Long Term Follow-Up After Administration of Human Gene Therapy Products

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Draft Guidance for Industry

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I. INTRODUCTION

We, FDA, are providing you, a sponsor who is developing a human gene therapy (GT) product,[1] recommendations regarding the design of long term follow-up observational studies (LTFU observations) for the collection of data on delayed adverse events following administration of a GT product. Often, GT products are designed to achieve therapeutic effect through permanent or long-acting changes in the human body. As a result of long term exposure to an investigational GT product, study subjects may be at increased risk of undesirable and unpredictable outcomes which may present as delayed adverse event(s). To understand and mitigate the risk of a delayed adverse event, subjects in gene therapy trials may be monitored for an extended period of time, which is commonly referred to as the "long term follow-up" (LTFU) period (of a clinical study). LTFU observations are extended assessments that continue some of the scheduled observations of a clinical trial past the active follow-up period, and are an integral portion of the study of some investigational GT products. LTFU observations are important to monitor long term safety of GT products. For GT products that present long term risks to subjects, LTFU/surveillance plan(s) should also be put in place post-licensure for monitoring of delayed adverse events (for details we refer you to section VI. of this document). Not all GT products will require LTFU observations; a risk assessment is performed by a sponsor based on several factors as outlined in this guidance.

In this guidance, we provide a brief introduction of the product characteristics, patient-related factors, and the preclinical and clinical data that should be considered when assessing the need for LTFU observations for your GT product. We also provide recommendations for the study design of LTFU observations with specific considerations for different gene therapy products and recommendations on patient monitoring for licensed GT products. Definitions of terms used throughout this guidance are provided in section VIII. of this document.

[1] See section VIII. Definitions: Human gene therapy product.

This draft guidance, when finalized, is intended to supersede the document entitled "Guidance for Industry: Gene Therapy Clinical Trials – Observing Subjects for Delayed Adverse Events" dated November 2006 (Ref. 1) (2006 Delayed Adverse Events). This draft guidance, when finalized, is also intended to supplement the guidance entitled "Testing of Retroviral Vector-Based Human Gene Therapy Products for Replication Competent Retrovirus during Product Manufacture and Patient Follow-up; Draft Guidance for Industry" dated July 2018.

FDA's guidance documents, including this draft 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. SCOPE

This guidance applies to all GT clinical studies and to licensed GT products for which LTFU observations are warranted based on analyses of available preclinical and clinical safety data for the GT product that raises concerns for delayed adverse events. The recommendations in this guidance apply to gene therapies that produce long lasting genetic effects (that is, gene therapy that represents more than just transient expression of a gene) and the performance of LTFU observations for evidence of delayed adverse events, i.e., adverse events that occur past the active follow-up period after exposure to the GT product, as described in the main study protocol.

III. BACKGROUND

A. Potential Risks of Delayed Adverse Events Following Exposure to Human Gene Therapy Products

Characteristics unique to human GT products that may be associated with delayed adverse events include:

1. The integration activity of the GT product: The biological activity of retroviral vectors[2] (e.g., vectors derived from gammaretrovirus, lentivirus, foamy virus etc.) and transposon elements is imparted by an integration event in the genome. In general, such integration is not directed to specific sites in the human genome, and this raises the potential for disruption of critical host (human) genes at the site of integration, or activation of proto-oncogenes near the integration site(s) and, thereby, the risk for malignancies.

[2] See section VIII. Definitions: Vector.

2. Genome editing activity: Genome editing based GT products impart their biological activity through site-specific changes in the human genome, but may also have off-target effects on the genome (Ref. 2). Similar to integrating vectors, genome editing may produce undesirable changes in the genome (whether *ex vivo* or *in vivo*), with the risk of malignancies, impairment of gene function, etc.

3. Prolonged expression: A GT product where the transgene (therapeutic gene) encodes growth factors, such as vascular endothelial growth factor (VEGF) or proteins associated with cell division such as p53, may raise the potential for unregulated cell growth and malignancies due to prolonged exposure to the therapeutic protein. Similarly, transgenes encoding immune recognition factors, such as chimeric antigen receptors or T-cell receptors, introduce the risk for autoimmune-like reactions (to self-antigens) upon prolonged exposure. For GT products that carry transcriptional regulatory elements (e.g., microRNA) or immune-modulatory proteins (e.g., cytokines) there is also the risk of unknown pleotropic effects, including altered expression of host (human) genes that could result in unpredictable and undesirable outcomes.

4. Latency: When the GT product has the potential for latency, such as a herpesvirus, there is the potential for reactivation from latency and the risk of delayed adverse events related to a symptomatic infection.

5. Establishment of persistent infections: GT products that are replication competent viruses and bacteria, such as listeria-based bacterial vectors, have the potential to establish persistent infections in immunocompromised patients leading to the risk of developing a delayed but serious infection.

In addition to product-related factors, the long term risk profile of a GT product should also take into consideration the target cell/tissues/organ, and the patient population (age, immune status, risk of mortality etc.), and the relevant disease characteristics.

B. History

The recommendations for LTFU monitoring in the 2006 Delayed Adverse Events guidance (Ref. 1) were based on extensive discussions among gene therapy stakeholders, and cumulative preclinical and clinical experience with GT products (Refs. 3, 4, 5) as summarized in this section. To discuss and solicit advice about long term risks to subjects exposed to such products, three separate meetings of the FDA advisory committee, Biological Response Modifiers Advisory Committee (BRMAC), were convened on November 17, 2000, April 6, 2001, and October 24, 2001 (Ref. 6).

A public workshop entitled "Long-term Follow-Up of Participants in Human Gene Transfer Research" was also held in June 2001, in association with the annual meeting of the American Society of Gene Therapy (ASGT). The workshop included a forum in which invited speakers discussed the challenges associated with LTFU of subjects in gene therapy clinical studies. The workshop organizers published a summary of the discussion (Ref. 7).

Taking these discussions into consideration, we provided detailed recommendations in the 2006 Delayed Adverse Events guidance document on the duration and design of LTFU observations (Ref. 1). The Agency advised sponsors to observe subjects for delayed adverse events for as long as 15 years following exposure to the investigational GT product, specifying that the LTFU observation was to include a minimum of five years of annual examinations, followed by ten years of annual queries of study subjects, either in person or by questionnaire.

