الهيئة العامة للغذاء والدواء
Saudi Food & Drug Authority

Guidance on Submission of Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Clinical Trial Applications

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Saudi Food & Drug Authority

Drug Sector

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Saudi Food and Drug Authority

Vision and Mission

Vision

To be a leading international science-based regulator to protect and promote public health

Mission

Protecting the community through regulations and effective controls to ensure the safety of food, drugs, medical devices, cosmetics, pesticides and feed

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Table of Contents

Acronyms and Abbreviations 6

I. Introduction 8

ii. Background 8

iii. Administrative information 10

1. Administrative documents 10

2. Labels 10

3. Previously Submitted Information 10

IV. Summary of quality information 11

1. General information 11

2. Drug Substance and Drug Product 11

3. Combination Products 12

4. Product Handling at the Clinical Site 12

V. Manufacturing process and control information (module 3 of the CTD) 13

1. Drug Substance (3.2.S) 13

2. Drug Product (3.2.P) 37

3. Appendices (3.2.A) 47

4. Regional Information (3.2.R) 47

Annex 1: General Considerations for CAR-T Cell Products 48

Acronyms and Abbreviations

AAV	ADENO-ASSOCIATED VIRUS
BHK21	Baby Hamster Kidney Cells
CAR-T	Chimeric Antigen Receptor T Cells
cGMP	current Good Manufacturing Practice
CMC	Chemistry, Manufacturing, and Control
CMO	Contract Manufacturing Organization
CMV	Cytomegalovirus
CoA	Certificate of Analysis
CoO	Certificate of Origin
CQA	Critical Quality Attribute
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
CTD	Common Technical Document
DP	Drug Product
DS	Drug Substance
EBV	Epstein-Barr Virus
EMA	European Medicines Agency
EOP	End of Production
FDA	Food and Drug Administration
GMP	Good Manufacturing Practice
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HEK293	Human Embryonic Kidney 293 Cells
HHV-6, HHV-7, HHV-8	Human Herpesvirus 6, 7, and 8
HIV	Human Immunodeficiency Virus

HPV	Human papillomavirus
HSA	Human Serum Albumin
HTLV-1, HTLV-2	Human T-Cell Lymphotropic Virus 1 and 2
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals For Human Use
IL-2	Interleukin-2
IP	Investigational Product
JC VIRUS	John Cunningham Virus
MCB	Master Cell Bank
MVB	Master Viral Bank
PCR	Polymerase Chain Reaction
QA	Quality Assurance
QC	Quality Control
RCA	Replication-Competent Adenovirus
RcAAV	Replication-Competent Adeno-Associated Virus
RCR	Replication-Competent Retrovirus
SFDA	Saudi Food and Drug Authority
SOP	Standard Operating Procedure
TSE	Transmissible Spongiform Encephalopathy
USP	United States Pharmacopeia
VCN	Vector Copy Number
WCB	Working Cell Bank
WVB	Working Viral Bank

I. INTRODUCTION

This guideline addresses the regulatory requirements for Chemistry, Manufacturing, and Control (CMC) Information in human gene therapy Clinical Trial Applications submitted to the Saudi Food and Drug Authority (SFDA).

The guideline reflects the SFDA's views and expectations considering the internationally recognized principles, best regulatory practices, and the following guidelines:

- Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Somatic Cell Therapy Investigational New Drug Applications (INDs)–U.S. Food and Drug Administration.
- Guideline on the requirements for quality documentation concerning biological investigational medicinal products in clinical trials)–European Medicines Agency.

The objective of this document is to provide a clear guidance on the CMC requirements for investigational human gene therapies to ensure that such products meet appropriate levels of quality and safety for clinical studies.

II. BACKGROUND

Human gene therapy products are classified as biological products in the SFDA Guideline on the Classification of Advanced Therapy Medicinal Products". According to the guideline, a gene therapy medicinal product is defined as a biological medicinal product with the following characteristics:

a) it contains an active substance which contains or consists of a recombinant nucleic acid used in or administered to human beings with a view to regulating, repairing, replacing, adding or deleting a genetic sequence;

b) its therapeutic, prophylactic or diagnostic effect relates directly to the recombinant nucleic acid sequence it contains, or to the product of genetic expression of this sequence. Gene therapy medicinal products shall not include vaccines against infectious diseases.

Any clinical trial application, including clinical trials for gene therapy products, is required to describe the CMC information for the investigational product including drug substance (DS) and the Drug Product (DP). The SFDA may place the product on clinical hold if the clinical trial application does not contain sufficient CMC information to assess the risks to subjects in the proposed studies.

The CMC information submitted in a clinical trial application describes the applicant's commitment to perform manufacturing and testing of the investigational product as stated. It is acknowledged that manufacturing changes may be necessary as product development proceeds, and the applicant should submit information on amendments to supplement the initial information submitted for the CMC processes. In addition, if manufacturing is changed, the applicant should submit the change for SFDA review prior to implementation.

SFDA recommends that applicants organize and categorize their CMC information in an investigational product (IP) dossier, in accordance with the electronic Common Technical Document (eCTD) format for Module 3. Applicants are advised to read this guidance in conjugation with The Saudi Clinical Trial Registry SCTR published guidance for submission.

The amount of CMC information to be submitted in a clinical trial application depends on the phase of investigation and the scope of the clinical investigation proposed. The emphasis for CMC review in all phases of development is product safety and manufacturing controls. It is expected that applicants may need to make modifications and additions to previously submitted information as clinical development progresses and additional product knowledge and manufacturing experience is collected.

The following sections provide detailed recommendations for submitting IP dossier.

III. ADMINISTRATIVE INFORMATION

1. Administrative documents

Administrative documents e.g., application forms, cover letters, reviewer guides, cross-reference, and labeling information should be included in the clinical trial application. The cover letter should include a brief explanation of the submission and its contents. When amendments are submitted to the clinical trial application for manufacturing changes, the cover letter should clearly describe the purpose of the amendment and highlight proposed changes. If numerous amendments or significant changes are made (e.g., manufacturing process, assays for critical quality attributes etc.), it is recommended to include a reviewer guide and a tracked-changes document.

2. Labels

The clinical trial application must include a copy of all labels and labeling to be provided to each investigator in the clinical study. It is recommended to include sample or mock-up labels in the administrative section of the application. The applicants should note that investigational products must bear a label indicating that it is for "investigational use only". Other important labeling information such as:

- For products derived from autologous sources the statement: "FOR AUTOLOGOUS USE ONLY", should be used. And,

- If donor testing and screening is not performed, the product should be labeled "NOT EVALUATED FOR INFECTIOUS SUBSTANCES"

3. Previously Submitted Information

If the applicant wishes to cross-reference another product, the applicant is required to submit a written statement in the clinical trial application that clearly identifies the previously submitted information. The applicant should describe the information and identify where that information is located in the previously submitted file.

IV. SUMMARY OF QUALITY INFORMATION

1. General information

The clinical trial application should include a general introduction to the quality aspects of the product (safety, identity, purity, potency/strength and stability) of the investigational product, mode of action, and proposed clinical use. A description of potential critical quality attributes (CQAs) that are relevant to the safety and biological activity of the product as they are understood by the applicant at the time of submission should be provided. A CQA is 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. SFDA acknowledges that as the applicant's knowledge about the product increases throughout the development stages. In the early clinical trial phases, understanding of the product's characteristics and CQAs is limited and as the clinical trials progress, understanding of the products is expected to advance, and therefore the identities and limits of CQAs should be refined accordingly. During the early stages, it is recommended to conduct extensive product characterization to help the applicant identify and understand CQAs, which in turn will help in defining manufacturing process controls, manufacturing consistency, and stability.

2. Drug Substance and Drug Product

The clinical trial application must contain a description of the Drug substance (DS) and Drugs product (DP), including the physical, chemical, or biological characteristics, manufacturing controls, and testing information, to ensure the DS and DP meet acceptable limits (safety, identity, purity, potency/strength and stability)

Drug substance (DS) means the same as "active pharmaceutical ingredient" which is further described as any substance that is intended for incorporation into a finished DP and is intended to furnish pharmacological 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.

Drug product (DP) is defined as "a finished dosage form" that contains an active drug ingredient generally, but not necessarily, in association with inactive ingredients. Additionally, a vector in its final formulation used for administration of the genetic material may also be an active ingredient, depending upon the manufacturing process and formulation of the finished dosage form.

It is recognized 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 a distinct DS. While others may consist of two or more different DSs that are combined to make the DP. When the manufacturing process includes more than one DS, it is recommended to provide separate DS sections for each active ingredient of the final product. A summary of the available stability data for the DS and the DP, along with the recommended storage conditions, and shelf life, if applicable, should also be included in each DS and DP section.

3. Combination Products

For submissions in which the gene therapy is a component of a combination product, it is recommended to briefly describe the combination product in the summary and state the regulatory status of each component.

