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Draft Guidance for Industry

This guidance document is for comment purposes only.

Submit one set of either electronic or written comments on this draft guidance by the date provided in the *Federal Register* notice announcing the availability of the draft guidance.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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Table of Contents

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This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA or Agency) on this topic.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 The purpose of this draft guidance is to inform sponsors how to provide sufficient CMC information required to assure product safety, identity, quality, purity, and strength (including potency)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This draft guidance, when finalized, will supersede the document entitled "Guidance for FDA Reviewers and Sponsors: Content and Review of Chemistry, Manufacturing, and Control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.

VI. REFERENCES

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