Herein, we update our recommendations in the guidance taking into account the clinical experience gained since 2006 in LTFU of investigational GT products (as described in the following section), and the development of novel GT products with emerging technologies such as genome-editing that may be associated with an increased risk of delayed adverse events (as described in section III.D of this document).

C. Experience Gained Through Long Term Follow-up of Subjects in Gene Therapy Trials

To date, leukemias have been reported in more than one trial where subjects have received genetically-modified cells that were manufactured using gammaretroviral vectors (Refs. 8-11). Advances in analytical approaches for integration site analysis in patient samples collected during LTFU have provided insight into the possible mechanisms involved in the occurrence of such delayed adverse events (Refs. 8-14).

Past clinical experience in LTFU monitoring, and significant improvements in analytical approaches to investigate the integration site have contributed greatly towards our understanding of the risks associated with integrating gene therapy vectors (Ref. 15). Such risks can be mitigated through improvements in vector design and the duration and design of LTFU observations. Because integrating gene therapy vectors can persist in the body over the life-span of the patient's transduced cells, vectors with an improved risk profile were desired, and have subsequently been developed for clinical use (Refs. 16, 17). These include gammaretroviral and lentiviral vectors modified:

1. To reduce the risk of activating host genes adjacent to the integration site (e.g., self-inactivating (SIN) vectors and vectors containing insulator sequences);

2. To be less genotoxic (e.g., carrying non-viral physiological promoters to drive the expression of the therapeutic gene); and

3. To reduce the potential for recombination, and thereby, the risk of generating replication competent, pathogenic variants.

D. Long Term Follow-up for Novel Gene Therapy Products

Novel GT products developed as a result of emerging technologies, such as transposon-based gene insertion and genome editing, also raise concerns for delayed adverse events due to the unique genome modifying activity of such products. Specifically, a vector with a transposon element can insert transgenes into the host chromosome randomly by a direct "cut-and-paste" mechanism, mediated by the transposases (enzyme) activity in the product (Ref. 18). A GT product with genome editing components (nucleases) can give rise to non-specific off-target changes in the genome (Ref. 2), and may be associated with unknown and unpredictable risks for developing delayed adverse events in study subjects and patients once approved. The LTFU observations for these novel GT products should be designed to take into account product-specific characteristics, the basic and translational knowledge generated in the field, and the product-specific preclinical data generated to enable investigational new drug application (IND) studies, as described in the following section.

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

A. Criteria to Assess Potential Delayed Risks of Gene Therapy Products

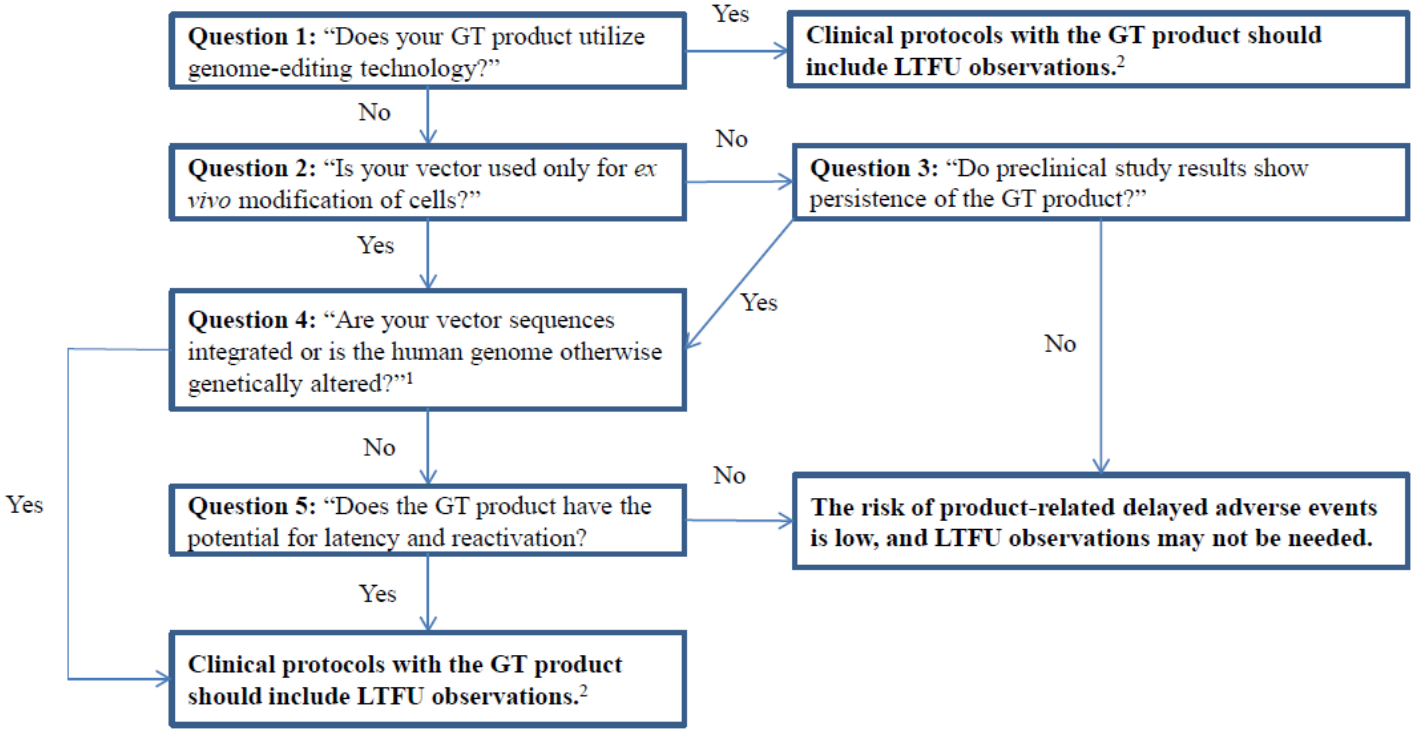
To assess the risk of delayed adverse events for a GT product, we recommend that you use available preclinical and clinical evidence, and current information about your product and similar products based on studies that you and others have performed. In general, when the risk of delayed adverse events is low following exposure to a GT product, LTFU observations are not recommended. We consider the assessment of risk to be a continuous process; in that, as more data accumulates, we recommend that you reassess the risk to your subjects and, if appropriate, revise an existing LTFU observations or initiate a LTFU observation, if previously allowed to proceed without LTFU observations.

