Guidance for Industry

Gene Therapy Clinical Trials – Observing Participants for Delayed Adverse Events

DRAFT GUIDANCE

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Contains Nonbinding Recommendations

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Guidance for Industry

Gene Therapy Clinical Trials – Observing Participants for Delayed Adverse Events

Paperwork Reduction Act

This guidance contains information collection provisions that are subject to review by the Office of Management and Budget (OMB) under the Paperwork Reduction Act of 1995 (44 U.S.C. 3501-3520). The information collection provisions in this guidance for the Investigational New Drug Application (IND) regulations (21 CFR Part 312) were approved under OMB Control No. 0910-0014; and the Good Laboratory Practice (GLP) regulations (21 CFR Part 58) were approved under OMB Control No. 0910-0119.

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I. INTRODUCTION

This guidance provides to you, sponsors of gene therapy studies, recommendations regarding the design of studies to include the collection of data on delayed adverse events in participants who have been exposed to gene therapy products. We, FDA, are recommending: (1) methods to assess the risk of gene therapy-related delayed adverse events following exposure to gene therapy products, (2) guidance for determining the likelihood that long-term follow-up observations on study participants will provide scientifically meaningful information, and (3) specific advice regarding the duration and design of long-term follow-up observations.[1] When the risks to human subjects presented by a gene therapy clinical trial continue into the long term, a gene therapy clinical trial must provide for long-term follow-up observations in order to mitigate those risks, because without such long-term follow-up observations, the study would expose the subjects to an unreasonable and significant risk of illness or injury (21 Code of Federal Regulations (CFR) 312.42(b)(1)(i) and (b)(2)(i)).

Exposure to gene transfer technology means any exposure to gene therapy products or to cells or tissue that has been transduced with gene therapy products ex vivo by any route of administration. Except as noted below, this guidance applies to all participants in clinical studies using gene transfer technology. The recommendations in this guidance are limited to the

[1] This guidance does not cover the following topics:

- Inadvertent germline gene transfer. (The term "germline" is used to designate genetic material destined to be transferred to gametes.) For a discussion of risks associated with inadvertent germline gene transfer for gene therapy products, we refer you to the following meeting transcripts:
  - December 15-16, 1997, Recombinant DNA Advisory Committee (RAC) meeting (http://www4.od.nih.gov/oba/rac/minutes/12151697.htm)
  - March 11-12, 1999, RAC meeting (http://www4.od.nih.gov/oba/rac/minutes/3-99RAC.htm)
  - November 16-17, 2000, Biological Response Modifiers Advisory Committee (BRMAC) meeting (http://www.fda.gov/cber/advisory/ctgt/ctgtmain.htm. November 17, 2000, 3664t2_b.pdf.).
- Vaccines used to prevent infectious diseases even if they use products analogous to those used for gene therapy (consult the Office of Vaccines Research and Review, Center for Biologics Evaluation and Research (CBER)).

performance of long-term observations for evidence of delayed adverse events, i.e., adverse events that occur more than one year after exposure to the gene therapy product.

This guidance supplements the recommendations in the "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" (Retroviral Vector guidance), dated October 2000 (Ref. 1), for study participant long-term follow-up. However, the recommendations in the Retroviral Vector guidance regarding the length of follow-up are superceded by this Gene Therapy Clinical Trials guidance.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the FDA's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in FDA's guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

A. Potential Risks of Delayed Adverse Events Following Exposure to Gene Transfer Technology

Study participants exposed to gene transfer technology may be at risk of delayed adverse events as a consequence of persistent biological activity of the genetic material or other components of the products used to carry the genetic material. The persistent biological activity may be required to provide a continuing clinical benefit. However, persistent biological activity could have adverse effects upon normal cell function, placing participants at risk for development of adverse events, some of which may be delayed by months or years.

Factors likely to increase the risk of delayed adverse events following exposure to gene transfer technology include persistence of the viral vector, integration of genetic material into the host genome, prolonged expression of the transgene, and altered expression of the host's genes. Persistence of the viral vector, sometimes associated with latency, could permit continued expression of the gene or delayed effects of viral infection. Integration of genetic material from a viral vector into the host cell genomic DNA raises the risk of malignant transformation (see section V.F for a discussion of risks of malignancy associated with retroviral vectors). Prolonged expression of the transgene may also be associated with long-term risks resulting from unregulated cell growth and malignant transformation, autoimmune-like reaction to self antigens, and unpredictable adverse events. Altered expression of the host genes could also result in unpredictable and undesirable biologic events.

B. Previous FDA Recommendations

We previously issued a guidance related to retroviral vector-mediated gene therapy (Ref. 1). We considered retroviruses to carry the highest known risk because of a reported case of new malignancy associated with a preclinical gene therapy study following exposure to cells transduced by a retroviral vector (Ref. 2), and therefore included in that guidance specific recommendations on performing long term observations of participants in trials of retroviral-mediated gene therapies.

We then sought additional information regarding gene-therapy related delayed adverse events following exposure to other gene-therapy products. Therefore, we convened three separate meetings of our Biological Response Modifiers Advisory Committee (BRMAC) to solicit advice about long-term risks to participants in gene therapy clinical trials exposed to other gene therapy products. The BRMAC meetings were held on November 17, 2000; April 5, 2001; and October 24, 2001.[2] Since 2001, and after reviewing BRMAC's recommendations, we have requested sponsors of studies involving gene transfer technology to submit to us their plans for long-term follow-up observations. We typically requested a time period of 15 years to observe participants for potential gene-therapy related delayed adverse events, and that the observations include a minimum of five years of annual examinations followed by ten years of annual queries of study participants, either in person or by questionnaire.

