Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs)

Draft Guidance for Industry

This guidance document is for comment purposes only.

Submit one set of either electronic or written comments on this draft guidance by the date provided in the *Federal Register* notice announcing the availability of the draft guidance. Submit electronic comments to https://www.regulations.gov. Submit written comments to the Dockets Management Staff (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. You should identify all comments with the docket number listed in the notice of availability that publishes in the *Federal Register*.

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For questions on the content of this guidance, contact OCOD at the phone numbers or email address listed above.

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This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff responsible for this guidance as listed on the title page.

I. INTRODUCTION

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. We, the FDA, are providing you, sponsors of a human gene therapy Investigational New Drug Application (IND), recommendations regarding chemistry, manufacturing, and control (CMC) information to be submitted in an IND. The purpose of this draft guidance is to inform sponsors how to provide sufficient CMC information required to assure product safety, identity, quality, purity, and strength (including potency) of the investigational product (21 CFR 312.23(a)(7)(i)). This guidance applies to human gene therapy products and to combination products[1] that contain a human gene therapy in combination with a drug or device.

This draft guidance, when finalized, will supersede the document entitled "Guidance for FDA Reviewers and Sponsors: Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs)," dated April 2008 (April 2008 guidance) (Ref. 1). The field of gene therapy has progressed rapidly since we issued the April 2008 guidance. Therefore, we are updating that guidance to provide you with current FDA recommendations regarding the CMC content of a gene therapy IND. This guidance is organized to follow the structure of the FDA guidance on the Common Technical Document (CTD). Information on the CTD can be found in the "Guidance for Industry: M4Q: The CTD – Quality," dated August 2001 (Ref. 2). For information on the submission of an electronic CTD (eCTD), please see the FDA website https://www.fda.gov/Drugs/DevelopmentApprovalProcess/FormsSubmissionRequirements/ElectronicSubmissions/ucm153574.htm.

[1] Combination products are comprised of any combination of a drug and a device; a device and a biological product; a biological product and a drug; or a drug, a device, and a biological product; see 21 CFR 3.2(e) for the complete definition of combination product. Combination products are assigned to a lead center for review; see 21 CFR 3.4.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidance describes 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 guidance means that something is suggested or recommended but not required.

II. BACKGROUND

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,[2] and ex vivo genetically modified human cells. Gene therapy products meet the definition of "biological product" in section 351(i) of the Public Health Service (PHS) Act (42 U.S.C. 262(i)) when such products are applicable to the prevention, treatment, or cure of a disease or condition of human beings.

The FDA requires all sponsors of investigational new drug products (DPs), including investigational gene therapy products, to describe the CMC information for the drug substance (DS) (21 CFR 312.23(a)(7)(iv)(*a*)) and the DP (21 CFR 312.23(a)(7)(iv)(*b*)). FDA may place the IND on clinical hold if the IND does not contain sufficient CMC information to assess the risks to subjects in the proposed studies (21 CFR 312.42(b)(1)(iv)).

The CMC information submitted in an IND is a commitment to perform manufacturing and testing of the investigational product, as stated. We acknowledge that manufacturing changes may be necessary as product development proceeds, and you should submit information amendments to supplement the initial information submitted for the CMC processes (21 CFR 312.23(a)(7)(iii)). The CMC information submitted in the original IND for a Phase 1 study may be limited, and therefore, the effect of manufacturing changes, even minor changes, on product safety and quality may not be known. Thus, if a manufacturing change could affect product safety, identity, quality, purity, potency, or stability, you should submit the manufacturing change prior to implementation (21 CFR 312.23(a)(7)(iii)).

We recently published a guidance document, entitled "Providing Regulatory Submissions in Electronic Format – Certain Human Pharmaceutical Product Applications and Related Submissions Using the eCTD Specifications; Guidance for Industry," dated April 2017, addressing the electronic submission of certain applications in the CTD format (eCTD) (Ref. 3). Beginning May 5, 2017, all New Drug Applications (NDAs), Abbreviated New Drug Applications (ANDAs), Biologics License Applications (BLAs), and Master Files must be submitted in eCTD, and commercial IND submissions must be submitted in eCTD, beginning

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

May 5, 2018 (Ref. 3). Excluded from the eCTD requirement are INDs for devices under section 351 of the PHS Act and products that are not intended to be distributed commercially. Investigator-sponsored INDs and expanded access INDs (e.g., emergency use INDs and treatment INDs) are also excluded from the eCTD requirement. In preparation for meeting these requirements, we recommend that sponsors begin to organize and categorize their CMC information, according to the CTD format.

You are not required to complete all CTD sections in your original IND submission. The amount of CMC information to be submitted in your IND depends on the phase of investigation (21 CFR 312.23(a)(7)(i)) and the scope of the clinical investigation proposed. The emphasis for CMC review in all phases of development is product safety and manufacturing control. We expect that sponsors may need to make modifications to previously submitted information as clinical development proceeds and additional product knowledge and manufacturing experience is collected.

We are providing detailed recommendations for submitting CMC information in Module 3 of your IND. We have structured these recommendations to follow the outline of the FDA "Guidance for Industry: M4Q: The CTD – Quality," dated August 2001 (Ref. 2). We are also providing general recommendations regarding administrative and quality summary information for Modules 1 and 2, respectively, of the CTD structure.

III. ADMINISTRATIVE INFORMATION (MODULE 1 OF THE CTD)

A. Administrative Documents

Administrative documents (e.g., application forms, such as Form FDA 1571, cover letters, reviewer guides, and cross-reference authorization letters), claims of categorical exclusion, and labeling information should be included in Module 1 of CTD submissions. The cover letter of your submission should include a brief explanation of your submission and its contents. When amendments are submitted to the IND for manufacturing changes, your cover letter should clearly describe the purpose of the amendment and highlight proposed changes. For amendments containing numerous or significant changes, we recommend that you include a "Reviewer's Guide," as described in FDA's "eCTD Technical Conformance Guide: Technical Specifications Document," dated November 2017 (Ref. 4), and that you allow sufficient lead time (e.g., 30 days) for FDA review before release of a new lot of clinical trial material.

B. Labels

Your IND must contain a copy of all labels and labeling to be provided to each investigator in the clinical study (21 CFR 312.23(a)(7)(iv)(*d*)). We recommend that you include sample labels in Module 1 of the CTD. Please note that IND products must bear a label with the statement, "Caution: New Drug--Limited by Federal (or United States) law to investigational use" (21 CFR 312.6). For products derived from autologous

donors and other situations described in 21 CFR 1271.90(a) for which a donor eligibility determination is not required, you must include the required labeling in 21 CFR 1271.90(c), as applicable. For example, for cells intended for autologous use, you must label the product "FOR AUTOLOGOUS USE ONLY" (21 CFR 1271.90(c)(1)) and "NOT EVALUATED FOR INFECTIOUS SUBSTANCES" if donor testing and screening is not performed (21 CFR 1271.90(c)(2)).

C. Environmental Analysis

Your IND must contain either an environmental analysis or a claim for categorical exclusion (21 CFR 312.23(a)(7)(iv)(*e*)). Please note that, under ordinary circumstances, most INDs are eligible for categorical exclusion under 21 CFR 25.31(e) (Ref. 5). This information can be submitted in Module 1 of the CTD.

D. Previously Submitted Information

For INDs, you generally are not required to resubmit information that you have previously submitted to the Agency, and you may incorporate such information by reference. You may submit a written statement in your IND that appropriately identifies previously submitted information (21 CFR 312.23(b)). We recommend you describe the information that you are referencing and identify where that information is located in the previously submitted file.

You may also reference information previously submitted by another individual if proper authorization has been granted. Proper authorization may be granted with a Letter of Authorization (LOA) from the individual who submitted the information (21 CFR 312.23(b)). We recommend that the LOA include a description of the information being cross-referenced (e.g., reagent, container, vector manufacturing process) and identify where that information is located (e.g., file name, reference number, volume, page number). Please note that this LOA only allows you to cross-reference the information outlined in the LOA and submitted by the author of the LOA. The LOA does not provide you permission to cross-reference information that was submitted by another individual and cross-referenced by the author of the LOA. In other words, you may not cross-reference information that is cross-referenced by the author of the LOA. You are required to submit an LOA for all information submitted by another individual (21 CFR 312.23(b)).

In addition to including LOAs in Module 1 of the CTD, you should list these files in the IND cover sheet (i.e., Form FDA 1571) of each IND submission. If the LOA is absent or inadequate or the information in the cross-referenced file is inadequate for the purpose cited, we will notify you that the information in the cross-referenced file is not sufficient to support your IND. In the event a cross-referenced IND is placed on clinical hold or is withdrawn, your IND may also be placed on clinical hold if critical cross-referenced information is no longer available or adequate.

IV. SUMMARY OF QUALITY INFORMATION (MODULE 2 OF THE CTD)

A. General Information

Your IND should contain a general introduction to the gene therapy product under investigation, including a description of its active ingredient(s), mode of action, and proposed clinical use. This summary should include an overview of the manufacturing process, controls in place to ensure product quality, and general information regarding the qualification of components and starting materials. You should describe the composition of the DS and DP. You should indicate if the DS is formulated into a DP for administration or if the DS is used for ex vivo genetic modification of cells.

Your summary should also include a description of critical quality attributes (CQAs) that are relevant to the safety and biological activity of the product as they are understood at the time of submission. For additional information regarding establishing CQAs, please see Guidance for Industry: "Q8(R2) Pharmaceutical Development," dated November 2009 (Ref. 6), and "Q11 Development and Manufacture of Drug Substances," dated November 2012 (Ref. 7). A CQA is defined as a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality. CQAs apply to DS and DP as well as to excipients and in-process materials. Information to support a CQA and results from specific studies or published literature may be included in Module 3 of the CTD "Pharmaceutical Development" (section 3.2.P.2) (Ref. 2) or linked to the relevant nonclinical or clinical sections of the application in the CTD.

As product development progresses, CQAs may be used to define DS and DP specifications. Understanding and defining product characteristics that are relevant to the clinical performance of the gene therapy may be challenging, particularly during early stages of product development. Therefore, we recommend that you evaluate a number of product characteristics during early clinical development to help you identify and understand the CQAs of your product. This will also help ensure your ability to assess manufacturing process controls, manufacturing consistency, and product stability as product development advances. This is especially important for sponsors of gene therapy products who are pursuing expedited product development programs (Ref. 8).

B. Drug Substance and Drug Product

Your IND must contain a description of the DS (21 CFR 312.23(a)(7)(iv)(*a*)) and DP (21 CFR 312.23(a)(7)(iv)(*b*)), including the physical, chemical, or biological characteristics, manufacturing controls, and testing information, to ensure the DS and DP meet acceptable limits for identity, strength (potency), quality, and purity. For the purpose of this guidance, a DS is defined as an active ingredient that is intended to furnish biological activity or other direct effect in the diagnosis, cure, mitigation,

treatment, or prevention of disease or to affect the structure or any function of the human body. Further, a DP is defined as the finished dosage form that contains the DS, generally, but not necessarily in association with one or more other ingredients (e.g., excipients).

We recognize that distinguishing a DS from a DP may be difficult for some gene therapy products, due to the complex nature of the manufacturing processes. Some gene therapy products may not have defined DS. Others may consist of two or more different DSs that are combined to make the DP. This guidance does not recommend how sponsors should distinguish the DS and DP. However, we do recommend that you provide an explanation to support your DS/DP distinction in the summary information in Module 2 of CTD submissions and that you submit the required information for each DS and DP, as outlined in Module 3 of the CTD (Ref. 2).

When the manufacturing process includes more than one DS, we recommend that you provide separate DS sections for each active ingredient of the final product. The CTD DS sections should follow the format and numbering scheme recommended in Module 3 of FDA "Guidance for Industry: M4Q: The CTD – Quality," dated August 2001 (Ref. 2), and the sections should be distinguished from one another by including the DS name and manufacturer in the heading (e.g., section 3.2.S.1 General Information [name, manufacturer]).

A summary of the available stability data for the DS and the DP, recommended storage conditions, and tentative expiry date, if applicable, should also be included in this section. Information on stability protocols and stability data should be included in the appropriate sections of Module 3.

C. Combination Products

For submissions in which the gene therapy is a component of a combination product, as defined in 21 CFR 3.2(e), we recommend that you briefly describe the combination product in the summary of your product and briefly state the regulatory status of each component. To clearly delineate the different components of a combination product, you should include manufacturing and engineering information for the gene therapy and drug or device in separate entries of the CTD submission, as described in the FDA "eCTD Technical Conformance Guide: Technical Specifications Document," dated November 2017 (Ref. 4).

D. Product Handling at the Clinical Site

Proper control of the finished DP is critical to your investigational studies. Therefore, your IND should also include a description of how the product will be shipped to, received, and handled at the clinical site to ensure safety, product quality, and stability. Your IND should also include information on shipping conditions, storage conditions, expiration date/time (if applicable), and chain of custody from the manufacturer to the

site of administration in the summary information of the CTD. Your summary in Module 2 should also include information for product handling at the clinical site prior to administration (such as thawing, washing, or the addition of diluent or adjuvant, loading into a delivery device, and transport to the bedside) and summary information on product stability prior to and during administration (e.g., in-device hold times and temperatures).

Details regarding product stability after preparation for delivery and delivery device compatibility data should be included in Module 3 (sections 3.2.P.8 and 3.2.P.2.6, respectively) of the CTD (Ref. 2). Instructions for drug handing and preparation for administration at the clinical site (e.g., Pharmacy Manual or Instructions for Use) should be provided in the "Clinical Study Reports" section of your IND (section 5.3 of the FDA "M4E(R2): The CTD – Efficacy; Guidance for Industry," dated July 2017 (Ref. 9)). Detailed information about the delivery device may be included in "Regional Information" (section 3.2.R of the CTD) (Ref. 2).

V. MANUFACTURING PROCESS AND CONTROL INFORMATION (MODULE 3 OF THE CTD)

The headings and text below include CTD section numbers in parentheses for reference (Ref. 2).

A. Drug Substance (3.2.S)

1. General Information (3.2.S.1)

a. Nomenclature (3.2.S.1.1)

You should provide the name of the DS(s). If the name of the DS has changed during clinical development, you should provide the names used to identify the DS at all stages of development. If the United States Adopted Name (USAN) Council has given it a nonproprietary name, you may provide it here.

b. Structure (3.2.S.1.2)

You should submit information on the molecular structure (including genetic sequence) and/or cellular components of the DS. The genetic sequence can be represented in a schematic diagram that includes a map of relevant regulatory elements (e.g., promoter/enhancer, introns, poly(A) signal), restriction enzyme sites, and functional components (e.g., transgene, selection markers). Please note that you should also submit information on your sequence analysis and the annotated sequence data in your IND. We recommend that your sequence data, including any data collected to support the genetic stability of your vector, be submitted in "Elucidation of Structure and other Characteristics" (section 3.2.S.3.1 of

the CTD). More information on our recommendations for sequence analysis is described in "Control of Materials (3.2.S.2.3)" (section V.A.2.c. of this guidance).

Some examples of additional information for structure and structural elements of different gene therapy products are outlined below:

- For viral vectors, you should include a description of the composition of the viral capsid and envelope structures, as appropriate, and any modifications to these structures (e.g., modifications to antibody binding sites or tropism-changing elements). We recommend that you include biophysical characteristics (e.g., molecular weight, particle size) and biochemical characteristics (e.g., glycosylation sites). You should also describe the nature of the genome of viral vectors, whether single-stranded, double-stranded, or self-complementary, DNA or RNA, and copy number of genomes per particle.

- For bacterial vectors, you should include defining physical and biochemical properties, growth characteristics, genetic markers (e.g., auxotrophic or attenuating mutations, antibiotic resistance) and the location (e.g., on plasmid, episome, or chromosome) and description of any inserted foreign genes and regulatory elements. For additional details on microbial vectors, please see the FDA's Guidance for Industry "Recommendations for Microbial Vectors used for Gene Therapy," dated September 2016 (Ref. 10).

- For ex vivo genetically modified cells, you should describe the expected major and minor cell populations as well as the vector that contains the transgene cassette that is transferred into the cell. For cells that have been genetically modified using genome editing, you should describe the gene(s) that are altered and how the change(s) was made (i.e., the gene editing technology used).

c. General Properties (3.2.S.1.3)

You should provide a section in the IND that describes the composition and properties of the DS, including the biological activity and proposed mechanisms of action.

2. Drug Substance Manufacture (3.2.S.2)

a. Manufacturer(s) (3.2.S.2.1)

You must provide the name and address of each manufacturer, including contract manufacturer(s), involved in the manufacture, testing, and storage of the DS (21 CFR 312.23(a)(7)(iv)*(a)*). You should indicate the responsibility of each manufacturer. Your IND should contain complete information on the DS manufacturer, regardless of whether the process is performed by you or by a contract manufacturing organization (CMO). As the sponsor of the IND, you are ultimately responsible for the safety of subjects in the clinical investigation (21 CFR 312.3); therefore, we recommend that you and the CMO understand and document your respective responsibilities for ensuring product quality. Additional information on quality agreements can be found in FDA's Guidance for Industry, "Contract Manufacturing Arrangements for Drugs: Quality Agreements," dated November 2016 (Ref. 11).

b. Description of Manufacturing Process and Process Controls (3.2.S.2.2)

Your description of the DS manufacturing process and process controls should include all of the following, as applicable: cell culture; transduction; cell expansion; harvest(s); purification; filling; and storage and shipping conditions. Your description should also accurately represent your process and process controls. Changes and updates to this information should be submitted as an amendment to the IND prior to implementation (21 CFR 312.23(a)(7)(iii)), as indicated in section II. Background of this guidance.

i. Batch and Scale

A description of how you define each manufacturing run (i.e., batch, lot, other) should be submitted with an explanation of the batch (or lot[3]) numbering system. You should clearly state whether any pooling of harvests or intermediates occurs during manufacturing. If pooling is necessary during production, we recommend that you control the storage conditions (e.g., time, temperature, container) for each pool and that you describe the testing that is performed prior to pooling to ensure the quality of each pool.

[3] For purpose of this guidance, batch and lot are used interchangeably.

We also recommend that you provide an explanation for how the batch scale is defined (e.g., bioreactor volume, cell processing capacity) and how the DS is quantified (e.g., vector genomes, transducing units, infectious particles, mass, number of gene modified cells). When known, please include the yield expected per batch.

ii. Manufacturing Process

The description of your manufacturing process should include a flow diagram(s) and a detailed narrative. Your description should clearly identify any process controls and in-process testing (e.g., titer, bioburden, viability, impurities) as well as acceptable operating parameters (e.g., process times, temperature ranges, cell passage number, pH, CO_2, dissolved O_2, glucose level).

We recommend the evaluation of operating parameters on a periodic basis to ensure process control and allow for trending and statistical analyses if deemed appropriate to monitor process consistency. You should clearly describe any environmental controls as well as tracking and segregation procedures that are in place to prevent cross-contamination throughout the manufacturing process.

iii. Cell Culture

The description of all cell culture conditions should contain sufficient detail to make understandable any of the process steps that apply, process timing, culture conditions, hold times and transfer steps, and materials used (e.g., media components, bags/flasks). You should describe whether the cell culture system is open or closed and any aseptic processing steps. If extensive culture times are needed, you should outline the in-process controls you have in place to monitor cell quality (e.g., viability, bioburden, pH, dissolved O_2). Expectations for media components and cell bank qualification are outlined in this guidance under "Control of Materials (3.2.S.2.3)" (section V.A.2.c. of this guidance).

iv. Vector Production

For the manufacture of gene therapy vectors (e.g., viral vectors, bacterial plasmids, mRNA), you should provide a description of all production and purification procedures. Production procedures should include a description of the cell substrate, cell culture and expansion steps, transfection or infection procedures, harvest steps,

hold times, vector purification (e.g., centrifugation, column purification, density gradients), concentration or buffer exchange steps, and the reagents/components used during these processes. You should outline any in-process testing to ensure vector quality as appropriate (e.g., titer, impurities).

You should describe whether the DS will be formulated into the DP for direct administration or whether it will be formulated for ex vivo genetic modification of cells, as outlined in section IV.B. As an active ingredient, the same level of control should be applied to each DS, and each DS should be manufactured under appropriate Good Manufacturing Practice (GMP) conditions. For more information on your Quality Unit and GMP manufacturing, see "Process Validation and/or Evaluation (3.2.S.2.5)" (section V.A.2.e. of this guidance).

v. Genetically Modified Cell Production

If your product consists of genetically modified cells, your cell processing description should contain sufficient detail to make understandable any of the following process steps that apply: source material (e.g., autologous or allogeneic cells); collection of cellular source material (e.g., leukapheresis, biopsy); storage at the collection site; shipping to and handling at the manufacturing facility; cell selection, isolation, or enrichment steps (including methods, devices, reagents); cell expansion conditions; hold times and transfer steps; and cell harvest, purification, if any, and materials used.

You should also provide a complete description of all procedures used for gene modification (such as transfection, infection or electroporation of vectors, or genome editing components) and any additional culture, cell selection, or treatments after modification.

vi. Irradiated Cells

If your product contains or is processed with irradiated cells, you should provide documentation for the calibration of the irradiator source and provide supporting data to demonstrate that the irradiated cells are rendered replication-incompetent, while still maintaining their desired characteristics.

vii. Filling, Storage, and Transportation (Shipping)

You should provide a detailed description and identify any associated process controls for formulation, filling, storage, and shipping of the DS, if applicable. You should also describe the container used for storage and shipping of the DS. We recommend that you describe procedures that are in place to ensure appropriate storage and transport (as needed).

c. Control of Materials (3.2.S.2.3)

You must provide a list of all materials used in manufacturing (21 CFR 312.23(a)(7)(iv)(*b*)) and a description of the quality and control of these materials. This information may be provided in tabular format and include the identity of the material, the supplier, the quality (e.g., clinical-grade, FDA-approved), the source of material (e.g., animal, human, insect), and the stage at which each material is used in the manufacturing process (e.g., culture media, vector purification). This includes information on components, such as cells, cell and viral banking systems, and reagents, as described in more detail below; it also includes raw materials and equipment, such as culture bags, culture flasks, chromatography matrices, and tubing, that come into contact with the product.

