Recommendations for Microbial Vectors used for Gene Therapy

Draft Guidance for Industry

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U.S. Department of Health and Human Services
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Contains Nonbinding Recommendations

Draft – Not for Implementation

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This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA or Agency) on this topic.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

I. INTRODUCTION

We, FDA, are providing you, investigational new drug application (IND) sponsors, with recommendations concerning IND submissions for microbial vectors used for gene therapy (MVGTs) in early-phase clinical trials. MVGTs meet the definition of "biological product" in section 351(i) of the Public Health Service (PHS) Act (42 U.S.C. 262), when such products are applicable to the prevention, treatment, or cure of a disease or condition of human beings. This draft guidance focuses on the chemistry, manufacturing, and control (CMC) information that you should submit in an IND for MVGTs and provides an overview of preclinical and clinical considerations for these products.

This draft guidance, when finalized, will supplement the guidance entitled, "Guidance for FDA Reviewers and Sponsors: Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs)," dated April 2008 (Ref. 1).

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

II. BACKGROUND

MVGTs include bacterial vectors such as Salmonella, Listeria, or *E. coli* genetically modified to express human tumor antigens, cytokines, growth factors, enzymes, therapeutic proteins, or nucleotides. For example, the bacterial vectors may be modified to express human tumor antigens, cytokines, growth factors, enzymes, therapeutic proteins, or nucleotides. MVGTs may also be generated by the modification (deletion, truncation, or point mutation) of chromosomal

or episomal genes and by the insertion of foreign genetic material into the chromosome, or into naturally occurring episomes; or by the introduction of one or more plasmids. In addition to the genetic modifications that may alter an MVGTs therapeutic profile, MVGTs may also be modified to alter their growth characteristics. For example, MVGTs may be may be live, dead or replication restricted as a result of specific biochemical requirements or genetic modification.

Gene therapies are defined in the FDA guidance document entitled, "Gene Therapy Clinical Trials – Observing Subjects for Delayed Adverse Events" dated November 2006 as "[p]roducts that mediate their effects by transcription and/or translation of transferred genetic material and/or by integrating into the host genome and that are administered as nucleic acids, viruses, or genetically engineered microorganisms. The products may be used to modify cells in vivo or transferred to cells ex vivo prior to administration to the recipient." (Ref. 2)

III. PRODUCT MANUFACTURING AND CHARACTERIZATION

Under § 312.23(a)(7)(i) (21 CFR 312.23(a)(7)(i)), you must, in your IND, as appropriate for the particular investigations covered by the IND, submit a section describing the chemistry, manufacturing and control of the drug substance and the drug product. Under § 312.23(a)(7)(iv)(*b*), you must also include in your submission a list of all components used in the manufacturing of your product. In addition, under § 312.23(a)(11), if requested by FDA, you are required to submit any other relevant information needed for review of the application.

In accordance with these regulations, and as described in the following sections, we recommend that you provide a detailed description of the complete MVGT manufacturing process in your IND submission. You should also describe all of the components used during the manufacture of the MVGT product, such as microbial vector, microbial cell bank systems, plasmid(s)/phage(s) harbored by the microbial cells, reagents used in the manufacture, and any excipients. In addition, you should describe all procedures used during the manufacturing process. This information will allow FDA to assess[1] the identity, quality, purity, and potency of your product.

A. Product Manufacturing – Components

You should provide the following information about your microbial product in the IND:

1. MVGT Seed Stock

   A description of the history and detailed derivation of the MVGT including:

[1] As a general matter, Office of Cellular, Tissue, and Gene Therapies (OCTGT) reviews clinical studies for MVGTs and related products such as oncolytic bacteria. The Office of Vaccine Research and Review (OVRR) in FDA's Center for Biologics Evaluation and Research (CBER) reviews the clinical studies for live biotherapeutic products (LBPs), as described in the FDA guidance entitled, "Early Clinical Trials with Live Biotherapeutic Products: Chemistry, Manufacturing, and Control Information," dated February 2012.

a. Selection of the MVGT clone

You should include a description of the history, source, and derivation of the MVGT. The MVGT seed stock should be derived from a single, well-isolated colony. You should ensure that antibiotic selection markers, such as beta lactamase, are not introduced into the MVGT for the purpose of propagation of the MVGT. Attenuation of MVGT should be through redundant and robust means, which may be achieved by mutation at more than one site on the MVGT genome and by more than a point mutation.