C. Concerns Raised by the Gene Therapy Community

Members of the gene therapy community asked that the issue of long-term follow-up following exposure to gene transfer technology be discussed in a public forum. Accordingly, in June 2004 a public workshop was held in association with the annual meeting of the American Society of Gene Therapy (ASGT). The workshop was entitled "Long-Term Follow Up of Participants in Human Gene Transfer Research" and was co-sponsored by the ASGT, Biotechnology Industry Organization (BIO), the Center for Biologics Evaluation and Research (CBER), the NIH Office of Biotechnology Activities (OBA), and Pharmaceutical Research and Manufacturers of America (PhRMA). The workshop included a forum in which invited speakers discussed the challenges associated with long-term follow-up of participants in gene therapy clinical studies. The workshop organizers published a summary of the discussion (Ref. 3).

[2] If you desire background information regarding prior recommendations from the BRMAC about gene therapy trials and long-term follow-up observations, we refer you to the transcripts for the November 17, 2000; April 5, 2001; and October 24, 2001 BRMAC meetings. The references can be located at http://www.fda.gov/cber/advisory/ctgt/ctgtmain.htm by searching under the year of the meeting.

Key issues identified by workshop participants include the following:

- Not all products classified as gene therapy products convey the same risks of delayed adverse events. Uniform recommendations for long-term follow-up did not take product characteristics into account.
- Some study participants appear unsuitable for meaningful long-term follow-up observations because of high short-term mortality, poor general health, or exposure to mutagenic agents.
- Our recommendations regarding duration and design of long-term follow-up have not been sufficiently specific.

These issues are addressed in sections IV and V of this guidance.

III. DEFINITIONS AND ABBREVIATIONS

The following definitions apply to this guidance:

Gene therapy products:
All products that mediate their effects by transcription and/or translation of transferred genetic material and/or by integrating into the host genome and that are administered as nucleic acids, viruses, or genetically engineered microorganisms. The products may be used to modify cells in vivo or transferred to cells ex vivo prior to administration to the recipient.

Gene transfer:
The transfer of genetic material into a cell.

Gene transfer system:
The combination of the vector, regulating elements, vector formulation, and the route and method of vector delivery.

Gene transfer technology:
The use of genetic material either alone or in a suitable transfer medium, such as lipids, viruses, or other microorganisms, to mediate an effect by transcription, translation, or integration into the host genome or any combination of these processes. Exposure to gene transfer technology may result from direct administration of the product to a study participant or through use of cells or tissues exposed to such products ex vivo prior to administration to a study participant.

IND:
Investigational New Drug Application, as described in 21 CFR Part 312.

Integration (of DNA):
The process whereby exogenous DNA sequences become incorporated into a genome.

Latency (of a viral infection):
A period of time during which a virus is present in the host without producing overt symptoms.

Long-term follow-up observations:
Long-term follow-up observations are extended assessments that continue some of the scheduled observations of a customary clinical trial. Long-term follow-up observations are an integral portion of the study of investigational products, such as gene therapy, that are considered to be high risk of producing delayed adverse events.

Maximum feasible dose (MFD) (in preclinical studies):
The highest dose that can be administered to a non-human animal. Limitations may be due to animal size, administration site, or product characteristics. The MFD may not be equivalent to the clinically relevant dose.

Persistence:
With respect to transferred genetic material, the continued presence of evidence of genetic sequences in the host after acute exposure to a transfecting agent, whether due to integration of the genetic sequence into the host genome or to latent infection with the viral vector bearing the genetic sequence.

Preclinical Study:
An investigational study performed in non-human animals or in isolated cells or tissue from humans or other animals. Preclinical studies may be performed prior to or during clinical studies.

Reactivation (of a viral infection):
The re-emergence of a symptomatic viral infection following a period of latency.

Transgene:
An exogenous gene that is introduced into a host genome.

Vector Sequences:
Refers to specific sequences of nucleotides, either DNA or RNA, that have been introduced into a gene therapy vector.

Viral Vector:
A virus that has been modified to transfer genetic material.

IV. PRECLINICAL DATA USED FOR ASSESSMENT OF DELAYED RISKS IN GENE THERAPY CLINICAL TRIALS

A. Criteria to Assess Potential Delayed Risks of Gene Therapy

We generally will not require long-term follow-up observations following exposure to gene transfer technology when the risk of delayed adverse events is low. To assess the risk related to your product, we recommend that you use available preclinical and clinical evidence. To assess the risks of delayed adverse events, you may use current information about your product and similar products based on studies that you and others have performed. As more data accumulates, it is important to reassess the risk to your participants and, if appropriate, revise your protocol as it relates to long-term follow-up observations.

We consider the assessment of risks to be a continuous process. New information may support the need for long-term follow-up observations or the revision of an existing study. For example, if recently reported evidence suggests a newly identified risk associated with your product or similar products, long-term follow-up observations may be necessary to mitigate long-term risks to subjects receiving these vectors. Similarly, if sufficient data accumulate to suggest that your product is not associated with delayed risks, it may be appropriate to reduce or eliminate provisions for long-term follow-up observations.

Pertinent previous preclinical and clinical experience with your product or similar products is highly relevant in the assessment of delayed adverse events. Experience with products in the same vector class, administered by a similar route, and given for the same clinical indication may contribute helpful information.