You should provide documentation that the materials used for manufacturing meet standards appropriate for their intended use (e.g., test results, certificates of analysis (COAs), package inserts). COAs for materials can be provided in "Facilities and Equipment" (section 3.2.A.1 of the CTD) and hyperlinked to relevant sections of your IND. We recommend that you use FDA-approved or cleared or other clinical-grade materials, when they are available. If the material is not FDA-approved or cleared (or in the absence of recognized standards), additional information on the manufacturing and/or testing may be needed to evaluate the safety and quality of the material. The extent of testing will depend on the specific material and the manner in which it is used in the manufacturing process.

i. Reagents

For purpose of this guidance, reagents (or ancillary materials) are those materials used for manufacturing (e.g., cell growth, differentiation, selection, purification, or other critical manufacturing steps) that are not intended to be part of the final product. Examples include fetal bovine serum, digestive enzymes (e.g., trypsin, collagenase, DNase/RNase, restriction

endonucleases), growth factors, cytokines, monoclonal antibodies, antibody-coated beads, antibiotics, media, media components, and detergents. These reagents can affect the safety, potency, and purity of the final product, especially by introducing adventitious agents or other impurities.

For biologically sourced reagents, including human, bovine, and porcine-derived materials, we recommend that you refer to the FDA Guidance for Industry: "Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications," dated February 2010 (Ref. 12). Animal-derived materials increase the risk of introducing adventitious agents. Certain animal-derived materials, such as sera, are complex mixtures that are difficult to standardize, and such materials may have significant batch-to-batch variations that may affect the reproducibility of your manufacturing process or the quality of your final product. We recommend that you use non-animal-derived reagents whenever possible (for example, serum-free tissue culture media and recombinant proteases).

ii. Bovine

We recommend that you provide information on any bovine material used in manufacturing, including the source of the material; information on the location where the herd was born, raised, and slaughtered; and any other information relevant to the risk of transmissible spongiform encephalopathy (TSE). If serum is used, we recommend that it be γ-irradiated to reduce the risk of adventitious agents.

Bovine materials used in production of reagents, which are, in turn, used in manufacturing a product, should also be identified, and the source and qualification of bovine material should be documented.

You should provide COAs for all bovine material lots used in the manufacture and establishment of cell and virus banks to document that these materials are compliant with the requirements for the ingredients of animal origin used for production of biologics described in 9 CFR 113.53.

iii. Porcine

You should provide COAs for all porcine material lots used in manufacture and establishment of cell and virus banks to document that these materials are compliant with the requirements for the ingredients of animal origin used for production of biologics described in 9 CFR 113.53. In addition, porcine reagents should be tested for porcine circovirus (PCV) 1 and 2 and porcine parvovirus.

iv. Murine or Monoclonal Antibodies

Monoclonal antibodies used in manufacturing that have product contact should be tested as per the recommendations described in the FDA "Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use," dated February 1997 (Ref. 13). Alternatively, you may provide a letter of authorization to cross-reference this information in a different regulatory submission (IND or MF). You should also consider that many monoclonal antibodies and recombinant proteins (such as cytokines) used during the manufacture of gene therapy products may be purified by affinity chromatography, using antibodies generated from mouse hybridomas. This may introduce the risk of contamination with adventitious agents from rodents.

v. Human Source

If human albumin is used, you should use FDA-approved products and have procedures in place to ensure that no recalled lots were used during manufacture or preparation of the product.

If human AB serum is used (e.g., for ex vivo genetically modified cells), you should ensure the serum is processed from blood or plasma collected at FDA licensed facilities. Source Plasma, which is often used to make human AB serum, must be collected as described in 21 CFR Part 640, Subpart G. Source Plasma is not tested as extensively as blood products intended for infusion, and we recommend that you ensure the AB serum used in your manufacturing does not have the potential to transmit infectious disease. For example, if your serum is derived from Source Plasma, you may reduce the risk of infectious disease by conducting additional testing for relevant transfusion-transmitted infections. Alternatively, including viral inactivation or clearance steps in the production of AB serum from Source Plasma may be an acceptable alternative.

For all other reagents that are human-derived, you should identify whether the reagent is a licensed product (e.g., HSA, IL-2) or is clinical or research grade and provide a COA or information regarding testing of the donor or reagent.

vi. Cells - Autologous and Allogeneic Cells or Tissue

For autologous or allogeneic cells or tissue, you should provide a detailed description of the cell source, the collection procedure, and any related handling, culturing, storage, and testing that is performed prior to use in manufacture. Your description should include the following information:

- materials used for collection (including devices, reagents, tubing, and containers);
- method of cell collection (i.e., standard blood draw or apheresis);
- enrichment steps, if performed;
- labeling and tracking of collected samples;
- hold times; and
- transportation conditions to the manufacturing facility.

As an example, for cells collected by leukapheresis: you should provide a detailed description of the collection device(s); operating parameters; volumes or number of cells to be collected; and how the collected material is labeled, stored, tracked, and transported to the manufacturing facility.

For multi-center clinical trials, establishing standardized procedures for cell collection and handling across all collection sites is critical to assuring the quality and safety of the final product as well as ensuring control of the manufacturing process. In your IND, you should include a list of collection sites, their FDA Establishment Identifier, and any accreditations for compliance with established standards (e.g., Foundation for the Accreditation of Cellular Therapy (FACT)), if applicable.

A. Autologous Cells

You are not required to make a donor eligibility determination or to perform donor screening on autologous cells or tissues (21 CFR 1271.90(a)(1)). However, you should determine whether your manufacturing procedures increase the risk to the patient by further propagation of pathogenic agents that may be present in the donor. You should also describe precautions to prevent the spread of viruses or other adventitious agents to persons other than the autologous recipient (Ref. 14).

B. Allogeneic Cells

For allogeneic cells or tissues, you must perform donor screening and testing, as required in 21 CFR Part 1271, Subpart C, except for those cells and tissues that meet the exceptions in 21 CFR 1271.90(a). Donors of all types of cells and tissues must be screened for risk factors and clinical evidence of relevant communicable disease agents and diseases, including: human immunodeficiency virus (HIV); hepatitis B virus (HBV); hepatitis C virus (HCV); human TSE, including Creutzfeldt-Jakob disease; and *Treponema pallidum* (syphilis) (21 CFR 1271.75). In addition, donors of viable leukocyte-rich cells or tissues should be screened for human T-lymphotropic virus (HTLV). You must also test a specimen of donor cells or tissue for evidence of infection due to relevant communicable disease agents, including: HIV-1; HIV-2; HBV; HCV; syphilis; and if the material is leukocyte-rich cells or tissue, HTLV-1, HTLV-2, and cytomegalovirus (21 CFR 1271.85). For donor eligibility testing, you must use appropriate FDA-licensed, approved, or cleared donor screening tests (21 CFR 1271.80(c)). You should also refer to recent Center for Biologics Evaluation and Research (CBER) guidance documents on donor eligibility for additional information on testing for emerging relevant communicable disease agents and diseases (e.g., West Nile virus (WNV), Zika virus). If cord blood or other maternally-derived tissue is used, you must perform screening and testing on the birth mothers, as described in 21 CFR 1271.80(a).

Allogeneic cells from a single donor or source tissue may sometimes be expanded and stored for greater consistency and control in manufacturing. In these situations, we generally recommend that you qualify allogeneic master and working cell banks in the same way as cell banks used for production of viral vectors (see "Banking Systems," below), provided that you have sufficient material for this testing. In these situations, we are most concerned about the introduction of adventitious agents (e.g., viruses, bacteria, mycoplasma) during the bank manufacturing process, especially from any bovine or porcine materials, animal feeder cells, other animal-derived reagents, or human AB serum, if used. If your allogeneic cell bank is small, we may recommend abbreviated cell bank qualification. In this case, please consult with the Quality Reviewer of your file for more information on appropriate qualification of small scale allogeneic cell banks.

vii. Banking Systems (Starting Materials)

A banking system improves control and consistency in the manufacturing of many biologics. Banking assures an adequate supply of equivalent, well-characterized material for production over the expected lifetime of production. For these reasons, banked materials are a common starting point for many routine production applications. We outline our current thinking for the qualification of different banking systems below, including banks of cell substrates for production of viral vectors, banks of bacterial/microbial cells, and banks of viral vectors. We recommend that you provide a summary of the testing and COAs in this section. Information on bank qualification and adventitious agent testing should also be included in your comprehensive "Adventitious Agents Safety Evaluation" (section 3.2.A.2 of the CTD).

viii. Master Cell Banks Used as Substrates for Production of Viral Vectors

Prior to selecting a cell line for viral vector manufacturing, you should carefully consider characteristics of the cells that may impact the safety of the final product (such as presence of tumorigenic sequences). This is especially important when the viral vector co-packages non-vector sequences, such as adeno-associated virus (AAV) (see "Impurities (3.2.S.3.2)" section V.A.3.b. of this guidance). We also recommend that you consider

cell attributes that can affect production capacity (e.g., growth characteristics, vector production capacity), prior to generation of a cell bank.

In your IND, you should provide a description of the history and detailed derivation of the source material for the cell bank. Your description should include information on cell source (including species of origin); how the bank was generated (e.g., from a single colony isolate or through limiting dilution); testing performed to characterize the bank; and if applicable, materials used to genetically modify the source material (i.e., packaging cell line).

When a cell substrate has been genetically modified (for example, to provide viral proteins to allow virus replication or packaging), you should provide a description of the materials used for the genetic modification, including information on the quality and control of the vectors used to introduce the genetic changes. Materials used to manufacture process intermediates should be sufficiently characterized to ensure safety and purity of the final gene therapy product. For more information regarding plasmid intermediates that are used for further manufacture, please see "Control of Critical Steps and Intermediates (3.2.S.2.4)" (section V.A.2.d. of this guidance).

For the banked material, itself, we recommend that you provide information on how the cell banks are stored and maintained as well as detailed information on qualification to adequately establish the safety, identity, purity, and stability of the cells used in your manufacturing process. Additional sources of information regarding qualification of cell substrates can be found in the FDA guidance "Q5D Quality of Biotechnological/Biological Products: Derivation and Characterization of Cell Substrates Used for Production of Biotechnological/Biological Products" (63 FR 50244, September 21, 1998) (Ref. 15) and FDA's Guidance for Industry: "Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications," dated February 2010 (Ref. 12).

Cell bank qualification includes tests to:

- Ensure absence of microbial contamination, including sterility, mycoplasma (and spiroplasma for insect cells), and in vivo and in vitro testing for adventitious viral agents. For cell lines used for production of vectors, we

recommend that you test for retroviral contamination, using reverse transcriptase (RT) assays and transmission electron microscopic (TEM) analysis. The presence of an adventitious viral agent in your bank should be vigorously investigated, and re-derivation of the bank should be considered.

- For additional information on the analytical methods used for cell bank qualification, please see "Analytical Procedures (3.2.S.4.2)" (section V.A.4.b. of this guidance).

- For cell lines that have been exposed to bovine or porcine components (e.g., serum, serum components, trypsin), appropriate testing would include testing for bovine or porcine adventitious agents. See further discussion on bovine and porcine reagents, above.

• Ensure absence of species-specific pathogens.

- For human cells, this may include testing for cytomegalovirus (CMV), HIV-1 & 2, HTLV-1 &-2, human herpesvirus-6 and -8 (HHV-6 & -8), JC virus, BK virus, Epstein-Barr virus (EBV), human parvovirus B19, HBV, human papillomavirus (HPV), and HCV, as appropriate.

- For other animal or insect cells, we recommend tests for species-specific viruses, as appropriate. For instance, for Vero cells, we recommend testing for simian polyomavirus SV40 and simian retrovirus.

- For insect cells, you may evaluate the presence of arboviruses in a susceptible cell line, such as baby hamster kidney (BHK21) cells. Insect cell lines with known viral contamination should be avoided.

• Identify cells. Identify your cells through tests that distinguish them from other cell lines used in your facility. For cell lines that you have purchased from a type collection or received from another investigator, we recommend master cell bank (MCB) testing to confirm the

purity of the cells by genetic analysis (i.e., short tandem repeat analysis or other profiling analysis).[4]

- Establish stability of the cell bank. Stability can be assessed by measuring viability of cells over time after cryopreservation. We also recommend a one-time test of end of production cells (EOP) or mock production cells of similar passage history, to be tested for their suitability to produce your vector. For stable retroviral vector producer cells, we recommend that you test the genetic stability of the gene insert in the EOP cells.

- Assess the ability of new cell lines to form tumors. We recommend that you perform tumorigenicity tests for cell lines that have not been previously characterized for their potential to form tumors. This test would not be necessary for cells known to form tumors; please see additional information on testing for process-related impurities under "Drug Substance Characterization (3.2.S.3)" (section V.A.3.b.i. of this guidance).

ix. Working Cell Banks

A Working Cell Bank (WCB) may be derived from one or more vials of the MCB. The information needed to document qualification and characterization for a WCB is less extensive than that needed for the MCB. WCB testing should include but is not limited to sterility, mycoplasma, identity, and in vitro adventitious agent tests. For additional information on the analytical methods used for WCB qualification, please see "Analytical Procedures (3.2.S.4.2)" (section V.A.4.b. of this guidance).

x. Bacterial or Microbial Master Cell Banks

For all bacterial or microbial (e.g., yeast) MCBs, you should describe the genotype and source of the microbial cells. Bacterial MCBs are frequently used as a starting material to generate plasmid DNA, which can be used as a vector for gene transfer or as a manufacturing intermediate for other gene therapy products, such as the AAV or lentiviral vectors. Microbial MCBs also may be used to generate a microbial vector for gene therapy. You should

[4] Reid Y, Storts D, Riss T, Minor L. Authentication of Human Cell Lines by STR DNA Profiling Analysis. In: Sittampalam GS, Coussens NP, Brimacombe K. et al., editors. Assay Guidance Manual. Bethesda (MD): Eli Lilly & Company and the National Center for Advancing Translational Sciences; 2004. https://www.ncbi.nlm.nih.gov/books/NBK144066/.

provide a detailed description of the history and derivation of the materials used to generate the cell bank, including information on how plasmid vectors were designed and constructed. For the bank material, itself, you should provide information on how the material was generated and how the bank is stored and maintained as well as detailed information on qualification of the bank (including cell bank COAs) to adequately establish the safety, identity, purity, and stability of the microbial cell preparation used in the manufacturing process.

For bacterial cell banks used to manufacture a DNA plasmid, we recommend MCB testing include:

- Bacterial host strain identity;
- Plasmid presence, confirmed by bacterial growth on selective medium, restriction digest, or DNA sequencing;
- Bacterial cell count;
- Bacterial host strain purity (no inappropriate organisms, negative for bacteriophage);
- Plasmid identity by restriction enzyme (RE) analysis;
- Full plasmid sequencing. We recommend that you fully sequence plasmid vectors and submit an annotated sequence for the vector, as described in more detail in the section below on viral vector banks; and
- Transgene expression and/or activity.

For microbial cell banks used to manufacture a microbial vector, our recommendations for MCB testing are outlined in the Guidance for Industry, "Recommendations for Microbial Vectors used for Gene Therapy," dated September 2016 (Ref. 10).

xi. Master Viral Banks

Viral banks may be expanded for viral vector production, or they may be used as helper viruses for manufacturing non-replicating vectors (e.g., AAV or gutless adenovirus). You should provide a detailed description of the history and derivation of the source or

seed materials for these banks. You should describe how the seed stock was generated and what cells and animal-derived materials were used in the derivation process.

A gene map of the final vector and vector intermediates is useful when describing the history and derivation of recombinant viral vectors. We recommend that you state whether the seed material was plaque-purified, purified by limiting dilution, or rescued from DNA or RNA clones and how many times it was passaged, during expansion.

For the banked material, itself, you should describe the manufacturing process and the conditions under which the banked material was generated, for example, in a research laboratory or a GMP facility. We recommend that you list animal-derived materials used in the generation of the bank and state whether the master virus bank (MVB) is expected to represent a single clone or a distribution of viral variants or sequences.

We also recommend that you provide information on how the bank is stored and maintained as well as detailed information on the qualification of the bank to adequately establish the safety, identity, purity, and stability of the virus preparation used in the manufacturing process. If a COA is available, it should be submitted to the IND. For additional information on the analytical methods used for MVB qualification, please see "Analytical Procedures (3.2.S.4.2)" (section V.A.4.b. of this guidance).

Viral vector bank qualification includes tests to:

- Ensure absence of contamination, including sterility, mycoplasma, and in vivo and in vitro testing for adventitious viral agents.

- Ensure absence of specific pathogens that may originate from the cell substrate, such as human viruses if the cell line used to produce the MVB is of human origin, or pathogens specific to the origin of the production cell line (e.g., murine, non-human primate, avian, insect).

- Ensure absence of replication competent virus in replication incompetent vectors.

- Ensure viral titer or concentration.

- Ensure sensitivity to anti-viral drugs, as applicable, for example, herpes simplex virus (HSV) sensitivity to ganciclovir.

- Ensure transgene activity, if appropriate.

- Identify the viral vector and therapeutic transgene (e.g., Southern blot or restriction endonuclease analysis), as needed.

- Ensure the correct genetic sequence. We recommend that you fully sequence all vectors that are 40 kb or smaller, analyze the sequence, and submit an annotated sequence of the entire vector. You should provide an evaluation of the significance of all discrepancies between the expected sequence and the experimentally determined sequence and an evaluation of the significance of any unexpected sequence elements, including open reading frames. We have the following recommendations, regarding sequence analysis:

    - We recommend that viral vectors be sequenced from the MVB, when possible.

    - For integrating viral vectors, we recommend that you perform DNA sequencing on the integrated vector. The material for sequencing can be collected from the producer cell line or, in the case of vectors generated by transient transfection, from material collected from cells that you have transduced after isolation of a vector lot.

    - For other situations in which no MVB exists, sequencing should be performed from the DS or DP. For example, AAV vectors are typically made by plasmid transfection, and the AAV vector is harvested directly from transfected cells to produce a DS. In this situation, we recommend that you sequence one or more lots (either material from DS or DP) to confirm that the vector sequence is stable, during manufacturing.

    - For viral vectors greater than 40 kb, you should summarize the extent and results of sequence analysis that you have performed, including any

testing performed by restriction endonuclease analysis. You should perform sequence analysis of the gene insert, flanking regions, and any regions of the vector that are modified or could be susceptible to recombination. The entire vector sequence will be necessary to confirm identity for licensure.

xii. Working Viral Banks

A working viral bank (WVB) may be derived from one or more vials of the MVB, and the information needed to document qualification and characterization of the WVB is less extensive than that needed for the MVB. You should describe the process used to generate the WVB and whether animal-derived materials were used. Testing for WVB should include but is not limited to sterility, mycoplasma, identity, and in vitro adventitious agent tests.

d. Control of Critical Steps and Intermediates (3.2.S.2.4)

You should describe the control of critical steps and intermediates in the manufacturing process. Critical control steps include those outlined in the "Description of Manufacturing Process and Process Controls" (section 3.2.S.2.2 of the CTD and section V.A.2.b. of this guidance). We recommend that you also consider any steps in which in-process tests with acceptance criteria are performed as critical control steps.

You should provide information on the quality and control of intermediates. Manufacturing intermediates should be defined by the manufacturer. Intermediates may include material from collection or hold steps, such as temporary storage of bulk harvest, concentration steps, or purification intermediates (e.g., column fractions or eluate). The duration of production steps and hold times should be controlled and recorded to facilitate the establishment of process limits and to allow for future validation of each step and hold time within the proposed limits in support of a license application.

Intermediates in gene therapy manufacturing may also include DNA plasmids that are used in the manufacture of other gene therapy products, such as AAV or lentiviral vectors. We recommend that DNA plasmid intermediates be derived from qualified banks, as described in more detail above and in "Control of Materials (3.2.S.2.3)" (section V.A.2.c. of this guidance). In addition, we recommend that you provide information on the plasmid manufacturing procedures, reagents, and plasmid specifications for use. In general, we recommend that this testing include

assays to ensure the identity, purity, potency, and safety of the final product. For a DNA plasmid, this may include sterility, endotoxin, purity (including percent of supercoiled form and residual cell DNA, RNA, and protein levels), and identity testing (restriction digest and sequencing if sequencing was not performed on the bacterial bank). A COA documenting plasmid quality testing should be included in the IND.

e. Process Validation and/or Evaluation (3.2.S.2.5)

Process validation studies are generally or typically not required for early stage manufacturing, and thus, most original IND submissions will not include process performance qualification. We recommend that you use early stage manufacturing experience to evaluate the need for process improvements and to support process validation studies in the future.

INDs at all stages of development should have established written standard operating procedures (SOPs) to ensure proper manufacturing control and oversight. Manufacturing oversight is usually performed by a dedicated Quality Unit, the duties of which include implementing procedures to prevent microbial contamination, cross-contamination, and product mix-ups. Your Quality Unit should have procedures in place to investigate lot failures, out-of-specification results, and ways to implement corrective actions. Your IND should include a description of your Quality Unit, including the manner in which quality control testing and oversight are separated from the manufacturing unit.

Additional information on quality systems and process validation can be found in the following FDA guidance documents: "Guidance for Industry: CGMP for Phase 1 Investigational Drugs," dated July 2008 (Ref. 16); "Quality Systems Approach to Pharmaceutical CGMP Regulations," dated September 2006 (Ref. 17); and "Process Validation: General Principles and Practices," dated January 2011 (Ref. 18). The application of current good manufacturing practices (CGMPs) is required under section 501(a)(2)(B) of the Federal Food, Drug, and Cosmetic Act at all stages of clinical investigation. However, the CGMP regulations (21 CFR Part 211) are not required for the manufacture of most investigational new drugs under Phase 1 INDs (See Ref. 16).

f. Manufacturing Process Development (3.2.S.2.6)

You should provide a description and discussion of the developmental history of the manufacturing process described in "Description of Manufacturing Process and Process Controls" (section 3.2.S.2.2 of the CTD).