b. Physical properties and growth characteristics

The IND submission should describe the physical properties of the organism, such as shape under a microscope, colony morphology, and staining properties including their ability to take up gram stain or acid fast staining (staining should be done on a bacterial culture in a log-phase growth). Growth characteristics include dividing time under optimal growth conditions, and any specific characteristics of the organism used to evaluate its growth such as an evaluation of bacterial motility.

c. Defining the genetic make-up of MVGT

You should provide information regarding the MVGT's chromosomal genetic marker(s) and distinguishing genomic restriction pattern(s). If the MVGT contains a naturally occurring plasmid(s), you should include a description of the plasmid(s), and a method to assay for the presence of the plasmid(s) in the Master Cell Bank (MCB) and final product. You should describe the MVGT's selection markers/selection method.

d. Growth conditions

The IND should include a description of the growth media and growth conditions, such as media composition, growth supplements, antibiotics, growth temperature requirements, and aeration/bioreactor/anaerobic requirements.

e. Episomal/Plasmid based foreign gene inserts

If the MVGT contains a plasmid vector, you should describe the method used to introduce the plasmid vector into the parental cells to establish the MVGT (e.g., use of an intermediate bacterial

"transfer" strain, and/or the use of techniques such as electroporation, transformation, conjugation, etc.). In addition, you should provide the following information on the derivation of the MVGT clone in your IND package:

- A diagram of the plasmid/phage that identifies the gene insert, regulatory elements such as the promoter, ribosomal binding sites/Shine-Dalgarno sequences, and transcriptional terminators, and pertinent restriction endonuclease sites, such as multiple cloning sites (MCS);
- Information on any codon optimizations that were performed; and
- Details of any signature sequences/linker sequences introduced into the plasmid/phage vector.

f. Chromosomally inserted foreign genes

You should provide a detailed description of the methods used to insert foreign genes into the chromosome. We recommend that you provide a flow chart of the derivation of the final clone containing a chromosomally integrated gene insert, and describe in the text any intermediate clones that were generated in the process of derivation of the final clone.

2. MVGT Banking System

The IND should contain information, as described below, on the microbial cell bank system (i.e., master cell bank, working cell bank).

a. Master cell bank (MCB)

Your IND should include information regarding MCB characterization, including testing to adequately establish the safety, identity, purity, and stability of the cells. The information should address the following:

- *Purity of the strain:* We recommend that the microbial MCB be derived from a single isolated colony (sequentially isolated after at least two rounds of colony selection). All further amplification of the bacterial products should be derived from this MCB without further single colony selection.

- *Microbial purity*[2]*:* For an MVGT that is a live bacterial product, if conventional application of sterility testing as outlined in § 610.12 (21 CFR 610.12) is not feasible because the live vector strain will show growth, testing for microbial purity by monoculture assays may be appropriate (see section III.C.1 microbial purity testing). The test method should be capable of detecting slow-growing microbial contaminants.
- *Identity of the bacterial cells (and plasmids/phages if applicable):* Your development program should include tests to distinguish the specified cells through physical or chemical characteristics (i.e., phenotype, genotype, deoxyribonucleic acid (DNA) sequence, or other markers), selection marker(s), and antibiotic resistance.
- *Sequencing of genetically modified plasmids 50kb or less:* You should provide the full sequence, along with a description of the sequencing method for all genetically modified plasmids introduced into the microbe to derive the MVGT MCB.
- *Annotate sequence information:* You should summarize the plasmid sequence information with appropriate annotations, identifying all open reading frames (ORF), whether expected or unexpected, and encoded genes and their regulatory elements. You should indicate whether there is sequence alignment between the MVGT derived plasmid/phage sequences and sequences identified by a search in a relevant current database.
- *Sequencing of chromosomally inserted foreign genes:* We recommend that you perform and provide sequence analysis of the gene insert and at least 0.5kb of flanking regions, and provide a diagram of the insert and its regulatory elements and any other regions of the genome that are modified.
- *Activity*: You should assay for product specific characteristics such as expression of the introduced gene, duration of gene expression, localization of the expressed gene product (such as the percentage of gene products that are either secreted/membrane bound or intracellular), and growth rate/dividing time (in vivo and in vitro).
- *Antibiotic sensitivity:* Antibiotic sensitivity tests should cover a panel of antibiotics, including at least two first line antibiotics and two second line antibiotics for that class of microbial agent.