We recommend you refer to the series of questions in Figure 1, "Framework to Assess the Risk of Gene Therapy-Related Delayed Adverse Events" to help you assess the level of risk. When the risk of delayed adverse events is low based on your answers to these questions, a plan for long-term follow-up observations may not be necessary to mitigate risks to subjects. Evidence from preclinical studies will help you answer questions 1 – 3. Include all of the primary data relevant to the assessment of the risk of delayed events when you submit your IND to us (see 21 CFR 312.23(a)(8), (10)(iv), (11)).

We suggest you use the framework in Figure 1 by answering the questions in sequence as follows:

- **Question 1:** "Is your gene therapy product used only for ex vivo modification of cells?"

  If the answer is "no," go to Question 2. If the answer is "yes," go to Questions 3 and 4.

- **Question 2:** "Do preclinical study results show persistence of vector sequences?"

  If the answer is "no," the risk of gene therapy-related delayed adverse events is low, and long-term follow-up observations may not be needed. If the answer is "yes," go to questions 3 and 4.

  If it is unknown whether your vector persists, for the purpose of assessing risk, we recommend that you either assume that it does persist, or perform a preclinical study to assay for vector persistence in a relevant animal species. Please refer to Section IV. B, "Considerations for Preclinical Study Design to Assess Vector Persistence," for help with preclinical trial design and details on the use and expected sensitivity of polymerase chain reaction (PCR) assay for biodistribution studies. In assays performed after the final administration of vector, persistence is indicated by detectable levels of vector sequences above the threshold level in the PCR assay and absence of an apparent downward trend over several time points. In contrast, persistence is unlikely if you cannot detect vector sequences with a sensitive PCR assay or if the assay for vector sequences demonstrates a downward trend over time.

- **Question 3:** "Are vector sequences integrated?"

  If the answer is "no," go to question 4. If the answer is "yes," we would require that clinical protocols with the product include clinical long-term follow-up observations.

- **Question 4:** "Does vector have potential for latency and reactivation?"

  If the answer is "no," the risk is low that exposure to your gene transfer technology will be followed by gene therapy-related delayed adverse events. Long-term follow-up observations may not be needed. If the answer is "yes," we would require that all your clinical protocols with the product include clinical long-term follow-up observations.

Laboratory and preclinical evidence of the low risk of delayed adverse events following exposure to a similar product may show that long-term follow-up observations are not needed. If you provide data from a similar product, we can assess the relevance to your product if you provide a clear explanation.

We provide the following two examples:

- Your product is a plasmid and the similar product also is a plasmid, but has different coding sequences for the proposed therapeutic gene product. The similar product has been used in preclinical and clinical studies, administered by an identical route and in an identical final formulation to that proposed in the prospective studies. Reference to a published study demonstrating lack of persistence of the vector for the similar

product may adequately address concerns regarding the persistence of the proposed vector.

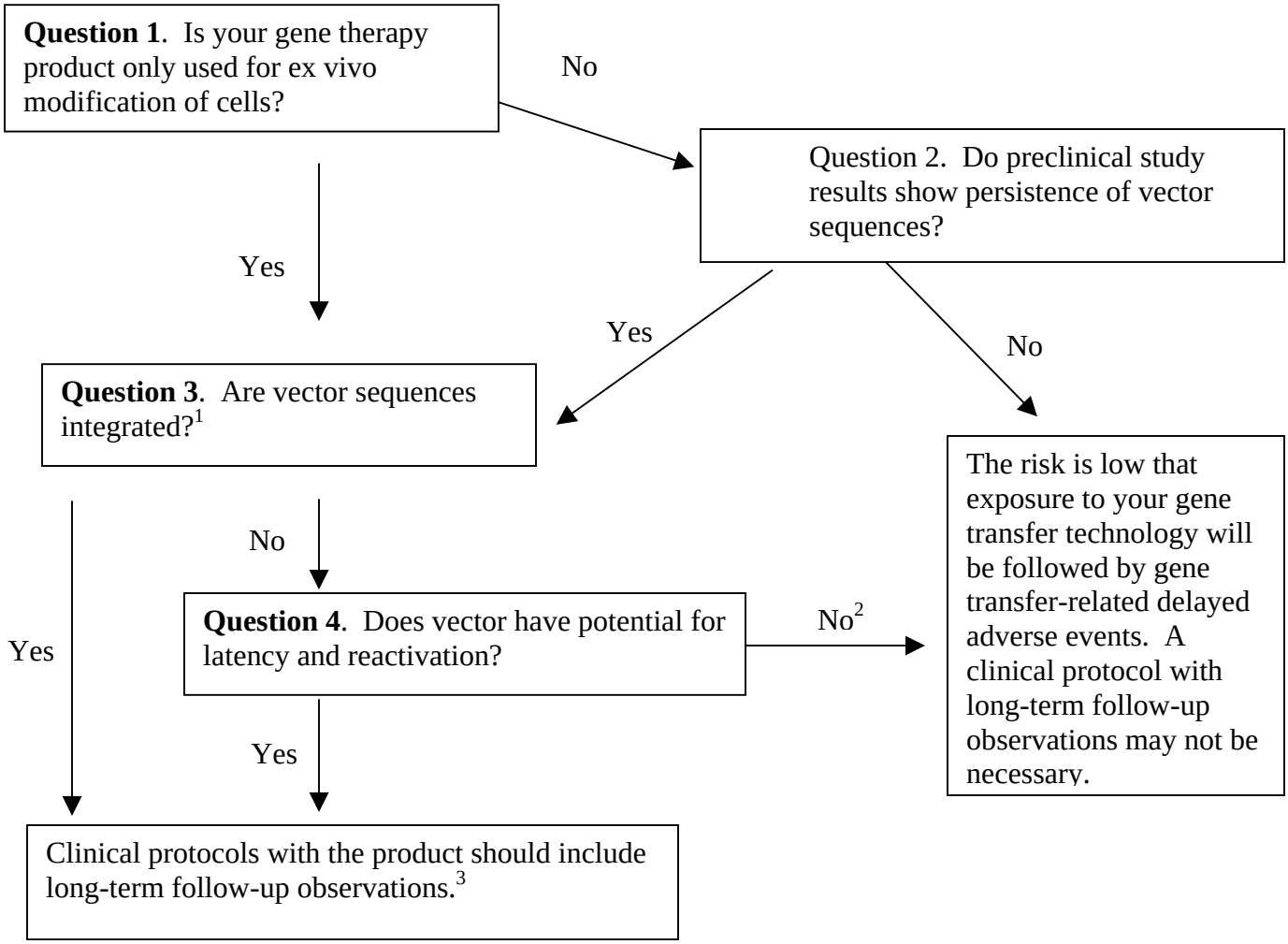
- Your proposed product and the similar product differ only with respect to route of administration. The similar product was administered into tumors (intratumorally). The proposed product is to be given intravenously. The data from the studies with the similar product are not sufficiently relevant, since there was no intended systemic exposure to the product. Thus, there is insufficient similarity to conclude that long-term follow-up observations are not necessary to mitigate long-term risks to subjects. In the absence of relevant data from a study involving a similar product, we recommend that you assess the risk of vector persistence with a preclinical study with the proposed product administered by the intravenous route.