For early stage INDs, there may be differences between the manufacturing and testing of the toxicology lots and the material you plan to use in the clinical studies. For later stage INDs, there may be changes to the manufacturing process as part of process development or optimization. In both situations, we recommend that you describe how manufacturing differences are expected to impact product performance. If you make significant manufacturing changes, then comparability studies may be necessary to determine the impact of these changes on the identity, purity, potency, and safety of the product. The extent of comparability testing will depend on the manufacturing change, the ability of analytical methods to detect changes in the product, and the stage of clinical development. For first-in-human studies, any differences between toxicology lots and clinical lots should be assessed for their impact on product safety. For later phase studies, especially those designed to measure product efficacy, differences in clinical lots should be assessed for their impact on product safety and activity.

Please note that it is important to retain samples of the DS and manufacturing intermediates, when possible, in the event that comparability studies are necessary during future product development.

3. Drug Substance Characterization (3.2.S.3)

a. Elucidation of Structure and Other Characteristics (3.2.S.3.1)

We recommend that you include annotated sequence data for your vector in the original IND submission. In addition, we recommend that you provide any further information confirming the primary, secondary, or higher order structure; post-translational modifications; and/or distribution of cell types for the DS if it has not already been described in "Structure" (section 3.2.S.1.2 of the CTD).

b. Impurities (3.2.S.3.2)

We recommend that your manufacturing process be designed to remove process- and product-related impurities and that you have tests in place to measure levels of residual impurities. You should describe your test procedures in the IND with appropriate limits. Your initial specification for impurities may be refined with additional manufacturing experience. We recommend that you measure impurities throughout product development, as this will help ensure product safety, contribute to your understanding of the manufacturing process, and provide a baseline for potential manufacturing changes in the future.

i. Process-Related Impurities

We recommend testing for process-related impurities. These include but are not limited to residual cell substrate proteins, extraneous nucleic acid sequences, helper virus contaminants (i.e., infectious virus, viral DNA, viral proteins), and reagents used during manufacture, such as cytokines, growth factors, antibodies, selection beads, serum, and solvents.

A common process-related impurity for many vector preparations is residual nucleic acid, such as cell substrate DNA, which can co-purify with the vector. Some vectors, including AAV, can also package (i.e., inside the viral capsid) a large amount of plasmid DNA sequences (used during transfection) as well as cellular DNA. The presence of these impurities may have adverse effects on product quality and safety. We recommend that you optimize your manufacturing process to reduce non-vector DNA contamination in your product. Additionally, you should monitor and control the amount of extraneous nucleic acid sequences in your product.

Since some cell substrates also harbor tumorigenic genetic sequences or retroviral sequences that may be capable of transmitting infection, we recommend that you take steps to minimize the biological activity of any residual DNA associated with your vector. This can be accomplished by reducing the size of the DNA to below the size of a functional gene and by decreasing the amount of residual DNA. We recommend that you limit the amount of residual DNA for continuous non-tumorigenic cells to less than 10 ng/dose and the DNA size to below approximately 200 base pairs.

If you are using cells that are tumor-derived (e.g., Hela) or with tumorigenic phenotypes (e.g., 293, also known as HEK293T) or other characteristics that give rise to special concerns, more stringent limitation of residual DNA quantities may be needed to assure product safety. In addition to controlling host cell DNA content and size, as described above, you should also control the level of relevant transforming sequences in your product with acceptance criteria that limit patient exposure. For example, products made in 293 cells should be tested for adenovirus E1 and SV40 Large T antigen sequences. Your tests should be appropriately controlled and of sufficient sensitivity and specificity to determine the level of these sequences in your product.

Some vectors, including AAV, can package a large amount of non-vector DNA (e.g., plasmid DNA, helper virus sequences, cellular DNA), and it may not be possible to remove or reduce this DNA from the product to a level sufficient to assure safety. Therefore, we strongly recommend that the cell lines and helper sequences used to make viral vectors that package non-vector DNA, such as AAV, be carefully chosen to reduce the risks of the product.

ii. Product-Related Impurities

Typical product-related impurities for viral vectors may include defective interfering particles, non-infectious particles, empty capsid particles, or replicating recombinant virus contaminants. These impurities should be measured and may be reported as a ratio, for example, full:empty particles or virus particles:infectious units.

For ex vivo genetically modified cells, product-related impurities include non-target cells, which may be present after selection or enrichment, and unmodified target cells, which may be present after the ex vivo modification step. We recommend that you evaluate the nature and number of non-target cells and measure the percentage of cells that have been genetically modified. As you develop a greater understanding of the cellular phenotypes present in your product during clinical development, you may also consider adding impurity tests for specific cell populations in order to establish greater manufacturing control.

4. Control of Drug Substance (3.2.S.4)

a. Specification (3.2.S.4.1)

You should list DS specifications in your original IND submission. Specifications are defined as a list of tests, references to analytical procedures, and appropriate acceptance criteria used to assess quality. Acceptance criteria should be established and justified, based on data obtained from lots used in preclinical and/or clinical studies, data from lots used for demonstration of manufacturing consistency, data from stability studies, and relevant development data.

For products in the early stages of clinical development, very few specifications are finalized, and some tests may still be under development. However, the testing plan submitted in your IND should be adequate to describe the physical, chemical, or biological characteristics of

the DS necessary to ensure that the DS meets acceptable limits for identity, strength (potency), quality, and purity (21 CFR 312.23(a)(7)(iv)(*a*)).

Your IND should include specifications with established acceptance criteria for safety testing at Phase 1. Safety testing includes tests to ensure freedom from extraneous material, adventitious agents, microbial contamination, and replication competent virus. Information on some common safety test methods is provided in more detail in the following section (see "Analytical Procedures (3.2.S.4.2)," section V.A.4.b. of this guidance). To maximize the sensitivity of safety testing, it is important that you perform each test at the stage of production at which contamination is most likely to be detected. For example, tests for mycoplasma or adventitious viruses (in vivo or in vitro) should be performed on cell culture harvest material (cells and supernatant) prior to further processing, e.g., prior to clarification, filtration, purification, and inactivation.

Your IND should also include specifications for measuring an appropriate dose level (i.e., strength or potency) at Phase 1. Assays used to determine dose (e.g., vector genome titer by quantitative polymerase chain reaction (qPCR), transducing units, plaque-forming units, transduced cells) should be well-qualified prior to initiating dose escalation studies. Information on how to qualify your dose determining assay is provided in "Validation of Analytical Procedures (3.2.S.4.3)" (section V.A.4.c. of this guidance).

Additional testing will depend on the type of gene therapy product and the phase of clinical development. These tests may include assays to assess product characteristics, such as identity, purity (including endotoxin and contaminants, such as residual host cell DNA, bovine serum albumin (BSA), DNase), and potency/strength. For additional information on potency tests, please refer to the FDA's Guidance for Industry "Potency Tests for Cellular and Gene Therapy Products," dated January 2011 (Ref. 19).

Please note that not all testing listed in this section of the guidance is required for release of both the DS and DP. In some cases, repeat testing may be good practice; however, redundant testing may not always be feasible or practical. In this case, we recommend that you provide a rationale to support the selection of testing performed for release of either DS or DP.

We provide some additional comments regarding tests for product characterization and impurities under "Specifications (3.2.P.5.1)" (section V.B.5.a. of this guidance).

b. Analytical Procedures (3.2.S.4.2)

You should provide a description of all the analytical procedures used during manufacturing to assess your manufacturing process and product quality. In your original IND submission, your descriptions should have sufficient detail so that we can understand and evaluate the adequacy of your procedures. We recommend that you develop detailed SOPs for how your analytical procedures are conducted at early stages of product development as a part of your quality system. We acknowledge that, during product development, analytical methods may be modified to improve control and suitability. However, assay control is necessary during all phases of clinical development to ensure product quality and safety and to allow for comparability studies, following manufacturing changes.

Documentation submitted in support of your analytical procedures should describe in detail how a procedure is performed and should specify any reference standards, equipment, and controls to be used. Submission of information, such as individual SOPs or batch records, will generally not be necessary, provided descriptions of your analytical procedures are sufficiently detailed in your IND. Contractor test reports are acceptable, provided there is adequate description of the analytical procedure, test sensitivity, specificity, and controls.

i. Safety Testing

Safety testing on the DS should include microbiological testing, such as bioburden (or sterility, as appropriate), mycoplasma, and adventitious viral agent testing, to ensure product quality. Guidelines and/or procedures for many safety tests have been described in detail, elsewhere (e.g., bioburden,[5] sterility,[6] mycoplasma (Ref. 20), adventitious agent testing, and tests for specific pathogens (Ref. 12)). Analytical procedures different than those outlined in the United States Pharmacopeia (USP), FDA guidance, or Code of Federal Regulations (CFR) may be acceptable under IND if you provide adequate information on your test specificity, sensitivity, and robustness. Examples of

[5] USP<61> describes membrane filtration, plate count, and most probable number methods that can be done to quantitatively determine the bioburden of non-sterile DPs. Although 21 CFR 211.110(a)(6) does not specify a test method, it requires that bioburden in-process testing be conducted pursuant to written procedures during the manufacturing process of DPs.

[6] Sterility testing may be performed on the DS when it cannot be performed on the DP, as outlined in the final rule: Amendments to Sterility Test Requirements for Biological Products (May 3, 2012; 77 FR 26162 at 26165). Sterility tests are described in 21 CFR 610.12 and USP<71> Sterility Tests.

alternative methods, which may be needed for live cells, include rapid sterility tests, rapid mycoplasma tests (including PCR-based tests), and rapid endotoxin tests. We recommend that you plan to demonstrate equal or greater assurance of your test methodology, compared to a compendial method, prior to licensure, as required under 21 CFR 610.9. We provide some additional comments regarding these tests under "Specifications (3.2.P.5.1)" (section V.B.5.a. of this guidance) as well as comments regarding replication competent virus and wild-type oncolytic virus testing, below.

ii. Replication Competent Virus

For many gene therapy viral vectors, we recommend specific testing, due to the potential for these vectors to recombine or revert to a parental or wild-type (WT) phenotype at a low frequency. Tests for replication-competent, parental, or wild-type viruses that may be generated during production (e.g., replication-competent adenovirus (RCA) and replication-competent retrovirus (RCR)) should be performed on material collected at the appropriate stage of the manufacturing process. For example, we recommend testing banked material for the presence of replication-competent viruses and as a specification for in-process or release testing of DS or DP, as appropriate (please see further details, below, within this section).

A. Replication-Competent Retrovirus (RCR) Testing

Retroviral-based products (including lentivirus and foamy virus-based products) used for most gene therapy applications are designed to be replication defective. To ensure the absence of RCR, you should perform testing for RCR at multiple points, during production of a retroviral vector. For further information on retroviral testing, refer to "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," dated November 2006 (Ref. 21). This guidance will be superseded by "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 (Ref. 22), when finalized.

B. Replication-Competent Adenovirus (RCA) Testing

The adenoviral-based products used for most gene therapy applications are designed to be replication defective. A notable exception is oncolytic adenoviruses (see "Wild-Type Oncolytic Virus Testing" in section V.A.4.b.ii.*D*. of this guidance). RCA may be generated at a low frequency as a result of homologous recombination between viral vector sequences and viral sequences present in the cell substrate, during manufacturing. Therefore, for most adenoviral-based products, we recommend that you qualify your MVB for RCA and test either the DS or DP of each production lot for RCA. We recommend a maximum level of 1 RCA in 3×10^{10} viral particles.

C. Replication-Competent AAV (rcAAV) Testing

Preparations of AAV vectors can be contaminated with helper virus-dependent rcAAV, also referred to as wild-type AAV or pseudo wild-type AAV. These rcAAV are generated through homologous or non-homologous recombination events between AAV elements present on the vector and AAV rep and cap sequences that are present, during manufacture. While wild-type AAV has no known associated pathology and cannot replicate without helper virus, expression of cap or rep genes in infected cells can result in unintended immune responses, which can reduce effectiveness and may have unintended safety risks.

Therefore, we recommend that you test for rcAAV, which could potentially replicate in the presence of helper virus, and report these results. A number of methods have been published for evaluating the level of rcAAV, including amplification of AAV in the presence of helper virus, followed by PCR for rep/inverted terminal repeats (ITR) junctions, and PCR for rep and cap sequences, following DNase digestion of the vector preparation. We do not recommend a specific method for determining rcAAV in this guidance. You should describe your test method and assay sensitivity in the IND.

D. Wild-Type Oncolytic Virus Testing

Most oncolytic viruses used in gene therapy applications not only carry transgenes but also have been attenuated or adapted from a parental virus strain to grow selectively in cancer cells. It may be possible for these attenuated or adapted viruses to either recombine or revert to a parental (or WT) genotype, during manufacture. Therefore, we recommend that you conduct tests to determine whether the parental virus sequences are present in your product. In addition, we recommend that you select production cells that do not contain viral sequences that may allow homologous recombination with the product. For example, we do not recommend 293 cell substrates for the manufacture of E1-modified oncolytic adenoviruses, due to the potential for homologous recombination with E1 sequences in the 293 cells.

c. Validation of Analytical Procedures (3.2.S.4.3)

Validation of analytical procedures is usually not required for original IND submissions for Phase 1 studies; however, you should demonstrate that test methods are appropriately controlled. In general, scientifically sound principles for assay performance should be applied (i.e., tests should be specific, sensitive, and reproducible and include appropriate controls or standards). We recommend that you use compendial methods when appropriate and qualify safety-related tests prior to initiation of clinical trials.

To ensure safety of gene therapy products, you should also qualify the assays used to determine dose (e.g., vector genome titer by qPCR, transducing units, plaque forming units) prior to initiating dose escalation studies. In your original IND submission, you should provide a detailed description of the qualification protocol (e.g., samples; standards; positive/negative controls; reference lots; and controls evaluated, such as operators, reagents, equipment, dates) and data supporting the accuracy, reproducibility, sensitivity, and specificity of the method. Also critical to ensuring safety is the ability to compare the dose used for preclinical evaluations to the dose to be used for clinical studies. One way to ensure that the doses compare is to use the same qualified method to quantitate preclinical and clinical lots. If it is not possible to use the same qualified method, we recommend that you retain sufficient quantities of preclinical material to enable side by side testing with the clinical material, using the

same qualified method. In addition, you should validate tests used to determine dose prior to initiating clinical studies to demonstrate efficacy or support licensure.

Assays used to measure RCR and RCA should also meet our current recommendations for sensitivity at an early stage of development (see descriptions "RCR Testing" and "RCA Testing" (section V.A.4.b.ii.*A.* and *B.* of this guidance). We recommend that you include relevant positive and negative controls when conducting these tests and include positive controls spiked in the test article to assess whether there are any inhibitory effects of the test article on detection.

For all analytical procedures, we recommend that you evaluate assay performance throughout product development, have a validation plan in place during later phase clinical studies, and complete validation before BLA submission. For more information on validation of analytical methods, please see the FDA's Guidance for Industry: "Q2B Validation of Analytical Procedures: Methodology," dated November 1996 (Ref. 23).

d. Batch Analysis (3.2.S.4.4)

You should include a table with test results for all of the batches (or lots) of DS that you have manufactured. For early stage INDs, this may include only toxicology lots or developmental batches and a single manufacturing run for clinical grade material. Please note that batches manufactured in different ways should be clearly identified in the submission. We recommend that you annually update this section of your IND as new batches are produced. You should indicate any batches that fail to meet release specifications and any action taken to investigate the failure (as outlined in "Process Validation and/or Evaluation (3.2.S.2.5)" (section V.A.2.e. of this guidance). We recommend that you retain samples of all production lots for use in future assay development, validation, or comparability studies.

e. Justification of Specification (3.2.S.4.5)

You should provide justification for the DS specifications in your IND. We recognize that acceptance criteria may be adjusted throughout the product development stages, based on both manufacturing and clinical experience. For early stage clinical studies, production lots may be more variable than those used in later phase investigations.

For later stage investigational studies in which the primary objective is to gather meaningful data about product efficacy, we recommend that acceptance criteria be tightened to ensure batches are well-defined and consistently manufactured.

5. Reference Standards or Materials (3.2.S.5)

You should provide information on the reference standards or reference materials used for testing the DS in your original IND submission. We recommend that you provide the source and lot number; expiration date; certificates of analyses, when available; and/or internally or externally generated evidence of identity and purity for each reference standard.

Three types of reference standards are generally used: 1) certified reference standards (e.g., USP compendial standards); 2) commercially supplied reference standards obtained from a reputable commercial source; and/or 3) other materials of documented purity, custom-synthesized by an analytical laboratory or other noncommercial establishment. In some cases, the reference material for an assay will be a well-characterized lot of the gene therapy product, itself. In this case, we recommend that you reserve and maintain a sufficient amount of material (e.g., part of a production lot) to serve as a reference material.

6. Container Closure System (3.2.S.6)

You should describe the type(s) of container and closure used for the DS in your original IND submission, including the identity of materials used in the construction of the container closure system. We recommend that you determine whether the containers and closures are compatible with the DS. For an original IND submission, compatibility with a gene therapy product may be evaluated during stability studies or may be based on historical data and experience, using similar products. You should indicate whether the container is an approved or cleared device and/or the information is cross-referenced to a master file, as described in section III. "Administrative Information" of this guidance.

7. Stability (3.2.S.7)

a. Stability Summary and Conclusions (3.2.S.7.1)

We recommend that you describe in your original IND submission the types of stability studies (either conducted or planned) to demonstrate that the DS is within acceptable limits. The protocol should describe the storage container, formulation, storage conditions, testing frequency, and specifications (i.e., test methodologies and acceptance criteria). Please

note that stability studies may evolve with product development, and if DS is immediately processed into DP, long term DS stability data may not be needed.

Your stability analysis may include measures of product sterility (or container integrity), identity, purity, quality, and activity or potency. We recommend that you provide justification for the test methods and acceptance criteria used in the stability analysis. It is often helpful to demonstrate that at least one or more of the test methods in your stability analysis are stability-indicating. You may demonstrate a test is stability-indicating, using forced degradation studies, accelerated stability studies, or another type of experimental system that demonstrates product deterioration. Information to help you design your stability studies may be found in the following guidance documents: FDA "Guideline for Industry: Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products," dated July 1996 (Ref. 24); FDA "Guidance for Industry: Q1A(R2) Stability Testing of New Drug Substances and Products," dated November 2003 (Ref. 25); and FDA "Guidance for Industry: Q1E Evaluation of Stability Data," dated June 2004 (Ref. 26).

b. Post-Approval Stability Protocol and Stability Commitment (3.2.S.7.2)

We do not recommend that you provide a post-approval stability protocol and stability commitment in the IND. However, as you progress with product development, you may want to consider which stability studies would be required to determine an expiry date for the approved product or to support post-approval changes to expiry. We recommend the discussion of these items at your late phase IND meetings.

c. Stability Data (3.2.S.7.3)

We recommend that you provide the results of your stability studies in your IND and update this information on a regular basis (e.g., annual reports). Information on the qualification of analytical procedures used to generate stability data should be included in your original IND submission.

B. Drug Product (3.2.P)

1. Drug Product Description and Composition (3.2.P.1)

You should provide a description of the DP and its composition (21 CFR 312.23(a)(7)(iv)*(b)*). This includes a description of the dosage form and a list of

all of its components (active and inactive), the amount on a per unit basis, the function, and a reference to quality standards for each component (e.g., compendial monograph or manufacturers' specifications). If a drug or device will be used with your gene therapy as a combination product, we recommend that quality information for the drug or device be included in section 3.2.P of the CTD with appropriate hyperlinks to section 3.2.R of the CTD, as described in the FDA "eCTD Technical Conformance Guide: Technical Specifications Document," dated November 2017 (Ref. 4). If a placebo treatment is used in the clinical trial, a separate DP section should be provided for the placebo. In addition, you should provide a description of any accompanying reconstitution diluents and a description of the container and closure used for the dosage form and accompanying reconstitution diluent in a separate DP section, if applicable.

2. Pharmaceutical Development (3.2.P.2)

The Pharmaceutical Development section should contain information on the development studies conducted to establish that product formulation, manufacturing process, container closure system, microbiological attributes, and instructions for use are appropriate for the stage of clinical development. The studies described here are distinguished from routine control tests conducted, according to specifications. Additionally, this section should identify and describe the formulation and process attributes (critical parameters) that can influence batch reproducibility, product performance, and DP quality. Supportive data and results from specific studies or published literature can be included within or attached to the Pharmaceutical Development section. Additional supportive data can be referenced to the relevant nonclinical or clinical sections of the application.

a. Components of the Drug Product (3.2.P.2.1)

i. Drug Substance (3.2.P.2.1.1)

You should describe the compatibility of the DS with the components listed in "Description and Composition of the Drug Product" (section 3.2.P.1 of the CTD) and the key characteristics of the DS (e.g., concentration, viability, aggregation state, viral infectivity) that can influence the performance of the DP.

ii. Excipients (3.2.P.2.1.2)

You should describe in your original IND submission the choice of excipients and inactive components of the DP listed in "Description and Composition of the Drug Product" (section 3.2.P.1 of the CTD), their concentration, and the characteristics of these excipients that can influence DP performance.

b. Drug Product (3.2.P.2.2)

i. Formulation Development (3.2.P.2.2.1)

You should briefly describe the development of the DP formulation, taking into consideration the proposed route of administration and usage in your IND.

We recommend that you describe any other formulations that have been used in clinical or preclinical studies and provide a reference to such studies, if applicable. If formulation changes were needed for stability, device compatibility, or safety concerns, this information can be reported here.

ii. Overages (3.2.P.2.2.2)

In your IND, you should describe whether gene therapy product in excess of your label claim is added during formulation to compensate for degradation during manufacture or a product's shelf life or to extend shelf life. We do not recommend the use of overages, and we recommend that you provide justification for an overage, as described in Guidance for Industry: "Q8(R2) Pharmaceutical Development," dated November 2009 (Ref. 6).

iii. Physicochemical and Biologic Properties (3.2.P.2.2.3)

You should describe the parameters relevant to the performance of the DP in your IND. These parameters include physicochemical or biological properties of the product (e.g., dosing units, genotypic or phenotypic variation, particle number and size, aggregation state, infectivity, specific activity (ratio of infectious to non-infectious particles or full to empty particles), biological activity or potency, and/or immunological activity). Understanding these parameters and how they affect product performance usually occurs over the course of product development. More information on pharmaceutical development and consideration in establishing critical quality attributes during the clinical research phase can be found in Guidance for Industry: "Q8(R2) Pharmaceutical Development," dated November 2009 (Ref. 6).