[2] For the purposes of this draft guidance, the term "microbial purity" is defined as freedom from microbes other than MVGT. Microbial purity may be evaluated by a monoculture assay (an assay designed to demonstrate the presence of a single strain of microbe in the test sample).

- *Characterization of attenuating mutation:* You should describe the size and stability of the attenuation. The attenuation site and at least 0.5kb of sequence on either side of the attenuation should be sequenced.
- *Bacterial growth:* You should describe bacterial growth conditions and include documentation of all media and reagents/components used during production, with copies of relevant certificates of analysis (COA). You should also include timing and duration of any induction conditions used to drive gene expression, such as change in temperature, pH, or other growth conditions or addition of inducing agents.
- *Cryopreservation, storage, and recovery:* The IND should include details on cryopreservation, storage, and recovery of the MCB, including information pertaining to cell density, number of vials frozen, storage temperature, and cell bank location.
- *Genetic and phenotypic stability:* A description of the genetic and phenotypic stability of the MCB after multiple passages, as well as viability of cells after cryopreservation, should be included in the IND.
- *Bacteriophage testing:* The MCB should be qualified by culture-based tests under conditions conducive for production of the bacteriophage lytic cycle to assay for the presence of bacteriophages in the bacterial product. The assay(s) should include positive and negative controls to evaluate test sensitivity. When available, positive controls for phage testing should include well-characterized bacteriophages specific to the MVGT.

b. Working cell bank (WCB)

The amount of information needed to document characterization of the WCB is usually less extensive than that needed to document characterization of the MCB. If there is a two tiered cell bank system in place, we recommend that you test the WCB for:

- Purity
- Limited identity testing (e.g., target specific polymerase chain reaction (PCR), for the presence of deletions or insertions)
- Test for plasmids (in case of MVGT products containing natural or introduced plasmids)
- Antibiotic sensitivity profile

- Viability
- Cell number
- Stability

3. Reagents

You must list in your IND any reagents used in manufacturing the product (§ 312.23(a)(7)(iv)(*b*)). For the purpose of this guidance, reagents are those components that are essential for bacterial growth, selection, purification, or other critical manufacturing steps, but are not intended to be part of the final product. Reagents can affect the safety, potency, and purity of the final product, especially by introducing adventitious agents. Examples of reagents include media and media components, antibiotics, and gene expression inducing agents. In addition to the list of reagents, the following information should be submitted in your IND:

a. Tabulation of reagents used in manufacture

For each reagent used in the manufacture of the MVGT, the following information should be submitted:

- Concentration of the reagent.
- The manufacturing step at which it is used.
- Vendor/supplier information, including the product identifying number and lot number.
- Information on the source of the reagent (i.e., whether it is synthetic, recombinant, or plant, animal, or human-derived). (Ref. 1).
- *Reagent quality*: We recommend that FDA-approved products or clinical grade reagents be used whenever they are available.

b. Qualification of reagents used in manufacture

The COA provided by the vendor of the reagent should be reviewed for each lot. Additional testing may be needed to help ensure the safety and quality of the reagent for use in the manufacture of your MVGT. We recommend that you establish a qualification program that includes safety testing (purity, endotoxin, mycoplasma, and adventitious agents), functional analysis, and assays to demonstrate the absence of potentially harmful substances (e.g., residual solvent testing). We believe that the appropriate extent of testing will depend on how the specific reagent is used in the manufacturing process.

c. Determination of removal of reagents from drug product

You should test the drug product for residual manufacturing reagents with known or potential toxicities. The IND should describe the test procedures used to detect residual levels of these reagents in the final product. We recommend that you determine whether a qualification study is sufficient to document their removal, or whether lot release testing is appropriate prior to initiation of clinical trials.

d. Other concerns

We recommend that you not use antibiotics for bacterial selection. Specifically, beta-lactam antibiotics such as penicillin should not be used during the manufacturing of a therapeutic product for humans. If antibiotics are used, we recommend that you quantitate the amount of residual antibiotics present in the final product and describe precautions to prevent hypersensitivity reactions.

