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

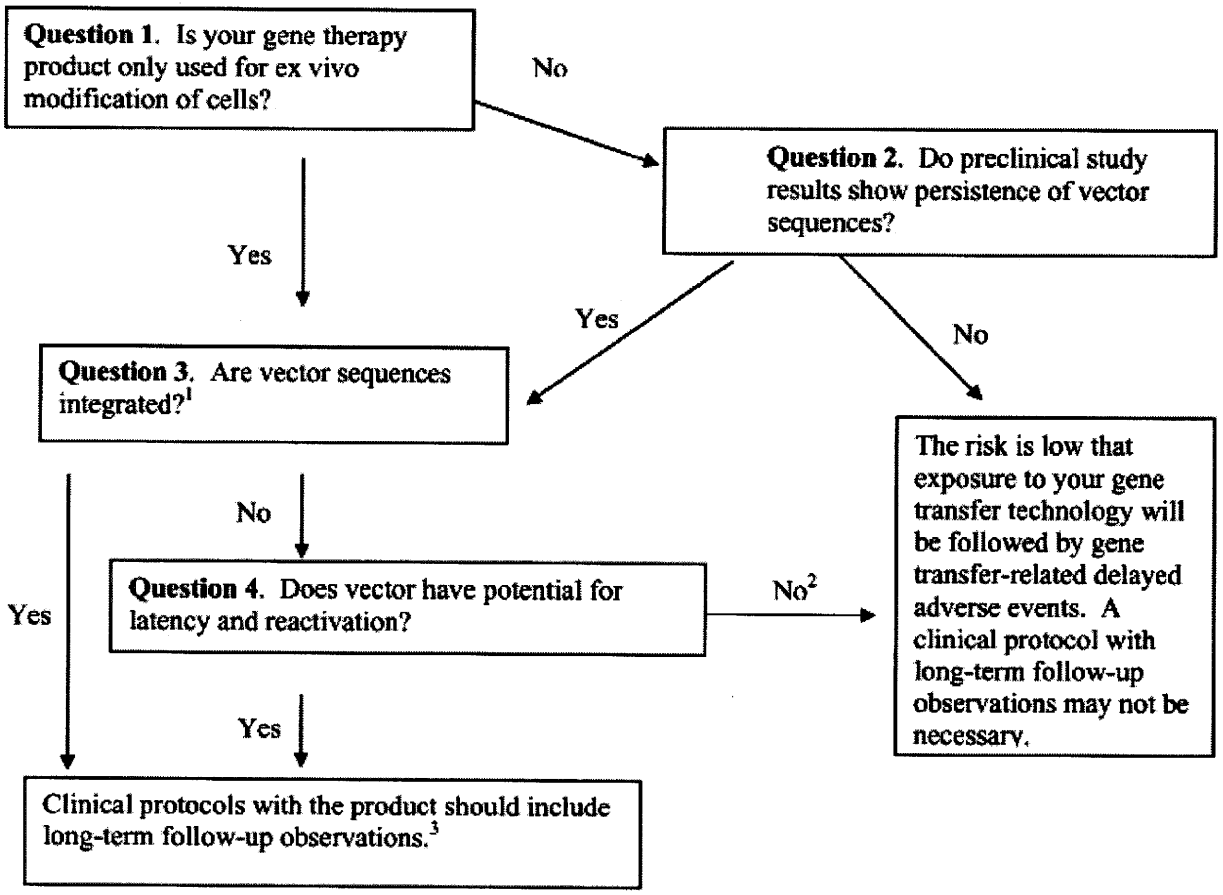
- Your proposed product and the similar product differ only with respect to route of administration. The similar product was administered into tumors (intratumorally). The proposed product is to be given intravenously. There is a published study demonstrating the lack of persistence of the vector when administered intratumorally. 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 in 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 Biodistribution and 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 subjects 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

- 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 if safety endpoints have not been evaluated already in a separate toxicity study using the same animal model. 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

- 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 PCR assay to analyze the samples for vector sequences. You should submit data to your IND to demonstrate that your assay methodology is capable of specifically detecting vector sequence in both animal and human tissues. We recognize that PCR technology is constantly changing, and encourage you to discuss the assay methology with us before initiating sample analysis. Current recommendations include the following:

  - The assay should have a demonstrated limit of quantitation of $\leq$50 copies of vector/1 μg genomic DNA, so that your assay can detect this limit with 95% confidence.
  - Use a minimum of three samples per tissue. One sample of each tissue should include a spike of control DNA, including a known amount of the vector sequences, in order to assess the adequacy of the PCR assay reaction. The spike control will determine the specified PCR assay sensitivity.
  - Provide a rationale for the number of replicates for testing per tissue, taking into account the size of the sample relative to the tissue you are testing.

3. Other Considerations

We encourage you to discuss with FDA your study design before starting the trial to ensure that the trial will adequately assess both biodistribution and vector persistence. There are many variables that will affect the outcome and interpretation of the in vivo assessment 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. 5-9). Another example would be changes in the methods used to introduce plasmid DNA vectors into cells that result in higher integration frequencies (Ref. 10). 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 data to your IND from preclinical studies to assess vector persistence in an appropriate model. As stated in Section IV.B.3, we encourage you to discuss with FDA your study design before starting the trial.