You should update this section on the physiochemical and biological properties of your product as you gain a better understanding of the CQA, during development.

c. Manufacturing Process Development (3.2.P.2.3)

You should describe the selection and optimization of the DP manufacturing process (described in "Description of Manufacturing Process and Process Controls," section 3.2.P.3.3 of the CTD) if development studies have been performed.

d. Container Closure System (3.2.P.2.4)

You should describe the suitability of the container closure system, which you have described in the "Container Closure System" (section 3.2.P.7 of the CTD), for the storage, transportation (shipping), and use of the DP.

We recommend that you consider choice of materials, protection from moisture and light, compatibility with the formulation (including adsorption to the container and leaching), safety of materials, and performance. For more information on container closure systems, refer to FDA's "Guidance for Industry: Container Closure Systems for Packaging Human Drugs and Biologics," dated May 1999 (Ref. 27).

In the selection of your container closure system, we also recommend that you consider how lots of your product will be tested for final product release. For gene therapy products that are manufactured in small lot sizes (e.g., autologous cell products or products vialed at very high dose levels), it may be challenging or not possible to dedicate a final container or multiple vials for lot release testing. In this case, we recommend that you consider a final container that can be sampled for release testing or that you consider alternatives to final container testing.

e. Microbiological Attributes (3.2.P.2.5)

We recommend, for live products intended to be sterile, that you provide details on measures taken to ensure aseptic processing, describe the final product microbial testing, and address how the integrity of the container closure system to prevent microbial contamination will be assessed.

f. Compatibility (3.2.P.2.6)

You should discuss the compatibility of the DP with the diluent used for reconstitution or the delivery device, as appropriate.

We recommend that compatibility studies include measures of both product quantity and product activity (e.g., for viral vectors, a measure of physical particles and infectivity to assess both adsorption and

inactivation). This in-use and in-device stability data should support recommended hold times and conditions outlined in the clinical protocol for patient administration.

3. Manufacture (3.2.P.3)

a. Manufacturers (3.2.P.3.1)

You should provide the name, address, and responsibility of each manufacturer, including contractor manufacturer(s), involved in the manufacture and testing of the DP.

For gene therapy-device combination products, we recommend that you list the manufacturing facilities for the device components and describe the assembly and testing processes taking place at each site, as described in FDA's eCTD Technical Conformance Guide (Ref. 4). You should also identify whether facilities follow the combination product streamlined manufacturing approach (as described in FDA's Guidance for Industry and FDA Staff: "Current Good Manufacturing Practice Requirements for Combination Products," dated January 2017 (Ref. 28) and identify the specific set of regulations (i.e., 21 CFR Part 211 or Part 820).

b. Batch Formula (3.2.P.3.2)

You should provide a batch formula that includes a list of all components of the dosage form, their amounts on a per-batch basis, and a reference to their quality standards.

c. Description of Manufacturing Process and Process Controls (3.2.P.3.3)

You should provide a detailed description of the DP manufacturing process and identify process controls, intermediate tests, and final product controls. Your description should include both flow diagram(s) and narrative description(s) as well as packaging, product contact materials, and equipment used. This process can include manufacturing steps, such as final formulation, filtration, filling and freezing, and process controls and release testing. For ex vivo genetically modified cells that are administered immediately after manufacturing, an in-process sterility testing on sample taken 48 to 72 hours prior to final harvest is one part of the sterility testing recommended for product release. Please see "Microbiological Attributes (3.2.P.2.5)" (section V.B.2.e. of this guidance) for more information on final product sterility testing for fresh cells.

d. Controls of Critical Steps and Intermediates (3.2.P.3.4)

You should describe the control of critical steps and intermediates in the manufacturing process. Critical steps should include those outlined in the "Description of Manufacturing Process and Process Controls" (section 3.2.P.3.3 of the CTD) to ensure control as well as steps in which tests with acceptance criteria are performed. We recommend that you provide justification for acceptance criteria or limits set for these tests. In addition, you should provide information on the quality and control of intermediates of the manufacturing process. Manufacturing intermediates are defined by the manufacturer and may include material from collection steps or hold steps.

e. Process Validation and/or Evaluation (3.2.P.3.5)

Process validation is not required for early stage manufacturing, and thus, most original IND submissions will not include this information. However, we do recommend that early stage INDs have information on methods used to prevent contamination, cross-contamination, and product mix-ups. For more information on functions of the Quality Unit under IND, please see "Process Validation and/or Evaluation (3.2.S.2.5)" (section V.A.2.e. of this guidance).

4. Control of Excipients (3.2.P.4)

a. Specifications (3.2.P.4.1)

You should provide specifications for all excipients listed in "Excipients" (section 3.2.P.2.1.2 of the CTD). For purpose of this guidance, an excipient is any component, in addition to the active ingredient, that is intended to be part of the final product (e.g., human serum albumin or Dimethyl Sulfoxide (DMSO)).

b. Analytical Procedures (3.2.P.4.2)

You should describe your analytical procedures for testing excipients.

c. Validation of Analytical Procedures (3.2.P.4.3)

Validation of analytical procedures is usually not required for original IND submissions. We recommend that you provide any available validation information for the analytical procedures used to test excipients.

d. Justification of Specifications (3.2.P.4.4)

You should provide justification for the proposed excipient specifications.

e. Excipients of Human or Animal Origin (3.2.P.4.5)

For excipients of human or animal origin, you should provide information regarding source, specifications, description of testing performed, and viral safety data. For human serum, we recommend that you submit information documenting donor suitability as well as appropriate infectious disease testing. You should ensure that collection is performed by a licensed blood bank and that testing meets the requirements described in 21 CFR Part 640.

f. Novel Excipients (3.2.P.4.6)

For excipients used for the first time in a DP or used for the first time in a route of administration, you should provide full details of manufacture, characterization, and controls, with cross-references to supporting safety data (nonclinical and/or clinical).

5. Control of Drug Product (3.2.P.5)

a. Specifications (3.2.P.5.1)

You should list DP specifications in your original IND submission. Your testing plan should be adequate to describe the physical, chemical, or biological characteristics of the DP necessary to ensure that the DP meets acceptable limits for identity, strength (potency), quality, and purity (21 CFR 312.23(a)(7)(iv)(*b*)). Product lots that fail to meet specifications should not be used in your clinical investigation without FDA approval. For early phase clinical studies, we recommend that assays be in place to assess safety (which includes tests to ensure freedom from extraneous material, adventitious agents, and microbial contamination) and dose (e.g., vector genomes, vector particles, or genetically modified cells) of the product. Additional information on safety testing and measuring product dose is described in "Specification (3.2.S.4.1)" (section V.A.4.a. of this guidance).

We recommend that product release assays be performed at the manufacturing step at which they are necessary and appropriate. For example, mycoplasma and adventitious agents release testing is recommended on cell culture harvest material, as discussed in "Specification (3.2.S.4.1)" (section V.A.4.a. of this guidance). In

addition, sterility, endotoxin, and identity testing are recommended on the final container product to ensure absence of microbial contamination or to detect product mix-ups that might have occurred during the final DP manufacturing steps (e.g., buffer exchange, dilution, or finish and fill steps). DP specifications should be further refined as a part of product development under IND. We recommend that sponsors establish or, in some cases, tighten acceptance criteria, based on manufacturing experience as clinical development proceeds. Acceptance criteria should also be established, based on clinical lots shown to be safe and effective, when appropriate. We also recommend that sponsors develop testing to assess product potency and have this assay in place prior to pivotal studies. For licensure, a complete set of specifications to ensure the safety and effectiveness of the product must include the general biological products standards, as outlined in 21 CFR Part 610.

b. Analytical Procedures (3.2.P.5.2)

You should describe the analytical procedures used for testing the DP. If the analytical procedures are the same as those for the DS, you do not need to repeat this information unless there is a matrix effect from the DP on assay performance. Please reference the appropriate section of your IND, where this information can be found (e.g., Drug Substance "Analytical Procedures," section 3.2.S.4.2 of the CTD). We have the following additional comments regarding these tests:

i. Sterility

We recognize that the compendial sterility test may not be suitable for all products. As mentioned in "Analytical Procedures" (section 3.2.S.4.2 of this guidance), rapid sterility tests may be needed for ex vivo genetically modified cells administered fresh or with limited hold time between final formulation and patient administration.

For ex vivo genetically modified cells that are administered immediately after manufacturing, in-process sterility testing on sample taken 48 to 72 hours prior to final harvest is recommended for product release. For such products, aside from an in-process sterility test, we also recommend that sponsors perform a rapid microbial detection test, such as a Gram stain, on the final formulated product and a sterility test, compliant with 21 CFR 610.12, on the final formulated product.

Under this approach, the release criteria for sterility would be based on a negative result of the Gram stain and a no-growth result

from the 48 to 72 hour in-process sterility test. Although the results of the sterility culture performed on the final product will not be available for product release, this testing will provide useful data. A negative result will provide assurance that an aseptic technique was maintained. A positive result will provide information for the medical management of the subject and trigger an investigation of the cause of the sterility failure. The sterility culture on the final formulated product should be continued for the full duration (usually 14 days) to obtain the final sterility test result, even after the product has been administered to the patient.

In all cases where product release is prior to obtaining results from a full 14-day sterility test, the investigational plan should address the actions to be taken in the event that the 14-day sterility test is determined to be positive after the product is administered to a subject. You should report the sterility failure to both the clinical investigator and FDA. We recommend that you include results of investigation of cause and any corrective actions in an information amendment submitted to your IND within 30 calendar days after initial receipt of the positive culture test result (21 CFR 312.31).

In the case of a positive microbial test result, the clinical investigator should evaluate the subject for any signs of infection that may be attributable to the product sterility failure. If the patient experiences any serious and unexpected adverse drug event that could be from administration of the non-sterile gene therapy product, then you must report this information to FDA in an IND safety report no more than 15 calendar days after your initial receipt of the information (21 CFR 312.32). If you determine that an investigational drug presents an unreasonable and significant risk to subjects of a positive microbial test result or for any other reason, you must discontinue those investigations that present the risk and notify FDA, all Institutional Review Boards, and all investigators (21 CFR 312.56(d)).

In addition, please be aware that a product may sometimes interfere with the results of sterility testing. For example, a product component or manufacturing impurities (e.g., antibiotics) may have mycotoxic or anti-bacterial properties. Therefore, we recommend that you assess the validity of the sterility assay using the bacteriostasis and fungistasis testing, as described in USP <71> Sterility Tests.

If you freeze DP before use, we recommend that you perform sterility testing on the product prior to cryopreservation so that results will be available before the product is administered to a patient. However, if the product undergoes manipulation after thawing (e.g., washing, culturing), particularly if procedures are performed in an open system, you may need to repeat sterility testing.

We recommend that you incorporate the results of in-process sterility testing into your acceptance criteria for final product specifications.

ii. Identity

We recommend that identity assays uniquely identify a product and distinguish it from other products in the same facility. This test is performed on the final labeled product to verify its contents (21 CFR 610.14). Sometimes, a single test is not sufficient to distinguish clearly among products, and therefore, it is good practice to use different types of test methods (e.g., vector genome restriction digest and protein capsid analysis).

If the final product is ex vivo genetically modified cells, we recommend that identity testing include an assay to measure the presence of vector (i.e., expression assay, restriction digest) or genetic change and an assay specific for the cellular composition of the final product (e.g., cell surface markers).

iii. Purity

Product purity is defined as the relative freedom from extraneous matter in the finished product, whether or not it is harmful to the recipient or deleterious to the product (21 CFR 600.3). Purity testing includes assays for pyrogenicity or endotoxin and residual manufacturing impurities, as outlined under "Impurities (3.2.S.3.2)" (section V.A.3.b. of this guidance) of drug substance, which include but are not limited to proteins; DNA; cell debris; reagents/components used during manufacture, such as cytokines, growth factors, antibodies, and serum; and in the case of ex vivo genetically modified cells, any unintended cellular populations.

Although the rabbit pyrogen test method is the current required method for testing licensed biological products for pyrogenic substances (21 CFR 610.13), we generally accept alternative test methods, such as the Limulus Amebocyte Lysate (LAL), under

IND. For any parenteral drug, except those administered intrathecally, we recommend that the upper limit of acceptance criterion for endotoxin be 5 EU/kg body weight/hour. For intrathecally-administered drugs, we recommend an upper limit of acceptance be set at 0.2 EU/kg body weight/hour.

iv. Potency

You should describe and justify in your IND all assays that you will use to measure potency. A potency assay is not required to initiate early phase clinical studies, but we recommend that you have a well-qualified assay to determine dose, as described below and in "Validation of Analytical Procedures (3.2.S.4.3)" (section V.A.4.c. of this guidance). For additional information on potency assays, please see FDA's "Guidance for Industry: Potency Tests for Cellular and Gene Therapy Products," dated January 2011 (Ref. 19).

v. Viability

You should establish minimum release criteria for viability, where appropriate. For ex vivo genetically modified cells, we recommend a minimum acceptable viability of at least 70 percent. If this level cannot be achieved, we recommend that you submit data in support of a lower viability specification, demonstrating, for example, that dead cells and cell debris do not affect the safe administration of the product and/or the therapeutic effect.

vi. Cell Number or Dose

Your dose-determining assay is an important part of your product specifications. For additional information on your dose-determining assay, please see "Specification (3.2.S.4.1)" (section V.A.4.a. of this guidance). If your final product is a genetically modified cell therapy, you should have an acceptance criterion for the minimum number of modified cells in a product lot. We recommend that the product dose be based on the total number of genetically modified cells.

c. Validation of Analytical Procedures (3.2.P.5.3)

Validation of analytical procedures is usually not required for original IND submissions, but we do recommend that you qualify certain safety-related or dose-related assays, even at an early stage of development (see

"Validation of Analytical Procedures (3.2.S.4.3)," section V.A.4.c. of this guidance). If they are the same as those listed for DS testing, you do not need to repeat them but should reference that section of your IND.

d. Batch Analyses (3.2.P.5.4)

You should provide final product COA(s) or a description of batches and results of batch analyses for the DP.

e. Characterization of Impurities (3.2.P.5.5)

You should provide information on characterization of impurities if not previously provided in "Impurities" (section 3.2.S.3.2 of the CTD).

f. Justification of Specifications (3.2.P.5.6)

You should provide justification for the DP specifications. See "Justification of Specification (3.2.S.4.5)" (section V.A.4.e. of this guidance) for additional details.

6. Reference Standards or Materials (3.2.P.6)

You should provide information on the reference standards or reference materials used in testing the DP if not previously provided in "Reference Standards or Materials" (section 3.2.S.5 of the CTD).

7. Container Closure System (3.2.P.7)

You should provide a description of the container closure systems, including identity of materials of construction or each primary packaging component and its specification. You should also provide information on how the container is sterilized.

Please see "Container Closure System (3.2.P.2.4)" (section V.B.5.d. of this guidance) for more information and recommendations, regarding the suitability of different final product containers.

If the final container is an FDA-cleared device, we recommend that you reference the 510(k) number for the device in your submission. For device combination products, we recommend that you include a table of contents for the combination product (with reference links to other files) in this section, as described in FDA's eCTD Technical Conformance Guide (Ref. 4).

8. Stability (3.2.P.8)

a. Stability Summary and Conclusion (3.2.P.8.1)

You should summarize the types of studies conducted, protocols used, and the results of the studies. Your summary should include, for example, conclusions regarding storage conditions and shelf life as well as in-use and in-device storage conditions.

If a short-term clinical investigation is proposed, or if a continuous manufacturing process with limited product hold times is used, stability data submitted may be correspondingly limited. For early stage INDs, stability data for the gene therapy may not be available to support the entire duration of the proposed clinical investigation. Therefore, we recommend that you submit a prospective plan to collect stability information and update this information to the IND in a timely manner (e.g., in an annual IND update).

b. Post-Approval Stability Protocol and Stability Commitment (3.2.P.8.2)

We do not recommend that you provide a post-approval stability protocol and stability commitment in your IND submission. However, as product development continues, we recommend that you consult with your Quality Reviewer to determine the type of studies that will be necessary to support product expiration dates for commercial manufacturing.

c. Stability Data (3.2.P.8.3)

You should provide results of the stability studies in your IND in an appropriate format (e.g., tabular, graphic, narrative). Information on the analytical procedures used to generate the data should also be included, and this may be referenced to other sections of your submission (e.g., "Analytical Procedures," section 3.2.P.5.2 of the CTD).

C. Appendices (3.2.A)

1. Facilities and Equipment (3.2.A.1)

You should provide a diagram, illustrating the manufacturing flow of the manufacturing areas, information on all developmental or approved products manipulated in this area, a summary of product contact equipment, and information on procedures and design features of the facility, to prevent contamination or cross-contamination.

A description of the Quality Unit and the quality control (QC) and quality assurance (QA) responsibilities may be included in this section.

COAs for all raw materials and reagents described in your IND may be put in this section.

2. Adventitious Agents Safety Evaluation (3.2.A.2)

You should provide information assessing the risk of potential contamination with adventitious agents. For non-viral adventitious agents, we recommend that you provide detailed information on the avoidance and control of transmissible spongiform encephalopathy agents, bacteria, mycoplasma, and fungi. This information can include certification and/or testing of components and control of the production process. For viral adventitious agents, we recommend that you provide information on viral safety studies. Study reports and data to support qualification of your manufacturing components (such as adventitious agents test reports for banked materials) may be submitted as a part of this appendix. These studies should demonstrate that the materials used in production are considered safe and that the approaches used to test, evaluate, and eliminate potential risks, during manufacture, are suitable.

Data collected (i.e., study reports) for adventitious agent testing can be placed in this section.

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

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Draft Guidance for Industry

This guidance document is for comment purposes only.

Submit one set of either electronic or written comments on this draft guidance by the date provided in the *Federal Register* notice announcing the availability of the draft guidance. Submit electronic comments to https://www.regulations.gov. Submit written comments to the Dockets Management Staff (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. You should identify all comments with the docket number listed in the notice of availability that publishes in the *Federal Register*.

Additional copies of this guidance are available from the Office of Communication, Outreach and Development (OCOD), 10903 New Hampshire Ave., Bldg. 71, Rm. 3128, Silver Spring, MD 20993-0002, or by calling 1-800-835-4709 or 240-402-8010, or email ocod@fda.hhs.gov, or from the Internet at https://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.

For questions on the content of this guidance, contact OCOD at the phone numbers or email address listed above.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
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Contains Nonbinding Recommendations

Draft – Not for Implementation

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

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Draft Guidance for Industry

This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff responsible for this guidance as listed on the title page.

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

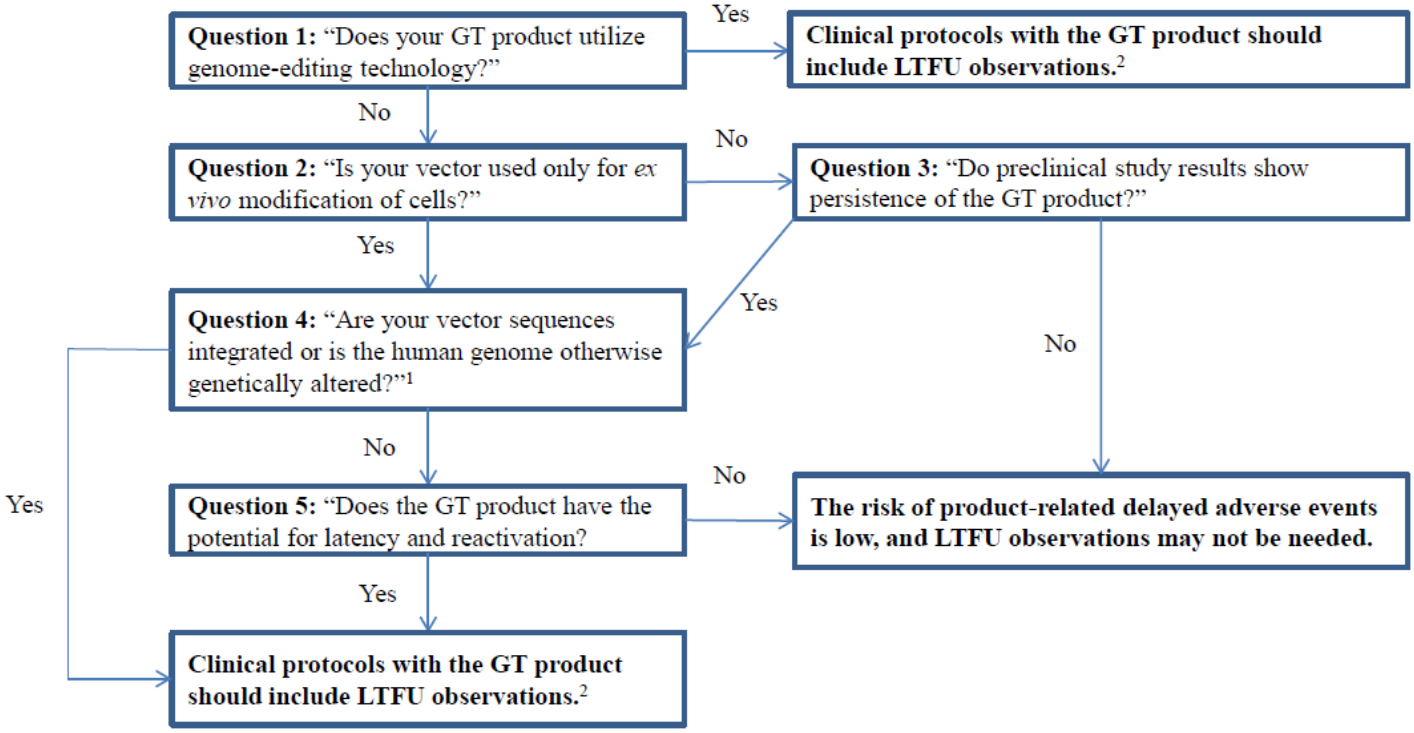
To assess the risk of delayed adverse events for a GT product, we recommend that you use available preclinical and clinical evidence, and current information about your product and similar products based on studies that you and others have performed. In general, when the risk of delayed adverse events 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)

Testing of Retroviral Vector-Based Human Gene Therapy Products for Replication Competent Retrovirus During Product Manufacture and Patient Follow-up

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Draft Guidance for Industry

This guidance document is for comment purposes only.