B. Product Manufacturing – Procedures

You should include in the IND a detailed description of all procedures used during the derivation, production, and purification of an MVGT product. We believe that inclusion of a schematic of the production and purification process, and in-process and final product testing, will help to provide this information more clearly.

1. MVGT Production/Purification

You should describe the procedures used during the production of the MVGT, including:

- Culturing procedures, media components (including animal derived components) used, and antibiotics used during MVGT propagation.
- Cell growth conditions, cell density, method of monitoring cell growth, pH adjustments, bioreactor specifications, etc. Include a description of culture systems (shake flasks, bioreactors, etc.), and state whether the system is closed or open. Describe any defrothing agents used during culture, cell harvest conditions (e.g., membrane filtration, centrifugation, etc.).
- Harvest conditions, including the volume, timing (duration of culture) and optical density of the culture at the time of harvest.
- All purification steps, such as centrifugation, column purification, and density gradients, listed in the order of conduct.

- Process timing and intermediate storage: We recommend that you report the approximate time elapsed for each step from the end of cell growth to filtration/wash steps and final harvest. It is important to know the time limit of each step in production to determine what, if any, in-process testing to perform.

2. Inactivated/Killed Cells

If the final product is an inactivated/killed MVGT, you should describe the method and conditions of cell inactivation/killing. The IND should also include data to demonstrate that the bacteria are rendered replication incompetent, but still maintain their desired characteristics after inactivation. You should evaluate cell killing by sterility assays that include media and growth conditions used in the manufacture of the MVGT product and by sterility assays, as described under § 610.12.

3. Final Harvest

You should provide a detailed description of the final harvest. You should describe whether the final MVGT harvest is concentrated prior to final formulation, and if so, describe the wash conditions and buffers/media used. If the MVGT is lyophilized prior to storage, describe the lyophilization conditions and stabilizers used for lyophilization. If the MVGT Drug Substance (DS) or Drug Product (DP) is cryopreserved, you should include this information along with any stability studies initiated (see section III.B.4 of this guidance). You should also describe the time and conditions of storage (if any) between cell growth and final harvest and between final harvest and lyophilization. If the manufacturing process includes intermediate storage steps between final harvest and final formulation, the storage conditions and the length of storage should be described.

4. Final Formulation

You must describe the formulation of the final product as prescribed under § 312.23(a)(7)(iv)(*a*). In addition, the IND should describe whether excipients such as growth factors, buffers and salt stabilizers are included in the final formulation and state their source (see section III.A.3 of this guidance). You should also identify the vendor and final concentration of these excipients.

The MVGT cell density or concentration (live cells vs. killed cells) used in the final product should be described. If the final product is delivered to the clinical site frozen, you should include a description of how the product will be shipped and data to show that the product can be thawed

with consistent results. If the final product is to be reconstituted, you should describe the volume and composition of the reconstitution medium/or buffer and provide data to show bacterial viability when reconstituted. The viability data should be from at least two different product batches tested independently in triplicates.

C. Product Testing

Product testing for MVGTs includes microbiological tests to help ensure safety, and assessments of product characteristics such as identity, purity (including endotoxin), viability, and potency.

You should perform testing throughout the manufacturing process, including the manufacture of MCB/WCB, to evaluate the manufacturing process itself and to ensure the quality and consistency of the product. You should describe the specifications used for intermediate acceptance criteria and final product release criteria. Specifications are the quality standards (i.e., tests, analytical procedures, and acceptance criteria) that confirm the quality of products and other materials used in the production of a product. Acceptance criteria mean numerical limits, ranges, or other criteria for the tests described. Specifications should be appropriate to the stage of product development, because release criteria are generally refined and tightened as product development progresses toward licensure (Ref. 1). Table-1 in Appendix 1 of this guidance provides examples of suggested testing for MVGT products.

You must, under § 312.33(b)(7), provide an annual report to your IND that includes a summary of any significant manufacturing or microbiological changes made during the past year. Your annual report should contain manufacturing updates that include results related to stability testing of MCB/WCB, and results of characterization testing and lot release testing for lots manufactured during the reporting period. The results may be submitted in a tabular form and should include the lot number or identifier, date of manufacture, test used, test method, the sensitivity and specificity of test methods when appropriate, release criteria, and test results. All assay methods should be adequately sensitive, specific, and reproducible for the intended purpose.