    - 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.

        - 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.

  - 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, 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 subjects 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 subjects 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 risks 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, you should include in your IND 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 the 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 reduced utility in assessing and mitigating subject risk when the population selected for the trial has characteristics, 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 impact if the subjects have widespread disease, or extensive exposure to agents with potential for delayed adverse events such as radiation or chemotherapy. In contrast, long-term follow-up observations could have greater value in assessing and mitigating the risks to subjects who have limited disease or are disease-free, and who have few co-morbidities and limited exposures to other agents with potential for delayed adverse events. In those cases where the gene therapy intervention alters life expectancy or co-morbidities, initial assessments regarding the suitability of long-term follow-up observations in a particular clinical trial may need to be reconsidered.

C. Recommended Duration of Follow-up Observations

The duration of long-term follow-up observations should be sufficient to observe the subjects 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 November 17, 2000, April 6, 2001, and October 24, 2001, discussed several different time periods for the performance of long-term follow-up observations, including a 15 year period (See Section II.B for reference). Based on the BRMAC advice, we also recommend a minimum 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 6, 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 subjects 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 subject administered the investigational drug or employed as a control in the investigation (see 21 CFR 312.62(b)). These records would include a baseline history prior to exposure to the product in which all diseases, conditions and physical abnormalities are recorded. You are encouraged to develop a template for health care providers who are not investigators or subinvestigators (for example, the subject's physician, physician assistant, or nurse practitioner) to use in recording and reporting such observations to the investigator. 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 subject.

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 subject, including history, physical examination, or laboratory testing at minimum intervals of one year;
- Establish a method for investigators to record the emergence of new clinical conditions, including:
  - 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 subjects 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 subjects 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 subjects by telephone or written questionnaire rather than by office 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 subjects 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 subject compliance and improve the quality of data collection, we suggest that you encourage study subjects to monitor themselves and assist in reporting adverse events. Devices that study subjects could use to report events to the investigator include subject 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 (required to be provided to investigators and 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 subjects of the risk of the adverse experiences. We may also request that investigators contact previously treated study subjects to inform them of the new risk.

3. Annual Reports to the IND/Summary Information: While the IND is in effect and until long-term follow-up observations are concluded, you must file an annual report. In that report, submit information obtained during the previous year's clinical and nonclinical investigations, including, among other things, 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. Amendments to Your Clinical Protocol: If clinical data suggest that your product is not associated with delayed risks, you may want to consider changing the clinical protocol regarding long-term follow-up of study subjects. However, before implementation of this change, you must submit to FDA a protocol amendment to your IND indicating the relevant changes (21 CFR 312.30(b)(1), (d), and (e)).

5. 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 subject exposed to your product or an analogous product develops a rapidly progressive, potentially reversible delayed adverse event, and there is a reasonable possibility that the event 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.

6. Vector Sequences: During long-term follow-up, we recommend that you test study subjects 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 the 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

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 subjects 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 Integrating Vectors

The recommendations in this section apply exclusively to subjects in clinical trials who received integrating vectors, such as 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. 11 and 12). In addition, in one clinical study, three out of a total of 11 human subjects 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. 13 and 14). One of the three subjects died (Ref. 14). These leukemias were the result of the retroviral vector-derived DNA integrating into the subjects' cellular DNA. The observation that children with X-SCID developed a malignancy after exposure to a retroviral vector (Ref. 13) has prompted us to provide additional recommendations for collection of data in studies in which subjects are exposed to integrating vectors, at this time best exemplified by 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 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 subjects in gene therapy clinical trials involving

integrating 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 could serve as a surrogate for testing for vector persistence if hematopoietic stem cells were the target of your gene therapy. In those cases where peripheral blood is the surrogate, analyses on purified subsets of hematopoietic cells (e.g., lymphocytes vs. granulocytes) may be performed, if deemed appropriate to the study by you or FDA. As an alternative example, if the integrating vector is used for in vivo transduction of liver hepatocytes, you may not need to perform this analysis, since terminally differentiated hepatocytes are non-dividing cells under normal circumstances, and there is no reasonable surrogate that allows for non-invasive testing of vector persistence. Please refer to the following recommendations for developing methods and plans for performing these analyses.

(a) The choice of method to assess the pattern of vector integration sites should be based upon data with appropriate positive and negative controls (i.e., target cells with a known number and sites of vector copies integrated vs. target cells with no vector integrants). Studies should be performed to provide information about the assay sensitivity, specificity, and reproducibility.

(b) 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.

(c) We recommend that you test for vector sequences by PCR in subject 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.

(d) We recommend that you perform an analysis to determine the site of vector integration if the analysis of a subject'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.

(e) 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.

(f) While we recognize that oligoclonality or even monoclonality itself will not a priori result in a malignancy (Refs. 15 and 16), we also recognize that these changes increase the risk of a malignancy, and therefore, we recommend that you institute a plan to monitor the subject 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.

