

BIOLOGICAL RESPONSE MODIFIERS ADVISORY COMMITTEE
Briefing Document For May 10th, 2002
Issues Pertaining To Inadvertent Germline Transmission Of Gene Transfer
Vectors

Introduction

The unintended modification of genetic material in host germ cells by gene transfer vectors during clinical trials remains a theoretical but real concern. For the purposes of this briefing document, such an event is referred to as Inadvertent Germline Transmission (IGLT). IGLT is of particular concern in clinical trials in which gene transfer vectors with the potential to integrate into the host chromosome are administered by systemic routes. While germline transmission has not been observed in clinical trial participants to date, findings obtained from a recent study have renewed concern regarding IGLT.

Data from a phase I safety study involving administration of an adeno-associated virus (AAV) vector carrying the factor IX gene into the hepatic artery of subjects with hemophilia B were presented at two recent meetings of the Recombinant DNA Advisory Committee (RAC). In this study, vector sequences were detected in semen samples from the first two subjects to which the vector was administered. In the context of these findings, the FDA is seeking guidance on how to further develop policies regarding IGLT, not just for this particular study, but in general for all clinical trials involving systemic administration of gene transfer vectors.

1. BACKGROUND

Pre-clinical animal studies

The data from animal studies regarding directed attempts to alter germ cells with gene transfer vectors have provided equivocal results regarding the ability of these agents to facilitate vertical gene transmission to progeny. Using cationic lipid-encapsulated plasmid DNA vectors encoding bacterial β -galactosidase, human growth hormone, or green fluorescent protein, gene transfer to spermatozoa was observed, both *in vitro* and following direct injection of vector into gonadal tissues (testes, epididymes, or vas deferens; [1-4]). When injected male mice were mated 2 to 3 days later with super-ovulated female animals, vector sequence could be detected in the F0 progeny, in some cases in as many as 50% of the fetuses [5,6]. Similarly, a study using retroviral vectors injected into the hearts of spontaneously hypertensive, newborn rats has demonstrated positive evidence of gene transfer, as determined by PCR and Southern blot analysis, as well as functional correction of the gene defect in the F1 and F2 offspring [7].

Data on risks associated with germline gene transfer are available from studies performed with retroviruses, where these viruses were in fact used as a tool to derive mouse strains with recessive lethal mutations induced by provirus mutations (for review, see [8]). To assess the mutagenic effect of retrovirus insertion, heterozygous animals were intercrossed in order to obtain homozygous animals at each locus. Combining results from a number of studies, two out of 48 crosses produced recessive lethal mutations [9].

However, similar attempts to create transgenic animals using adenoviral vectors, either after *in vitro* exposure of mature sperm to the virus or by direct injection into testicular tissues have failed to show significant gene transfer to either spermatozoa or offspring [10,11]. In a study utilizing an adenoviral vector, murine spermatogenic cells were exposed immediately prior to or during *in vitro* fertilization. When examining exposed testes or pre-implantation embryos, no evidence of germline transmission was observed. Additionally, treatment of either male, female, or animals of both genders with high doses of adenoviral vectors by intravenous injection (i.v.) failed to result in detectable sequences in offspring, even when the gonadal tissues of the parental animals were highly positive for vector sequence by PCR analyses [12].

Safety studies of AAV vectors, including the one used in the present clinical trial, have failed to demonstrate germline transmission in animal models [13,14]. When AAV vector encoding canine or human factor IX, or a null AAV vector, were injected into either the muscle or hepatic artery of four different species of animals (mice, rat, dog, rabbit), vector dissemination to the testis was detected, although the signal decreased over time. However, vector signal was localized to the testis basement membrane and the interstitial spaces [13]. In contrast to the results obtained from the current AAV FIX human trial, semen obtained from the dogs exposed i.v. to AAV in this study had no detectable vector sequences present by PCR analysis.

Taken together, these data suggest that the risks of IGLT are more than theoretical, but are difficult to accurately assess in the available animal models.

Previous public discussions on IGLT

During the March 1999 RAC meeting, pre-clinical data on gonadal biodistribution and the potential for IGLT were discussed (<http://www4.od.nih.gov/oba/rac/minutes/3-99RAC.htm>). The consensus from this meeting was that the risk of IGLT occurring in a gene transfer clinical trial is low. Further discussion of IGLT became necessary when data from a gene transfer trial conducted by Chiron Corporation revealed the detection of vector sequences in the semen of a study subject. The data from this study was presented at the BRMAC meeting held Nov 17, 2000. This trial involved i.v. administration of a murine gammaretroviral vector carrying a factor VIII gene to

subjects with hemophilia A. At week nine post administration, DNA extracted from a semen sample was positive for vector sequences as detected in 1/10 polymerase chain reaction (PCR) replicates. No positive signals were detected in subsequent semen samples. Murine gammaretroviruses transduce only dividing cells, thus mature sperm cells are unlikely targets for transduction by these viruses. However, spermatogonial stem cells, which are rapidly dividing, theoretically could be transduced by a retroviral vector via hematogenous spread. Considering the physical barriers that a systemically administered vector would need to cross, type A and B spermatogonia would be accessible for transduction since these progenitor germ cells are on the blood side of the Sertoli cell barrier (for review see [15]). Since one cycle of spermatogenesis takes approximately 64-74 days, the timing of the appearance of a transduced progenitor's daughter cells in the semen is predictable. The protocol by Chiron to test study subjects' semen samples took this timing into account. The BRMAC committee agreed with the FDA instituting a clinical hold when vector sequences are detected in semen samples from study subjects. In order for the clinical hold to be lifted, the FDA approach was to require that the sponsor demonstrate that three consecutive semen samples, collected monthly, were negative for the presence of detectable vector sequence. The committee also recommended that fractionation of semen samples be performed as a follow up to a positive semen sample. Using fractionation methods such as density separation, potential contaminating transduced white blood cells can be removed from sperm cell fractions.