Under § 312.23(a)(7)(i), you must provide a section describing the composition, manufacture, and control of the drug substance and the drug product. Although in each phase of the investigation, you are required to submit sufficient information to assure the proper identification, quality, purity, and strength of the investigational drug, the amount of information needed to make that assurance will vary with the phase of the investigation, the proposed duration of the investigation, the dosage form, and the amount of information otherwise available. As such, you must provide information in your IND for MVGT products to assure their: (1) freedom from other contaminating organisms (microbial purity and sterility); (2) identity; (3) purity (freedom from extraneous process related material); and (4) potency. In accordance with these requirements, we recommend the following testing:

1. Microbial Purity and Sterility Testing for MVGT Products

   - Live MVGT products should be assayed for freedom from causative agents of other diseases or conditions. The bacterial growth medium, growth conditions and amount of DP used to evaluate purity should be described and justified.[3] Tests of microbial purity and test components (growth promoting medium, conditions) should be validated to demonstrate that the test is capable of reliably and consistently detecting the presence of viable contaminating microorganisms. For examples of common bacterial agents for which the assay(s) should be capable of detecting, please refer to the Centers for Disease Control and Prevention (CDC) list of invasive bacterial pathogens (Ref. 3), and specific pathogens described in the United States Pharmacopeia (USP), Chapter 62,[4] as appropriate. We also recommend evaluating for environmental agents isolated from the manufacturing facility. For early phase clinical trials, when a dedicated live microbe manufacturing facility is not feasible, tests for microbial purity should include tests for other microbial products manufactured in the same manufacturing facility.
   - For killed MVGT product or an MVGT product that is incapable of host-free growth, such as bacteriophages or empty bacterial envelopes (mini cells), you should perform sterility testing of the DP. Sterility testing should include assays for both aerobic and anaerobic bacterial contaminants and fungal contaminants. For these products, the MCB, DS and in process samples should also be tested to demonstrate microbial purity.

2. Identity

   You should test for identity with respect to the MCB and DP by assays that will be specific for each product in a manner that will adequately identify the MVGT (§ 312.23(a)(7)(i)) and distinguish it from any other products being processed in the same manufacturing facility. For the final product, identity testing is important to ensure that the contents of the vial are labeled appropriately.

[3] Additional information can be found in the USP Chapter 61<USP61>: Microbiological examination of non-sterile products.

[4] The specific pathogens and detection methods described under USP Chapter 62 may not be appropriate for all MVGT products. We recommend that you discuss with FDA the appropriateness of test methods and test organisms during the Pre-IND meeting.

For bacterial strain identification, we recommend at least two complementary methods[5] of identity assays, such as the following tests:

- Gram staining.
- Colony morphology/cell culture nutrient dependent colony identification.
- DNA based PCR assays/Restriction Fragment Linked Polymorphism (RFLP) of the chromosomal DNA/16s ribonucleic acid (RNA) based analysis/analysis of house-keeping genes by sequencing.
- The host genotypic and phenotypic properties should be assayed using DNA based assays and phenotypic assays to detect the presence of genetic markers and or deletions.
- PCR or similar sensitive method to identify the number, size, and sequence of native and introduced plasmids (if any).

3. Purity

Product purity is defined as freedom from extraneous material, except that which is unavoidable in the manufacturing process described in the approved biologics license application § 600.3(r) (21 CFR 600.3(r)). Product purity must be described in the IND (§ 312.23(a)(7)(i)). Purity testing for MVGT products should include assays for pyrogenicity/endotoxin and a test for monoculture to demonstrate that the product is free from unintended bacterial and fungal organisms. Tests for purity should be included as a part of seed stock, MCB and WCB qualification tests, and as a release test for the final DP.

Suggested purity tests specific to MVGT products include the following:

- Residual host cellular protein/DNA content (for bacteriophage/mini cells).
- Strain purity: Presence of colonies of a single strain of bacteria biochemically identifiable as similar to each other. If the final product is a mixture of more than one type of MVGT, each MVGT component should be tested for purity. A minimum of 10 random colonies (number based on an estimate of 100 isolated colonies per plate) should be evaluated to confirm purity of the final product.
- Endotoxin assay with a specification of < 5 EU/kg/dose is required for all parenterally administered MVGT products.