Pertinent previous preclinical and clinical experience with your product or similar products is highly relevant in the assessment of the risk for delayed adverse events. For example, experience with GT products in the same vector class, administered by a similar route, or given for the same clinical indication may contribute helpful information. However, for novel products such information may not be available or pertinent, or may be limited, in which case data from well-designed preclinical studies (as described in section IV.B of this document) should be used in assessing the risk of delayed adverse

events. Primary data and information relevant to the assessment of the risk of delayed events should be submitted in your IND along with other preclinical data (see 21 CFR 312.23(a)(8), 312.23(a)(10)(iv), and 312.42(a)(11)).

GT product knowledge is critical in assessing the level of risk for delayed adverse events and the need for LTFU observations. To help you in this process, we refer you to section III.A of this document, and to the series of questions in Figure 1, "Framework to Assess the Risk of Gene Therapy-Related Delayed Adverse Events."

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



[1] If you have evidence that suggests that the product may integrate or if the product was intentionally designed to facilitate integration (please refer to Table 1, section IV.C of this document); the answer is "yes."

[2] See section V. of the text for recommendations on how to perform clinical LTFU observations.

Note, that evidence from preclinical studies will help you answer questions 3 through 5 below and in Figure 1. When the risk of delayed adverse events is low based on your answers to these questions, a plan for LTFU observations may not be necessary to mitigate risks to subjects.

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

Question 1: "Does your GT product utilize genome-editing technology?"

If the answer is "no," go to Question 2. If the answer is "yes," all your clinical protocols proposing administration of the GT product should include LTFU observations for appropriate human subject protections (see section V. for recommendations on how to perform clinical LTFU observations).

Question 2: "Is your vector used only for *ex vivo* modification of cells?"

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

Question 3: "Do preclinical study results show persistence of the GT product?"

If the answer is "no," the risk of product-related delayed adverse events is low, and LTFU observations may not be needed. If the answer is "yes," go to Question 4.

If it is unknown whether your GT product persists, for the purpose of assessing the risk of delayed adverse events, we recommend that you either assume that the GT product does persist, or perform preclinical studies to assay for the GT product persistence in a relevant animal species. For the design and details of such preclinical studies, please refer to section IV.B of this document; specifically, the polymerase chain reaction (PCR) assay for determining vector persistence in biodistribution studies. Following administration of the product, persistence is indicated by detectable levels of GT product sequences above the threshold level of the PCR assay, and absence of an apparent downward trend over several time points. In contrast, persistence is unlikely if product sequences cannot be detected with a sensitive assay such as PCR or if the assay for GT product sequences demonstrates a downward trend over time. We encourage you to consult with the Office of Tissues and Advanced Therapies (OTAT) at the Center for Biologics Evaluation and Research (CBER) for specific advice regarding determination of GT product persistence and biodistribution in your test system.

Question 4: "Are your vector sequences integrated or is the human genome otherwise genetically altered?"

If the answer is "no," go to Question 5. If you have evidence that suggests that the product may integrate or if the product was intentionally designed to facilitate integration (please refer to Table 1, section IV.C of this document); the answer is "yes." If the answer is "yes," all your clinical protocols proposing administration of the GT product should include LTFU observations for appropriate human subject protections (see section V. for recommendations on how to perform clinical LTFU observations).

> **Question 5:** "Does the GT product have the potential for latency and reactivation?"
>
> If the answer is "no," the risk of product-related delayed adverse events is low, and LTFU observations may not be needed. If the answer is "yes," all your clinical protocols with the GT product should include LTFU observations for appropriate human subject protections (see section V. for recommendations on how to perform clinical LTFU observations).

Laboratory and preclinical evidence of a low risk of delayed adverse events following exposure to a similar GT product may show that LTFU observations for your GT product are not needed. When such data/information is made available for review, we can assess their relevance to your product if you provide adequate details and a clear explanation of similarities and differences between the two products. For additional guidance, we provide the following two examples:

- Your GT product is a plasmid, and the similar product is also 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 in your program. In this case, reference to a published study demonstrating lack of persistence of the vector sequence for the similar (plasmid) product may adequately address concerns regarding the persistence of the proposed vector (your plasmid).

- Your GT product and the similar product differ only with respect to route of administration. The similar product was administered into tumors (intratumorally). Your GT product is to be administered intravenously. There is a published study demonstrating the lack of persistence of the similar product when administered intratumorally. In this case, the data is not sufficiently relevant to the GT product under study, since there was no intended systemic exposure to the product. Thus, there is insufficient similarity to conclude that LTFU observations are not necessary in your proposed study to mitigate the 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 product persistence in a preclinical study with the proposed GT 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 either the GT product is unlikely to persist in human hosts, or the vector sequence does not integrate into the human genome and the GT product does not have the potential for latency and reactivation, you may decide to submit a clinical protocol that does not provide for LTFU observations. We will review such submissions and, if based upon our review of your submission or other additional information, we

conclude that LTFU observations for delayed adverse events are necessary to mitigate long term risks, and that without LTFU observations, the study presents an unreasonable and significant risk to study subjects, we may place your study on clinical hold (21 CFR 312.42(b)(1)(i) and 312.42(b)(2)(i)).

We provide the following examples of evidence obtained from investigation of a product that may warrant our recommendation of LTFU observations for delayed adverse events:

- A preclinical toxicology study indicates that expression of the therapeutic gene (the transgene in your product) is associated with delayed toxicity.

- The therapeutic gene provides functional replacement of a host gene that is otherwise not expressed, and the therapeutic protein is potentially immunogenic.

- Data collected in a clinical study with your GT product indicates product persistence, even though data from your preclinical studies suggested that the product did not persist.

- Data collected in a clinical study with your GT product identifies an increased risk of delayed adverse events.

