoring and oversight of the clinical investigations. In response to the letter, some sponsors who have completed all studies asked to withdraw their IND, leading to questions about how the long-term data required for some applications (see below) would be collected.

Properties of Vector Classes that Raise Long-Term Risks to Human Subjects

This section summarizes the known and potential risks from integrating vectors to provide the committee data supporting the need for long-term follow-up of subjects participating in gene transfer clinical trials. Of the gene transfer vectors currently being evaluated in clinical trials, only retroviral vectors and adeno-associated virus vectors have demonstrated capacity for integration into the genome of target cells with a reliably high frequency. Other classes of vectors integrate either at lower frequencies or under certain conditions (details are discussed below). CBER is seeking guidance from the committee regarding the properties of a gene transfer vector necessitating long-term follow-up of subjects in clinical trials.

Risks Associated with Integration of Retroviral Vectors

Murine retroviruses related to those used in gene transfer clinical trials can cause tumors in mice. The mechanism of tumorigenesis for these retroviruses is via proviral insertional mutagenesis. This mechanism seems to be associated with and presumably requires extensive viral replication. High levels of virus replication leads to a high number of sites of provirus integration eventually causing disruption of a locus with pathogenic effects. In other words, most proviral insertions are benign. In the small subset of insertions that are not benign, provirus insertion causes gene dysregulation by one of several mechanisms [1]:

1. The strong viral promotor or enhancer of the retrovirus may cause inappropriate levels of expression at downstream or sometimes even distal sites from the site of provirus integration.
2. The site of provirus insertion may disrupt certain control sequences, such as sequences found in the 3' untranslated regions that regulate levels of mRNA.
3. The site of provirus insertion may destroy regulatory elements such as silencers that control gene transcription.

While retroviral vectors carry the potential to cause similar disrupted gene expression leading to pathogenic effects, the frequency of integration events is significantly lower when a replication-defective vector of limited titer is used. For example, one estimate is that with a typical ex vivo transduction protocol that the integration events would be once every 700 KB in the genome [2]. Howard Temin has estimated that the risk of activating a particular proto-oncogene by retrovirus integration is approximately 10^{-5} /integration event [3].

Risks Associated with Integration of AAV Vectors

The pathogenic consequences of AAV vector integration are unknown. Wildtype (WT) AAV is not associated with any human disease. However, the replication cycle of WT AAV differs from that of AAV vectors: WT AAV integrates at a specific locus on chromosome 19 [4], while AAV vectors do not. An in vitro study of AAV vector integration in human leukemia and human lymphoma cell lines used fluorescence in situ hybridization to identify integrated AAV vector DNA on chromosomes 1, 2, 3, 8, 14, 15, 19, and Y [5]. In vivo, the integration properties of AAV may vary depending upon the target tissue. After injection of AAV into skeletal muscle of mice, AAV DNA was detected in various forms, but the data did not definitively demonstrate whether the DNA was present in episomal or integrated forms [6]. In contrast, portal vein injection of an AAV vector into mice produced clear evidence of integrated vector sequences in the liver [7]. Of note, the latter study also reported occasional rearrangement of cellular DNA sequences at the site of vector DNA integration. The significance of these observations as they relate to potential pathogenic consequences is unknown.

Other Vector Classes

Other vector classes currently in clinical trials either are not known to integrate or do so at frequencies measured to be orders of magnitude lower than the frequencies for retroviral vectors. For example, a study of helper-dependent (gutless) adenovirus vector and E1-deleted classical adenovirus vectors demonstrates that adenovirus vectors integrate, but at a low frequency of 10^{-3} to 10^{-5} per cell [8]. Similarly, plasmid DNA can integrate, but the frequency is significantly lower than for retroviral vectors. In addition, manipulations of vector sequences or the conditions of gene transfer may alter the frequency of integration events. Two examples follow:

1. A modified adenovirus vector containing the long terminal repeat sequences of a retrovirus was reported to integrate into 10-15% of exposed cells in vitro and up to 5% of rat spleen cell in vivo [9].
2. Treatment of cells with camptothecin (an inhibitor of topoisomerase I) after electroporation with a plasmid resulted in 4 to 33-fold increase in plasmid integration [10].

It is unclear what frequency of integration is related to development of disease. Using the tumorigenic non-oncogene carrying murine retroviruses as a model, the assumption is that a high frequency and high number of integration events are required to increase the risk of integration into a locus of the genome that would result in altered gene regulation and disease. Additional support for this concept comes from the study of naturally occurring insertions via retrotransposition. Considering the estimated frequency of retrotransposition in 1 in every 50-100 germ cells compared to the documented identification of 14 retrotranspositions that have resulted in disease [11], it appears that integration events into the genome may be generally well tolerated.

Current recommendations for long term follow up

FDA's current long-term testing and patient monitoring recommendations focus on retroviral vectors. The rationale for recommending long-term follow-up in patients participating in gene transfer clinical trials using retroviral vectors is based on the fact that these vectors are known to integrate into the genome. The long-term consequences of life-long exposure to the gene product or to the introduced genetic sequences can only be assessed through the careful follow-up of these patients. In addition, use of retroviral vectors carries the potential for exposure of patients to replication competent retroviruses (RCR). In 1993, a report of lymphoma in 3/10 immunosuppressed non-human primates that received retrovirally transduced bone marrow cells with high titre RCR led to recommendations for patient testing. Those recommendations, have recently been refined (see "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", provided in attachments) as follows:

..analysis of patient samples at the following time points: pretreatment, 3, 6, mos, 1 year after treatment, and yearly thereafter. If all post treatment samples for the first year are negative, remaining samples can be archived..... At time of collection of yearly samples, a brief clinical history should be obtained and targeted towards determination of clinical outcomes suggestive of retroviral disease, such as cancer, neurologic disorders, or hematologic disorders.....If patients die or develop neoplasms during a gene therapy trial, every effort should be made to assay for RCR in a biopsy sample of the neoplastic tissue or the pertinent autopsy tissue.

To date, data on RCR testing has been negative. FDA is currently reviewing all retroviral vector IND to assess their status regarding the long term follow, including the completeness of the data and obstacles to data collection.

References:

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DRAFT Questions to the committee

1. FDA currently asks that gene transfer trials using vectors with demonstrated potential for genome integration would include plans for long term follow up.

What characteristics of gene transfer methods should trigger the need for long term follow up?

2. FDA recommends study participants who receive retroviral vector products be evaluated with particular attention to the development of oncologic, neurologic, or hematologic events.

Please comment on the recommendations for long-term clinical assessments of patients who receive retroviral gene therapy products.

3. Currently, sponsors and investigators commit to life long follow up of subjects who receive retroviral vectors. Practical issues, such as companies going out of business, academic investigators leaving their institutions, and/or patients losing interest in being studied, may limit the completeness of such data collection. Even with good intentions and efforts, long-term follow-up is likely to be significantly incomplete, and as sponsors' and investigators' and patients' interest in the study declines over time, the quality and quantity of follow-up data will likely decline.

Are there approaches which might help improve collection and submission of long-term data? Are concerns regarding long-term safety of integrating vectors sufficient to warrant stopping such research until these issues can be better addressed?