If you believe you have evidence from studies on a similar product that is adequate to support conclusions that the vector is unlikely to persist in human hosts and that the vector's DNA does not integrate into the human genome, you may decide to submit a clinical protocol that does not provide for long-term follow-up observations. We will review such submissions and, if we disagree based upon our review of your submission or other additional information, we may conclude that long-term follow-up observations for delayed adverse events are necessary to mitigate long-term risks, and that without long-term follow-up observations, the study presents an unreasonable and significant risk to study subjects (21 CFR 312.42(b)(1)(i) and (b)(2)(i)).

We provide the following examples of evidence that might cause us to require you to perform long-term follow-up observations for delayed adverse events:

- A preclinical toxicology study indicates that expression of the transgene is associated with delayed toxicity.
- The transgene provides functional replacement of a host gene; the transgene product is potentially immunogenic.
- Data collected in your short-term clinical study indicate vector persistence, even though data from your preclinical studies suggested that the vector did not persist.

Figure 1. Framework to Assess the Risk of Gene Therapy-Related Delayed Adverse Events.



[1] If you have evidence that suggests that the vector may integrate or if the vector was intentionally designed to facilitate integration (please refer to Table 1, section IV.C), the answer is "yes." If you have no evidence regarding integration, we recommend that you include preclinical study in your development plan to address this question.

[2] If you or others identify an increased risk of delayed adverse events from persistent gene expression or from exposure to your product based on additional information reported after your protocol is accepted, you should plan to perform long-term follow-up observations even if the answer to these questions is "No". See Section IV.A of the text for examples.

[3] See section V of the text for recommendations on how to perform clinical long-term follow-up observations.

B. Considerations for Preclinical Study Design to Assess Vector Persistence

As discussed in Section IV.A, vector persistence heightens the risk of delayed adverse events following exposure to gene transfer technology. Indeed, the longer the vector persists, the greater the duration and degree of risk of delayed adverse events. We recommend that you perform preclinical biodistribution studies using methods that are shown to be sensitive and quantitative to detect vector sequences. Such studies would be designed to determine the distribution of your vector in nontarget tissues and the persistence of the vector in both nontarget and target tissues following direct in vivo administration of the vector product. If possible and applicable, we recommend that the studies employ an animal species that permits vector transduction and/or vector replication and that the animal species be biologically responsive to the specific transgene of interest (Ref. 4). The duration of the preclinical studies will vary, depending on the animal model employed. Projections of delayed adverse reactions in human participants may be derived from assessment of data from appropriate long-term observational studies in animals, when possible.

A biodistribution study in animals can be performed either as a separate study or as a component of a toxicology study. Consider the following points in your animal study design to permit evaluation of vector localization and persistence (Ref. 5).

1. Animal Study Design

We recommend that you:

- Use the product in the final formulation proposed for the clinical study because changes in the final formulation may alter biodistribution patterns.
- Use both genders or justify the use of a single gender.
- Use at least 5 animals per gender per group per sacrifice time point for rodents, and between 3-5 animals per gender per group per sacrifice time point for nonrodents.
- Consider factors in the study design that might influence or compromise the vector distribution and/or persistence such as the animal's age and physiologic condition.
- Use the intended clinical route of vector administration if possible.
- Assess vector biodistribution in a vehicle control group and a group of animals that receives the MFD or clinically relevant dose (defined in section III). Studies at additional dose levels might provide dose-dependent information.
- Include appropriate safety endpoints in your biodistribution study in order to assess any potential correlation between vector presence/persistence and adverse findings. These safety endpoints should include clinical observations, body weights, clinical pathology, gross organ pathology, and histopathology.

- Include several sacrifice intervals to characterize the kinetics of vector distribution and persistence. We recommend sacrifice at the expected time of peak vector detection and at several later time points to evaluate clearance of vector sequences from tissues.