Submit one set of either electronic or written comments on this draft guidance by the date provided in the *Federal Register* notice announcing the availability of the draft guidance. Submit electronic comments to https://www.regulations.gov. Submit written comments to the Dockets Management Staff (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. You should identify all comments with the docket number listed in the notice of availability that publishes in the *Federal Register*.

Additional copies of this guidance are available from the Office of Communication, Outreach and Development (OCOD), 10903 New Hampshire Ave., Bldg. 71, Rm. 3128, Silver Spring, MD 20993-0002, or by calling 1-800-835-4709 or 240-402-8010, or email ocod@fda.hhs.gov, or from the Internet at https://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.

For questions on the content of this guidance, contact OCOD at the phone numbers or email address listed above.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
July 2018

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Testing of Retroviral Vector-Based Human Gene Therapy Products for Replication Competent Retrovirus During Product Manufacture and Patient Follow-up

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Draft Guidance for Industry

This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff responsible for this guidance as listed on the title page.

I. INTRODUCTION

The potential pathogenicity of replication competent retrovirus (RCR) requires vigilant testing to exclude the presence of RCR in vector-based human gene therapy products (Ref. 1). We, the FDA, are providing you, sponsors of retroviral vector-based human gene therapy products, recommendations regarding the testing for RCR during the manufacture of retroviral vector-based gene therapy products, and during follow-up monitoring of patients who have received retroviral vector-based gene therapy products. Recommendations include the identification and amount of material to be tested as well as general testing methods. In addition, recommendations are provided for monitoring patients for evidence of retroviral infection after administration of retroviral vector-based gene therapy products.

The *Retroviridae* family is composed of two subfamilies: *Orthoretrovirinae*, which consists of six genera of viruses: *Alpharetrovirus*, *Betaretrovirus*, *Gammaretrovirus*, *Deltaretrovirus*, *Epsilonretrovirus*, and *Lentivirus*, and *Spumaretrovirinae* (foamy viruses) which has recently been updated to consist of five genera of viruses: *Bovispumavirus*, *Equispumavirus*, *Felispumavirus*, *Prosimiispumavirus*, and *Simiispumavirus* (Refs. 2, 3). RCR can be generated during the manufacture of a retrovirus vector from any of these genera. At this time, the most common retrovirus-based vectors are constructed from gammaretroviruses or lentiviruses, and therefore further details are provided for these genera. Historically, lentivirus RCR is referred to as replication competent lentivirus (RCL).[1]

This guidance, when finalized, is intended to supersede the guidance entitled, "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" dated November 2006 (2006 RCR Guidance) (Ref. 4). This guidance, when

[1] RCR and RCL are synonymous for the purposes of this guidance.

finalized, is also intended to supplement the following two guidances: the "Long Term Follow-Up After Administration of Human Gene Therapy Products; Draft Guidance for Industry" dated July 2018 (Long Term Follow-up Draft Guidance) and "Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs); Draft Guidance for Industry" dated July 2018 (CMC Draft Guidance).[2]

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

II. BACKGROUND

FDA's Center for Biologics Evaluation and Research (CBER) recommendations for RCR testing during retroviral vector production and patient monitoring were originally developed at a time when clinical experience was limited to a small number of studies using gammaretrovirus vectors (Ref. 5). At that time, the overriding safety concerns associated with the use of retroviral vectors were exemplified by the findings of an animal study involving administration of gammaretroviral vector-transduced bone marrow progenitor cells that had been inadvertently exposed to high-titer RCR, and administered to severely immunosuppressed rhesus monkeys (Ref. 1). In this setting, 3/10 animals developed lymphomas and died within 200 days. The RCR was presumed to be etiologically associated with the disease by virtue of the presence of multiple murine RCR sequences in the lymphomas and an inverse correlation between anti-retroviral antibodies and development of disease (Refs. 6, 7). In contrast, another study in moderately-immunosuppressed cynomolgus monkeys exposed intravenously to RCR showed no signs of disease (Refs. 8, 9).

More than two decades of experience has generated a substantial amount of data on the safety of retroviral vectors in clinical applications for gene therapy, including experience with different vector designs, vector producing cells, RCR detection assays, and lack of positive results from RCR testing of vector lots, ex vivo transduced cells, and patient samples collected during monitoring. These data have provided the basis for public discussions, including Retroviral Breakout Sessions at the 1996 and 1997 FDA/National Institutes of Health (NIH) Gene Therapy Conferences, the 2010 Cellular, Tissue, and Gene Therapies Advisory Committee meeting (Ref. 10), and the 2014 American Society of Gene and Cellular Therapy (ASGCT) Breakout Session

[2] When finalized, these guidances will represent FDA's current thinking on the topics.
The Long Term Follow-up Draft Guidance is available at this website:
https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM610797.pdf
The CMC Draft Guidance is available at this website:
https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM610795.pdf

on Replication Competent Virus (Ref. 11). In addition, FDA scientists published an evaluation of RCR testing methods associated with the use of retroviral vectors (Ref. 12). During this time, the gene therapy community has improved retroviral vector design to reduce the likelihood of generating RCR during the manufacturing process (Refs. 13, 14). For instance, the likelihood that recombination will generate RCR is reduced by manufacturing vectors using a split plasmid design, where the vector genome is on a separate plasmid from the envelope protein and packaging functions. RCR generation can be further reduced by using more than two plasmids for vector production. Lentiviral vectors have been further modified to remove genes encoding accessory and regulatory proteins, which would cripple the functionality of an RCR in the event an RCR may be generated (Refs. 15, 16).

Summary of Revisions from the 2006 RCR Guidance:

With consideration of the accrued scientific evidence of safety associated with retroviral vector design and testing, we are revising our current recommendations for RCR testing during retroviral vector-based gene therapy product manufacture and patient monitoring. More specifically, we are no longer recommending RCR testing on working cell banks for retroviral producer cells. We have also revised our recommendations regarding the amount of vector that should be tested (section III.B and Appendix 1-1 of this document). Briefly, rather than testing based on production lot size we are recommending that you test a sufficient amount of vector to demonstrate that your vector contains <1 RCR per patient dose. Additionally, we are recommending that all retroviral vector transduced cell products be tested for RCR, including those cultured for 4 days or less. We have found no convincing evidence that the length of culture time influences the likelihood of RCR development in transduced cells. However, if you have accumulated manufacturing and clinical experience that demonstrates that your transduced cell product is consistently RCR-negative (section III.A.3 of this document), we recommend that you provide this data to support reduction or elimination of testing ex vivo genetically modified cells for RCR. Finally, we have revised our advice for active monitoring of patients following administration of retroviral vector-based products (section IV of this document), and added post-licensure considerations for RCR testing and risk assessment (section VI of this document).

III. RECOMMENDATIONS FOR PRODUCT TESTING

A. Material for Testing

Generally, retroviral vectors are manufactured by collection of supernatant following transient or stable production from cultured cells. RCR may develop at any step during manufacturing, from the initial transfection or transduction steps through production of the retroviral vector supernatant. In addition, the expansion of ex vivo transduced cells in culture provides the potential for amplification of an RCR contaminant that may be below the level of detection in the retroviral vector supernatant. Therefore, current recommendations include testing of material from multiple stages of product manufacture (see Table of this document).

When the vector is produced by transient transfection, the cell banks should be qualified according to the CMC Draft Guidance. Retroviral vector RCR-specific testing requirements are outlined below for the vector supernatant (section III.A.2 of this document), end of production cells (section III.A.2 of this document), and ex vivo transduced cells (section III.A.3 of this document), if applicable.

We recommend use of a stably-transfected Vector Producer Cell (VPC) bank system, when possible, in order to ensure an adequate and consistent supply of retroviral vector. The generation of a Master Cell Bank (MCB) for the VPC allows for the collection of cells of uniform composition derived from a single cell clone. The Working Cell Bank (WCB) is derived from the MCB, following expansion by serial subculture to a specified passage number (refer to "Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals" dated May 1993)[3]. When the vector is collected from VPC banks, RCR-specific testing of the VPC MCB (section III.A.1 of this document) is recommended in addition to vector supernatant (section III.A.2 of this document), end of production cells (section III.A.2 of this document), and ex vivo transduced cells (section III.A.3 of this document), if applicable.

1. Vector Producer Cell Master Cell Bank

Both cells and supernatant from the VPC MCB should be tested for RCR using a cell line permissive for the RCR that could potentially be generated in a given producer cell line. For example, VPC containing envelopes such as gibbon ape leukemia virus (GALV) envelope or vesicular stomatitis virus glycoprotein (VSV-G) are typically tested on a human cell line. Other retroviral envelopes should be tested on a cell line permissive for infection by the relevant RCR.

If the VPC MCB was produced using a retroviral vector pseudotyped with an envelope distinct from the clinical vector product, for example, an ecotropic Murine Leukemia Virus (MLV), the potential exists for introduction of an RCR with that distinct envelope. Even though an ecotropic MLV RCR may present a minimal direct safety risk to humans, the presence of any replication-competent genome in the VPC MCB is problematic because of the increased probability of generating an RCR with a human host range through recombination with elements within the VPC.

Therefore, in cases where VPC are derived, at any step, by transduction with an ecotropic retroviral vector, testing of the MCB for the presence of ecotropic RCR is recommended, in addition to amphotropic RCR testing. For example, VPC possibly containing ecotropic MLV envelope should be tested for RCR on an appropriate cell line, such as that derived from *Mus dunni*, which is permissive to

[3] https://www.fda.gov/downloads/BiologicsBloodVaccines/SafetyAvailability/UCM162863.pdf.

infection by ecotropic MLV-like RCR (Ref. 17), except in the case of Moloney murine leukemia virus (MoMLV). Insufficient testing of the VPC MCB may necessitate additional RCR testing of the working cell bank, if applicable.

2. Retroviral Vector Supernatant Product and End of Production Cells

Both retroviral vector supernatant lots and end of production (EOP) cells should be tested for RCR. EOP cells are defined as cells from which a single bulk harvest of retrovirus-containing supernatant is taken or cells from which the last of a serial set of supernatant harvests is taken. This recommendation is based on data and experience reported at the 1997 FDA/NIH Gene Therapy Conference, where it was reported that RCR in vector production lots was not always consistently detected in both vector supernatant and EOP cells. These data support the position that dual testing provides a complementary approach to assuring RCR-free retroviral supernatant.

3. Ex Vivo Transduced Cells

It is possible that RCR may be present in your vector at undetectable levels, which could be amplified during the manufacture of ex vivo transduced cells. Therefore, we recommend that each lot of ex vivo transduced cells and culture supernatant be tested for RCR. This recommendation applies regardless of the length of time that the cells are cultured after transduction, because the length of culture time (e.g., greater than 4 days) has not been shown to strongly influence the likelihood of RCR development.

However, experience with vectors that have been deliberately designed to minimize the likelihood of recombination suggests that amplification of RCR in transduced cells is unlikely for many vectors. If you have accumulated manufacturing and clinical experience that demonstrates that your transduced cell product is consistently RCR-negative (section III.A.3 of this document), we recommend that you provide this data to support reduction or elimination of testing ex vivo genetically modified cells for RCR. We recommend you include a discussion of safety features in the vector design that reduces the likelihood of generating RCR, a description of vector testing in accordance with current guidance, and your experience manufacturing RCR-free cell products. You may provide information supporting removal of RCR testing for lot release of ex vivo transduced cells in your IND (i.e., in the section titled: Manufacturing Process Development Section 3.2.S.2.6 or 3.2.P.2.3 of the electronic Common Technical Document (eCTD)) or discuss with the FDA during your pre-IND meeting.

If the ex vivo transduced cell product is not tested for RCR at lot release, we recommend archiving a sample for at least 6 months after the product expiration date. We recommend that you retain a sufficient amount (section III.B.2 and Appendix of this document) of the cell product to perform RCR testing in the

future if necessary (section IV of this document). Samples should be archived with appropriate safeguards to ensure long-term storage (e.g., a monitored freezer alarm storage system) and an efficient system for the prompt linkage and retrieval of the stored samples with the medical records of the patient and the production lot records.

Table. Recommendations for Product Testing

Material to be Tested	Frequency of Testing	Testing for Expected RCR[1]	Testing for Ecotropic RCR
		Cells and Supernatant	Cells and Supernatant
MCB -Derived by transduction with ecotropic vector -Derived by transfection of retroviral vector plasmid	One-time	 Yes Yes	 Yes NA[2]
Vector Harvest Material -EOP cells -Vector supernatant	Lot release	 Yes Yes	NA
Ex vivo Transduced Cells	Lot release	Yes OR archive[3]	NA

[1] RCR testing should be based on the type of vector envelopes used. Consult text in section III.A.1 of this document for details.
[2] NA, not applicable.
[3] If an agreement reached with FDA to discontinue testing; consult text in section III.A.3 of this document.

B. Amounts for Testing

1. Supernatant Testing

Historically, we have recommended that it would be appropriate to test at least 5% of the total supernatant, or 300 mL, to ensure absence of RCR. This volume was set based on our experience at the time with gammaretrovirus vector production lot size, reference material, and patient dosing. From this, we have concluded that current manufacturing experience indicates that <1 RCR/dose equivalent is a tolerable and achievable level for retroviral vector preparations intended for clinical use. We recommend that sufficient supernatant be tested to ensure a 95% probability of detection of RCR if present at a concentration of 1 RCR/dose equivalent. A more detailed explanation of the rationale and the mathematical formulas applied is found in Appendix 1-1 of this document. Using

the provided formula, you should detail the amount to be tested in the description of RCR testing procedures included in your IND (in the eCTD section: Analytical Procedures 3.2.S.4.2 or 3.2.P.4.2).

To support the underlying assumption that a single retrovirus will be detected, one should determine a volume in which a single RCR can be detected by an individual RCR assay. Based on the determination of this volume, the total test volume should then be divided into replicate samples, each containing the volume demonstrated to detect a single RCR. When large volumes or high titer retroviral vector preparations are used, interference in RCR detection may occur. Sponsors are encouraged to develop more sensitive detection methods that overcome the interference effect of high titer retroviral vector preparations in order to use the alternative approach.

2. Cell Testing

We recommend that you test 1% or 10^8 (whichever is less) pooled vector-producing cells or ex vivo transduced cells by co-culture with a permissive cell line. This recommendation is unchanged from previous recommendations and is consistent with public consensus expressed at the 1996 and 1997 FDA/NIH Gene Therapy Conferences.

C. Assays for Testing

Vector supernatant assays should include culture of supernatant on a permissive cell line for a minimum of five passages in order to amplify any potential RCR present. Similarly, cell testing should be accomplished by co-culture with a permissive cell line for a minimum of five passages in order to amplify any potential RCR present. Sponsors are encouraged to develop RCR assays that support virus entry, amplification, and particle production specific to vector design (e.g., *Mus dunni* for ecotropic MLV (Ref. 17), C8166 cells for VSV-G pseudotyped HIV-1 (Ref. 18), or 293F-DCSIGN-CD4 cells for E1001 enveloped HIV-1 (Ref. 19). The amplified material may then be detected in an appropriate indicator cell assay (e.g., PG-4 S+L- (Ref. 20), XC (Ref. 21)), or by PERT (Ref. 22), or by psi-gag or VSV-G polymerase chain reaction (PCR) (Ref. 23), or by a commercially available p24 ELISA. All assays should include relevant positive and negative controls to assess specificity, sensitivity, and reproducibility of the detection method employed. Each lot of retroviral vector supernatant should be tested for inhibitory effects on detection of RCR by using positive control samples that are added to vector supernatant.

Alternative methods, such as PCR, may be appropriate for lot release testing of ex vivo transduced cells in lieu of culture based methods; particularly, when time constraints are present or when you have accumulated sufficient data with the culture based methods.

Any alternative methods should be developed in consultation with CBER. Data on sensitivity, specificity and reproducibility should be provided to support the use of alternative methods.

For assay development, you should develop a reference standard for use as a positive control and for method validation. The reference standard can be used for determination of the volume in which a single RCR can be determined. A gammaretrovirus RCR standard has been developed, its infectious titer has been determined, and it is available through the American Type Culture Collection (ATCC). Refer to Appendices 1-2 and 1-3 of this document for detailed information about the gammaretrovirus RCR standard and how it can be used to determine the replicate size and number for RCR detection. Standards have not yet been developed for other retrovirus vectors. We recommend that you develop an in-house reference standard that represents your clinical vector attributes, including, the genetic background, envelope protein, and deletion of accessory proteins. The reference standard should be characterized for growth kinetics in the cells used during the RCR assay and tested for stability. For more information on reference materials, please refer to FDA's "Analytical Procedures and Methods Validation for Drugs and Biologics; Guidance for Industry," dated July 2015.[4]

IV. RECOMMENDATIONS FOR PATIENT MONITORING

Previous FDA guidance for active patient monitoring recommended RCR testing and/or archiving of patient samples at regular intervals for fifteen (15) years. To date, RCR or delayed adverse events related to RCR have not been reported in patients who have received retrovirus-based gene therapies (Refs. 5, 25, 26, 27, 28).

A. RCR Testing Schedule

We recommend the monitoring schedule to include analysis of patient samples at the following time points: pre-treatment, followed by testing at three, six, and twelve months after treatment, and yearly for up to fifteen (15) years. However, if all post-treatment assays are negative during the first year, collection of the yearly follow-up samples may be discontinued. If any post-treatment samples are positive, further analysis of the RCR, and more extensive patient follow-up should be undertaken, in consultation with CBER.

After you have accumulated patient monitoring data with your product, you may provide a rationale to discontinue active testing of patient samples for RCR in the safety monitoring section of your clinical protocol. The rationale may include a discussion of safety features in the vector design that reduce the likelihood of generating RCR, as well as results of your previous clinical testing experience.

[4] https://www.fda.gov/downloads/drugs/guidances/ucm386366.pdf

As part of the long-term follow-up protocol, a yearly long-term follow-up clinical report[5] should be submitted to the IND. This history should be targeted towards determination of clinical outcomes suggestive of retroviral disease, such as cancer, neurologic disorders, or other hematologic disorders. Relevant clinical samples should be collected and tested for RCR upon development of an adverse event suggestive of a retrovirus-associated disease. If patients die or develop neoplasms during a gene therapy trial, every effort should be made to assay for RCR in a biopsy sample of the neoplastic tissue or the pertinent autopsy tissue. Sample collection and storage should be compatible with the expected testing strategy. Additional recommendations for long-term follow-up of patients in clinical trials using retroviral vectors are discussed in the Long-Term Follow-up Draft Guidance.

B. Recommended Assays

We recommend two methods that are currently in use for detecting evidence of RCR infection in patients: 1) serologic detection of RCR-specific antibodies; and 2) analysis of patient peripheral blood mononuclear cells by PCR for RCR-specific DNA sequences. The choice of assay may depend on the vector, mode of vector administration, and the clinical indication. For example, it has been shown that direct administration of VPC or repeat direct injection of a vector can result in vector-specific antibodies that do not correlate with the presence of RCR (Refs. 29, 30). Therefore, in cases where vector or VPCs are directly administered, a PCR assay may be preferable over serologic monitoring. Additionally, monitoring of patient samples by PCR may be preferable over serologic monitoring if the patients are immunocompromised to an extent that antibody production may be minimal or not at all. In either situation, all confirmed positive results should be pursued by direct culture assay to obtain and characterize the infectious viral isolate.

V. DOCUMENTATION OF RCR TESTING RESULTS

RCR testing results from production lots and patient monitoring should be documented in amendments to the IND file. Positive results from patient monitoring should be reported immediately as an adverse experience in the form of an IND safety report (21 CFR 312.32). Negative results should be reported by way of the IND annual report (21 CFR 312.33). In addition, to enhance the accumulation of data on RCR testing assays, CBER encourages members of the gene therapy community to publish data and/or discuss data publicly regarding their experience with different vector producer cell lines, patient monitoring, and safety.

[5] For more information, refer to section V of the Long Term Follow-up Draft Guidance ("Recommendations for Protocols for Long Term Follow-Up Observations: Clinical Considerations").

VI. POST-LICENSURE CONSIDERATIONS

We recommend that labeling for retroviral vector-based gene therapy products incorporate relevant data and information to clearly present the immediate and long-term risks associated with RCR. As a critical safety test for retroviral vectors, testing for RCR during vector manufacture and release should continue after licensure.

At the time of submission of your Biologics License Application (BLA),[6] you should have accumulated sufficient manufacturing and clinical safety data to determine whether there is a significant risk of RCR developing with your product. This risk assessment may be used to propose that periodic patient monitoring for RCR would not be warranted for your product post-licensure. However, you should include a provision in the BLA to collect relevant clinical samples from patients for RCR testing upon development of an adverse event suggestive of a retrovirus-associated disease. In the event patients die or develop neoplasms following product administration, every effort should be made to assay for RCR in a biopsy sample of the neoplastic tissue or the pertinent autopsy tissue.

We also recommend continued long term patient follow-up, up to fifteen (15) years, after licensure of retroviral-based gene therapy products to monitor for delayed adverse events. For more information, refer to section VI of the Long Term Follow-up Draft Guidance ("General Considerations for Post-Marketing Monitoring Plans for Gene Therapy Products").