4. Product Handling at the Clinical Site

Proper control of the finished DP is critical to the investigational studies. Therefore, the clinical trial application should 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. The clinical trial application 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. Information for product handling at the clinical site prior to administration (such as thawing, 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)

should be provided. Details regarding product stability after preparation for delivery and delivery device compatibility data should be provided in IP dossier (sections 3.2.P.8 and 3.2.P.2.6)-respectively. Instructions for drug handing and preparation for administration at the clinical site (e.g., Pharmacy Manual, Instructions for Use, Investigator's Brochure) should be provided in the appropriate section of the clinical trial application.

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

The headings and text below include IP dossier section numbers in parentheses for reference (Ref. 1).

1. Drug Substance (3.2.S)

1.1. General Information (3.2.S.1)

a. **Nomenclature (3.2.S.1.1)**

The applicant should provide the name of the DS(s). If the name of the DS has changed during clinical development, the applicant should list all names used to identify the DS at all stages of development.

b. **Structure (3.2.S.1.2)**

The applicant should submit information on the molecular structure, including the genetic sequence if applicable, 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 (RE) sites, and functional components (e.g., transgene, selection markers). The applicant should also submit information on the sequence analysis and the annotated sequence data in the application. It is recommended that sequence data, particularly data supporting the genetic stability of the vector, be included in the submission.

Some specific examples of structure and structural elements of different gene therapy products are outlined below:

- **For viral-based gene therapies**: the applicant 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). It is recommended to include biophysical characteristics (e.g., molecular weight, particle size) and biochemical characteristics (e.g., glycosylation sites). The applicant should also describe the nature of the genome of viral vectors, whether single-stranded, double-stranded, or self-complementary, deoxyribonucleic acid (DNA) or RNA, and the copy number of genomes per particle.

- **For microbial-based gene therapies**: the applicant 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, if applicable.

- **For ex vivo genetically modified cell-based gene therapies:** such as Chimeric Antigen Receptor modified T cells (CARTs), the applicant 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, the applicant should describe the genes that is altered and how the changes were made (i.e., the gene editing technology used).

c. **General Properties (3.2.S.1.3)**

The applicant should provide a section in the clinical trial application that describes the composition and properties of the DS, including the biological activity and proposed mechanisms of action.

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

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

The applicant must provide the name and address of each manufacturer, including contract manufacturer(s), involved in the manufacture, testing, and storage of the DS. The applicant should indicate the responsibility of each manufacturer. The clinical trial application should contain complete information on the DS manufacturer, regardless of whether the process is performed by the applicant or by a contract manufacturing organization (CMO).

The applicant is ultimately responsible for the safety of subjects in the clinical investigation; therefore, it is recommended that the applicant and the CMO ensure product quality.

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

The description of the DS manufacturing process and process controls should include the following, as applicable: cell culture; transduction; cell expansion; harvest(s); purification; filling; storage and shipping conditions. The description should also accurately represent the process and process controls. It is acknowledged that information on process controls may be limited early in development, and it is recommended that applicants provide additional information and updates as product development proceeds. Changes and updates to the DS manufacturing process and process controls should be submitted as an amendment to the clinical trial application prior to implementation for investigational use in clinical studies.

- Batch and Scale

A description of how the applicant defines each manufacturing run (i.e., batch, lot, other) should be submitted, along with an explanation of the batch numbering system. The applicant should clearly state whether any pooling of harvests or intermediates occurs during manufacturing. If pooling is necessary during production, it is recommended that storage conditions (e.g., time, temperature, container closure system) for each pool be controlled, and that testing performed on each pool be described in the clinical trial application.

It is also recommended that the applicant provide an explanation of 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, or number of gene modified cells) to facilitate review and allow a better understanding of the manufacturing process. When known, the expected yield per batch should also be included.

- Manufacturing Process

The description of the manufacturing process should include a process flow diagram(s) and a detailed narrative. The 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). It is acknowledged that this information may be collected over the course of product development and may be submitted in a stage appropriate manner.

SFDA recommends that the applicant monitor process performance parameters for process consistency. Process trend analysis and evaluation of process parameters and materials will help determine and establish process control strategies. The applicant should clearly describe any controls for cleaning and changeover as well as tracking and segregation procedures that are in place to prevent cross- contamination throughout the manufacturing process.

- Cell Culture (for Vector Production)

The description of all cell culture conditions should contain sufficient detail to make any of the applicable process steps understandable, including process timing, culture conditions, hold times/transfer steps, and materials used (e.g., media components, bags/flasks). The applicant should describe whether the cell culture system is open or closed and any aseptic processing steps. If extensive culture times are needed, the applicant should outline the in-process controls used to monitor cell quality (e.g., viability, bioburden, pH, dissolved O2).

- Vector Production

For the manufacture of gene therapy vectors (e.g., virus, bacteria, plasmids), the applicant should provide a description of all production and purification procedures.

Production procedures should include the cell culture and expansion steps, transfection or infection procedures, harvest steps, hold times, vector purification (e.g., density gradient centrifugation, column purification), concentration or buffer exchange steps, and the reagents/components used during these processes. The applicant should outline any in-

process testing to ensure vector quality as appropriate (e.g., titer, impurities).

The applicant should describe whether the vector DS will be formulated into the DP for administration of the genetic material or whether it will be formulated as a bulk DS for ex vivo genetic modification of cells. As an active pharmaceutical ingredient, an appropriate level of control should be applied to each DS, and each DS should be manufactured under current Good Manufacturing Practice (cGMP) conditions that are appropriate for the stage of development.

- Genetically Modified Cell Production

If the gene therapy product consists of genetically modified cells, the cell processing description should contain sufficient detail to make any of the following applicable process steps understandable: source material (e.g., autologous or allogeneic cells, donor eligibility when applicable); 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; cell harvest and purification -if any-, and materials used.

The applicant 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. The vector used should be described in detail as indicated above.

- Irradiated Cells

If the product contains or is processed with irradiated cells, the applicant should provide information on the irradiator source, documentation of 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.

- Filling, Storage, and Transportation (Shipping)

The applicant should provide a detailed description and identify any associated process controls for formulation, filling, storage, and shipping of the DS, if

applicable. The applicant should also describe the container closure system used for storage and shipping of the DS. It is recommended that the applicant describe procedures that are in place to ensure appropriate storage and transport (as needed).

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

The applicant must provide a list of all materials used in manufacturing and a description of the quality or grade of these materials. This information, which is recommended to be provided in tabular format, should include the identity of the material, the supplier (e.g., batch, lot, other identifier), the quality (such as clinical grade) the source of material (e.g., animal, human, insect), and the stage at which each material is used in the manufacturing process (e.g., cell culture, 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 that come into contact with the product, such as culture bags, culture flasks, chromatography matrices, and tubing. It is important to note that information relevant to materials and their control should be provided under the Control of Materials section in the drug substance and drug product as appropriate.

The applicant should establish a risk-based materials qualification program and 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). The nature and extent of materials qualification, including the testing required, will depend on the specific material and its risk potential, the manner in which it is used in the manufacturing process, and the stage of clinical development.

- Reagents

For the purposes of this guidance, reagents (or raw materials) are those materials used in the manufacturing process, but 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, culture media and 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 those of human or animal origin, special consideration must be given to the risk of introducing adventitious agents. Animal-derived materials, such as sera, are complex mixtures that are difficult to standardize. These materials may exhibit significant batch-to-batch variations which can affect the reproducibility of the manufacturing process and compromise the quality of the final product.

SFDA recommends the use of non-animal-derived reagents wherever possible for example, serum-free tissue culture media or recombinant proteases. However, it should be noted that recombinant reagents may still pose risks related to adventitious agents, depending on their source materials and manufacturing process.

For biological materials such as material derived from human or animal sources, SFDA may require information on the manufacturing and/or testing to evaluate the safety and quality of the material. For example:

- **Bovine derived materials:**

The applicant should provide information on any bovine material used in the manufacturing, including: source of the material, 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, SFDA recommends that it be γ-irradiated to reduce the risk of adventitious agents. This information may be included on the Certificate of Analysis (CoA) and Certificate of Origin (CoO) provided from the supplier.

- **Recombinant proteins (such as cytokines):**

  Qualification of recombinant proteins should be risk-based. SFDA-approved pharmaceutical-grade recombinant proteins do not require further adventitious agent testing, but may require testing to demonstrate performance in manufacturing the gene therapy product. Other recombinant proteins may require targeted adventitious agent

testing, depending on the cell lines and materials used in their production and purification. The applicant should consider that many recombinant proteins (such as cytokines) and monoclonal antibodies 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, which should be controlled by the supplier.

- **Murine or Monoclonal Antibodies:**

  The applicant should determine and, if necessary, address the risk of contamination with adventitious agents from rodents.