2. Tissue Collection and Analysis

We recommend that you:

- Sample and analyze the following panel of tissues, at a minimum: blood, injection site(s), gonads, brain, liver, kidneys, lung, heart, and spleen. Consider other tissues for evaluation, depending on the vector type and the transgene, as well as the route of administration (e.g., draining lymph nodes and contralateral sites for subcutaneous/intramuscular injection, bone marrow, eyes, etc.).
- Choose a method for tissue collection that avoids the potential for contamination among different tissue samples.
- Use a quantitative, sensitive, state-of-the-art PCR assay to analyze the samples for vector sequences. The detection method should adequately and specifically detect vector sequence in both animal and human tissues. Currently, we recommend the following as the minimum criteria for assay performance:

  – Use three samples per tissue.
  – Each sample should contain at least 1 μg genomic DNA, or test sufficient replicates to equal a total of 3 μg if the assay capacity is less than 1 μg DNA per sample.
  – Analysis of one sample should include introduction of vector DNA control while two of the three samples collected should be tested in the absence of any introduced vector DNA.
  – The assay should be able to detect $\leq$100 copies of vector/1 μg genomic DNA.

3. Other Considerations

We encourage you to discuss your study design with us before starting the trial because there are many variables that will affect the outcome and interpretation of the in vivo biodistribution of each vector type.

C. Vector Integration Potential and Reactivation as Risks for Delayed Adverse Events

Three gene therapy vectors currently under study (i.e., Gammaretrovirus, Lentivirus, and Herpesvirus) possess characteristics that we consider to pose high risks of delayed adverse events. Accordingly, we believe that clinical long term follow-up observational studies would be necessary to mitigate long-term risks to subjects receiving these vectors. Gammaretrovirus and Lentivirus have a documented ability to integrate and Herpesvirus has a documented potential for latency and reactivation. In this section, we discuss those risks and the relatively low risks associated with gene transfer technology with vectors that lack those properties.

Most vectors used in gene therapy clinical trials can be categorized according to their propensity to integrate into host cell DNA. Please refer to Table 1, "Integration Properties of Current Commonly Used Gene Therapy Vectors in Clinical Trials." As shown in Table 1 and reflected in the answer to question 3 in Figure 1, "Framework to Assess the Risk of Gene Therapy-Related Delayed Adverse Events," vectors that have a potential to integrate present sufficient risk that long-term follow-up observations are necessary to mitigate long-term risks to subjects receiving these vectors.

Because of its potential for latency and reactivation, a Herpes virus-based gene transfer vector also presents a risk of delayed adverse events related to its use as a vector in gene therapy products. During latency, the virus and its gene products remain inactive. Reactivation may be delayed for months or years following initial exposure.

We are aware that the potential of vectors to integrate may be modified to increase their utility as gene therapy agents. For example, an adenovirus vector can be modified to induce integration of its DNA (Refs. 5-8). Another example would be changes in the methods used to introduce plasmid DNA vectors into cells that result in higher integration frequencies (Ref. 9). In those cases where a modification of the gene therapy system may have altered the persistence or integration properties, we recommend that you take one of the following actions:

- Submit to your IND data from preclinical studies to assess vector persistence in an appropriate model.
  - If the vector is not persistent, the predicted risk of delayed adverse events would be low. Long term follow-up observations would be at your discretion.
  - If the vector is persistent, we recommend that you perform preclinical studies to assess vector integration, as well as the potential for vector latency and reactivation.

    1. If the studies show no evidence for persistence due to integration of the genetic material or development of latency, the predicted risk of delayed adverse events would be low. Long term follow-up observations would be at your discretion.
    2. If the studies show no evidence for integration of the genetic material but studies for latency and reactivation are inconclusive, cannot be performed, or show evidence of latency and/or reactivation, the predicted risk of delayed adverse events is indeterminate. We would require long-term follow-up observations.
  - If preclinical studies of vector integration are not feasible or if the genetic material integrates, or if the vector is shown to persist in a latent state that may be reactivated, the risk of delayed adverse events is high or unknown, and long-term follow-up observations in study participants are warranted.
- If vector integration studies are not performed, we recommend that you provide other evidence to support an assessment that your vector does not pose high risks of delayed adverse events, including the following:
  - A discussion of why vector integration studies were not performed.
  - The evidence supporting your assessment of the risk of delayed adverse events posed by your product.

Plasmids, poxvirus, adenovirus, and adeno-associated virus-based vectors (AAV) are vectors that do not have a propensity to integrate or reactivate following latency and, in the absence of evidence to the contrary, present a low risk of gene therapy-related delayed adverse events. However, even if your vector has a low propensity to integrate or reactivate, preclinical or clinical data showing persistence of the vector raise concerns about a risk of delayed adverse events, and follow-up observations would be necessary to mitigate long-term risks to subjects receiving these vectors. For example, if an AAV vector is shown to have persistent transgene expression, the risk of a delayed aberrant immune response should be considered because of the potential for autoimmune phenomena.

We also note that some vectors currently considered to pose delayed risks might be modified in order to reduce those risks. Therefore, data supporting claims of a decreased risk for delayed adverse events with novel vector types could provide the basis for reassessing the need for performing long-term follow-up observations in participants exposed to those vectors.

Table 1. Integration Properties of Current Commonly Used Gene Therapy Vectors in Clinical Trials.