[6] 21 CFR 601.2

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APPENDIX

1-1. Derivation of Recommendation for Test Volume for RCR Detection

Assuming the RCR are present in the production lot at a concentration (**c**) and that an assay will detect a single retrovirus in the sample, the probability (**p**) of detecting retrovirus in a volume (**Vt**) is given by the formula: **p = 1-exp(-cVt)**, because the number of RCR in **Vt** follows a Poisson distribution with a parameter **cVt**. Solving for **Vt**, one gets the following equation:

$$\mathbf{Vt = -\ (1/c)\ ln\ (1\text{-}p)}$$

where **ln** denotes the natural logarithm.

Value for p

For the use of this formula, it is recommended that the value for **p** be set at 0.95. With the recommended replicate size and number defined in Appendix 1-3 of this document, **p** becomes the probability of detecting an RCR in the production lot.

Value for c

We recommend that the value for **c** be set no higher than 1 RCR/dose equivalent. If the concentration of RCR in the production lot is 1 RCR/dose equivalent or greater, then the probability of detection is at least 0.95. If the production lot contains RCR at a concentration of <1 RCR/dose equivalent, the RCR may not be detected and would be administered to the patient. We also recommend that a dose equivalent be defined as the maximum amount of vector expected to be administered at one time. For ex vivo genetically modified cells, a dose equivalent is the amount of vector used to transduce the maximum number of target cells for each production lot.

Value for Vt

With the recommended value for **p** and **c**, the total volume of retroviral supernatant to be tested, independent of lot size, is calculated as follows:

Vt = - (1 / (1 RCR/dose equivalent)) ln (1 -0.95)

Direct administration example:

If your product is administered at $1x10^{10}$ TU (transducing unit)

Vt = - (1 / ($1/1x10^{10}$ TU)) ln (1 -0.95) = $3x10^{10}$ TU

Ex vivo genetic modification example:

If you aim to transduce up to $1x10^{8}$ cells at an MOI (multiplicity of infection) of 0.5 with a titer of $5x10^{7}$ TU/mL:

Dose equivalent = ($1x10^{8}$ cells) (0.5 TU/cell) / ($1x10^{7}$ TU/mL) = 5 mL

Vt = - (1 / (1/5 mL)) ln (1 -0.95) = 15 mL

Proposals to use smaller volumes should be developed and reviewed in consultation with CBER.

1-2. Empirical Determination of Assay Sensitivity

In collaboration with the ATCC, a standard gammaretroviral stock (ATCC # VR-1450) has been established for use in determination of sensitivity and validation of assays used to detect the presence of RCR which would be produced from VPC containing an amphotropic envelope. This stock can be used to determine the relative assay sensitivity for detecting RCR. This information can subsequently be used to determine the size of replicates of retroviral supernatant to be tested that will ensure detection of a single retrovirus and thus, the number of replicates to ensure an adequate total volume, **Vt,** as specified in this guidance (Appendix 1-3 of this document). The virus stock is derived from a cell line which has been transfected with a molecular clone encoding MoMLV with a substitution of the envelope coding region from the 4070A strain of amphotropic MLV (Ref. 31). Therefore, this virus stock represents a typical recombinant virus that could be generated in a retroviral packaging cell line containing coding sequences for a MLV envelope.

The standard virus stock and its infectious titer can be used as a positive control to empirically determine the relative sensitivity of assay methods used for detection of RCR in retroviral vectors. In particular, this stock will allow investigators to determine the largest test volume in which a single RCR can be detected. The determination should be performed in the presence of a retroviral vector supernatant typical of a production lot in order to control for inhibitory effects of the retroviral vector particles on detection of RCR. Availability of this standard should allow individual investigators to establish this methodology in their own laboratories, as well as allow exploration of alternative methods for detection of RCR.

1-3. Formula to Determine Replicate Size and Number

Depending on the volume in which a single RCR can be detected by an individual RCR assay (as determined by use of the RCR standard, Appendix 1-2 of this document), it may be necessary to divide the total test volume into several replicate samples to ensure the detection of RCR in the sample. The number of replicates (**r**), can be determined using the formula,

$$\mathbf{r = Vt / Vs}$$

where **Vs** is the volume in which one RCR can be consistently detected (Appendix 1-1 of this document for determination of **Vt**).

Human Gene Therapy for Hemophilia

Draft Guidance for Industry

This guidance document is for comment purposes only.

Submit one set of either electronic or written comments on this draft guidance by the date provided in the *Federal Register* notice announcing the availability of the draft guidance. Submit electronic comments to https://www.regulations.gov. Submit written comments to the Dockets Management Staff (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. You should identify all comments with the docket number listed in the notice of availability that publishes in the *Federal Register*.

Additional copies of this guidance are available from the Office of Communication, Outreach and Development (OCOD), 10903 New Hampshire Ave., Bldg. 71, Rm. 3128, Silver Spring, MD 20993-0002, or by calling 1-800-835-4709 or 240-402-8010, or email ocod@fda.hhs.gov, or from the Internet at https://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.

For questions on the content of this guidance, contact OCOD at the phone numbers or email address listed above.

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Food and Drug Administration
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Human Gene Therapy for Hemophilia

Draft Guidance for Industry

This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff responsible for this guidance as listed on the title page.

I. INTRODUCTION

This guidance is intended to assist stakeholders developing human gene therapy (GT)[1] products for the treatment of hemophilia. This guidance provides recommendations on the clinical trial design and related development of coagulation factor VIII (hemophilia A) and IX (hemophilia B) activity assays, including how to address discrepancies in factor VIII and factor IX activity assays. This guidance also includes recommendations regarding preclinical considerations to support development of GT products for the treatment of hemophilia. Additional clinical and preclinical recommendations are available through several other guidances.[2,3] This guidance does not provide recommendations for products for the treatment of hemophilia C (factor XI deficiency) or for the treatment of any bleeding disorders other than hemophilia A and B, because of the unique nature of those other bleeding disorders.

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.

[1] 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 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 (Ref. 1), and ex vivo genetically modified human cells. Gene therapy products meet the definition of "biological product" in section 351(i) of the Public Health Service (PHS) Act (42 U.S.C. 262(i)) when such products are applicable to the prevention, treatment, or cure of a disease or condition of human beings.

[2] Guidance for Industry: Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products, dated June 2015 https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM564952.pdf

[3] Guidance for Industry: Preclinical Assessment of Investigational Cellular and Gene Therapy Products, dated November 2013 https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM376521.pdf

The use of the word *should* in FDA's guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

Hemophilia therapy in the United States has progressed from replacement therapies for on-demand treatment of bleeding to prophylaxis to reduce the frequency of bleeding. Current replacement therapies utilize plasma-derived coagulation factor or recombinant factor concentrates. Prophylaxis has been shown to prevent joint damage in children and allows lower factor usage compared to on-demand therapy, and is currently the optimal treatment for hemophilia. Dosing intervals with prophylaxis are associated with peaks and troughs and aim at maintaining trough levels >1% between doses. Compliance with dosing is a necessary aspect of prophylaxis, and patients may experience breakthrough bleeding episodes that require treatment with replacement therapies for control of bleeding. The main adverse event associated with factor replacement therapy is the development of inhibitors (neutralizing antibodies) to factor VIII or factor IX, which requires use of alternative therapies to overcome the effect of the inhibitor.

GT products for the treatment of hemophilia are being developed as single-dose treatments that may provide long-term expression of the missing or abnormal coagulation factor in the patient at steady levels to reduce or eliminate the need for exogenous factor replacement. GT products in the advanced phase of clinical development may use a vector to deliver the coagulation factor gene to the liver. The coagulation factor that is expressed may be different from the wild type (normal) form. For example, the coagulation factor may be a truncated variant, such as B domain-deleted factor VIII, or a hyper-functional natural variant (such as the Padua variant of factor IX).

III. CONSIDERATIONS FOR PRODUCT DEVELOPMENT

The general chemistry, manufacturing and control (CMC) considerations for product manufacturing, testing and release of GT products for the treatment of hemophilia are the same as those described for other GT products (Ref. 2). For early-phase clinical trials, a sponsor should be able to evaluate the identity, purity, quality, dose, and safety of a GT product. A potency assay to assess the biological activity of the final product, with relevant lot release specifications, should be established prior to the initiation of clinical trials intended to provide substantial evidence of effectiveness for a marketing application. To support licensure of a GT product, manufacturing processes and all testing methods for product release must be validated (21 CFR 211.165(e)). Sponsors developing GT products for hemophilia are strongly encouraged to contact the Office of Tissues and Advanced Therapies (OTAT) in the Center for Biologics Evaluation and Research (CBER) early in product development to discuss product-specific issues.

IV. CONSIDERATIONS FOR FACTOR VIII/FACTOR IX ACTIVITY MEASUREMENTS ASSESSED BY DIFFERENT CLINICAL LABORATORY ASSAYS

One stage clotting (OC) assays and chromogenic (CS) assays have been used to measure factor activity; however, discrepancies in factor activity measurements between the OC and CS methods have been observed (Refs. 3-9). For example, in patients with hemophilia A treated with recombinant B-domain-deleted factor VIII products, CS assays indicate higher factor activity than OC assays. In contrast, for patients with hemophilia A who receive GT products that express a B-domain-deleted factor VIII transgene, OC assays indicate higher factor activity than CS assays. These contrasting results prevent us from generalizing our previous experience with recombinant factor VIII products to clinical benefits related to factor VIII levels produced by recipients of GT products. Similarly, for hemophilia B patients who receive GT products that express the Padua variant of factor IX, discrepancies between results of the OC and CS assays have been observed across products.

Factor activity assay discrepancies are not limited to differences between OC and CS assays, but are also observed between OC assays using different OC reagents. These discrepancies indicate structural and functional differences between the transgene proteins and normal factor proteins used as an assay standard. The discrepancies preclude reliable interpretation of factor activity measurements and present a challenge when factor activity levels are proposed as surrogate endpoints for hemostatic efficacy. Even if factor activity is not used as a surrogate endpoint to support accelerated approval, safe clinical management of patients in GT trials depends on an understanding of any assay discrepancies.

To better interpret these results, we recommend that sponsors consider:

- Performing animal or in vitro preclinical studies that compare the performance of OC and CS assays. Both assays should be calibrated in International Units (IU) of factor activity and should use a reference standard analogous to the expressed transgene, if available.[4]

- Using various clinical laboratory assays in preclinical animal studies and, where feasible, assays intended for human use.

We also recommend that sponsors perform analytical studies to clarify the biochemical root-causes for any discrepancies observed, addressing:

- Methodology (OC vs. CS)

[4] 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 or replace animal studies. Sponsors are encouraged to submit proposals and justify any potential alternative approaches, which we will evaluate for equivalency to animal studies.

- Reagents (phospholipids, activators, chromogenic substrates)
- Conditions (incubation times, temperature)
- Choice of reference standards
- Vendors/kits/lab being used
- Correlations between factor activity and antigen levels (by immunoassay)

Data from preclinical studies should inform the selection of assays used in early-phase clinical studies to:

- Measure factor activity intended to be used as a surrogate endpoint to support accelerated approval; and
- Guide exogenous replacement therapy for the treatment of bleeding.

During clinical trials, we recommend that sponsors consider:

- Performing a comparative field study with patient plasma samples using assays routinely performed in clinical laboratories to evaluate the range of discrepancies.
- Performing bridging studies on patient samples if changes to the assay(s) are initiated after a clinical trial is underway.

V. CONSIDERATIONS FOR PRECLINICAL STUDIES

A preclinical program that is tailored to the investigational product and planned early-phase clinical trial contributes to characterization of the product's benefit/risk profile for the intended patient population. The overall objectives of a preclinical program for a GT product include: 1) identification of a biologically active dose range; 2) recommendations for an initial clinical dose level, dose-escalation schedule, and dosing regimen; 3) establishment of feasibility and reasonable safety of the proposed clinical route of administration (ROA); 4) support of patient eligibility criteria; and, 5) identification of potential toxicities and physiologic parameters that help guide clinical monitoring for a particular investigational product.

Further details for general considerations in preclinical studies are available in a separate guidance document.[5] The following elements are recommended for consideration when

[5] Guidance for Industry: Preclinical Assessment of Investigational Cellular and Gene Therapy Products, dated November 2013 https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM376521.pdf

developing a preclinical program for an investigational GT product for treatment of hemophilia (some of which are not necessarily exclusive to GT products for treatment of hemophilia).

- Preclinical in vitro and in vivo proof-of-concept (POC) studies are recommended to establish feasibility and support the scientific rationale for administration of the investigational GT product in a clinical trial. Data derived from preclinical POC studies may guide the design of both the preclinical toxicology studies, as well as the early-phase clinical trials. Several hemophilia animal models are available in the literature (Ref. 10) and can be used to demonstrate biological activity of an investigational GT product and to help the evaluation of the human response.

- Biodistribution studies are conducted to assess the pharmacokinetic (PK) profile of a GT product. (Ref. 11) These data encompass the distribution, persistence, and clearance of the vector and possibly the expressed transgene product in vivo, from the site of administration to target and non-target tissues, including biofluids (e.g., blood, lymph node fluid). These data can determine extent of tissue transduction and transgene expression, evaluate whether expression is transient or persistent, and guide the design of the preclinical toxicology studies as well as the early-phase clinical trials.

- Toxicology studies for an investigational GT product should incorporate elements of the planned clinical trial (e.g., dose range, ROA, dosing schedule, evaluation endpoints, etc.), to the extent feasible. Study designs should be sufficiently comprehensive to permit identification, characterization, and quantification of potential local and systemic toxicities, their onset (i.e., acute or delayed) and potential resolution, and the effect of dose level on these findings.

- To support translation of effective and safe dose levels determined in preclinical studies to clinical trials, the assay for vector titer determination of the preclinical lots should be identical to the assay used for clinical lots. The assays for measuring factor activity in animals administered the GT product should be consistent to the assays used in humans. The factor activity assays are discussed in detail under section IV. of this document.

- As the clinical development program for an investigational GT product progresses to late-phase clinical trials and possible marketing approval, additional nonclinical studies may need to be considered to address: 1) the potential for reproductive/developmental toxicity and 2) any significant changes in the product manufacturing process or formulation changes for which product comparability may be an issue.

VI. CONSIDERATIONS FOR CLINICAL TRIALS

The fundamental considerations for clinical development programs of GT products for hemophilia are similar to those for other biologic products. Early-phase trials of GT products should not only evaluate safety and feasibility, but also gauge bioactivity and preliminary efficacy. Sponsors should evaluate the discrepancies between OC and CS assays early in the course of clinical development, prior to considering whether to pursue accelerated approval

using factor activity levels as a surrogate endpoint. Later-phase trials should be designed as adequate and well-controlled studiesthat can provide substantial evidence of effectiveness to support an application for marketing. For further details of general considerations for gene therapy clinical trials, please refer to relevant FDA guidance documents.[6, 7]

With respect to late-phase clinical trials that are intended to form the primary basis of an effectiveness claim for hemophilia GT products, we have the following recommendations:

A. Efficacy Endpoints

Sponsors may consider using the following efficacy endpoints as primary endpoints in clinical trials of GT products for hemophilia:

1. Traditional Approval

   - Annualized Bleeding Rate (ABR) as a primary endpoint to demonstrate clinical benefit.

2. Accelerated Approval

   - Factor activity may be considered as a surrogate endpoint[8] for primary efficacy assessment under the accelerated approval pathway.[9] (Ref. 12)

[6] Long Term Follow-Up After Administration of Human Gene Therapy Products: Draft Guidance for Industry, July 2018, (when finalized), https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM610797.pdf

[7] Guidance for Industry: Providing Clinical Evidence of Effectiveness for Human Drug and Biological Products, dated May 1998, https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM072008.pdf

[8] For the purposes of accelerated approval, a surrogate endpoint is a marker, such as a laboratory measurement, radiographic image, physical sign, or other measure, that is not itself a measure of clinical benefit, but is considered reasonably likely to predict clinical benefit.

[9] Section 506(c) of the Federal Food, Drug, and Cosmetic Act (FD&C Act); 21 CFR Part 314, Subpart H – Accelerated Approval of New Drugs for Serious and Life Threatening Illnesses; 21 CFR Part 601, Subpart E.

However, to support the use of this surrogate endpoint, we recommend that you:

- Resolve discrepancies in factor assay results from various assay methods prior to considering a target factor activity as a surrogate endpoint for primary efficacy assessment.
- Determine a target factor activity level within the range of factor activity of normal population.

B. Study Design

While designing the clinical study, sponsors should consider the following pre-and post-administration recommendations:

1. Pre-administration Considerations

   We recommend:

   - Enrolling patients who have not required dose adjustments to their prophylactic replacement therapy for at least 12 months as this may best facilitate efficacy determinations following administration.
   - Observing patients for 6 months (lead-in period) in-study to collect data for ABR rates. ABR rates based on retrospective data collection from medical records may be subject to recall bias and missing information. Collecting:
     - ABR on an optimized prophylactic regimen to allow for within-subject (paired) comparison, increasing the statistical power relative to a design with parallel control.
     - Data for supportive endpoints (e.g., utilization of exogeneous replacement therapy or trough levels of factor activity).
   - Enrolling patients who use on-demand therapy prior to study entry in a separate cohort. Analysis of efficacy in this cohort may provide evidence to support the primary endpoint results.

2. Post-administration Considerations

   We recommend:

   - Using the same exogenous replacement therapy as in the lead-in phase to prevent (or treat) bleeding during the interval from post-GT product administration to steady state factor levels.
   - Including a washout period following exogenous factor replacement therapy to measure factor activity.

- Including a pre-specified target factor activity level or duration from treatment that specifies the timing to discontinue exogeneous factor prophylaxis.
- Specifying when assessment of ABR rates and durability of response is to begin (e.g., 3 weeks after steady state levels of factor activity is reached and exogenous factor prophylaxis is discontinued).
- Collecting data for analyses of supportive endpoints as related to the pre-treatment phase.
- Including a plan for initiation, dosing and tapering of corticosteroids for management (treatment or prophylaxis) of immune-mediated liver dysfunction.
- Including an assessment plan to correlate factor activity and bleeding rates.

C. Study Population

Sponsors may consider the following recommendations when identifying the target population:

- Pre-existing antibodies to the GT product may block delivery of the coagulation factor gene to its target (e.g., liver cells), limiting its therapeutic potential. Therefore, sponsors may choose to exclude patients with pre-existing antibodies to the GT product. In such cases, the sponsor should strongly consider contemporaneous development of a companion diagnostic to detect antibodies to the GT product. (Ref. 13) If an *in vitro* companion diagnostic is needed to appropriately select patients for study (and later, once the GT product is approved, for treatment), then submission of the marketing application for the companion diagnostic and submission of the biologics license application for the GT product should be coordinated to support contemporaneous marketing authorizations. In addition, the clinical development plan should include studies to assess the effect of such pre-existing antibodies on the safety and efficacy of the product.
- Hemophilia affects both children and adults. Since many similar rare diseases are pediatric diseases or have onset of manifestations in childhood, pediatric studies are a critical part of drug development. However, treatment in pediatric patients cannot proceed without addressing ethical considerations for conducting investigations in vulnerable populations. Unless the risks of an investigational drug are no more than a minor increase over minimal risk (21 CFR 50.53), the administration of an investigational drug in children must offer a prospect of direct clinical benefit to individually enrolled patients, the risk must be justified by the anticipated benefit, and the anticipated risk-benefit profile must be at least as favorable as that presented by accepted alternative treatments (21 CFR 50.52). Additionally, adequate provisions must be made to obtain the permission of the parents and the assent of the child as per 21 CFR 50.55.

D. Statistical Considerations

To support a marketing application for traditional approval, we recommend a non-inferiority (NI) clinical trial design with ABR as the primary efficacy endpoint using a within-subject comparison design. We also recommend:

- Developing a NI margin (*M*) for comparing ABR of the investigational GT product to that of current prophylaxis therapies in the within-subject comparison trial.

- Proposing a statistical test to rule out that the ABR of the investigational GT product is more than *M* above the ABR of the within-subject comparator, taking into account the paired nature of the ABRs before and after GT for the same subject. One possible approach is to take the difference of each pair of ABRs, and then test that the median of the differences is less than *M* using the Wilcoxon Signed Rank test. We recommend that you also report a 95% confidence interval (CI) on the median of the ABR difference.

The within-subject comparison design provides an added advantage in evaluating the treatment effect of the investigational product by controlling for other factors that may also influence the bleeding outcomes. Additional information on general statistical and clinical considerations for these trials is described in FDA's guidance.[10]

E. Study Monitoring

The goal of the follow-up is to monitor the safety and durability of response. Sponsors may consider the following recommendations for short-term and long-term monitoring:

1. Short-Term Monitoring (first 2 years following GT product administration)

   We recommend:

   - Monitoring factor activity levels and liver function once or twice weekly in the interval between administration of the GT product and until steady state factor levels are reached.

   - Decreasing the frequency of monitoring of factor activity once steady state levels are achieved (for instance, monthly).

   - Periodic monitoring for levels of vector-related antibodies and assessing interferon-γ secretion from peripheral blood mononuclear cells by ELISPOT assay (more frequent monitoring may be appropriate if immune-mediated hepatic dysfunction is suspected).

[10] Non-Inferiority Clinical Trials to Establish Effectiveness; Guidance for Industry, dated November 2016, https://www.fda.gov/downloads/Drugs/Guidances/UCM202140.pdf

- Monitoring for inhibitor antibodies to factor VIII or factor IX.

- Assessing for viral shedding for products where a viral vector is used for gene transfer. (Ref. 15)

2. Long-Term Monitoring (≥2 years following GT product administration)

   We recommend:

   - Monitoring for adverse events for at least 5 years after exposure to non-integrating GT products and 15 years for integrating GT products. (Ref. 16)

   - Monitoring for adverse events to include: eliciting history of and non-invasive screening for hepatic malignancies; physical examination; and laboratory testing for hepatic function.

   - Monitoring for inhibitor antibodies to factor VIII or factor IX.

   - Monitoring for the emergence of new clinical conditions, including new malignancies and new incidence or exacerbation of pre-existing neurologic, rheumatologic, or autoimmune disorders.

   - Monitoring factor activity at least once every 6 months for 5 years.

F. Patient Experience

Patient experience data[11] may provide important additional information about the clinical benefit of a GT product. FDA encourages sponsors to collect patient experience data during product development, and to submit such data in the marketing application.