- **Human Source:**

  a. Human albumin

     It is recommended to use SFDA approved products, and to have procedures in place to ensure that no recalled lots were used during manufacture or preparation of the product.

  b. Human serum

     Human AB serum may be used in manufacturing the gene therapy product. However, SFDA recommends that the applicant ensure the AB serum used in manufacturing the product does not have the potential to transmit infectious disease.

     If the serum is derived from Source Plasma, which may be pooled from a large number of donors and is not tested as extensively as blood products intended for infusion, the applicant may reduce the risk of infectious disease transmission by conducting additional testing for relevant transfusion-transmitted infections.

     Alternatively, it may be acceptable to include viral inactivation or clearance steps in the production of human AB serum from Source Plasma.

  c. Other human-derived reagents

     For all other reagents that are human-derived, the applicant 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.

- Cells - Autologous and Allogeneic Cells or Tissue

For autologous or allogeneic cells or tissue, the applicant should provide a description of the cell source, the collection procedure, and any related handling, culturing, processing, storage, shipping, and testing that is performed prior to use in manufacturing. The 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, bone marrow aspiration, or apheresis).
- Enrichment steps and cryopreservation, if performed.
- Labeling and tracking of collected samples.
- Hold times
- Transportation conditions to the manufacturing facility.

As an example, for cells collected by leukapheresis: the applicant should provide a summary 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. Establishing well-designed process controls and standard operating procedures (SOPs) for manipulating and handling starting, and in-process materials can help reduce variability in the manufacturing process and ultimately in the DS and DP.

This is especially important for multi-center clinical trials, where establishing standardized procedures for cell collection and handling across all collection sites is critical to ensuring the quality and safety of the final product and maintaining control of the manufacturing process. The clinical trial application should include a list of collection sites that comply with SFDA regulations.

a. Autologous Cells

No donor eligibility screening is required for cells and tissues for autologous use. However, the applicant should determine based on patient information, whether the

manufacturing procedures could increase the risk to the patient by further propagation of pathogenic agents that may be present in the starting material (patient cellular material). SFDA recommends that the applicant describe precautions to prevent the spread of viruses or other adventitious agents to individuals other than the autologous recipient and to prevent cross contamination with another patient's material.

b. Allogeneic Cells

Allogeneic leukapheresis starting material requires a donor eligibility determination, including screening and testing for relevant communicable disease agents and diseases, please refer to the table below:

	Autologous	**Allogeneic**
Donor screening and testing	Not required	Required
List of tastings	-	HIV-1, HIV-2, hepatitis B virus (HBV), surface and core antigen), hepatitis C virus (HCV), human TSE, including Creutzfeldt-Jakob disease (CJD) and variant CJD (vCJD); and Treponema pallidum (syphilis)
Testing for Donors of viable leukocyte-rich cells or tissues	-	(HTLV-1, HTLV-2) and CMV
If cord blood other maternally derived tissue	-	Describe testing and screening performed on birth mothers.

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, SFDA generally recommends qualifying allogeneic master and working cell banks in the same way as cell banks used for the production of viral vectors (see Cell Banking Systems,

below), provided that sufficient cellular material is available for this testing. In these situations, SFDA is primarily concerned about the introduction of adventitious agents (e.g., viruses, bacteria, and mycoplasma) during the bank manufacturing process, especially from human or bovine materials, animal feeder cells, other animal-derived reagents, or human AB serum if used. If the allogeneic cell bank is small and limited material is available for testing, the applicant should consult with the SFDA for more information on appropriate cell bank qualification.

- 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 throughout development and commercialization. For these reasons, banked materials are a common starting point for many routine production applications.

It is recommended that the applicant provide a description of the testing of the cell bank and relevant CoA(s) in section 3.2.A.1 of the IP dossier. Information on bank qualification and adventitious agent testing should also be included in the comprehensive Adventitious Agents Safety Evaluation (3.2.A.1) section of the IP dossier.

a. **Master Cell Banks Used as Substrates for Production of Viral Vectors**
SFDA recommends that the applicant, when selecting a cell line for viral vector manufacturing, consider characteristics of the cells that may impact the safety of the final product. Safety-related characteristics, such as the presence of tumorigenic sequences, are especially important when the viral vector co-packages non-vector sequences, such as adeno-associated virus (AAV). Cell attributes that can affect production capacity, such as growth characteristics and vector production capacity, should also be considered. In the clinical trial application, the applicant should provide a description of the history and detailed derivation of the source material for the cell bank. The description should include information on: 1. **The cell source** (including species of origin). 2. **How the bank was generated** (e.g., from a single colony isolate or through limiting dilution). 3. **Testing performed to characterize the bank** (e.g., CoA); and if applicable, materials used to genetically modify the source material (e.g., packaging cell line). 4. **Information on the quality of the materials** (e.g., plasmids, viruses, gene-editing components)

used to introduce the genetic changes.

5. **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 the manufacturing process.**
6. **Ensure absence of adventitious agent, such as:**
    - Microbial contamination including sterility, mycoplasma (and spiroplasma for insect cells), and adventitious viral agents. The applicant should consider the cell line used to conduct appropriate testing for example testing for bovine adventitious agents, including viral clearance studies in case of adventitious viral agent presence in the bank.
7. **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, -7 and -8 (HHV-6, -7 & -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**, it is recommended to test for species-specific viruses, as appropriate. For instance, for Vero cells, testing for simian polyomavirus SV40 and simian retrovirus is recommended.
    - **For insect cells,** the applicant should 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.
8. **Cell testing including identity, genetic stability and tumorigenicity.** The applicant should identify the cells through tests that distinguish them from other cell lines used in the facility. For cell lines that the applicant has purchased from a type collection, vendor, or received from another investigator, SFDA recommends 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). Stability should be also assessed by measuring viability of cells over time after cryopreservation. For stable retroviral vector producer cells, it is recommended that the applicant test the genetic stability of the gene insert in the EOP cells. It is also recommended that the applicant perform tumorigenicity tests for cell lines that have not been previously characterized for their potential to form tumors.

b. **Working Cell Banks**

Since a Working Cell Bank (WCB) is derived from one or more vials of the MCB, the amount of characterization testing for a WCB is less extensive than that for the MCB. The testing should generally include sterility, mycoplasma, identity, and in-vitro adventitious-agent tests. Under certain conditions and with proper justification, the MCB may be used as WCB.

c. **Bacterial or Microbial Master Cell Banks**

Bacterial MCBs are frequently used as the starting material to generate plasmid DNA, which can be used as a gene therapy DS or used as a manufacturing intermediate to generate a DS for other gene therapy products such as AAV or lentiviral vectors. Bacterial MCBs also may be used to generate a microbial vector for gene therapy.

The establishment of a bacterial MCB is generally recommended, as it can provide a consistent starting material for the manufacture of plasmids or microbial vectors. However, MCBs are not always necessary for all manufacturing situations if the plasmid intermediate is appropriately qualified (e.g., for early phase studies when the plasmid is used to make a vector for ex vivo modification of cells). It is recommended that the bacterial MCBs be qualified, and sufficient detailed information be submitted for the qualification of the banked material, regardless of use.

The applicant should provide a 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, the applicant should describe the genotype and source of the microbial cells, provide information on how the material was generated, and how the bank is stored and maintained as well as information on the 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, the testing should 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 RE analysis

Full plasmid sequencing. It is recommended that the applicant fully sequence plasmids 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, as applicable

d. **Master Viral Banks**

Viral banks may be expanded to manufacture a viral vector DS (e.g., herpesvirus-based vectors, adenovirus-based vectors), or they may be used to generate helper viruses for manufacturing non-replicating vectors (e.g., AAV). The applicant should provide a detailed description of the history and derivation of the source or seed materials for these banks. The applicant should describe how the seed stock was generated and which 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. It is recommended that the applicant state whether the seed material was plaque-purified, purified by limiting dilution, or rescued from DNA or RNA clones and how many passages occurred during expansion.

For the banked material, the applicant should describe the manufacturing process and the conditions under which the banked material was generated, for example, in a research laboratory or a Good Manufacturing Practice (GMP) facility. The applicant should provide a list of 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.

It is also recommended that the applicant 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 IP dossier (section 3.2.A.1). For additional information on analytical methods used for MVB qualification, please see "Analytical Procedures (3.2.S.4.2)" section of this guidance.