Vector Type	Propensity to Integrate[1]	Long-term Follow-up observations[2]
Plasmid	No	No
PoxVirus	No	No
Adenovirus	No	No
Adeno-associated virus[3]	No	No
Herpesvirus	No, but may undergo latency/reactivation	Yes
Gammaretrovirus	Yes	Yes
Lentivirus	Yes	Yes

[1]Based on vector design (i.e., lack of any known mechanism to facilitate integration), as well as cumulative preclinical and clinical evidence suggesting that vector does not integrate or integrates only at very low frequencies.
[2]Specific circumstances showing persistent expression of the transgene, in the absence of integration, may be the basis for a conclusion that long-term follow-up observations are necessary to mitigate long-term risis to subjects receiving these vectors. This would depend on additional criteria, such as the transgene expressed or clinical indication, as described in the text.
[3]*Rep*-negative vectors only.

V. RECOMMENDATIONS FOR PROTOCOLS FOR LONG-TERM FOLLOW-UP OBSERVATIONS: CLINICAL CONSIDERATIONS

In this section, we recommend elements appropriate to the design and conduct of long-term follow-up observations.

A. Decision to Conduct Long-term Follow-up Observations

The recommendations in this section apply to protocols for which long-term follow-up observations appear advisable. Long-term follow-up observations may be necessary to mitigate long-term risks to subjects receiving these vectors if:

- The answers to the questions posed in Section IV, Figure 1. “Framework to Assess the Risk of Gene Therapy-Related Delayed Adverse Events” lead you to decide that the risks associated with your product are high or uncertain.
- The information about your product, taken as a whole, shows that long-term follow-up observations would mitigate the risks to human subjects. For examples of such circumstances please refer to the final paragraphs in section IV.A.

In selected instances where we would generally require long-term follow-up observations, you may determine that the observations would have no scientific value based on the suitability of your clinical trial population. If you make that determination and decide not to conduct long-term follow-up, we recommend that you include in the justification for your decision not to continue to observe your subject population.

The sections below provide information on criteria you may choose to use to determine the suitability of monitoring your clinical trial population to collect scientifically informative data by the performance of long-term follow-up observations. We also discuss our recommendations for minimum duration of follow-up observations and the minimum observations to be made during long-term follow-up.

B. Suitability of Clinical Trial Populations for Long-term Follow-up Observations

Long-term follow-up observations may have limited scientific value because of characteristics of the population selected for the trial, such as short life expectancy, multiple morbidities, and exposure to other agents that also could cause delayed adverse events. Thus, for example, long-term follow-up observations might have little scientific value if the participants have widespread disease, or extensive exposure to agents with potential for delayed adverse events such as radiation or chemotherapy. In contrast, participants who have limited disease or who are disease-free and who have few comorbidities and limited exposures might be appropriate candidates for scientifically productive long-term follow-up observations.

C. Recommended Duration of Follow-up Observations

The duration of long-term follow-up observations should be sufficient to observe the participants for risks that may be due to the characteristics of the product, the nature of the exposure, and the anticipated time of occurrence of delayed adverse events. The BRMAC on April 5, 2001, discussed several different time periods for the performance of long-term follow-up observations, including 15 year follow-up observations (See section II.B for reference). Based on the BRMAC advice, we also recommend a 15 year time period for follow-up observations. However, we recognize that shorter periods of observation may be appropriate in individual trials based on supporting evidence. Elements that will influence the determination of the duration of long-term follow-up observations include the following:

- The observed duration of in vivo vector persistence;
- The observed duration of in vivo transgene expression;
- The prior, concomitant, and post gene therapy exposures of the study population;
- The expected survival rates in the study population; and
- Other factors that may be relevant to the feasibility and scientific value of conducting long-term follow-up observations.

D. Elements of Follow-up Observations

Our recommendations on the nature of the follow-up observations are also based on the recommendations and discussions at the November 17, 2000, April 5, 2001, and October 24, 2001, BRMAC meetings (See section II.B for references). As more clinical data accumulate, our recommendations regarding the duration of long-term follow-up observations may change.

It is important that the design of long-term follow-up observations be appropriate to detect potential gene therapy-related delayed adverse events in the study participants enrolled in your clinical studies. In this document, we provide recommendations for general minimum elements for the long-term follow-up component of your study protocol.

The investigator is required to prepare and maintain adequate and accurate case histories that record all observations and other data pertinent to the investigation on each individual administered the investigational drug or employed as a control in the investigation (see 21 CFR 312.62(b)). We interpret this to include a baseline history prior to exposure to the product in which all diseases, conditions and physical abnormalities are recorded. If the investigator receives observations obtained by health care providers who are not investigators or subinvestigators (for example, the subject's physician, physician assistant, or nurse practitioner), you are encouraged to develop a template for them to use in recording and reporting such observations. Case histories should also include information from scheduled visits by a health care professional and test results for persistent vector sequences. The use of surrogate tests may be used to indicate vector persistence if direct sequence testing would require an invasive procedure for the patient.

In addition, for at least the first five years we recommend that you do the following:

- Implement methods for detection of gene therapy-related delayed adverse events;
- Assure that investigators maintain in the case history a detailed record of all exposures to mutagenic agents and other medicinal products and have ready access to information about their adverse event profiles;
- Design a plan for scheduled visits with a health care provider to elicit and record new findings for each study participant, including history, physical examination, or laboratory testing at minimum intervals of one year;
- Establish a method for investigators to record the emergence of the following clinical entities:
  - New malignancy(ies)
  - New incidence or exacerbation of a pre-existing neurologic disorder
  - New incidence or exacerbation of a prior rheumatologic or other autoimmune disorder
  - New incidence of a hematologic disorder; and

- Design a plan to elicit the cooperation of study participants and their health care providers in reporting delayed adverse events, including unexpected illness and hospitalization.