The treatment landscape for hemophilia is evolving. Therefore, the benefit-risk profile of the investigational product will be evaluated in the context of the treatment landscape at the time of our review of a marketing application.

[11] As defined in section 569(c) of the FD&C Act, the term "patient experience data" includes data that are:

- Collected by any persons (including patients, family members and caregivers of patients, patient advocacy organizations, disease research foundations, researchers, and drug manufacturers); and
- Intended to provide information about patients' experiences with a disease or condition, including the impact (including physical and psychosocial impacts) of such disease or condition, or a related therapy or clinical investigation, on patients' lives; and patient preferences with respect to treatment of such disease or condition.

Additional information on Patient-Focused Drug Development can be found on this website: https://www.fda.gov/drugs/developmentapprovalprocess/ucm579400.htm

VII. EXPEDITED PROGRAMS

There are several programs that may be available to sponsors of GTs intended to address unmet medical needs in the treatment of serious or life-threatening conditions that are intended to facilitate and expedite development and review of these therapies, including regenerative medicine advanced therapy designation, breakthrough therapy designation, fast track designation, accelerated approval, and priority review. In particular, regenerative medicine advanced therapy designation and breakthrough therapy designation call for earlier attention from FDA to these potentially promising therapies, offering sponsors earlier and more frequent interactions with FDA on efficient trial design and overall drug development. Further information on these programs is available in separate guidance documents.[12,13]

VIII. COMMUNICATION WITH FDA

FDA recommends communication with OTAT) early in product development, before submission of an investigational new drug application (IND). There are different meeting types that can be used for such discussions, depending on the stage of product development and the issues to be considered. These include pre-IND meetings and, earlier in development, INitial Targeted Engagement for Regulatory Advice on CBER producTs (INTERACT) meetings.[14]

Early nonbinding, regulatory advice can be obtained from OTAT through an INTERACT meeting, which can be used to discuss issues such as a product's early preclinical program, and/or through a pre-IND meeting prior to submission of the IND. (Ref. 17)

[12] Guidance for Industry; Expedited Programs for Serious Conditions – Drugs and Biologics, dated May 2014, https://www.fda.gov/downloads/Drugs/Guidances/UCM358301.pdf

[13] Expedited Programs for Regenerative Medicine Therapies for Serious Conditions; Draft Guidance for Industry, dated November 2017, when finalized, https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM585414.pdf

[14] Going forward, INTERACT meetings will serve in place of pre-pre-IND meetings. For additional information about INTERACT meetings, please see https://www.fda.gov/BiologicsBloodVaccines/ResourcesforYou/Industry/ucm611501.htm

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

Human Gene Therapy for Rare Diseases

Draft Guidance for Industry

This guidance document is for comment purposes only.

Submit one set of either electronic or written comments on this draft guidance by the date provided in the *Federal Register* notice announcing the availability of the draft guidance. Submit electronic comments to https://www.regulations.gov. Submit written comments to the Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. You should identify all comments with the docket number listed in the notice of availability that publishes in the *Federal Register*.

Additional copies of this guidance are available from the Office of Communication, Outreach and Development (OCOD), 10903 New Hampshire Ave., Bldg. 71, Rm. 3128, Silver Spring, MD 20993-0002, or by calling 1-800-835-4709 or 240-402-8010, or email ocod@fda.hhs.gov, or from the Internet at https://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.

For questions on the content of this guidance, contact OCOD at the phone numbers or email address listed above.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
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Human Gene Therapy for Rare Diseases

Draft Guidance for Industry

This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff responsible for this guidance as listed on the title page.

I. INTRODUCTION

This guidance provides recommendations to stakeholders developing a human gene therapy (GT) product[1] intended to treat a rare disease[2] in adult and/or pediatric patients regarding the manufacturing, preclinical, and clinical trial design issues for all phases of the clinical development program. Such information is intended to assist sponsors in designing clinical development programs for such products, where there may be limited study population size and potential feasibility and safety issues, as well as issues relating to the interpretability of bioactivity/efficacy outcomes that may be unique to rare diseases or to the nature of the GT product itself.

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] 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 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 (Ref. 1), and ex vivo genetically modified human cells. Gene therapy products meet the definition of "biological product" in section 351(i) of the Public Health Service (PHS) Act (42 U.S.C. 262(i)) when such products are applicable to the prevention, treatment, or cure of a disease or condition of human beings.

[2] A rare disease is defined by the Orphan Drug Act of 1983 as a disorder or condition that affects fewer than 200,000 persons in the United States. Public Law 97-414, 96 Stat. 2049 (1983). Amended by Public Law 98-551 (1984) to add a numeric prevalence threshold to the definition of rare diseases.

II. BACKGROUND

The National Institutes of Health (NIH) reports that nearly 7,000 rare diseases affect more than 25 million Americans. Approximately 80% of rare diseases are caused by a single-gene defect, and about half of all rare diseases affect children. Since most rare diseases have no approved therapies, there is a significant unmet need for effective treatments, and many rare diseases are serious or life-threatening conditions. As a general matter, developing safe and effective products to treat rare diseases can be challenging. For example, it might be more difficult to find and recruit patients with rare diseases into clinical trials. Additionally, many rare diseases exhibit a number of variations or sub-types. Consequently, patients may have highly diverse clinical manifestations and rates of disease progression with unpredictable clinical courses. These challenges are also present for the development of GT products. However, despite these challenges, GT-related research and development in the area of rare diseases continues to grow at a rapid rate.

III. CONSIDERATIONS FOR PRODUCT DEVELOPMENT

The general chemistry, manufacturing and control (CMC) considerations for product manufacturing, testing and release of GT products for rare diseases are the same as those described for other GT products (Ref. 2). However, some aspects of the development programs for rare diseases, such as limited population size and fewer lots manufactured, may make it challenging to follow traditional product development strategies. In traditional product development, critical quality attributes (CQA) of the product are evaluated during each phase of clinical development, and characterization data from many product lots are correlated to clinical outcomes. In addition, GT products may have CQA with higher variability than drugs or well-characterized biologics, which can add to CQA uncertainty. Smaller study populations may result in the need for fewer manufacturing runs, which can make it difficult to establish the critical process parameters (CPP) necessary for ensuring CQA. However, demonstrating process control to ensure a consistent product with predefined CQA for potency, identity and purity is required to demonstrate compliance with licensure and regulatory requirements.[3]

These factors make it even more critical that a sponsor of a GT product for a rare disease establish a well-controlled manufacturing process along with suitable analytical assays to assess product CQA as early in development as possible, optimally before administration of the GT product to the first subject. Importantly, as the phase 1 study may provide evidence of safety and effectiveness, characterization of product CQA and manufacturing CPP should be implemented during early clinical development, and innovative strategies such as the production of multiple small lots versus a single large product lot may be considered. Sponsors developing GT products for rare diseases are strongly encouraged to contact the Office of Tissues and Advanced

[3] Section 351(a)(2)(C)(i) of the PHS Act (42 U.S.C. 262(a)(2)(C)(i)); 21 CFR 601.2; 21 CFR 601.20; 21 CFR Part 610, Subpart B.

Therapies (OTAT) in the Center for Biologics Evaluation and Research (CBER) prior to investigational new drug application (IND) submission to discuss their product-specific considerations, which may include:

- Product-related variations, including those contributed by intrinsic differences among subjects' cells, may have a more pronounced effect on the interpretability of smaller rare disease studies. This is equally true of impurities such as empty and wild type viral particles that may be present in viral vectors. Establishment of assays for characterization of product-related variants and impurities will be important for program success.

- Potency assays are critical to assess product functional activity, consistency, stability, and to provide evidence of comparability after changes to the manufacturing process. Therefore, we strongly encourage the evaluation of multiple product characteristics that could be used to establish a potency test during initial clinical studies. As these assays are critical to product development, we recommend that a potency test that measures a relevant biological activity be qualified for suitability (i.e., accurate, precise, sensitive, specific) prior to conducting trials intended to provide substantial evidence of effectiveness for a marketing application, and validated for licensure (Ref. 3).

- Limited availability of starting materials (e.g., autologous cells) and reference materials to design suitable assays to measure CQA, as well as limited process understanding, can hamper manufacturing process development, comparability studies, and process validation (Ref. 4). Sponsors are encouraged to consider, where possible, implementing manufacturing changes needed for commercial-scale production and demonstrating product comparability prior to the initiation of clinical trials intended to provide substantial evidence of effectiveness for a marketing application. Importantly, if product comparability cannot be demonstrated, additional clinical studies may be needed.

IV. CONSIDERATIONS FOR PRECLINICAL STUDIES

A preclinical program that is tailored to the investigational product and planned early-phase clinical trial contributes to characterization of the product's benefit/risk profile for the intended patient population. The overall objectives of a preclinical program for a GT product include: 1) identification of a biologically active dose range; 2) recommendations for an initial clinical dose level, dose-escalation schedule, and dosing regimen; 3) establishment of feasibility and reasonable safety of the proposed clinical route of administration (ROA); 4) support of patient eligibility criteria; and, 5) identification of potential toxicities and physiologic parameters that help guide clinical monitoring for a particular investigational product. In addition, to justify conducting a first-in-human clinical trial in pediatric subjects that is associated with more than a minor increase over minimal risk, the preclinical program should include studies designed to

demonstrate a prospect of direct benefit (21 CFR 50.53) of the investigational GT product (refer to section V.A. of this document for further discussion). This objective is important when clinical evidence is not available from adult subjects with the same disease.

Further details for general considerations in preclinical studies are available in a separate guidance document (Ref. 5). Although not specific to rare diseases, the following elements are recommended in the development of a preclinical program for an investigational GT product:

- Preclinical in vitro and in vivo proof-of-concept (POC) studies are recommended to establish feasibility and support the scientific rationale for administration of the investigational GT product in a clinical trial. Data derived from preclinical POC studies can guide the design of both the preclinical toxicology studies, as well as the early-phase clinical trials. The animal species and/or models selected should demonstrate a biological response to the investigational GT product that is similar to the expected response in humans.

- Biodistribution studies should be conducted to assess the pharmacokinetic (PK) profile of a GT product (Ref. 6). These data encompass the distribution profile of the vector from the site of administration to target and non-target tissues, including biofluids (e.g., blood, lymph node fluid, cerebrospinal fluid (CSF)) as applicable. These data can determine extent of tissue transduction and transgene expression, evaluate whether expression is transient or persistent, and guide the design of the preclinical toxicology studies as well as the early-phase clinical trials.

- Toxicology studies for an investigational GT product should incorporate the elements of the planned clinical trial (e.g., dose range, ROA, dosing schedule, evaluation endpoints, etc.) to the extent feasible. Study designs should be sufficiently comprehensive to permit identification, characterization, and quantification of potential local and systemic toxicities, their onset (i.e., acute or delayed) and potential mitigation and resolution, and the effect of dose level on these findings. In some cases, additional assessments may also be important to consider, such as safety and feasibility of the proposed GT delivery system and procedure, and immune response directed against vector and expressed transgene product.

- The conduct of additional nonclinical studies[4] may be needed to address such factors as: 1) the potential for developmental and reproductive toxicity; and 2) significant changes in the manufacturing process or formulation that may impact comparability between the product administered in clinical trials and the product intended for licensure.

[4] 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 or replace animal studies. Sponsors are encouraged to submit proposals and justify any potential alternative approaches, which we will evaluate for equivalency to animal studies.

V. CONSIDERATIONS FOR CLINICAL TRIALS

Many rare disorders are serious, with no approved treatments and represent substantial unmet medical needs for patients. Because of phenotypic heterogeneity, disease manifestations are likely to vary in onset and severity. Information obtained from a natural history study can potentially provide critical information to guide every stage of drug development from drug discovery to determining effectiveness and safety of the drug in treating a disease (Ref. 7). However, there may be insufficient information on the natural history of the disease to inform the selection of a historical comparator or to inform clinical endpoint selection in future clinical trials.

In a majority of these disorders, clinical manifestations appear early in life, and there are ethical and regulatory considerations regarding enrollment of children in clinical trials. These considerations should factor into the design of both early- and late-phase clinical trials. Further details of general considerations for GT clinical trials are available in a separate guidance document (Ref. 8).

The following important elements are recommended for consideration during clinical development of investigational GT products intended for treatment of rare diseases (although they are not exclusively applicable to GT products for rare diseases).

A. Study Population

Selection of the study population should consider existing preclinical or clinical data to determine the potential risks and benefits for the study subjects. In addition, sponsors should consider whether the proposed study population is likely to provide informative safety and/or efficacy data (Ref. 8). The following points should be considered with respect to trials of GT products for rare diseases:

- If the disease is caused by a genetic defect, the sponsor should perform genetic test(s) for the specific defect(s) of interest in all clinical trial subjects. This information is important to ensure correct diagnosis of the disorder of interest. In addition, since many of these disorders can involve either deletions or functional mutations at any of several loci within a specific gene, safety and effectiveness may be linked to genotype in unpredictable ways. Given this, early understanding of such associations may help in planning future clinical trials. Therefore, if there are no readily available, reliable means of obtaining the needed genetic diagnosis, a companion diagnostic may be needed and therefore should be considered early in development.

- Pre-existing antibody to the GT product may limit its therapeutic potential. Sponsors may choose to exclude patients with pre-existing antibodies to the GT product. In such cases, the sponsor should strongly consider contemporaneous development of a companion diagnostic to detect antibodies to the GT product. If an *in vitro* companion diagnostic is needed to appropriately select patients for

study (and later, once the GT product is approved, for treatment), then submission of the marketing application for the companion diagnostic and submission of the biologics license application for the GT product should be coordinated to support contemporaneous marketing authorizations.

- Severity of disease should be considered in designing clinical GT trials (Ref. 8), as well as the anticipated risk and potential benefits to subjects. Subjects with severe or advanced disease might experience confounding adverse events that are related to the underlying disease rather than to the GT product itself; however, they may be more willing to accept the risk of an investigational GT product in the context of the anticipated clinical benefit.

- Since most rare diseases are pediatric diseases or have onset of manifestations in childhood, pediatric studies are a critical part of drug development. However, treatment in pediatric patients cannot proceed without addressing ethical considerations for conducting investigations in vulnerable populations. Unless the risks of an investigational drug are no more than a minor increase over minimal risk (21 CFR 50.53), the administration of an investigational drug in children must offer a prospect of direct clinical benefit to individually enrolled patients, the risk must be justified by the anticipated benefit, and the anticipated risk-benefit profile must be at least as favorable as that presented by accepted alternative treatments (21 CFR 50.52). Additionally, adequate provisions must be made to obtain the permission of the parents and the assent of the child as per 21 CFR 50.55.

- The risks of most GT products include the possibility of unintended effects that may be permanent, along with adverse effects due to invasive procedures that may be necessary for product administration. Because of these risks, it is generally not acceptable to enroll normal, healthy volunteers into GT studies. A well-written informed consent document is also essential.

B. Study Design

For rare diseases, there may be a limited number of patients who may qualify for enrollment into a clinical study. As a result, it is often not feasible to enroll unique subjects for all studies conducted under different phases of the clinical development program. Limitation in the number of prospective subjects warrants the collection of as much pertinent data (e.g., adverse events, efficacy outcomes, biomarkers) as possible from every subject, starting from the first-in-human study. All such data may be valuable

to inform the design of subsequent studies (e.g., selection of study populations and endpoints). Sponsors developing GT products for rare diseases should consider the following:

- The randomized, concurrent-controlled trial is generally considered the ideal standard for establishing effectiveness and providing treatment-related safety data. Randomization in early stages of development is strongly encouraged when feasible.

- Sponsors should consider designing their first-in-human study to be an adequate and well-controlled investigation that has the potential, depending on the study results, to provide evidence of effectiveness to support a marketing application.

- To promote interpretability of data for studies that enroll subjects with different disease stages or severities, sponsors should consider stratified randomization based on disease stage/severity.

- For some GT indications (e.g., a genetic skin disease), the use of an intra-subject control design may be useful. Comparisons of local therapeutic effects can be facilitated by the elimination of variability among subjects in inter-subject designs.

- A single-arm trial using historical controls, sometimes including an initial observation period, may be considered if there are feasibility issues with conducting a randomized, controlled trial.

- If use of a type of single-arm trial design with a historical control is necessary, then knowledge of the natural history of disease is critical. Natural history data may provide the basis of a historical control, but only if the control and treatment populations are adequately matched, in terms of demographics, concurrent treatment, disease state, and other relevant factors. In circumstances where randomized, concurrent controlled trials cannot be conducted and the natural history is well characterized, sponsors may consider the clinical performance of available therapies (if there are any) when setting the performance goal or criteria against which the product effect will be tested.

- A small sample size, together with high inter-subject variability in clinical course, diminishes a study's power to detect treatment-related effects. Therefore, alternative trial designs and statistical techniques that maximize data from a small and potentially heterogeneous group of subjects should be considered. Ideally, utilizing as an endpoint a treatment outcome that virtually never occurs in the natural course of the disease would greatly facilitate the design and cogency of small trials.

- Adequate measures to minimize bias should be undertaken. The preferred approach to minimize bias is to use a study design that includes blinding.

C. Dose Selection

- Dose selection should be informed by all available sources of clinical information (e.g., publications, experience with similar products, experience in related patient populations).

- Leveraging non-human data obtained in animal models of disease and in vitro data may be, in some cases, the only way to estimate a starting human dose that is anticipated to provide benefit. Additional dosing information can be obtained from predictive models based on current understanding of in vitro enzyme kinetics (including characterizing the enzyme kinetics in relevant cell lines), and allometric scaling.

- For early-phase studies, clinical development of GT products should include evaluation of two or more dose levels to help identify the potentially therapeutic dose(s). Ideally, placebo controls should be added to each dose cohort.

- Some GT products may have an extended duration of activity, so that repeated dosing may not be an acceptable risk until there is a preliminary understanding of the product's toxicity and duration of activity.

Efforts should be made early in the GT product development program to identify and validate biomarkers and to leverage all available information from published investigations for the disease of interest (or related diseases). Some biomarkers or endpoints are very closely linked to the underlying pathophysiology of the disease (e.g., a missing metabolite in a critical biosynthetic pathway). In this case, total or substantial restoration of the biosynthetic metabolic pathway may generally be expected to confer clinical benefit. Changes in such biomarkers could be used during drug development for dose-selection, or even as an early demonstration of drug activity.

D. Safety Considerations

- Clinical trials should include a monitoring plan that is adequate to protect the safety of clinical trial subjects. The elements and procedures of the monitoring plan should be based upon what is known about the GT product, including preclinical toxicology, as well as CMC information, and, if available, previous human experience with the proposed product or related products (Ref. 8).

- Innate and adaptive immune responses directed against one or more components of GT products (e.g., against the vector and/or transgene) may impact product safety and efficacy. Early development of appropriate assays to measure product-

directed immune responses may be critical to program success. Development of neutralizing and non-neutralizing immune responses that are directed against the product should be monitored throughout the clinical trial (Ref. 9).

- When there is limited previous human experience with a specific GT product, administration to several subjects concurrently may expose those subjects to unacceptable risk. Most first-in-human trials of GT products should stagger administration to consecutively enrolled subjects, for at least an initial group of subjects, followed by staggering between dose cohorts. This approach limits the number of subjects who might be exposed to an unanticipated safety risk (Ref. 8). The optimal dosing interval between consecutively enrolled subjects and dose cohorts should be discussed with OTAT prior to conduct of the trial.

- Because of the unique nature of the mechanism of action involving genetic manipulation, a potential exists for serious long-term effects that may not be apparent during development or even at the time of an initial licensure. The long-term safety of GT products is currently unknown. The appropriate duration of long term follow-up depends on the results of preclinical studies with this product, knowledge of the disease process, and other scientific information (Ref. 6).

- Early-phase GT clinical trial protocols should generally include study stopping rules, which are criteria for halting the study based on the observed incidence of particular adverse events. The objective of study stopping rules is to limit subject exposure to risk in the event that safety concerns arise. Well-designed stopping rules may allow sponsors to assess and address risks identified as the trial proceeds, and to amend the protocol to mitigate such risks or to assure that human subjects are not exposed to unreasonable and significant risk of illness or injury.

- The potential for viral shedding should be addressed early in product development (Ref. 10).

E. Efficacy Endpoints

Demonstration of clinical benefit of a GT product follows the same principles as for any other product. However, in some cases there may be unique characteristics of GT products (e.g., a protein that is expressed by a GT product may have different bioactivity than standard enzyme replacement therapy) that warrant additional considerations both pre-approval and post-marketing. Prior to commencing clinical trials of GT products for rare diseases, it is critically important to have a discussion with FDA about the primary efficacy endpoint(s). For many rare diseases, well-established, disease-specific efficacy endpoints are not available (Ref. 11). Endpoint selection for a clinical trial of a GT product for a rare disease should consider the following:

- Sponsors should utilize an understanding the pathophysiology and natural history of a disease as fully as possible at the outset of product development. Full understanding of mechanism of product action is not required for product approval; however, understanding of pathophysiology is important in planning clinical trials, including selection of endpoints.

- For sponsors that are considering seeking accelerated approval of a GT product for a rare disease pursuant to section 506(c) of the Federal Food, Drug, and Cosmetic Act (FD&C Act) based on a surrogate endpoint, it will be particularly important to understand the pathophysiology and natural history of the disease in order to help identify potential surrogate endpoints that are reasonably likely to predict clinical benefit.

- Sponsors should identify specific aspects of the disease that are meaningful to the patient and might also be affected by the GT product's activity (Ref. 12).

- Considerable information can be gained by collecting clinical measurements repeatedly over time. Such longitudinal profile allows the assessments of effect, largely based on within-patient changes, that otherwise could not be studied.

F. Patient Experience

Patient experience data[5] may provide important additional information about the clinical benefit of a GT product. FDA encourages sponsors to collect patient experience data during product development, and to submit such data in the marketing application.