Viral vector bank qualification should include tests to:

- Ensure absence of contamination, including sterility, mycoplasma, and in-vivo and in-vitro testing for adventitious viral agents.
- Ensure the 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 the 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. It is recommended that the applicant fully sequence all vectors that are 40 kb or smaller, analyze the sequence, and submit an annotated sequence of the entire vector. The applicant 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.
- Regarding sequence analysis, it is recommended to:
  - Sequence viral vectors from the MVB, when possible.
  - For integrating viral vectors, it is recommended that the applicant 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 the applicant has 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, it is recommended that the applicant 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, the applicant should summarize the extent and results of sequence analysis that have been performed, including any testing performed by restriction endonuclease analysis. The applicant should perform sequence analysis of the gene insert, flanking regions, and any regions of the vector that are modified or deleted or that could be susceptible to recombination. The entire vector sequence will be necessary to confirm identity for licensure.

e. **Working Viral Banks**

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

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

The applicant should describe the control of critical steps and intermediates in the manufacturing process. It is recommended that the applicant consider any steps in which in-process tests with acceptance criteria are performed as critical control steps. SFDA acknowledges that this information may be limited in the early phases of development and recommends that applicants provide additional information and updates as product development proceeds.

The applicant should define manufacturing intermediates and provide information on the quality and control of intermediates. 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.

It is recommended that the applicant provide information on the plasmid manufacturing procedures, reagents, and plasmid specifications for use, regardless of whether the

plasmids were made by the applicant or a contract manufacturer. In general, it is recommended 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 application.

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

SFDA does not require process validation studies for early-stage manufacturing (e.g., Phase I-II). It is recommended that the applicant use early-stage manufacturing experience to evaluate the need for process improvements and to support future process validation studies. At all stages of development, however, the manufacturer must have established written SOPs to ensure proper manufacturing control and oversight.

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

The applicant should provide a description and discussion of the developmental history of the manufacturing process. For later stages of manufacturing, there may be changes to the process as part of process development or optimization, and under certain conditions, there may be reprocessing steps. It is recommended that the applicant describe how manufacturing differences are expected to impact product safety and activity in the "Batch Analysis" section.

If the applicant makes 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. The comparability plan should be discussed with SFDA prior to beginning comparability studies.

SFDA strongly recommends the applicant retain samples of the DS and manufacturing intermediates, when possible, in case comparability studies are required in the future.

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

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

It is recommended that the applicant include annotated sequence analysis for the vector in the application, and any additional sequence information gathered during product development in subsequent submissions.

b. Impurities (3.2.S.3.2)

It is recommended that the manufacturing process be designed to remove process- and product-related impurities and that the applicant have tests in place to measure levels of residual impurities.

As the applicant optimizes manufacturing process the impurity profile including acceptance limits must be refined. SFDA recommends that the applicant measures impurities throughout product development, as this will help ensure product safety, contribute to a deep understanding of the manufacturing process, and provide a baseline for comparing product quality after manufacturing changes, if needed.

- Process-Related Impurities

Specifications should be set for materials used in the manufacturing process, unless process validation data have been provided to demonstrate that these residues are consistently reduced to acceptable levels.

For the release specifications, tests should be developed and relevant (upper) limits set to monitor the residual levels of contaminants. These include, but are not limited to, residual cell substrate proteins, extraneous nucleic acid sequences, helper virus contaminants (e.g., infectious virus, viral DNA, viral proteins), and reagents used during manufacture, such as cytokines, growth factors, antibodies, selection beads, serum, and solvents, as well as raw materials that may have been used during the production process such as resins or benzonase.

Common process-related impurities for viral preparations are listed in the table below:

Common process-related impurity for viral preparations		
Type of impurity	**Example**	**Recommendations**
Residual nucleic acid	**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.	Since some cell substrates also harbor tumorigenic genetic sequences or retroviral sequences that may be capable of transmitting infection, it is recommended that applicants minimize the biological activity of any residual DNA associated with the viral preparation. 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. It is also recommended that the amount of residual DNA in continuous non-tumorigenic cells be limited to less than 10 ng/dose and that the DNA size be reduced to below approximately 200 base pairs.
Tumor-derived cells	**Tumor-derived** such as (Hela) or have tumorigenic phenotypes such as (HEK293, HEK293T) or other characteristics that may give rise to special concerns.	The applicant should control the level of relevant **transforming sequences** in the product with acceptance criteria that limit patient exposure. For example, products made in **293T cells** should be tested for adenovirus **E1 and SV40 Large T antigen sequences**, and products made in Hela cells should be tested for **E6/E7 genes**. The tests should be appropriately controlled and **have** sufficient sensitivity and specificity to determine the level of these sequences in the product.
Some vectors, including AAV can package large amount of non-vector DNA	(e.g., plasmid DNA, helper virus sequences, cellular DNA)	It is strongly recommended 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 product-related risks. Applicants should provide the necessary quality data, risk assessments, and/or details of their process and product control strategies to address and mitigate potential risks posed by the manufacturing systems used.

- Product-Related Impurities

For viral vectors, typical product-related impurities 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 may 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. It is recommended that the applicant evaluate the nature and number of non-target cells and measure the percentage of cells that have been genetically modified. As the applicant develops a greater understanding of the cellular phenotypes present in the product during clinical development, the applicant may also consider adding impurity tests for specific cell populations to establish greater manufacturing control.

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

a. Specifications (3.2.S.4.1)

The applicant should list DS specifications in the application. 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, lots used to demonstrate manufacturing consistency, stability study data, and other 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 the application 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.

For later phases, the applicant should describe assays to assess product characteristics, product safety, and purity. To ensure product quality, the applicant should perform identity testing e.g., quantitative polymerase chain reaction (qPCR), safety testing, 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. The

applicant should also ensure consistent dosing and potency in the clinical investigations. Assays used to determine dose (e.g., vector genome titer by quantitative polymerase chain reaction (qPCR), transducing units, plaque-forming units, and flow cytometry for transduced cells) should be qualified as suitable for use prior to initiating clinical studies.

The applicant should note that the some types of tests listed in this section of the guidance may not be necessary for the release of both the DS and DP. In certain situations, the DS and DP may not be readily distinguishable due to the design of manufacturing process and control. In this case, it is recommended that the applicant provide a rationale to support the selection of testing performed for release of either the DS or DP.

b. Analytical Procedures (3.2.S.4.2)

The applicant should provide a description of the analytical procedures used during manufacturing to assess the manufacturing process and product quality. In the original clinical trial application submission, the descriptions should include sufficient detail, such as a description of system suitability controls, so that they can be understood and evaluated for the adequacy of the procedures. Documentation of analytical procedures should describe in detail how a procedure is performed and should specify any reference standards, equipment, and controls to be used.

Non-compendial (in-house testing) analytical procedures may be acceptable if the applicant provides adequate information about test method, including specificity, sensitivity, and robustness.

- Replication Competent Virus

For non-replicating gene therapy viral vectors, specific testing is recommended, because of 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, testing banked material for the presence of replication competent viruses is recommended and as

part of in process or release testing specifications of the DS or DP, as appropriate.

- Replication-Competent Retrovirus (RCR) Testing

Most **retrovirus-based** products (including lentivirus and foamy virus-based products) used for gene therapy applications are designed to be replication defective. To ensure the absence of RCR, the applicant should perform testing for RCR at multiple points during the production of retroviral vectors.

- Replication-Competent Adenovirus (RCA) Testing

Most adenoviral-based products used for gene therapy applications are designed to be replication defective. 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 adenovirus-based gene therapy products, it is recommended that the applicant qualify the MVB for RCA and test either the DS or DP of each production lot for RCA. A maximum level of 1 RCA per 3×10^{10} viral particles is recommended.

- 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 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 pose unintended safety risks. Therefore, it is recommended that the applicant test for rcAAV, which could potentially replicate in the presence of helper virus, and report these results in the application.

- 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. These attenuated or adapted viruses may recombine or revert to a parental (or WT)

genotype during manufacture. Therefore, it is recommended that the applicant conduct tests to determine whether the parental virus sequences are present in the product. In addition, it is recommended that the applicant select production cells that do not contain viral sequences that may allow homologous recombination with the product. For example, 293 cell substrates are not recommended 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 Phase 1 studies; however, the applicant 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 should include appropriate controls or standards). It is recommended that the applicant use compendial methods when appropriate and qualify safety-related tests prior to initiation of clinical trials.

To ensure safety of gene therapy products, the applicant should also qualify the assays used to determine dose (e.g., vector genome titer by qPCR, transducing units, plaque forming units, and transduced cells) prior to initiating clinical studies. The applicant should also 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.

For all analytical procedures, it is recommended that the applicant evaluate assay performance throughout product development, have a validation plan in place prior to initiating clinical studies, and complete validation before marketing authorization application approval.

d. Batch Analysis (3.2.S.4.4)

The applicant should include a table with test results for batches (or lots) of the DS that have been manufactured. For early-stage development, this may include toxicology lots,

developmental batches, engineering runs, or a single manufacturing run for clinical grade material. It is recommended that the applicant gain adequate experience with new clinical manufacturing processes prior to making clinical material. This is especially critical following technology transfer to a new manufacturing facility, when manufacturing changes occur during development, and when multiple manufacturing facilities are utilized. It should be noted that batches manufactured in different ways should be clearly identified in the submission. It is recommended that the applicant update this section of the application annually as new batches are produced. The applicant should indicate any batches that fail to meet release specifications and any action taken to investigate the failure. It is also recommended that the applicant retain samples of production lots for use in future assay development, validation, or comparability studies.