B. Considerations for Preclinical Study Design to Assess Biodistribution and Persistence of Gene Therapy Product

As discussed in section III.A of this document, product persistence heightens the risk of delayed adverse events following exposure to the GT product. Indeed, the longer the GT product persists, the greater the duration and degree of risk of delayed adverse events. We recommend that you perform preclinical biodistribution studies using methods shown to be sensitive and quantitative to detect product sequences. Such studies would be designed to determine the distribution of your product in non-target tissues and the persistence of the product in both non-target and target tissues following direct *in vivo* administration of the 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 or to therapeutic components in the product (e.g., for products that may not contain transgenes and only genome editing components) (Ref. 19). 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 such observational studies are 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 GT product localization and persistence (Ref. 20).

1. Animal Study Design

   a. Use the GT product in the final formulation proposed for the clinical study because changes in the final formulation may alter biodistribution pattern.
   b. Use both genders or justify the use of a single gender.
   c. 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 non-rodents.
   d. Consider factors in the study design that might influence or compromise the GT product distribution and/or persistence such as the animal's age and physiologic condition.
   e. Use the intended clinical route of GT product administration, if possible.
   f. Assess GT product biodistribution in a vehicle control group and a group of animals that receives the maximum feasible dose (MFD) or clinically relevant dose (defined in section VIII). Studies at additional dose levels might provide information on dose-dependent effects of your product.
   g. Include appropriate safety endpoints in your biodistribution study to assess any potential correlation between product presence/persistence and adverse findings if safety endpoints have not been evaluated already in a separate toxicology study using the same animal model. These safety endpoints should include clinical observations, body weights, clinical pathology, gross organ pathology, and histopathology.
   h. Include several sacrifice intervals to characterize the kinetics of GT product distribution and persistence. We recommend sacrifice of animals at the expected time of peak GT product detection and at several later time points to evaluate clearance of product sequences from tissues.

2. Tissue Collection and Analysis

   a. 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 product, vector type and tropism, and transgene(s), as well as the route of administration (e.g., draining lymph nodes and contralateral sites for subcutaneous/intramuscular injection, bone marrow, eyes, etc.).
   b. Choose a method for tissue collection that avoids the potential for cross contamination among different tissue samples.

c. Use a quantitative, sensitive assay like 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 analytical technologies are constantly changing, and encourage you to discuss the assay methodology with us before initiating sample analysis. Our current PCR recommendations include the following:

i. The assay should have a demonstrated limit of quantitation of ≤50 copies of product per 1 µg genomic DNA, so that your assay can detect this limit with 95% confidence.

ii. You should 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, to assess the adequacy of the PCR assay reaction. The spike control will determine the specified PCR assay sensitivity.

iii. You should 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

There are many variables that will affect the outcome and interpretation of the *in vivo* assessment of each GT product type. Hence, we encourage you to discuss with OTAT the study design for your GT product before initiating the preclinical biodistribution study to ensure that both biodistribution and persistence will be adequately assessed[3].

C. Vector Persistence, Integration, Reactivation and Genome Modification: Assessing Long Term Risks

GT products may or may not use technologies that modify the host genome. For products that do, such as integrating vectors (gammaretrovirus, lentivirus, foamy virus etc.), herpesvirus capable of latency-reactivation, and genome editing products (as described under sections III.A and III.D of this document, respectively), there is the risk of delayed

[3] The preclinical program for any investigational product should be individualized with respect to scope, complexity, and overall design, to maximize the contribution and predictive value of the resulting data for clinical safety and therapeutic activity. We encourage sponsors to explore opportunities for reducing, refining, and replacing animal use in the preclinical program. For example, it may be appropriate to use *in vitro* or *in silico* testing to complement replace animal studies. Sponsors are encouraged to submit proposals and justify any potential alternative approaches, which we will evaluate for equivalency to animal studies.

adverse events. Accordingly, as depicted in Table 1 of this document and in the answer to Question 4 in Figure 1, it is important to conduct LTFU observations to mitigate delayed risks to subjects receiving GT products with integrating activity.

We are aware that the potential of vectors to integrate may be modified to increase their utility as gene therapy agents; for example, a vector can be modified to induce integration of its DNA (Refs. 21-24). Another example would be changes in the methods used to introduce plasmid DNA vectors into cells that result in higher integration frequencies (Ref. 25). In those cases where a modification of the GT product may have altered its persistence or integration properties, we recommend that you submit data to your IND from preclinical studies to assess vector persistence in an appropriate model and take one of the following actions:

1. If the vector is not persistent, the predicted risk of delayed adverse events would appear to be low in which case LTFU observations may not be needed.

2. 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.

3. 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. LTFU observations may not be needed.

4. 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. LTFU observations may be recommended for human subject protections.

5. If preclinical studies of vector integration are not feasible, if the therapeutic gene/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 LTFU observations in study subjects are recommended for human subject protection.

6. If vector integration studies are not performed, we recommend that you provide other evidence to support an assessment that your product does not pose high risks of delayed adverse events, including the following:

    a. A discussion of why vector integration studies were not performed.
    b. The evidence supporting your assessment of the risk of delayed adverse events posed by your product.

As stated in section IV.B.3 of this document, we encourage you to discuss with FDA your study design before starting the trial.

GT products that are based on vectors such as plasmids, poxvirus, adenovirus, and adeno-associated virus vectors (AAV) that do not have a propensity to integrate or reactivate following latency, generally present a lower risk of delayed adverse events. Clinical data from LTFU observations of subjects that have received plasmids, poxvirus, adenovirus, and AAV in trials conducted since 2006, further supports the assessment of lower risk for these GT products. However, vector or product-specific modifications may alter the risk profile of products that are currently considered lower risk, for example a plasmid that is modified to carry genome editing components. Conversely, gene therapy vectors currently considered to pose delayed risks might be modified in order to reduce those risks. Hence, data supporting decreased or increased risk for delayed adverse events with novel GT products or vector types could provide the basis for sponsors to reassess our recommendations for performing LTFU observations. We encourage you to consult with OTAT regarding a reassessment of our recommendations for performing LTFU observations.