At the RAC meetings of December 22, 2001 and March 7, 2002 data were presented from a trial being conducted by Avigen Inc. (<http://www4.od.nih.gov/oba/RAC/meeting.html>). In this study, an AAV vector carrying the factor IX gene was administered into the hepatic artery of subjects with hemophilia B. As detected by PCR analysis of unfractionated semen, vector sequences have been found in the semen of the first two subjects treated. In the first subject treated, positive PCR signals were detected up to ten weeks post administration, but no signal was detected in multiple latter time-point samples. Only early time-point samples have been analyzed on the second subject. Conceivably, all subjects treated in this trial may have vector sequences that are transiently detected in semen. In fact, vector sequences may be detected in subjects' semen in all trials involving systemic administration of an AAV vector. Additionally, with new technologies allowing for higher titer vectors to be made, and with new vector types being tested (such as lentiviral vectors), detection of vector sequences subjects' semen might become more prevalent in future clinical trials.

Considerations for different vector classes

Due to the biology of the various gene transfer vectors being used, specific consideration may be needed in terms of procedures for patient follow-

up. For example, unlike murine gammaretroviral vectors, AAV does not have a strict requirement for dividing cells in order to stably transduce cells. Thus, in addition to transduction of the spermatogonial progenitor cells being a concern, transduction of mature sperm is also a theoretical risk. In addition to AAV, lentiviral and adenoviral vectors do not have a requirement for cell division in order to transduce cells. The potential for host chromosome integration for these vectors range from very likely to highly unlikely and must also be accounted for in considering the appropriate follow-up procedures for subjects receiving these vectors.

2. CURRENT FDA APPROACH

FDA pre-clinical recommendations to assess potential for germline transmission.

During the conduct of pre-clinical safety studies of gene transfer vectors, FDA recommends that the distribution of vector out of the intended site be evaluated by PCR analysis of DNA isolated from a panel of different tissues, including gonads (ovaries and testes). The FDA's current recommendations for the design and conduct of biodistribution studies are to include a minimum of 5 animals per gender, per dose group, and per time point of sacrifice for analysis. If a single gender is used in support of gender-specific indications (e.g. hemophilia in males), then at least ten animals per group should be evaluated. The route of administration of the vector should mimic the intended clinical route as closely as possible. Studies should include a vehicle control group, and preferably two or more dose levels of the test article to both establish a no-effect level for biodistribution, as well as to maximize exposure to the gene transfer agent. Tissue sampling should be performed at terminal sacrifice, and should, at a minimum, include at least one time point where peak gene expression occurs, as well as at least one later time point. The later time points are to be determined by the intended clinical use (e.g. if the clinical trial includes plans for re-treatment with the vector, the later time point in the animal study should approximate the day of re-administration). For integrating vectors, the later samples should be obtained at least 60 days after injection, to determine the potential for signal persistence through one complete cycle of spermatogenesis. To minimize the chances of contamination of the samples by leukocytes present in peripheral blood, every attempt should be made to harvest the tissues as cleanly as possible, and to remove peripheral blood as much as possible at necropsy.

PCR Assay Conduct

- Assays should be able to detect a minimum of 10-100 copies of vector signal per mcg of DNA
- A minimum of 3 mcg of DNA per tissue should be assayed
 - Two mcg of DNA per tissue sample to be assayed directly, with no

- further treatment
- One mcg of DNA per tissue to be “spiked” with positive control for vector amplification, as a control for effects of tissue factors on detection of signal

FDA current recommendations for patient follow up

In response to data from the AAV FIX trial, and subsequent discussions at the Dec 2001 RAC, the FDA has modified its approach, and will only institute clinical hold if the motile sperm from fractionated semen tests positive for vector sequences.

Current approach for follow-up of potential IGLT of gene transfer vector sequences to the sperm of clinical trial participants:

- If semen tests positive for vector sequences, the FDA will allow the clinical trial to continue, but request that the sponsor begin fractionating semen at time points appropriate for the detection of a transduced progenitor in semen. Testing of fractionated semen should span at least one complete cycle of spermatogenesis.
- If the motile sperm fraction tests positive for vector sequences, patient enrollment will be stopped and the clinical trial will be placed on hold.
 - Subsequent fractionated samples from the same patient must test negative over 3 consecutive monthly intervals for enrollment to resume.
- Use of barrier contraception is requested until 3 consecutive semen samples test negative.

The potential for assessing IGLT in women is especially difficult and, to date, no clinical studies have proposed sampling of female reproductive tissue.

Informed consent

If during pre-clinical animal studies, vector is found in gonadal tissue, this finding and the potential for gonadal dissemination should be included in informed consent documents. If vector sequences are found transiently in subject semen, sponsors will be asked to revise the informed consent document in order to describe the detection of vector DNA sequences within the semen of subjects tested.

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