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

The applicant should provide justification for the DS specifications in the application. It is recognized that acceptance criteria may be adjusted throughout the product development stages, based on manufacturing and clinical experience. For early-stage clinical studies, assays used to characterize 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, it is recommended that acceptance criteria be tightened to ensure that batches are well defined and consistently manufactured.

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

The applicant should provide information on the reference standards or reference materials used for testing the DS in the application. It is recommended that the applicant provide the source and lot number; expiration date; CoAs, when available; and 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 that are

custom synthesized by an analytical laboratory or other noncommercial establishment.

In some cases, the reference material for an assay may be a well-characterized lot of the gene therapy product itself, which is considered an in-house reference standard. In this case, it is recommended that the applicant reserve and maintain a sufficient amount of material (e.g., part of a production lot) to serve as a reference material to bridge to a new reference material as needed during product development. SFDA recommends the applicant to establish a protocol to evaluate the stability of the reference standard.

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

The applicant should describe the type(s) of container and closure used for the DS, including the identity of the materials used in the construction of the container closure system. It is recommended that the applicant determine whether the containers and closures are compatible with the DS. Compatibility with a gene therapy product may be evaluated during stability studies or may be based on historical data and experience with similar products.

1.7. Stability (3.2.S.7)

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

It is recommended that the applicant describe in application 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). The applicant should note that stability studies may evolve with product development, and if the DS is immediately processed into a DP, long-term DS stability data may not be needed. The stability analysis may include measures of product sterility (or container integrity), identity, purity, quality, and activity or potency. It is recommended that the applicant provide justification for the test methods and acceptance criteria used in the stability analysis. It is often helpful to demonstrate that one or more of the test methods in the stability analysis are stability-indicating. The applicant may demonstrate that a test is stability-indicating by using forced degradation studies, accelerated stability studies, or

another type of experimental system that demonstrates product deterioration.

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

As the applicant progresses with product development, it may be necessary to conduct stability studies required to determine an expiry date.

c. Stability Data (3.2.S.7.3)

It is recommended that the applicant provide the results of the stability studies and update this information on a regular basis (e.g., in annual reports) once the data are available. Information on the qualification of analytical procedures used to generate stability data should also be submitted.

2. Drug Product (3.2.P)

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

The applicant should provide a description of the DP and its composition. This includes a description of the dosage form and a list of all of its components (active and inactive), the amount per-unit basis, the function, and a reference to quality standard for each component (e.g., compendial monograph or manufacturers' specifications).

If a drug or device will be used with the gene therapy as a combination product, it is recommended that quality information for the drug or device also be included. In addition, the applicant should provide a description of any accompanying reconstitution diluents and a description and of the container and closure used for the dosage form and accompanying reconstitution diluent, if applicable.

2.2. 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 and microbiological attributes. 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. It is

understood that only limited information may be available at early stages of development; thus, SFDA recommends that the applicant provides additional information and updates as the product progresses into the late stage of development.

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

- Drug Substance (3.2.P.2.1.1)

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

- Excipients (3.2.P.2.1.2)

The applicant should describe in the application the choice of excipients and inactive components of the DP listed in the "Description and Composition of the Drug Product", including their concentration, and the characteristics of these excipients that can influence the DP performance.

b. Drug Product (3.2.P.2.2)

- Formulation Development (3.2.P.2.2.1)

The applicant should briefly describe the development of the DP formulation, taking into consideration the proposed route of administration and usage in the application. SFDA recommends that the applicant 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 made for stability, device compatibility, or safety concerns, this information should be reported here.

- Physicochemical and Biologic Properties (3.2.P.2.2.2)

The applicant should describe the parameters relevant to the performance of the DP, or reference relevant DS sections, as appropriate. These parameters include the 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). The applicant should update this section on the physiochemical and biological properties of the product as a better understanding is gained during development.

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

The applicant should describe the selection and optimization of the DP manufacturing process (described in the "Description of Manufacturing Process and Process Controls" (3.2.P.3.3) section of the IP dossier) if development studies have been performed. It is understood that only limited information may be available in the early stages of development; therefore, SFDA recommends that the applicant provide additional information and updates as the product progresses into the later stages of development.

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

The applicant should describe the suitability of the container closure system for the storage, transportation (shipping), and use of the DP. SFDA recommends that the applicant consider choice of materials, protection from moisture, gases, and light, compatibility with the formulation (including adsorption to the container and leaching), safety of materials, and performance.

e. Microbiological Attributes (3.2.P.2.5)

The applicant should describe the final product microbial-testing procedures and address how the integrity of the container-closure system will be assessed to prevent microbial contamination. SFDA will generally consider the submission of other innovative testing methods, such as rapid microbiological testing, if scientifically justified.

f. Compatibility (3.2.P.2.6)

The applicant should discuss the compatibility of the DP with the diluent used for reconstitution or the delivery device, if applicable. SFDA recommends that compatibility studies for drug products (DP) include evaluations of both product quantity and product activity. For example, in the case of viral vectors, these studies should measure both physical particles (quantity) and infectivity or potency (activity) to assess potential adsorption or inactivation effects.

Additionally, in-use and in-device stability data should be generated to support the recommended hold times and conditions specified in the clinical protocol for patient administration. A lack of understanding of in-use and in-device stability, as well as their potential impact on product performance, may not adequately justify the risks associated with clinical study treatments.

Therefore, it is highly recommended that applicants rigorously control and assess DP compatibility, as well as the final steps of product preparation and administration, to ensure product quality, safety, and efficacy throughout the clinical process.

2.3. Manufacture (3.2.P.3)

a. Manufacturers (3.2.P.3.1)

The applicant should provide the name, address, and responsibility of each manufacturer, including contract manufacturers, involved in the manufacture and testing of the DP.

For gene therapy-device combination products, it is recommended that the applicant list the manufacturing facilities for the device components and describe the assembly and testing processes that take place at each site.

b. Batch Formula (3.2.P.3.2)

The applicant should provide a batch formula that includes a list of all components of the dosage form, the appropriate amount or range per batch, and a reference to their quality standards.

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

The applicant should provide a detailed description of the DP manufacturing process and identify process controls and intermediate tests. The description should include both flow diagrams and narrative descriptions, as well as packaging, product-contact materials, and equipment used. This process should include manufacturing steps, such as final formulation, filtration, filling, and freezing, and process controls. Under certain conditions, repeating sterile filtration (if applicable) may be critical. SFDA recommends that the applicant clearly describe the conditions and justifications for each reprocessing procedure and demonstrate product consistency between reprocessed lots and normal production lots.

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

The applicant 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 to ensure control as well as steps in which tests with acceptance criteria are performed. It is recommended that the applicant provide justification for acceptance criteria or limits set for these tests. In addition, the applicant 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. It is understandable that only limited information may be available in the early stages of development; therefore, SFDA recommends that the applicant provide additional information and updates as the product progresses into the later stages of development.

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

SFDA does not require full process validation during the early stages of clinical development. However, based on the development stage of the product, SFDA may require the applicant to provide information that supports the manufacturing process consistency.

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

An excipient is any component, other than the active ingredient that is intended to be part of the final product (e.g., human serum albumin, Dimethyl Sulfoxide (DMSO)). The applicant should provide the following sections:

- Specifications for all excipients. If the excipient is compendial, information may not be required.
- Analytical procedures for testing excipients. If the applicant is performing any additional testing or qualification of compendial excipients, the applicant should describe that testing here.
- Process validation is not expected during the early stages of clinical development. However; SFDA recommends that the applicant provide any available validation or verification information for the analytical procedures used to test excipients.

SFDA may request the applicant to provide process validation as the product progresses into the late stages of development.

- The applicant should provide justification for the proposed excipient specifications, as appropriate.
- For excipients of human or animal origin, the applicant should provide information regarding source, specifications, description of testing performed, and viral safety data. If human serum is used, it is recommended that the applicant submit information documenting donor suitability as well as appropriate infectious disease testing.
- For excipients used for the first time in a DP or used for the first time in a route of administration (Novel Excipients), the applicant should provide full details of manufacture, characterization, and controls, with cross-references to supporting safety data (nonclinical and/or clinical) in a regulatory file submitted to SFDA, if available.

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

a. Specifications (3.2.P.5.1)

The applicant should list DP specifications in the original clinical trial application submission. The 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. Product lots that fail to meet specifications should not be used in the clinical investigation without providing protocol for out-of-specifications and obtaining SFDA approval. For early-phase clinical studies, it is recommended 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.