For the subsequent ten years, at a minimum, we recommend that you ensure that your investigators:

- Contact participants at a minimum of once a year. At your discretion, unless the long term follow-up observation plan provides for additional, specific screening, you may arrange to contact participants by telephone or written questionnaire rather than by face-to-face visits with a health care provider.

- Continue appropriate follow-up methods as indicated by previous test results. For example, it would be appropriate to monitor for vector sequences in participants who had previous test results demonstrating vector persistence.

Perform all long-term follow-up observations according to FDA regulations governing clinical trials (See http://www.fda.gov/oc/gcp/regulations.html). We provide additional specific recommendations and requirements for data collection and reporting of adverse events for long-term follow-up clinical observations as follows:

1. Detection of Adverse Events: To facilitate detection of delayed adverse events, we recommend that the protocol identify suitable health care professionals whose observations would be used in the assessment of the occurrence of adverse events in the study population. Suitable health care professionals might include physicians, physician's assistants, and nurse practitioners who were not otherwise associated with the clinical trial. You may arrange to have such individuals notified to provide prompt reports of adverse events to the investigators.

   To increase participant compliance and improve the quality of data collection, we suggest that you encourage study participants to monitor themselves and assist in reporting adverse events. Devices that trial subjects could use to report events to the investigator include patient diaries of health-related events, informational brochures, and laminated, wallet-sized cards with investigator contact information.

2. IND Safety Reports: You must follow applicable reporting requirements outlined in 21 CFR 312.32 for adverse experiences associated with the use of the product. As the long-term follow-up observations proceed, you must also notify each participating investigator of any adverse experience associated with the use of the gene therapy product that is both serious and unexpected (21 CFR 312.32(c)(1)(i)(A)), as well as any new observations discovered by, or reported to, you (21 CFR 312.55(b)). In each IND Safety Report filed with FDA, you must identify all safety reports previously filed concerning a similar adverse experience, and analyze the significance of the adverse experience in light of the

previous, similar reports (21 CFR 312.32(c)(1)(ii)). You must promptly investigate all safety information you receive (21 CFR 312.32(d)(1)). If the relationship of the adverse experience to the gene therapy product is uncertain, we may recommend that you perform additional investigations and revise your Informed Consent Document and Investigator Brochure to inform all study participants of the risk of the adverse experiences. We may also request that investigators contact previously treated study participants to inform them of the new risk.

3. Annual Reports to the IND/Summary Information: You must submit in your annual report information obtained during the previous year's clinical and nonclinical investigations, including a summary of all IND safety reports submitted during the past year, and a narrative or tabular summary showing the most frequent and most serious adverse experiences by body system (21 CFR 312.33(b)(1) and (2)).

4. Scheduled Physical Examinations: We recommend that long-term follow-up observations include scheduled physical examinations performed by a health care professional at least once a year during the first five years, unless the assessed risks associated with your protocol indicate that they should be done more frequently. For example, if a participant exposed to your product or an analogous product develops a rapidly progressive, potentially reversible delayed adverse event that may have been caused by the product, it may then become advisable to perform observations on a semi-annual or quarterly basis. Such periodic evaluation should include a brief history and focused examination designed to determine whether there is any evidence of emergence of clinically important adverse events. Appropriate laboratory evaluations, such as a hematology profile, should be included with the periodic physical examination. Long-term follow-up observations are intended for study purposes only, not to provide evaluation and treatment of health care problems that are not associated with the use of the product.

5. Vector Sequences: During long term follow up, we recommend that you test study participants at least annually for persistent vector sequences until they become undetectable. The assay should be sufficiently sensitive to detect vector sequences. We recommend that you sample the likely population of transduced cells without being overly invasive (e.g., peripheral blood is a suitable sample to test for presence of hematopoietic stem cells, rather than bone marrow biopsy). In those cases where the transduced cell population may require an invasive procedure, we recommend that you consider, instead, measuring a surrogate that may indicate vector persistence (e.g., the level of transgene product or some clinical effect). Data demonstrating lack of detectable vector may provide a rationale to revise the long-term follow-up elements of your study as an amendment to your IND. In any such protocol amendment, include an assessment

of risks associated with your product and an evaluation of the impact of the waning persistence of the vector on those risks (21 CFR 312.30(b), (d)(2)).

E. Informed Consent in Trials Involving Long-term Follow-up Observations

Under 21 CFR 50.25, the informed consent document must describe, among other things, the purposes of the research, the expected duration of the subject's participation and the procedures to be followed (21 CFR 50.25(a)(1)). Accordingly, the informed consent document must explain the purpose and duration of long-term follow-up observations, the time intervals and the locations at which you plan to request the participants to have scheduled study visits or be contacted by other means, and details as to what those contacts will involve (21 CFR 50.25).

We provide additional informed consent recommendations for retroviral vectors in section V.F.3 below.

F. Special Considerations Regarding Retroviral Vectors

The recommendations in this section apply exclusively to participants in clinical trials who received retroviral vectors or cells modified ex vivo by retroviral vectors. In at least two preclinical studies performed in mice, integration of genetic material from a retroviral vector into mouse cell DNA was reported to cause malignant transformation (Refs. 10 and 11). In addition, in one clinical study, three out of a total of 11 human participants with X-linked Severe Combined Immunodeficiency (X-SCID) have developed clonal T-cell proliferation after receiving hematopoietic cells that had been modified ex vivo with a retroviral vector (Refs. 12 and 13). One of the three participants died (Ref. 13). These leukemias were the result of the retroviral vector-derived DNA integrating into the participants' cellular DNA. The observation that children with X-SCID developed a malignancy after exposure to a retroviral vector (Ref. 12) has prompted us to provide additional recommendations for collection of data in studies in which participants are exposed to retroviral vectors, including products derived from either gammaretroviruses or lentiviruses.