VI. EXPEDITED PROGRAMS

There are several programs that may be available to sponsors of GTs intended to address unmet medical needs in the treatment of serious or life-threatening conditions that are intended to facilitate and expedite development and review of these therapies, including regenerative medicine advanced therapy designation, breakthrough therapy designation, fast track designation, accelerated approval, and priority review. In particular, regenerative medicine advanced therapy designation and breakthrough therapy designation call for earlier attention

[5] As defined in section 569(c) of the FD&C Act, the term "patient experience data" includes data that are:

- Collected by any persons (including patients, family members and caregivers of patients, patient advocacy organizations, disease research foundations, researchers, and drug manufacturers); and
- Intended to provide information about patients' experiences with a disease or condition, including the impact (including physical and psychosocial impacts) of such disease or condition, or a related therapy or clinical investigation, on patients' lives; and patient preferences with respect to treatment of such disease or condition.

Additional information on Patient-Focused Drug Development can be found on this website: https://www.fda.gov/drugs/developmentapprovalprocess/ucm579400.htm

406 from FDA to these potentially promising therapies, offering sponsors earlier and more frequent
407 interactions with FDA on efficient trial design and overall drug development. Further
408 information on these programs is available in separate guidance documents[6,7].
409
410
411 **VII. COMMUNICATION WITH FDA**
412
413 FDA recommends communication with OTAT early in product development, before submission
414 of an IND. There are different meeting types that can be used for such discussions, depending
415 on the stage of product development and the issues to be considered. These include pre-IND
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418 from OTAT through an INTERACT meeting, which can be used to discuss issues such as a
419 product's early preclinical program, and/or through a pre-IND meeting prior to submission of the
420 IND (Ref. 13).
421

[6] Guidance for Industry: Expedited Programs for Serious Conditions – Drugs and Biologics, dated May 2014, https://www.fda.gov/downloads/Drugs/Guidances/UCM358301.pdf

[7] Expedited Programs for Regenerative Medicine Therapies for Serious Conditions, Draft Guidance for Industry, dated November 2017, https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM585414.pdf

[8] Going forward, INTERACT meetings will serve in place of pre-pre-IND meetings. For additional information about INTERACT meetings, please see https://www.fda.gov/BiologicsBloodVaccines/ResourcesforYou/Industry/ucm611501.htm.

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

Human Gene Therapy for Retinal Disorders

Draft Guidance for Industry

This guidance document is for comment purposes only.

Submit one set of either electronic or written comments on this draft guidance by the date provided in the *Federal Register* notice announcing the availability of the draft guidance. Submit electronic comments to https://www.regulations.gov. Submit written comments to the Dockets Management Staff (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. You should identify all comments with the docket number listed in the notice of availability that publishes in the *Federal Register*.

Additional copies of this guidance are available from the Office of Communication, Outreach and Development (OCOD), 10903 New Hampshire Ave., Bldg. 71, Rm. 3128, Silver Spring, MD 20993-0002, or by calling 1-800-835-4709 or 240-402-8010, or email ocod@fda.hhs.gov, or from the Internet at https://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.

For questions on the content of this guidance, contact OCOD at the phone numbers or email address listed above.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
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Human Gene Therapy for Retinal Disorders

Draft Guidance for Industry

This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff responsible for this guidance as listed on the title page.

I. INTRODUCTION

This guidance provides recommendations to stakeholders developing human gene therapy (GT) products[1] for retinal disorders affecting adult and pediatric patients. These disorders vary in etiology, prevalence, diagnosis, and management, and include genetic as well as age-related diseases. These disorders manifest with central or peripheral visual impairment and often with progressive visual loss. This guidance focuses on issues specific to GT products for retinal disorders and provides recommendations related to product development, preclinical testing, and clinical trial design for such GT products.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe 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] 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 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, (Ref.1) and ex vivo genetically modified human cells. Gene therapy products meet the definition of "biological product" in section 351(i) of the Public Health Service (PHS) Act (42 U.S.C. 262(i)) when such products are applicable to the prevention, treatment, or cure of a disease or condition of human beings.

II. CONSIDERATIONS FOR PRODUCT DEVELOPMENT

There are multiple GT products being studied in clinical trials in the United States for retinal disorders. GT products are commonly delivered by intravitreal or subretinal injections through a medical delivery system. In some cases, the GT products are encapsulated in a device to be implanted intravitreally.

The general chemistry, manufacturing and control (CMC) considerations for product manufacturing, testing and release of GT products for retinal disorders are the same as those described for other GT products (Ref. 2). For early-phase clinical trials, a sponsor should be able to evaluate the identity, purity, quality, dose, and safety of a GT product. A potency assay to assess the biological activity of the final product, with relevant lot release specifications, should be established prior to the initiation of clinical trials intended to provide substantial evidence of effectiveness for a marketing application. To support licensure of a GT product, manufacturing processes and all testing methods for product release must be validated (21 CFR 211.165(e)). Sponsors developing GT products for retinal disorders are strongly encouraged to contact the Office of Tissues and Advanced Therapies in the Center for Biologics Evaluation and Research (CBER) early in product development to discuss product-specific issues.

Sponsors of GT products for retinal disorders should take into account general CMC considerations for all GT products (Ref. 2), as well as CMC considerations specific to the products intended for treatment of retinal disorders, including:

- Consideration of the final product formulation and concentration to meet the expected dose and volume requirement;

- The endotoxin limit for intraocular delivery is not more than (NMT) 2.0 Endotoxin Unit (EU)/dose/eye or NMT 0.5 EU/mL (USP <771>);

- GT vector-based final products should be tested for particulate matter, and the test method and release criteria should follow USP <789>;

- Product testing and release should include testing of the final product configuration;

- Compatibility of the GT product and the delivery system should be evaluated.

III. CONSIDERATIONS FOR PRECLINICAL STUDIES

A preclinical program that is tailored to the investigational product and the planned early-phase clinical trials helps characterize the product's benefit/risk profile for the intended patient population. Overall objectives of the preclinical program for a GT product include: 1) identification of a biologically active dose level range; 2) recommendations for an initial clinical dose level, dose-escalation schedule, and dosing regimen; 3) establishment of feasibility and

reasonable safety of the proposed clinical route of administration (ROA); 4) support of patient eligibility criteria; and, 5) identification of potential toxicities and physiologic parameters that help guide clinical monitoring.

Further details for general considerations in preclinical studies of these investigational GT products are available in a separate guidance document.[2] The following elements are recommended for consideration when developing a preclinical program for an investigational GT product intended for treatment of retinal disorders (some of which are not necessarily exclusive to GT products for retinal disorders):

- Preclinical in vitro and in vivo proof-of-concept (POC) studies are recommended to establish feasibility and support the scientific rationale for administration of the investigational GT product in a clinical trial. Data derived from preclinical POC studies may guide the design of both the preclinical toxicology studies, as well as the early-phase clinical trials. The animal species and/or models selected should demonstrate a biological response to the investigational GT product that is similar to the expected response in humans.

- Biodistribution studies should be conducted to assess the pharmacokinetic profile of a GT product (Ref. 3). These data encompass the distribution, persistence, and clearance of the vector and possibly the expressed transgene product in vivo, from the site of administration to target ocular and non-ocular tissues, intraocular fluids, and blood. These data can determine extent of tissue transduction and transgene expression, evaluate whether expression is transient or persistent, and guide the design of the preclinical toxicology studies as well as the early-phase clinical trials.

- Toxicology studies for an investigational GT product should incorporate elements of the planned clinical trial (e.g., dose range, ROA, dosing schedule, and evaluation endpoints, etc.), to the extent feasible. Study designs should be sufficiently comprehensive to permit identification, characterization, and quantification of potential local and systemic toxicities, their onset (i.e., acute or delayed) and potential resolution, and the effect of dose level on these findings. For any abnormal ophthalmic findings or lesions, sponsors should determine the frequency, severity, potential cause, and clinical significance. Inflammatory or immune responses should be further characterized to assess potential attribution to the vector or transgene.

- Animal models of retinal disorders are frequently developed in rat or mouse strains (e.g., transgenic or knockout models) and these models are often utilized to generate POC

[2] Preclinical Assessment of Investigational Cellular and Gene Therapy Products; Guidance for Industry, dated November 2013, https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM376521.pdf

data.[3] However, due to differences in ocular size and anatomy in rodents as compared to the human eye, animals with more 'human-like' eyes, such as rabbits, pigs, dogs, or nonhuman primates, may also provide applicable safety information. Inclusion of the larger animals also facilitates relevant experience with the surgical procedures and delivery systems intended for clinical use.

- Differences between the immune responses of animals and humans are important considerations when interpreting preclinical data. Retinal disorders typically are bilateral and chronic. However, a second administration of a GT product to either the contralateral eye or to the same eye may not be feasible due to an immunologic reaction against the vector and/or the transgene product. Therefore, clinical data, rather than preclinical data, may provide the most relevant safety information for repeat product administration.

- As the clinical development program for an investigational GT product advances to late-phase clinical trials and possible marketing approval, additional preclinical studies may be indicated. Further testing may be necessary to address factors such as any significant changes in the manufacturing process or formulation, which may affect comparability of the late-phase product to product administered in early-phase clinical trials.

IV. CONSIDERATIONS FOR CLINICAL TRIALS

The fundamental considerations for clinical development programs of GT products for retinal disorders are similar to those for other biological products. Early-phase trials of GT products should not only evaluate safety and feasibility, but also gauge bioactivity and preliminary efficacy. Later-phase trials should be designed as adequate and well-controlled studies that can provide substantial evidence of effectiveness to support an application for marketing. For further details of general considerations for gene therapy clinical trials, please refer to relevant FDA guidance documents.[4,5]

The following important elements are recommended for consideration during development of clinical programs of investigational GT products intended for treatment of retinal disorders.

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[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 or replace animal studies. Sponsors are encouraged to submit proposals and justify any potential alternative approaches, which we will evaluate for equivalency to animal studies.

[4] Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products; Guidance for Industry, dated June 2015, https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM564952.pdf

[5] Guidance for Industry: Providing Clinical Evidence of Effectiveness for Human Drug and Biological Products, dated May 1998, https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM072008.pdf

A. Natural History Studies

A thorough understanding of the natural history of a disease is an important element in all clinical development programs. Many degenerative retinal disorders are rare, and their natural history is often poorly characterized. Early in product development, sponsors should evaluate the depth and quality of existing natural history data. When such information is insufficient to guide clinical development, FDA recommends that a sponsor perform a careful natural history study to facilitate the product development program, although FDA does not require these studies. Early interactions between FDA and sponsors are welcome regarding the design of natural history studies (Ref. 4).

B. Study Design

To facilitate interpretation of clinical data, inclusion of a randomized, concurrent parallel control group is recommended for clinical trials whenever possible. Administration of the vehicle alone may serve as a control. In general, while intravitreal injection of the vehicle alone is often feasible as a placebo control, it may not be considered ethically acceptable unless the physical properties of an injection in a closed space have a potential therapeutic benefit. When ethically acceptable, such a control is especially helpful early in clinical development, to evaluate bioactivity of the investigational GT product and possibly to provide initial evidence of its clinical efficacy. However, FDA acknowledges the risks associated with intravitreal and subretinal injection procedures and vehicles; without any prospect of direct benefit, these risks may not be acceptable under certain circumstances, such as for pediatric patients (21 CFR Part 50, Subpart D). Other possibilities to vehicle controls include alternative dosing regimens, alternative dose levels, and existing products approved for the indication being sought.

Measurement of certain efficacy and safety endpoints such as visual acuity is subjective, and results can be influenced by effort on the part of the patient, leading to a potential source of bias in the clinical trial. For trials intended to form the primary basis of an efficacy claim to support a marketing application, concurrent parallel group(s) should be used as a control (placebo or active) to decrease potential bias.

To further reduce potential bias, sponsors should include adequately-designed masking procedures. Differences between the procedure used for product delivery and a sham procedure may enable patients to distinguish the eye which received the product from that which received the sham treatment. FDA recommends at least two treatment arms, utilizing different doses but the same product administration procedures, to minimize patients' ability to identify their treatment arm, in addition to a sham control group. In addition to facilitating masking, the second treatment arm has value as a dose-ranging control.

Although use of the contralateral eye to which the GT product is not administered as a control may potentially be considered, it is generally not recommended due to the following:

- For most indications in which GT products are likely to be used, the treated eye and contralateral eye are often at different stages of disease at the time of trial entry. In addition, disease progression in the two eyes is not necessarily similar over the relatively short duration of the trial.

- When a patient is exposed to different procedures in the two eyes (e.g., one eye receives a GT product and the other eye receives sham procedure), it frequently leads to unmasking, which can confound the interpretation of the study results, particularly for endpoints where patient effort can make a difference, such as visual function measures.

C. Study Population

For clinical trials of GT products providing gene replacement, the correct genetic diagnosis is essential for identifying potential participants. Thus, confirmation of the genetic mutation prior to enrollment is recommended as an important element of the clinical trial. If there are no readily available, reliable means of obtaining the needed genetic diagnostic testing, a companion diagnostic may be needed and therefore should be strongly considered early in development. If an *in vitro* companion diagnostic is needed to appropriately select patients for study (and later, once the GT product is approved, for treatment), then submission of the marketing application for the companion diagnostic and submission of the biologics license application for the GT product should be coordinated to support contemporaneous marketing authorizations.

Patients with severe visual impairment, or a disease that is likely to progress to severe visual impairment, may be more willing to accept the potential or unknown risks of a novel GT product, and those risks may be more readily justified in this population. However, in some cases – for example, a GT product designed to restore function to remaining viable retinal cells – severely affected patients may not benefit from administration of the GT product nor would use in these patients provide information about the effectiveness of the product. In general, first-in-human GT trials should enroll patients with severities of visual impairment that offer a favorable benefit-risk profile. If preliminary safety data supports further clinical development, sponsors may consider a broader patient population in future trials.

Many retinal disorders affect both children and adults. For diseases that affect both adults and children, trials in adult patients should be conducted prior to trials in pediatric patients, whenever feasible. Since most rare diseases are pediatric diseases or have onset of manifestations in childhood, pediatric studies are a critical part of drug development. However, treatment in pediatric patients cannot proceed without addressing ethical considerations for conducting investigations in vulnerable populations. Unless the risks

of an investigational drug are no more than a minor increase over minimal risk (21 CFR 50.53), the administration of an investigational drug in children must offer a prospect of direct clinical benefit to individually enrolled patients, the risk must be justified by the anticipated benefit, and the anticipated risk-benefit profile must be at least as favorable as that presented by accepted alternative treatments (21 CFR 50.52). Additionally, adequate provisions must be made to obtain the permission of the parents and the assent of the child as per 21 CFR 50.55.

D. Study Use

For early-phase trials, dose-ranging study designs are recommended. Comparing a range of doses can identify potential therapeutic doses for a wider group of patients. The choice of an initial dose and dose regimen should be supported by preclinical studies and/or available clinical information. Such data should indicate that the initial dose is not only reasonably safe, but also has therapeutic potential, particularly when the administration procedure carries substantial risks.

Most retinal indications for which GT products are studied involve bilateral disease; consideration, therefore, should be given during product development to the planned administration of the GT product in both eyes. Because of safety concerns related to the product, administration procedure, and any ancillary medications, administration to each eye for an individual patient should be performed sequentially, rather than simultaneously. While often the eye with more advanced disease receives the GT product initially, a rationale should be developed for deciding which eye will receive the GT product first. The time interval between administration in each eye should be carefully planned for each patient based on preclinical data and available human experience. For products intended for both eyes, the overall development plan prior to approval should include clinical trials in which both eyes receive the GT product.

To ensure consistency across study sites, sponsors should include in the study protocol a detailed description of the product delivery procedure and devices used for delivery.

A single administration of a GT product in each eye may not always be sufficient for a variety of reasons. In such cases, careful studies, especially trials in humans, are recommended to explore the feasibility of repeat administration in the same eye.

E. Safety Considerations

Intraocular administration (e.g., intravitreal or subretinal injection) may be the most efficient method to deliver GT products intended for treatment of retinal disorders. Risks of such procedures include intraocular infection, elevated intraocular pressure, media opacities, and retinal damage. Therefore, the procedure should be performed by individuals experienced in the method of planned delivery.

Local or systemic immune responses to GT products may pose important safety risks. For certain GT products, such as those using various viral vectors to introduce therapeutic transgene(s) in vivo, immune reactions also may decrease transduction efficiency and thereby diminish the treatment effect. Biomicroscopy and optical coherence tomography are recommended to detect inflammatory reactions within the globe. To monitor systemic immune reactions, immunoassays should be performed to measure cellular and humoral immune responses to the vector and the transgene-encoded protein.

To minimize immune responses, immunosuppressants such as corticosteroids may be considered before and after product administration. Immunosuppressant drugs may cause increased intraocular pressure, cataracts, and other adverse events. Patients should be closely monitored and treated as necessary to minimize the risk of developing glaucoma, vision loss, and other complications.

F. Study Endpoints

Early-phase clinical trials typically focus on safety. However, for trials of GT products, early assessment of potential clinical benefit is also important, particularly for rare diseases with a limited number of patients available to participate in clinical development. To guide further clinical development, FDA encourages sponsors to explore a wide spectrum of potential clinical endpoints and other clinical effects in early-phase trials. For example, sponsors may include endpoints based on retinal imaging (optical coherence tomography, retinal photography, fluorescein angiography), visual acuity (low and high luminance), visual fields, color vision, contrast sensitivity, other measures of visual function (i.e., how well the eye and visual system function), and functional vision (i.e., how well the patient performs vision-related activities of daily living). For later-phase trials intended to provide substantial evidence of effectiveness to support a marketing application, primary efficacy endpoints should reflect clinical benefit, such as improvement in function or symptoms.

Examples of established efficacy endpoints that can be used to evaluate clinical benefit of GT products intended for treatment of retinal disorders include:

- Best corrected distance visual acuity, measured with the Early Treatment of Diabetic Retinopathy Study (ETDRS) chart or other visual acuity charts with an equal number of letters per line and equivalent spacing between lines. A halving (or doubling) of the visual angle represented by a gain (or loss), respectively, of at least 15 letters on the ETDRS chart from baseline is considered clinically meaningful.

- Rate of photoreceptor loss, determined by measures such as optical coherence tomography or autofluorescence photography. The comparison should be made between the baseline and at least two subsequent area images, with intervals of 6 months or more between images. The best curve fit analyses demonstrating

reduction in the rate of photoreceptor loss exceeding measurement uncertainty are considered clinically meaningful.

FDA encourages sponsors to develop and propose novel endpoints to measure clinically meaningful effects in patients with retinal disorders. This can be especially pertinent to some rare retinal disorders for which the established efficacy endpoints may not be appropriate to assess clinically meaningful effect of an investigational product. Sponsors are welcome to engage FDA early in this process, and FDA is committed to working with sponsors to develop acceptable endpoints.

- For example, a novel primary efficacy endpoint measuring mobility under different levels of illumination was utilized to support marketing approval for voretigene neparvovec-rzyl (a recombinant adeno-associated vector (AAV) carrying the gene for human retinal pigment epithelium-specific 65 kDa protein). During the clinical trials, the sponsor worked with FDA to develop this clinically meaningful primary efficacy endpoint.

G. Follow-Up Duration

The length of follow-up to provide additional information regarding the safety and efficacy of the GT product depends on many aspects of a GT product, including vector persistence, genome integration, and transgene activity, and the goal of the follow-up (e.g., safety vs. durability of clinical effect). In addition to monitoring for safety, long-term follow-up is recommended to evaluate durability of the clinical effect. More detailed discussion of long-term follow-up is provided in a separate FDA guidance document (Ref. 3).

H. Patient Experience

Patient experience data[6] may provide important additional information about the clinical benefit of a GT product. FDA encourages sponsors to collect patient experience data during product development, and to submit such data in the marketing application.

[6] As defined in the section 569(c) of the Federal Food, Drug, and Cosmetic Act (FD&C Act), the term "patient experience data" includes data that are:

- Collected by any persons (including patients, family members and caregivers of patients, patient advocacy organizations, disease research foundations, researchers, and drug manufacturers); and
- Intended to provide information about patients' experiences with a disease or condition, including the impact (including physical and psychosocial impacts) of such disease or condition, or a related therapy or clinical investigation, on patients' lives; and patient preferences with respect to treatment of such disease or condition.

Additional information on Patient-Focused Drug Development can be found on this website: https://www.fda.gov/drugs/developmentapprovalprocess/ucm579400.htm

V. EXPEDITED PROGRAMS

There are several programs that may be available to sponsors of GTs intended to address unmet medical needs in the treatment of serious or life-threatening conditions that are intended to facilitate and expedite development and review of these therapies, including regenerative medicine advanced therapy designation, breakthrough therapy designation, fast track designation, accelerated approval, and priority review. In particular, regenerative medicine advanced therapy designation and breakthrough therapy designation call for earlier attention from FDA to these potentially promising therapies, offering sponsors earlier and more frequent interactions with FDA on efficient trial design and overall drug development. Further information on these programs is available in separate guidance documents.[7,8]

VI. COMMUNICATION WITH FDA

FDA recommends communication with OTAT early in product development, before submission of an investigational new drug application (IND).) There are different meeting types that can be used for such discussions, depending on the stage of product development and the issues to be considered. These include pre-IND meetings and, earlier in development, INitial Targeted Engagement for Regulatory Advice on CBER producTs (INTERACT) meetings.[9] Early nonbinding, regulatory advice can be obtained from OTAT through an INTERACT meeting, which can be used to discuss issues such as a product's early preclinical program, and/or through a pre-IND meeting prior to submission of the IND (Ref. 5).

[7] Guidance for Industry: Expedited Programs for Serious Conditions – Drugs and Biologics, dated May 2014, https://www.fda.gov/downloads/Drugs/Guidances/UCM358301.pdf

[8] Expedited Programs for Regenerative Medicine Therapies for Serious Conditions: Draft Guidance for Industry, dated November 2017, (when finalized), https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM585414.pdf

[9] Going forward, INTERACT meetings will serve in place of pre-pre-IND meetings. For additional information about INTERACT meetings, please see https://www.fda.gov/BiologicsBloodVaccines/ResourcesforYou/Industry/ucm611501.htm.

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