(g) 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, 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 in Trials Involving Retroviral Vectors

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 the informed consent documents (21 CFR 56.109(b) and (c), 312.66). For all clinical trials in which subjects are exposed to retroviral vectors, the informed consent documents should include, in layman's language, a complete and accurate disclosure of the development of leukemia in the children with X-SCID. We recommend that you include the following information, where applicable, in language understandable to the study subjects, 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.

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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

This guidance is for immediate implementation.

FDA is issuing this guidance for immediate implementation in accordance with 21 CFR 10.115(g)(4)(i). Submit written comments on this guidance at anytime to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. Submit electronic comments to http://www.fda.gov/dockets/ecomments. You should identify all comments with the title of this guidance.

Additional copies of this guidance are available from the Office of Communication, Training and Manufacturers Assistance (HFM-40), 1401 Rockville Pike, Suite 200N, Rockville, MD 20852-1448, or by calling 1-800-835-4709 or 301-827-1800, or from the Internet at http://www.fda.gov/cber/guidelines.htm.

For questions on the content of this guidance, contact the Office of Cellular, Tissues, and Gene Therapies at 301-827-5102.

U.S. Department of Health and Human Services
Food and Drug Administration
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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

This guidance represents the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the appropriate FDA staff. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

I. INTRODUCTION

This guidance document applies to the manufacture of gene therapy retroviral vector products intended for in vivo or ex vivo use and to follow-up monitoring of patients who have received retroviral vector products.[1] Guidance is provided for replication competent retrovirus (RCR) testing during manufacture, including timing, amount of material to be tested, and general testing methods. In addition, guidance is provided on monitoring patients for evidence of retroviral infection. This guidance document finalizes the draft guidance document "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" announced in the *Federal Register* of November 3, 1999 (64 FR 59783). The guidance document also supplements the guidance and recommendations pertaining to RCR testing given in the following documents: 1) "Guidance for Industry: Guidance for Human Somatic Cell Therapy and Gene Therapy," March 1998; and 2) a letter to Sponsors of INDs Using Retroviral Vectors, dated September 20, 1993. For general guidance on gene therapy refer to "Guidance for Industry: Guidance for Human Somatic Cell Therapy and Gene Therapy," March 1998.

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.

[1] This guidance issued in October 2000, and was updated in November 2006 to cross-reference the recommendations contained in the "Guidance for Industry: Gene Therapy Clinical Trials – Observing Subjects for Delayed Adverse Events," which supplements the recommendations in Section IV.A. of this document.

II. BACKGROUND

CBER's current recommendations for RCR testing during retroviral vector production and patient monitoring were developed in 1993, at a time when clinical experience with retroviral vectors was limited (Ref. 4). The overriding safety issues associated with the use of retroviral vectors are exemplified by the findings of an experiment involving administration of ex vivo transduced bone marrow progenitor cells that had been inadvertently exposed to high titer RCR contained in the retroviral vector material to severely immunosuppressed Rhesus monkeys. In this setting, 3/10 animals developed lymphomas and died within 200 days (Ref. 3). The RCR was presumed to be etiologically associated with the disease by virtue of the presence of multiple murine RCR sequences in the monkey lymphomas and the observed correlation between the lack of antiretroviral antibody response and the development of prolonged retroviremia and disease (Refs. 9, 11). Since 1993, accumulating experience with different vector producing cells, RCR detection assays and results from patient monitoring have allowed the generation of a small data base of information on the safety of the use of retroviral vectors in clinical applications of gene therapy. This information base has provided a framework for discussion of the RCR recommendations by Center for Biologics Evaluation and Research and the gene therapy community. Public discussion and development of these supplemental recommendations have taken place during the Retroviral Breakout Sessions at the 1996 and 1997 FDA/NIH Gene Therapy Conferences, with representatives of the gene therapy community, and through the publication of the FDA considerations on these issues (Ref. 12).

III. RECOMMENDATIONS FOR PRODUCT TESTING

A. When to Test

RCR may develop at any step during manufacturing from development of the initial master cell bank through production of the retroviral vector supernatant. In addition, the growth of ex vivo transduced cells provides the potential for amplification of any RCR contaminant which may be below the level of detection in the retroviral vector supernatant. Therefore, current testing recommendations include testing of material from multiple stages of product manufacture (see Table 1). Use of a cell bank system is recommended in order to ensure an adequate and consistent supply of vector producer cells (VPC). The Master Cell Bank (MCB) is a collection of cells of uniform composition derived from a single tissue or cell. The Working Cell Bank (WCB) is derived from one or more ampules of the MCB, expanded by serial subculture to a specified passage number (refer to Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals, 1993).

1. Testing of Vector Producer Cell Master Cell Bank (one time testing)

Both VPC and supernatant from production of a MCB should be tested for RCR using a cell line permissive for the RCR most likely to be generated in a given producer cell line. For example, VPC containing amphotropic Murine Leukemia Virus (MLV) envelope should be tested for RCR on a cell line such as *Mus dunni*