It is recommended that product release assays be performed at the manufacturing step where they are necessary and appropriate. For example, mycoplasma and adventitious agents release testing are recommended for cell culture harvest material. 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). SFDA also recommends establishing or, in some cases tightening 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. It is also recommended that applicants develop testing to assess product potency and have this assay in place prior to initiating studies used to support product efficacy.

b. Analytical Procedures (3.2.P.5.2)

The applicant should describe the analytical procedures used for testing the DP. If the analytical procedures are the same as those for the DS, there is no need for repeating this information unless there is a matrix effect from the DP on assay performance. Additional comments regarding analytical tests are provided below:

- Sterility

Non-compendial (in-house testing) analytical procedures may be acceptable if the applicant provides adequate information about the test method, including specificity, sensitivity, and robustness.

It is recognized that the compendial sterility tests (USP <71>; 610.12) may not be suitable for all products (e.g., those with limited shelf life). Rapid sterility tests may be acceptable 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, it is recommended that a negative test result from an in-process sterility test (on a sample taken 48 to 72 hours prior to final harvest) be available for the release of the DP. The sterility test on the final formulated product should be continued for the full duration (e.g., 14 days for USP method) to obtain the final sterility test result, even after the product has been administered to the patient. In all cases where product release occurs prior to obtaining results from the sterility test, the investigational plan should address the actions to be taken in the event that

the sterility test returns a positive result after the product has been administered to a subject. The applicant should report the sterility failure and adverse side effects to both the clinical investigator and SFDA according to the following guideline: *Regulations and Requirements for Conducting Clinical Trials on Drugs.*

If the applicant freezes the DP before use, SFDA recommends that the applicant perform sterility testing on a sample of 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, the applicant may need to perform additional release testing, including sterility and identity testing, to ensure product quality.

- Identity

This test is performed on the final labeled product to verify its contents. SFDA recommends using different types of test methods as a single test is not sufficient to clearly distinguish among products. If the final product is ex-vivo genetically modified cells such as such as CAR-T cell therapy products, it is recommended that identity testing include an assay to measure the presence of vector or genetic change and an assay specific for the cellular composition of the final product.

- Purity

Product purity is the extent to which the final product is free from any additional substances, regardless of whether they are harmful to the recipient or damaging to the product itself.

Purity testing includes assays for residual manufacturing impurities of the DS, which may include, but are not limited to host cell proteins and DNA, reagents used during manufacture, such as cytokines and growth factors.

In the case of ex vivo genetically modified cells such as CAR-T cell therapy products, any unintended cellular populations should be reported. The assays used to demonstrate product purity should be phase-appropriate and may evolve during development as the applicant develops greater understanding of the impurities

present in the product, or as any manufacturing process changes are made.

Although the rabbit pyrogen test method is the current required method for testing certain licensed biological products for pyrogenic substances, alternative test methods are generally accepted, such as the Limulus Amebocyte Lysate (LAL) test.

- Potency

The applicant should describe and justify in the application all assays used to measure potency. A potency assay is not required to initiate early-phase clinical studies, but it is recommended that the applicant have a well-qualified assay to determine dose.

- Viability

The applicant should establish minimum release criteria for viability, where appropriate. For ex vivo genetically modified cells such as CAR-T cell therapy products, it is recommended that there be a minimum acceptable viability of at least 70 percent. If this level cannot be achieved, it is recommended that the applicant 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.

- Cell Number or Dose

The dose-determining assay an important part of the DP specifications and should be qualified as suitable for use prior to initiating clinical studies. If the final product is ex vivo genetically modified cells, the applicant should define an acceptance criterion for the minimum number of genetically modified cells in a product lot. It is recommended that the product dose for such products be determined based on the total number of genetically modified cells.

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

SFDA does not usually require validation of analytical procedures during the original submission of the clinical application. However, based on the development stage of the product, SFDA may require the applicant to provide information to support manufacturing

process consistency. Also, it is recommended that the applicant provide information to support the qualification of certain safety-related or dose-related assays. If f these assays are the same as those listed for DS testing, the applicant does not need to repeat them but should reference that section of the application.

d. Batch Analyses (3.2.P.5.4)

The applicant should provide final product CoA(s), if available, or a description of the batches generated to date, and the results of any batch analyses for the DP used in toxicology studies, engineering runs, or clinical studies. Applicants may also include supportive developmental batches, if appropriate.

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

The applicant should provide information on the characterization of impurities if this has not been previously provided in the "Impurities – Drug Substance" section.

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

The applicant should provide justification for the DP specifications.

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

The applicant should provide information on the reference standards or reference materials used for testing the DP, if this has not been previously provided in the "Reference Standards or Materials – Drug Substance" section.

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

The applicant should provide a description of the container closure systems, including the identity of the construction materials for each primary packaging component (where primary packaging is defined packaging in direct contact with the product itself) and its specifications. The applicant should also provide information on how the container is sterilized.

2.8. Stability (3.2.P.8)

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

Summary table of all batches used in stability studies should be listed along with

corresponding protocols and results based on that stage of development or clinical trial phase. Stability protocols may differ depending on the product type and manufacturing hold time. A long-term stability plan should be submitted as part of the protocol. The summary should include storage conditions and shelf life as well as in-use storage conditions.

b. Stability Data (3.2.P.8.2)

The applicant should provide the results of the stability studies in the clinical trial application in an appropriate format (e.g., tabular, graphic or narrative). Information on the analytical procedures used to generate the data should also be included.

3. Appendices (3.2.A)

3.1. Adventitious Agents Safety Evaluation (3.2.A.1)

The applicant is recommended to assess the risk of potential contamination with adventitious agents as follows:

- For non-viral adventitious agents (such as transmissible spongiform encephalopathy agents, bacteria, mycoplasma, and fungi). Submission of certification and/or testing of components and control of the production process is recommended.
- For viral adventitious agents, viral safety studies should be submitted. Study reports and data to support qualification of the manufacturing components (such as adventitious agents test reports for banked materials) should be included as a part of this appendix.

4. Regional Information (3.2.R)

Information that is specific to a regulatory region can be put in the regional section of the application. This would include environmental risk assessment, commitments, protocol, and other information as requested.

Annex 1: General Considerations for CAR-T Cell Products

Introduction

Chimeric antigen receptor (CAR)-T cell products are regulated as gene therapy (GT) products under SFDA's existing framework for biological products. This annex of the guidance addresses general considerations for CAR-T product manufacturing and testing but is not designed to be a stand-alone CMC guidance. **Please refer to *Guidance on Submission of Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Clinical Trial Applications*.**

CAR-T cells consist of a vector, a CAR construct, and cellular starting material, which are critical components for delivering pharmacological activity for the treatment of disease.

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

CAR construct: CARs generally contain two types of domains: antigen recognition and signaling. Antigen recognition domains allow CAR-T cells to bind to one or more target antigen(s).

Cellular Starting Material: The starting material for CAR-T cell manufacture is generally obtained by **leukapheresis** of patients (for autologous products) or healthy donors (for allogeneic products). Other cellular starting materials can include cord blood cells or induced pluripotent cells (iPSCs).

A. Vector Manufacturing and testing considerations

Release of viral vector should be based on specifications that ensure:

- Identity: To ensure genomic integrity, this includes structure, vector design, and genetic development information, as well as general characteristics.
- Biological activity: This includes both transgene activity and transduction efficiency, which can be linked to final CAR-T cell potency profile. While transgene activity is measured by the release of biological markers, vector performance ability is amount of

vector added to produce the required transduction efficiency, while maintaining a targeted safe vector copy number.

- Purity: Applicants are advised to include testing of residual amount of any raw materials used such as benzonase. Other expected impurities should be tested such as residual host cell DNA or RNA and host cell proteins.
- Safety: Safety testing which includes sterility, mycoplasma, and endotoxin testing must be submitted within the clinical trial application. Adventitious agent testing is different based on type of vector used, as well as the replication competent virus assay as appropriate.
- Stability: Studies to be conducted to establish storage conditions and expiration time of the vector.
- All vectors used in clinical studies must be manufactured according to according to the following reference: **SFDA guide to good manufacturing practice for medicinal product** (SFDA GMP Guide) (Annex 2A): manufacture of advanced therapy medicinal products for human use.

B. Starting material

Cellular starting material such as leukapheresis must be well characterized. Autologous leukapheresis starting material does not require a donor eligibility determination but you may consider a risk-based approach for screening or testing. However, allogeneic leukapheresis starting materials or starting materials that were derived from cord blood or induced pluripotent stem cells require a donor eligibility determination, including screening and testing for relevant communicable disease agents and diseases.

Collection, handling, and stability

Collection, handling, storage (whether fresh or cryopreserved), and transportation from collection centers to manufacturing sites should be well documented.

The stability of the starting material during storage and shipment must be demonstrated using shipping-validation studies. These studies should provide information on

shipping container and temperature-control measures, such as data loggers.