Table 1. Propensity of Commonly Used Gene Therapy Products/Vectors to Modify the Host Genome

Product/Vector Type	Propensity to Modify Genome[1]	Long Term Follow-up Observations[2]
Plasmid	No	No
RNA	No	No
Poxvirus	No	No
Adenovirus	No	No
Adeno-associated virus[3]	No	Product specific (2-5 years)
Herpesvirus	No, but may undergo latency/reactivation	Yes
Gammaretrovirus	Yes	Yes
Lentivirus	Yes	Yes
Transposon elements	Yes	Product specific
Microbial vectors for gene therapy (MVGT)[4]	No, but may persist and undergo reactivation	Product specific
Genome editing products	Yes; permanent changes to the host genome	Yes

[1] Based on product design (i.e., lack of any known mechanism to facilitate integration or genome editing), as well as cumulative preclinical and clinical evidence suggesting that a GT product does not integrate into or edit the genome or integrates in/modifies the genome at very low frequencies.

[2] Specific circumstances that indicate persistent expression of the transgene, in the absence of integration or genome editing, may be the basis for a conclusion that LTFU observations are recommended 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 this section.

[3] Replication-negative vectors only.

[4] For additional guidance we refer you to "Recommendations for Microbial Vectors used for Gene Therapy; Guidance for Industry" dated September 2016, https://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/default.htm.

D. Considerations for Preclinical Evaluation of Products that Involve Genome Editing

Genome editing, whether *ex vivo* or *in vivo*, introduces the risk for delayed adverse effects, due to 1) the permanent nature of change; 2) the potential for off-target genome modifications that can lead to aberrant gene expression, chromosomal translocation, induce malignancies, etc.; 3) the risk for insertional mutagenesis when integrating vectors are used to deliver the genome editing components, and the associated risk of tumorigenicity; and/or 4) the possibility of an immune response to the genome-editing components or the expressed transgene. Preclinical safety evaluation of genome editing products should consider: 1) the technology used to edit the genome; 2) the cell type that is modified *ex vivo*; 3) the vector used to deliver the genome-editing components; and 4) the clinical route of administration. Preclinical studies evaluating these factors can inform the scope of the clinical LTFU observations.

For guidance on the biodistribution studies when considering the vector type in the genome edited product, and the related long term risks with integrating vectors, we refer you to sections IV.B and IV.C of this document.

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 LTFU observations for delayed adverse events in study subjects receiving investigational GT products. Typically, LTFU observations are conducted under a protocol (LTFU protocol) that is separate from the main study protocol, and may begin immediately after the main study protocol ends.

A. Goals of the Long Term Follow-up Observations

The objective of LTFU observations in clinical development of a GT product is to identify and mitigate the long term risks to the patients receiving the GT product. The LTFU protocol for GT trials is primarily designed to capture delayed adverse events in study subjects as well as to understand the persistence of the GT product. As a sponsor, you may consider designing the LTFU protocol to assess the long term clinical efficacy, and durability of your product. For additional guidance on trial design for GT products we refer you to FDA's guidance document "Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products; Guidance for Industry" dated August 2015 (Ref. 26). Please refer to Appendix 1 of this document for a LTFU Annual Report Template.

B. Clinical Trial Populations for Long Term Follow-up Observations

When a GT product is deemed to pose a risk for delayed adverse events (based on the recommendations/discussions provided under sections III and IV of this document) and a decision to perform LTFU observations is made, all study subjects who receive the GT product are expected to be enrolled in the LTFU protocol after signing an informed consent document. LTFU observations may have reduced utility in assessing and mitigating subject risk when the population selected for the trial has characteristics that could confound the observation of the delayed adverse events, such as short life expectancy, multiple co-morbidities, and exposure to other agents such as radiation or chemotherapy. In contrast, LTFU 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. Hence, characteristics of the patient population and the disease to be treated should be considered when designing a LTFU protocol.

C. Duration of Long Term Follow-up Observations

It is important that the design of LTFU observations be appropriate to detect potential gene therapy-related delayed adverse events in the study subjects enrolled in your clinical studies. The duration of LTFU 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. Elements that will influence the determination of the duration of LTFU observations include the following:

- The observed duration of *in vivo* product persistence.
- The observed duration of transgene expression.
- Product characteristics *in vivo*.
- Route of administration.
- The expected survival rates and the known background rates of the events of interest occurring in the study population.
- Other factors that may be relevant to the feasibility and scientific value of conducting LTFU observations; for example, the durability of the clinical effect.

In general, our current recommendations for the duration of a LTFU protocol based on product type are as follows:

- Fifteen years for integrating vectors such as gammaretroviral and lentiviral vectors and transposon elements.
- Up to fifteen years for genome editing products.
- Up to five years for AAV vectors.

Additionally, a risk-based approach for determining the duration of a LTFU protocol may be considered for vectors capable of latency (e.g., Herpesvirus) or long term expression without integration (e.g., AAV).

Although these recommendations are broadly based on GT product type, you should also consider the elements listed above, in this section, as it applies to your GT product, disease characteristics, and the patient population, in addition to the discussions in sections III. and IV. of this document.

To reduce the unnecessary burden to study subjects and to you as the study sponsor, it may be appropriate to modify the duration of the LTFU observation based on your ongoing assessment of product persistence, transgene expression, and clinical findings. If you intend to modify the duration of the follow-up, you may submit an amendment to your IND justifying the change to your LTFU protocol, and communicate with FDA to reach a final decision (we refer you to section V. of this document for additional guidance regarding amendments to the clinical protocol).

D. Elements of Long Term Follow-up Observations

We recommend that at least the following general elements be part of the LTFU protocol:

- You should establish a dedicated clinical LTFU protocol detailing patient visit schedules, sampling plan (for patient test samples, such as blood), methods of monitoring tests, and clinical events of interest that will be monitored over the entire LTFU observation.

- 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 investigational product in which all diseases, conditions and physical abnormalities are recorded. A template for health care providers (HCPs) who are not investigators or sub-investigators (for example, the subject's physician, physician assistant, or nurse practitioner) to use in recording and reporting such observations to the investigator may be helpful for such HCPs. Case histories should also include information from scheduled visits with a HCP and test results for persistent vector sequences. The use of surrogate tests may be necessary to indicate vector persistence if direct sequence testing involves an invasive procedure for the subject. If surrogate tests are considered, we recommend that you consult with FDA regarding the types and characteristics of the surrogate tests you intend to use before including them in your study.

In addition, for the first five years or more (as applicable to your product), we recommend that you do the following:

- Assure that investigators maintain, in the case history, a detailed record of exposures to mutagenic agents and other medicinal products, and have ready access to information about their adverse event profiles.
- Establish a method for investigators to record the emergence of new clinical conditions, including, but not limited to:
  - 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.