1. Data Collection

We recommend that you perform assays to assess the pattern of vector integration sites in relevant surrogate cells (e.g., determine whether cells carrying integrated retroviral vector sequences are polyclonal, oligoclonal, or monoclonal, with respect to vector integration patterns). We consider an assessment of the vector integration pattern to be relevant in participants in gene therapy clinical studies involving retroviral vectors if: (1) the target cells are known to have a high replicative capacity and long survival, and (2) a suitable surrogate is accessible for assay. For example, hematopoietic stem cells have a high replicative capacity and long survival; peripheral blood mononuclear cells could serve as a surrogate for testing for vector persistence if hematopoietic stem cells were the target of

your gene therapy. Please refer to the following recommendations for developing methods and plans for performing these analyses.

(a) We recommend that you perform an analysis to assess the pattern of vector integration sites if at least 1% cells in the surrogate sample are positive for vector sequences by PCR. As an alternative, you may base the decision to analyze for clonality of vector integration sites on an evaluation of the sensitivity of the assay system used to detect clonality.

(b) We recommend that you test for vector sequences by PCR in participant surrogate samples obtained at intervals of no greater than six months for the first five years and then no greater than yearly for the next ten years, or until such time that no vector sequences are detectable in the surrogate sample.

(c) We recommend that you perform an analysis to determine the site of vector integration if the analysis of a participant's surrogate cells suggests a predominant clone (e.g., oligoclonal pattern of vector insertions) or monoclonality. In addition, if you detect a predominant integration site, test for persistence by performing another analysis for clonality no more than three months later.

(d) When the nucleotide sequence adjacent to the site of the vector integration has been determined, we recommend that you compare the identified integration site sequence with known human sequences in the human genome database and other databases that document oncogenes to determine whether the identified sequences are known to be associated with any human cancers.

(e) We recommend that you institute a plan to monitor the participant closely for signs of malignancy if any of the following conditions pertain:

- Persistent monoclonality;
- Clonal expansion (e.g., the per cent cells positive for a particular vector integration site is shown to increase over multiple timepoints); or
- Evidence of vector integration near or within a locus known to have oncogenic activity.

(f) To screen for specific disease entities, we recommend that you use established methods and/or seek advice from clinicians with expertise in screening for the health care risks to which, according to your evidence, your subjects may be exposed.

2. Data Reporting

If no evidence of oligo- or monoclonality is observed, we recommend that you report a summary of all analyses for the pattern of vector integration sites in narrative or tabular form in the annual report to your IND (21 CFR 312.33(b)(5)). However, if evidence of oligo- or monoclonality is observed, and this serious adverse experience is unexpected, you must file an IND safety report with FDA and all participating investigators (21 CFR 312.32(c)(1)(i)(A)). If evidence of oligo- or monoclonality is observed, but is an expected serious adverse experience, submit this essential information in an information amendment to the IND (21 CFR 312.31(a)). We recommend that you submit this amendment within 30 days.

3. Informed Consent

Each subject in an investigation must be provided with a description of any reasonably foreseeable risks from participating in the investigation (21 CFR 50.25(a)(2)). Investigators must submit for Institutional Review Board approval your informed consent documents (21 CFR 56.109(b) and (c), 312.66). We believe that the informed consent document should include a complete and accurate disclosure of the development of leukemia in the children with X-SCID in layman's language for all clinical trials in which participants are exposed to retroviral vectors. We recommend that you include the following information, where applicable, in language understandable to the study participants, in the section describing the risks associated with the study agent:

- Description of study agent - The study involves giving a person some cells that have been changed by a retroviral vector. A retroviral vector is a virus that can insert genetic material into cells.
- Mechanism of action for retroviral vectors - When retroviral vectors enter a normal cell in the body, the deoxyribonucleic acid (DNA) of the vector inserts itself into the normal DNA in that cell. This process is called DNA integration.
- Effect of DNA integration - Most DNA integration is expected to cause no harm to the cell or to the patient. However, there is a chance that DNA integration might result in abnormal activity of other genes. In most cases, this effect will have no health consequences.
- Discussion of cancer occurring in animal studies - In some cases, abnormal activity of a normal gene may cause an uncontrolled growth of the cell that sometimes results in a cancer. This type of event has occurred in animal studies in which retroviral vector DNA integration appeared to cause cancers in mice and monkeys.
- Discussion of delayed adverse event, leukemia-like malignancy, occurring in human studies - It is important that you know about some cancers that

occurred in another gene therapy research study. The study, conducted in France, involved a disease called X-linked Severe Combined Immunodeficiency (SCID). Years after receiving cells that were modified by a retroviral vector, a significant number of the children in this small study developed a leukemia-like malignant disease (cancer). At least one child died from the cancer. A group of experts in this field studied the results from tests performed on these children's blood cells. They concluded that the leukemia-like malignancy was caused by the retroviral vector DNA. However, most of the children with X-linked SCID who have received experimental gene therapy have not been found to have a leukemia-like disease at this time. Although they appear healthy, we still do not know whether they, too, will develop a malignant growth.

- Risk of malignancy for this study - We do not know if the retroviral vector used in this protocol might cause a new malignancy. However, you should be aware that the DNA contained in retroviral vectors will integrate into your DNA and that under some circumstances, this has been known to cause malignant (cancerous) growth months to years later.

VI. REFERENCES

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