Cellular material variability

Cellular starting-material variability between donors is the main cause of CAR-T cell product lot-to-lot variability. To limit this variability, applicants are advised to refine the acceptance criteria of the starting material as more manufacturing experience is gained throughout the clinical trial phases. Common parameters to be tested include cell number, cell viability, identity and purity of target cell population.

Traceability and labeling system

The applicant must establish a labelling system that maintains the chain of identity from collection through to finished product labelling and administration. Collection bags must be labelled according to the approved policy of the collection center. A minimum of three unique identification parameters is recommended (for example donor name, date of birth and donation identification number). In addition, it is important to clearly describe batch size and batch-number genealogy.

Considerations for use of surrogate material

In many cases, applicants must use healthy-donor material as surrogate for patient material for characterization and/or comparability purposes mainly due to scarcity of clinical material and patient disease states. Healthy-donor materials should be segregated from patient materials in the storage area.

C. CAR-T Cell manufacturing and testing recommendations

Manufacturing process control

Donor-to-donor variability is a major source of variability in the cellular starting material. Therefore, manufacturing process should be well controlled thorough in-process control of process parameters, in-process testing and final product critical quality attributes CQAs, as appropriate for the phase of product development. CAR-T

cell manufacturing relies heavily on specialized reagents, such as selection and activation reagents, antibodies, cytokines, serum, and growth factors. Variability between reagent lots and their stability can pose risks to the quality and safety of the final CAR-T cell product. SFDA recommends that manufacturers implement comprehensive qualification programs and incoming-material qualification programs, including quarantine, Certificate of Analysis (CoAs) and Certificate of Origin (CoOs) assessment, visual inspection, and testing, as appropriate. Additionally, manufacturing equipment—such as centrifuges, washers, selection devices, and incubators, including automated systems—should be qualified by the applicants as suitable for their intended use. SFDA requires applicants to submit amendment application prior to making changes of manufacturing process, materials and/or equipment submitted on the clinical trial application.

The applicant should develop in-process controls to minimize lot-to-lot CAR-T cell manufacturing variability. Examples include

- Using a fixed bead: cell ratio at the activation stage.
- Using a constant amount of vector per cell (e.g., a fixed multiplicity of infection for viral vectors) and maintaining a fixed duration at the gene transfer step.
- Using fixed electroporation settings.
- Monitoring cell expansion in culture and maintaining an optimal cell density by media addition.

Appropriate in-process testing at relevant time points is vital to achieve and maintain control of the manufacturing process. In-process testing regimens for CAR-T cells typically assess multiple parameters (e.g., viability, cell number, cell phenotype, and CAR expression). Results from in-process tests can be used to guide manufacturing decisions at critical steps, such as when to change culture media or when the CAR-T cells are ready to harvest.

Due to the nature of CAR-T cells, terminal sterilization and/or filtration is not possible.

SFDA recommends that applicants to manufacture CAR-T cell product in an aseptic environment, according to the following reference: *SFDA guide to good manufacturing practice for medicinal product (SFDA GMP Guide)(Annex 13): manufacture of investigational medicinal product.*

D. Analytical testing

To ensure the safety and efficacy of CAR-T cell therapies, rigorous testing is essential. This involves analyzing the product to confirm its identity, purity, potency, and safety. Given the complex nature of CAR-T cells, specialized assays are often needed to characterize the product. SFDA recommends that the applicant begin developing these assays early in the clinical trial process to characterize their product. As the clinical trials progress, the understanding of the product is expected to advance, therefore the identities of critical quality attributes (CQAs) and their limits should be refined.

Information about assays should include:

- A description of the assay
- A brief summary of instrument calibration, maintenance and quality-control activities
- A list of assay controls
- Established and implemented written procedures
- Assay qualification and/or comprehensive validation study (before licensure) for lot release

Analytical testing specific recommendation for CAR-T:

- **Identity:** It is recommended that identity testing of CAR-T cells include assays to verify the presence of transgene (e.g., CAR expression by flow cytometry, gene detection by PCR) and an assay specific for the desired T cell markers.
- **Potency:** The applicant should describe and justify in the application all assays used to measure potency. A qualified potency assay is not required to initiate early phase clinical studies; however, it must be fully validated before marketing

authorization. Consideration for potency determination of CAR-T cells may account for their multiple mechanisms of action. To assess their potency, various tests, such as cell killing assays, transduction efficiency measurements, and cytokine secretion assays, can be used. Characterizing CAR-T cell function throughout clinical trial phases will support comparisons and help determine the most suitable assays.

If the CAR-T cells express multiple transgene elements, each transgene may contribute to product safety and efficacy and therefore should be adequately controlled. A potency assay to measure the intended biological activity of each element may be needed, depending on the contribution of each transgene to the product's activity. Justification for the proposed assays should be supported by characterization studies. For example:

- If the CAR-T cell targets multiple antigens (e.g., CD19 and CD22), the applicant should assess the activity of the CAR-T cells against each individual target antigen because T cell activation upon engagement with either antigen is required for the product's function.
- If the CAR-T cell includes a cytokine transgene to enhance the CAR activity, the applicant should assess the activity of the CAR-T cells against the target antigen and the production of the transgenic cytokine because the cytokine is not primarily responsible for the CAR-T cell activity specific to the target antigen.
- If the CAR-T cell includes a transgene conferring drug resistance, the applicant should assess drug resistance and CAR-T cell activity because they have independent mechanisms of action.

- **Purity:** Purity testing includes assays for pyrogenicity or endotoxin and residual manufacturing impurities, which may include, but are not limited to proteins; DNA; cell debris; and reagents or components used during manufacture, such as cytokines, growth factors, antibodies, and serum. SFDA recommends reporting any

unintended changes in cell numbers or types in the CAR-T cell product. The assays used to demonstrate product purity should be phase appropriate and may evolve during development as the applicant gains greater understanding of the impurities present in the product, or as any manufacturing process changes are made.

While the rabbit pyrogen test method remains the standard for testing pyrogenic substances, alternative test methods, such as the Limulus Amebocyte Lysate (LAL) method is a viable option for pyrogen detection in many biological products including CAR-T cell products. Vector integration can potentially alter the expression of cellular genes and contribute to tumorigenicity. Therefore, vector integration in the drug product (DP) is an important safety attribute to measure for CAR-T cell release. For integrating vector systems, the average number of integrations per CAR-positive cell, generally referred to as the vector copy number (VCN), should be determined and reported on the Certificate of Analysis (CoA) for each lot. Determining the VCN relative to total cells includes CAR-negative cells in the denominator and thus lowers the reported vector integration rate.

Using the percentage of CAR-positive cells, the average VCN per CAR-positive cell can be calculated. VCN as a function of CAR-positive cells provides a more accurate representation of the VCN in modified cells and, therefore a more accurate representation of product risk for insertional mutagenesis. It is recommended that the manufacturing process be optimized to control VCN while meeting the target CAR-positive cell frequency.

For CAR-T cells manufactured without extended culture, determining the stably integrated VCN at the time of lot-release testing may be difficult (e.g., due to the persistence of episomal copies of non-integrated vectors). In some cases, an interim VCN assessment at the time of lot release, followed by subsequent VCN assessment(s) on cultured CAR-T cells, may be needed to determine the stably integrated VCN. The appropriate duration of extended culture for the stably

integrated VCN (and other release assays, as applicable) is product-specific and should be determined experimentally.

Although all the purity considerations mentioned above focus on viral based CAR-T Cell therapies, other consideration may be required for non-viral-based CAR-T cell therapies.

- **Sterility:** To ensure product safety, CAR-T cells should be free of viable contaminating microorganisms; however, the final DP cannot be sterilized by filtration or terminally sterilized because cells need to be fully viable and functional. Therefore, manufacturing should be conducted using qualified aseptic processing under cGMP requirements, and aseptic processing must be validated. Product safety is further supported by the use of sterility testing performed in accordance with international pharmacopeia or compendial methods. However, SFDA may consider the submission of other innovative testing methods, such rapid microbiological testing, if scientifically justified.
- **Viability and Cell Number or Dose:** SFDA recommends submitting data supporting 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. For CAR-T cell products, SFDA recommends that the applicant establish an acceptance criterion for the minimum number or percentage of genetically modified viable cells in a product lot.

E. Validation studies

SFDA does not require full process validation during the early stage of clinical development for Phase I studies. However, based on the development stage of the product, SFDA may require the applicant to provide information to support the manufacturing-process consistency. It is Also recommended to provide information to support the qualification of certain safety-related or dose-related assays.

When changing an assay, a risk assessment should be performed to determine how the assay change impacts CAR-T cell evaluation. If there are major changes to assay methodology, it is recommended that the assay be requalified to ensure that its assay performance characteristics remain acceptable. If an assay is replaced with a new assay that measures the same attribute in the same way (e.g., a change to another ELISA-kit vendor), the assay should be qualified, and a study may be requested to demonstrate that the new assay yields results that are equivalent to the old assay. It is recommended that these studies include side-by-side analysis of the old and new assays using the same test samples. If an assay is replaced with a new assay that measures an attribute in a fundamentally different way (e.g., potency assay changed from a cell killing assay to a cytokine release assay), the new assay should be qualified, and data to support any associated changes to assay acceptance criteria or the impact on stability should be provided to SFDA prior to implementation.