- Design a plan for scheduled visits with an HCP to elicit and record new findings for each study subject, including history, physical examination, or laboratory testing.

- Such a plan needs to facilitate reporting of delayed adverse events, including unexpected illness and hospitalization by study subjects and HCPs.

For the subsequent ten years (applicable to products for which such length LTFU is needed), 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 LTFU protocol provides for additional specific screening, you may arrange to contact subjects by telephone or written questionnaire rather than by office visits with an HCP.

- 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 LTFU observations according to FDA regulations governing clinical trials (Ref. 27).

We provide additional specific recommendations and requirements for data collection, recording, and reporting of adverse events for LTFU observations as follows:

1. Detection of Adverse Events and Coordination of Data Collection

    a. To facilitate detection of delayed adverse events, we recommend that the LTFU protocol identify suitable HCPs whose observations would be used in the assessment of the occurrence of adverse events in the study population. Suitable HCP 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.

    b. To increase subject compliance and improve the quality of data collection, we suggest that you encourage study subjects to be proactive in reporting adverse events. Tools 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.

    c. To determine the causality of potential related adverse events (such as tumor formation) associated with your GT product, you should propose a clinical program for follow-up procedures. Such a program would lay out the efforts that would be needed among the study subjects, HCPs, investigators, and the sponsor for study coordination. This includes the collection of tissue samples for follow-up analysis, obtaining informed consent for a biopsy or autopsy (see section V.E. of this document), communicating with the study subject, and preserving and analyzing the tissues/samples according to the LTFU protocol. You may propose specific tests to enable causality analyses such as general blood work, cytogenetic and histological analysis, PCR, HLA typing, or deep sequencing.

2. IND Safety Reports

    You must follow applicable reporting requirements outlined in 21 CFR 312.32 for adverse events associated with the use of the investigational product. As the LTFU observations proceed, you must notify FDA and each participating investigator of any serious and unexpected suspected adverse reaction (21 CFR 312.32(c)(1)(i)), and findings from other studies (21 CFR 312.32(c)(1)(ii)). 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 finding, and analyze the

significance of the adverse finding in light of the previous, similar reports (21 CFR 312.32(c)(1)). You must promptly investigate all safety information you receive (21 CFR 312.32(d)(1)). If the relationship of the adverse event to the GT product is uncertain, additional investigations may be needed. You must also revise your informed consent document and Investigator Brochure to include the new adverse event(s) that may be associated with the product or study procedures (21 CFR Part 50, 21 CFR 312.55(b)). You must inform all clinical investigators of the newly identified risk (21 CFR 312.32(c)(1)).

3. Annual Reports to the IND/Summary Information

While the IND is in effect and LTFU observations are ongoing, you must file an annual report. It is recommended that the annual report contain a subtitle for Long Term Follow-Up (See Appendix 1 of this document). In that report, you should submit 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)). If adverse reactions are reported and determined to be related to your product or delivery procedure, you should provide causal analyses based on evidence from clinical, laboratory, molecular, cytogenetic, histological, or HLA analysis, or deep sequencing data. In lieu of annual reports, you may submit a Development Safety Update Report (DSUR). In this case, you should provide the LTFU information in a subsection with a subtitle for LTFU in your DSUR report (Ref. 28).

4. Amendments to the Clinical Protocol

If clinical data suggest that your GT product is not associated with delayed risks or there is no evidence of vector persistence, you may want to consider revising the clinical protocol regarding LTFU of study subjects. However, before implementation of this change, we recommend that you consult with FDA and provide your rationale with supporting clinical and laboratory data (we refer you to section V.C of this document for additional guidance). 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 LTFU observations include scheduled physical examinations performed by a HCP once a year during the first five years (or until the completion of LTFU if the LTFU is less than five years),

unless the assessed risks associated with your GT product indicate that they should be done more frequently. For example, if a subject exposed to your GT 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. LTFU observations are intended to collect data on delayed adverse events related to the GT product, and are not intended to provide evaluation or treatment data for the underlying disease.

6. GT Product Persistence

During LTFU observations, we recommend that you test study subjects at least annually for persistent vector sequences until they become undetectable. More frequent testing may be necessary as outlined in section V.G of this document. 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 collecting the transduced cell population may involve 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 LTFU protocol as a protocol amendment to your IND. In any such protocol amendment, include an assessment of risks associated with your GT product and an evaluation of the impact of the waning persistence of the vector on those risks (21 CFR 312.30(b) and (d)(2)).

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

Each subject in a clinical investigation must be provided with a description of any reasonably foreseeable risks from participating in the investigation (21 CFR 50.25(a)(2)). 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 LTFU 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).

When appropriate, the informed consent document must be updated to describe any adverse reactions that may be associated with the product from your trial or other human or animal (preclinical) studies (21 CFR 50.25(b)(5)). If the sponsor intends to store blood or tissue samples for future testing, the informed consent document must convey this information (21 CFR 50.25(a)(1)). The informed consent should also convey that an autopsy may be requested to test vector persistence, transgene expression, and related adverse reactions at the molecular, cellular or tissue level if there are deaths during the LTFU observation. Sponsors must ensure that investigators submit the informed consent documents for Institutional Review Board approval (21 CFR 312.53(c)(1)(vi)(*d*)).

We provide additional informed consent recommendations for retroviral vectors in section V.G.3 of this document.

F. Special Considerations Regarding Integrating Vectors

The recommendations in this section apply exclusively to subjects in clinical trials who received GT products that are integrating vectors, such as transposon elements, gammaretroviral, lentiviral, other retroviral vectors, or GT products that are cells modified *ex vivo* by integrating vectors or transposon-based vectors. See section VI. for post licensure considerations. Because of the risk of developing leukemias and premalignant conditions (clonal cell expansion) due to integration of gammaretroviral vectors and lentiviral vectors (as described in sections III.B and III.C of this document), we are also providing additional recommendations (as listed below) for collection of data in studies in which subjects are exposed to integrating vectors.

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 when: (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 are 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. 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. 29, 30), 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:

i. Persistent monoclonality;
ii. Clonal expansion (e.g., the percent cells positive for a particular vector integration site is shown to increase over multiple time points); or
iii. 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.

For retroviral (e.g., gammaretroviral and lentiviral) vector-based GT products, additional follow-up monitoring for the presence of replication competent retrovirus (RCR) may be necessary. For details regarding duration of the follow-up monitoring for RCR and methods, please refer to the document "Testing of Retroviral-Based Human Gene Therapy Product for Replication Competent Retrovirus During Product Manufacture and Patient Follow-up; Draft Guidance for Industry" dated July 2018.

We recommend that GT products with transposon elements should be monitored in a similar way as gammaretroviral or lentiviral vectors. This recommendation is based on the potential safety risk of insertional mutagenesis due to the random integration directed by the transposon, and due to the potential for remobilization of a transposon (secondary transposition-insertion event) as a result of the continuing presence of the transposase enzyme in target cells. Yet, if your GT product contains transposon elements you may propose shorter LTFU observation by providing adequate supporting data/information related to your product.

2. Data Reporting

If no evidence of oligoclonality 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 oligoclonality or monoclonality is observed, you must 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 of receiving the report of such an observation.

3. Informed Consent in Trials Involving Retroviral Vectors

Please see section V.E for general consideration of LTFU observation informed consent. In accordance with 21 CFR 50.25(a)(2), for all clinical trials in which subjects are exposed to retroviral vectors, the informed consent documents must include current, complete and accurate disclosure

of the development of leukemias in the clinical trials where such adverse events were reported. Further, the information that is given to the subject or his/her representative must be in language understandable to the subject or representative (21 CFR 50.20). We provide the following list as information and language we recommend be included in the informed consent document, where applicable, in the section describing the risks associated with the study agent:

a. 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.

b. 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.

c. 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. However, in some cases, abnormal activity of a gene may cause unpredictable harm such as the development of cancer.

d. 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. Clinical studies were conducted in France and United Kingdom to treat 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). 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 cancer 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 cancer at this time. Although they appear healthy, we still do not know whether they, too, will develop cancer.

e. Risk of malignancy for this study - We do not know if the retroviral vector used in this protocol might cause cancer. 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 cancer months to years later.

G. Special Considerations Regarding Product Involving Genome Editing

While the general principles for LTFU observations of GT products also apply to LTFU observations of genome editing products, we recommend that you consider the following:

1. Propose a specific plan to monitor for delayed adverse events based on the off-target activities noted in your preclinical studies (e.g., *in vivo, in vitro* and *in silico* analysis such as INDEL, (insertion and deletion of bases in a genome). For example, if the off-target activity involves a tumor suppression gene in liver cells, you may propose a monitoring plan for evaluation of occurrence of liver cancer as part of the LTFU observation.

2. Propose a monitoring plan regarding the adverse events from the specific organ system that the genome editing targets, that may include history and physical examination, general and specific laboratory tests, and imaging studies.

3. If direct monitoring of the target tissue is not ethical or feasible, such as, the brain tissue, you may propose an alternative plan for monitoring of the product's effects.

4. Quantitate the relationship between the off-target and on-target activities, and use the measured level of on-target activity to predict the level of off-target activity and, if appropriate, establish a follow-up plan;

5. If the genome editing product is delivered via systemic administration, clinical safety monitoring may be directed not only to off-target activity of the target organ or tissue, but also to other off-target effects that may occur in other tissues and organs. Accordingly, you may include appropriate monitoring tests with a rationale for the proposed monitoring in your LTFU protocol.

VI. GENERAL CONSIDERATIONS FOR POST-MARKETING MONITORING PLANS FOR GENE THERAPY PRODUCTS

The number of subjects receiving GT products is typically limited during clinical investigations. In addition, the recommended LTFU (e.g., 15-year period) will often not elapse for all subjects who received an investigational GT product in the pre-marketing program before the product is licensed. Considering that, the safety data generated during clinical trials may not capture all possible delayed adverse events. Therefore, continuing LTFU observations is often essential even after a product's licensure. Consequently, we recommend that at the time of your BLA submission you submit a Pharmacovigilance Plan (PVP) as described in the FDA Guidance for Industry; E2E Pharmacovigilance Planning (Ref. 31). The contents of PVP for a particular GT

product will depend on its safety profile and will be based on data, which includes the pre-licensure clinical safety database, published literature, and known product-class effects, among other considerations.

Routine surveillance for licensed biological products includes adverse event (AE) reporting in accordance with 21 CFR 600.80 (reporting of expedited and non-expedited AEs as well as periodic safety reports). Submission of reports for serious, life-threatening and unexpected adverse events may also be required in an expedited manner beyond routine required reporting.

Additional pharmacovigilance elements may be needed, such as those described in the FDA Good Pharmacovigilance Practices and Pharmacoepidemiologic Assessment; Guidance for Industry dated March 2005 (Ref. 32), for LTFU of patients treated with GT products. For instance, we may recommend that you establish a registry to systematically capture and track data from treated patients with solicited sample collection, and follow-up of adverse events to resolution or stabilization to collect additional pertinent data. It may be necessary to establish a registry system to specifically capture adverse event data from treated patients who receive a GT product. This registry system can be a part of the PVP plan and reviewed at the time of licensure.

For any proposed or required post-marketing observational studies or clinical trials, we recommend that you include in your BLA submission the study protocol, statistical analysis plan, and a projected schedule of anticipated study milestones. Your study protocol should include specific adverse events of interest that you intend to evaluate, and the duration of observation for all patients enrolled in your post-marketing study.

During our review of your BLA, we will also assess whether a Risk Evaluation and Mitigation Strategy (REMS) is necessary to ensure that the benefits of your product outweigh its risks. If you consider that risk mitigation measures are necessary for the safe use of your product, you may voluntarily submit your proposed REMS as desecribed in Format and Content of a REMS Document; Draft Guidance for Industry; Drug Safety dated October 2017 (Ref. 33).

VII. LONG TERM FOLLOW-UP UNDER SPECIAL CIRCUMSTANCES

A sponsor may cease to operate or may decide to inactivate, transfer or withdraw an IND before completion of LTFU observations for all subjects exposed to the GT product under its IND. Under such circumstances, prior to inactivating, transferring or withdrawing an IND, or ceasing to operate, we recommend that a sponsor consult with OTAT on the plans for completion of LTFU observation.