F. Stability studies

- Apheresis material (fresh or cryopreserved) should be assessed for storage stability before CAR-T cell manufacturing. In addition, it is recommended to assess at least the cell viability of apheresis material during storage. In case of cryopreserved apheresis material, sterility testing should be considered.
- Initial stability studies may use products from healthy donors (surrogate materials).
- In-use stability studies: in-use stability studies should be performed to evaluate the time between the final formulation and administration for fresh products or the time between thawing and administration for cryopreserved products, to support the intended hold time.
- Hold-time stability: stability studies should be performed on CAR-T cells to determine appropriate holding times.
- Long term stability accelerated stability studies, and stress-testing studies are recommended to determine appropriate storage periods.

G. Label recommendations

- It is recommended that the applicants include sample or mock-up labels.
- It is recommended that the label includes the product name, manufacturer information, and, as applicable, the warnings “Do not use leukoreduction filters” and “Do not irradiate”. Other labelling considerations must conform to the requirements of SFDA guidelines.
- CAR-T cells manufactured from autologous starting material must be labeled “FOR AUTOLOGOUS USE ONLY”. It is recommended that the label must state “NOT EVALUATED FOR INFECTIOUS SUBSTANCES,”
- CAR-T cells manufactured from allogenic starting material must be labeled “EVALUATED FOR INFECTIOUS SUBSTANCES,”
- It is recommended that the label includes at least two unique identifiers to confirm patient identification prior to administration.
- CAR-T cells must also be labelled with the biohazard legend.
- If the results of any screening or testing performed indicate the presence of relevant communicable disease agents and/or risk factors for, or clinical evidence of relevant communicable disease agents or diseases, labelling must also bear the statement “WARNING: Reactive test results for (name of disease agent or disease),” in the case of reactive test results

H. CAR-T Cells Manufacturing changes

SFDA acknowledges that changes may be needed to the CAR-T cell design, manufacturing process, or manufacturing facility during product development or post-approval. Changes to the final container, cytokines used during culture, or the duration of cell expansion, may impact product quality, safety, efficacy, or stability. However, there are some changes (e.g., changes to the CAR construct or changing from an autologous to allogeneic product) which would generally result in a new product that requires a new clinical trial application**.**

Due to the essential role of the vector in CAR-T cell activity, the impact of vector manufacturing process changes should be assessed on both the vector and the CAR-T cells. SFDA recommends considering the following when planning on substantial changes to the vector and/or CAR-T cell manufacturing process.

- The complexity of comparability assessments may differ depending on the extent of the change to the vector or CAR-T cell manufacturing process for example, a small change in the volume of culture media used to manufacture CAR-T cells can generally be supported by cell viability and expansion data. In contrast, a more robust comparability study should be conducted for a change to the concentration or type of growth factors or supplements in the culture media.

  Note that the term "comparability" does not mean that pre- and post-change products are identical, but rather that they are highly similar and that any differences in product **critical quality attributes (CQAs)** have no adverse impact on CAR-T cell quality, safety, or efficacy.

- CAR-T cells manufactured with pre- and post-change vector should be assessed using the same cellular starting material (e.g., splitting the leukapheresis starting material from the same donor).
- When the CAR-T cells or vector manufacturing facility is changed, **comparability** between manufacturing facilities should also be assessed.

1. **Consideration for changes through different product development:**

A. Analytical comparability

- The stage of product development may impact whether an analytical comparability study is required. During early-stage development, the primary consideration should be to avoid any impact of the change on product safety. However, when considering changes at later stages of product development, the applicant should evaluate the impact of the change on both safety and efficacy.

- Depending on the type of change, the applicant should consider its impact on product stability, and the proposed control strategy should be submitted as an amendment to the clinical-trial application before the implementation of the change.
- Analytical comparability of CAR-T cells may be assessed by following the general principles described in "Comparability of Biotechnological/Biological Products" Guidance on Biotechnological/Biological Products Subject to Changes in their Manufacturing Process (ICH Q5E).
- SFDA emphasizes the importance of analytical comparability depending on the type of change to ensure there is no negative impact on CAR-T cell quality, safety, or efficacy. Nonclinical or clinical studies may otherwise be required.

B. Product comparability

- Early establishment of CQAs by characterization studies facilitates the design of comparability studies, which helps ssupport the progression of product development.
- SFDA recommends that CAR-T cell comparability be assessed using the same cellular starting material, when possible

2. Comparability of CAR-T Cell Manufacturing facilities:

CAR-T cells may be manufactured at several facilities which may contribute to product variability. In this case, applicants should demonstrate the following:

1. That comparable product is manufactured at each location to support the analysis of the clinical trial results.
2. Analytical methods are comparable across the different sites, if applicable.
3. Each manufacturing site follows cGMPs.

4. Using same standard operating procedures (SOPs), personnel-training program, reagents, reference materials and equipment across manufacturing facilities, when possible.
5. That the clinical trial application describes any differences in the manufacturing process across the manufacturing sites.
6. Assay transfer protocol to ensure that non-compendial testing performed at each site is suitable for the intended purpose and is reproducible among all testing sites. Compendial testing methods do not require comparability studies, but feasibility should be demonstrated for each site.

3. Other modalities

1. Allogenic CAR-T cells

In addition to the clinical considerations applicable to autologous CAR-T cell therapies, allogeneic CAR-T products introduce further complexities. One key aspect involves donor-related requirements, beginning with stringent eligibility criteria that include thorough medical-history assessments, physical examinations, and mandatory testing for infectious agents such as HIV, HBV, HCV, CMV, and EBV.

Informed consent must be obtained in accordance with ethical standards. Moreover, complete traceability from the point of cell collection to patient administration is required and must be documented and submitted to the SFDA. From a manufacturing and quality control standpoint, allogeneic CAR-T cells are generally derived from healthy donors, although alternative sources like umbilical-cord blood or induced pluripotent stem cells (iPSCs) may also be used. Genetic modifications can be introduced through viral or non-viral vector-mediated approaches using advanced gene-editing tools such as CRISPR/Cas9, TALENs, or zinc-finger nucleases (ZFNs) to introduce the CAR transgene into T cells and, in some cases, to delete T-cell receptors (TCRs) and human leukocyte antigens (HLAs).

These modifications help minimize the risk of graft-versus-host disease (GvHD) and immune rejection. To ensure product quality, SFDA requires data on off-target effects, validation of gene edits, and long-term genomic stability.

Furthermore, release testing must confirm key quality attributes, including identity, purity, potency, viability, sterility, absence of endotoxins, and lack of replication-competent virus. Potency assays should correlate with expected clinical outcomes. Evaluating immunogenicity and rejection potential is also critical and must be supported by comprehensive nonclinical studies. Lastly, demonstrating comparability and manufacturing consistency across product batches is essential. This requires robust in-process controls, donor-variability assessments, and validated stability of the cell banks used.

2. **In vivo CAR-T cells**

In vivo CART cell therapies, also known as “off-the-shelf” CAR-T products, are gene therapies delivered using viral vectors, DNA or mRNA nanoparticles that can transfect and engineer T cells in vivo. Concerns associated with in vivo CAR-T therapies include the risk of off-target cell transfection. Key factors to consider include the disease indication, choice of delivery method, targeting mechanism, target-cell population, and route of administration.

3. **Non-Viral CAR-T based Therapy:**

Examples of non-viral methods that enable stable genomic integration of CAR transgenes in primary T cells include transposon-based systems (e.g., Sleeping Beauty or PiggyBac) and CRISPR/Cas9-mediated knock-in. Transposon systems use a transposase enzyme to integrate the transgene and can be delivered as proteins, mRNAs, or plasmid DNA. In contrast, CRISPR/Cas9 typically uses linear or circular DNA for delivery.

Non-viral gene-editing systems, such as electroporation, liposome-mediated transfection, or nanoparticle-based delivery, are used to introduce genetic modifications into primary T cells. However, non-viral methods typically yield lower CAR delivery and expression levels than viral approaches, necessitating a final enrichment step to ensure purity and avoid manufacturing failures.

Enrichment strategies must be non-immunogenic, GMP-compatible, and scalable. Techniques include surface marker targeting or drug selection systems used to isolate modified T cells.

CRISPR/Cas9 knock-in carries risks of off-target effects and chromosomal translocations, while transposon/transposase systems require monitoring of transgene copy numbers due to the associated risk of secondary malignancies resulting from random integration patterns. Both methods demand rigorous testing and optimization to ensure safety and efficacy.