VIII. DEFINITIONS

The following definitions apply to this guidance:

Engineered site-specific endonucleases: Enzymes that are capable of precisely cleaving (cutting) DNA based on specific recognition of the DNA sequence at or near the site of DNA cleavage.

Genome editing: The processes by which the genome sequence is changed by adding, replacing, or removing DNA base pairs using engineered site specific nucleases.

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

Human gene therapy: Human gene therapy seeks to modify or manipulate the expression of a gene or to alter the biological properties of living cells for therapeutic use.

Human gene therapy product: Human gene therapy products are defined as all products that mediate their effects by transcription or translation of transferred genetic material, or by specifically altering host (human) genetic sequences. Some examples of gene therapy products include nucleic acids, genetically modified microorganisms (e.g., viruses, bacteria, fungi), engineered site-specific nucleases used for human genome editing[4], and *ex vivo* genetically modified human cells.

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 clinical symptoms.

Maximum feasible dose (MFD) (in preclinical studies): The highest dose that can be administered to an 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 or altered genetic material, the continued presence of transferred or modified genetic sequences in the host after acute exposure to a gene therapy agent, whether due to integration of the genetic sequence into the host genome, deletion, insertion, or otherwise modified following genome editing, or to latent infection with the viral vector bearing the genetic sequence.

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

[4] Human Genome Editing: Science, Ethics, and Governance. The National Academies Press; 2017. https://www.nap.edu/read/24623/chapter/1#xvii

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

Vector sequences: Refers to specific sequences of nucleotides, either DNA or RNA, that have been introduced into a gene therapy product and includes the vector backbone, transgene(s), and regulatory elements.

Vector: A vehicle consisting of, or derived from, biological material that is designed to deliver genetic material. Examples include plasmids, viruses, and bacteria that have been modified to transfer genetic material.

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*****When finalized, this guidance will represent FDA's current thinking on this topic.

APPENDICES

APPENDIX 1: INFORMATION FOR LONG TERM FOLLOW-UP (LTFU) OBSERVATION ANNUAL REPORT

Category	Required LTFU Data	Rationale
Protocol Title	**"Long Term Follow-Up Observation Annual Report"**	**The placement of this title will facilitate FDA to search for LTFU data in our database**
LTFU Protocol Status	Total length (years) Starting date Total number of subjects enrolled Subjects that have completed LTFU observation Remaining subjects on LTFU observation	This will serve as a brief summary.
Product Information	Vector persistence Clonality analyses RCR On and off-target analyses for products that involve genome editing	This is the focus of the product safety assessment in the LTFU protocol and provides important information for monitoring, and for determination of the length of the LTFU observation.
Preclinical Information	New preclinical data Relevant findings from the literature	This provides data and signals to guide the direction of LTFU observation.
Clinical Information	Any related delayed adverse event with brief narrative Oncological, neurological, hematological, auto-immune or other disorder Causal analyses based on evidence from clinical, laboratory, molecular, cytogenetic, histological, HLA analysis, deep sequencing data Serious adverse events Evidence for persistence of the product/therapeutic protein/sequences, and durability of the clinical effects	This is the focus of the product safety assessment in LTFU observation, and serves as a guide for the types of AE, organ systems, and methodology to attribute AE/Serious Adverse Event (SAE) to the GT product. The durability of clinical effect also allows for an assessment of product efficacy in the LTFU observation report, but inclusion of such data is at the sponsor's discretion.
Revision of LTFU protocol	Rationale for modifying LTFU observation FDA agreement to revised LTFU protocol: synopsis of meeting(s) discussion/email communication Discussion and date of discontinuation	This will provide an opportunity for revising the content and length of the LTFU observation based on data collected in the studies or other relevant information.

APPENDIX 2: SAMPLE TEMPLATE: LONG TERM FOLLOW-UP (LTFU) OBSERVATION ANNUAL REPORT

Category	List of LTFU data	Annual reporting
Protocol title	"Long Term Follow-Up Observation Annual Report"	[product name]: LTFU2017 annual report for protocol [#]
LTFU protocol status	Total length (years):	15 years
	Starting date:	October 30, 2009
	Total number of subjects enrolled:	30
	Subjects that have completed LTFU observation:	0
	Remaining subjects on LTFU observation:	20 (2 deaths, 5 lost to flu, 3 drop outs)
Product information	Vector persistence:	PCR[1] of [name] transgene positive in 17 of 20 subjects still on study at 5 yrs and 3 subjects at 7 yrs.
	Clonality analyses:	No clones more than 1% for more than 1 testing period
	RCR	ND[2], request to discontinue RCR testing
	On and off-target analyses for products that involve genome editing	NA[3]
Preclinical information	New preclinical data	Final study report for large reproductive toxicity study in normal SD rats (study report [#]). Published in [journal citation]. No additional studies ongoing at this time.
	Relevant findings from the literature	No new literature on [x] disease at this time.
Clinical information	Any related delayed adverse event with brief narrative	One case of rash that resolved with steroids. No other symptoms. PCR of rash biopsy was negative for vector.
	Oncological, neurological, hematological, auto-immune or other disorder	Secondary tumor on left ear, negative for vector sequences by PCR. Unrelated, melanoma.

	Causal analyses based on evidence from clinical, laboratory, molecular, cytogenetic, histological, HLA analysis, deep sequencing data	NA
	Serious adverse events	2 deaths due to sepsis, related to underlying disease. No other unexpected SAE reported
	Evidence for persistence of the product/therapeutic protein/sequences, and durability of the clinical effects	20 subjects are still on study with vector persists in BM and PBMC samples, and clinical benefit observed. All twenty subjects have reconstituted immune system, with some b cell aphasia and low platelet counts in three subjects, however no transfusions needed to date.
Revision of LTFU Protocol	Rationale for modifying LTFU observation	All RCR testing results negative (n=150 samples). Risk assessment determined very low risk of RCR developing in subjects at this time.
	FDA agreement to revised LTFU protocol: synopsis of meeting(s) discussion/email communication	Revision to LTFU discussed during pre-BLA meeting [date]. RCR testing will no longer performed for LTFU protocol [#]
	Discussion and date of discontinuation	NA

[1] polymerase chain reaction
[2] none detected (ND)
[3] not applicable (NA)