[5] Depending on the MVGT product, additional identity tests may be required. We recommend that you discuss your specific product and the types of identity tests you propose to conduct during your Pre- IND meeting with FDA.

4. Potency

You should describe and justify the assay(s) you will use to measure potency. We recommend the use of a quantitative assay(s) to measure potency. An alternative approach using a matrix of assays, including quantitative and qualitative measures of potency, may be considered (Ref. 4). Potency assay should be included as a part of the MCB, qualification and DP release tests. For Phase 1/Phase 2 studies, an assay to quantify transgene expression, along with assays to quantitate the amount of live and dead organisms, may be acceptable as a surrogate measurement of potency. For a Phase 3 study, you should include in vivo or in vitro assay(s) that measure an appropriate biological activity of the MVGT. Note that potency assays must be validated prior to licensure (21 CFR 211.165(e)).

5. Other

a. Viability

For live bacterial products, we recommend that you establish minimum release criteria for viability. We recommend that the DP specification be set at > 60% live/viable cells[6] (Ref. 5). Viability should be tested at the MCB, WCB, DS and DP stages.

b. Cell number/dose

If the final MVGT product is administered parenterally, we recommend that you establish a dose level based on the number of live and dead bacteria in the final product considering dead bacteria can also contribute to antigen-specific and vector-specific immune responses.

c. Tests for aggregates

The presence and size of microbial aggregates is a potential safety concern in parenterally administered MVGT products. The final product should be tested for the presence of aggregates at the time of manufacture and at periodic intervals as a part of the stability protocol. A lot release specification with acceptance limits should be set for the presence and size of particulates in the DP.

[6] If your specific MVGT product is unable to meet this specification, you are encouraged to discuss the issue with FDA during Pre-IND discussions.

D. Drug Substance

Drug substance is an active ingredient that is intended to furnish pharmacological activity. An MVGT that is processed further (e.g., heat killed, lyophilized, encapsulated, made into a tablet, etc.,) to obtain the final DP, should be assessed for purity, the presence of plasmids (if applicable), viability, cell number etc., and this information should be included in the IND, along with test specifications, acceptance limits and a description of test methods. Some of the other testing parameters to be considered for inclusion in the IND are described below in section III.F.2 of this guidance. This information may be obtained by testing the DS, or if the final product is manufactured without additional manipulations other than aliquoting, the DP (see Table-1 in Appendix 1 of this guidance for a list of suggested test parameters for DS and DP).

E. Drug Product

The drug product is the final formulated product used for patient administration. In the case of an MVGT product, this would include, for example, the vialed microbial vector. Drug product release criteria testing should be performed on each lot of product manufactured. We recommend that you use a table format to provide all of your proposed specifications (tests for safety, purity, potency, and identity as described in section III.C of this guidance).

F. Stability Testing

As provided in § 312.23(a)(7)(ii), you must conduct stability testing in all phases of the IND to demonstrate that the product is within acceptable chemical and physical limits for the planned duration of the proposed clinical investigation. Stability testing is performed during early phases of the clinical trial to establish that the product is sufficiently stable for the time period required by the study. Data supporting a final formulation and dating period will be necessary for licensure.

You should submit a stability protocol and data for both in-process material and the final MVGT product. A proposed stability protocol should include a measure of product identity, purity, quality, and potency. For each test conducted you should describe the test method, sampling time points (there should be a zero-time point), testing temperature, and other appropriate information, including your justification of the assays used to indicate product stability, measuring these parameters for the duration of storage required by the clinical protocol. For further information, refer to International Conference on Harmonisation (ICH) Q5C Guideline for Industry: Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products (Ref. 6).

1. In-process Stability Testing

   If the MVGT is cryopreserved or lyophilized, your stability protocol should be designed to ensure that the product is stable during the period of cryopreservation/lyophilization, and to measure the parameters described above, as appropriate. In order to assess the biological function of the MVGT, we recommend that evaluation of the duration of lag phase for post-storage revival be conducted in rich medium under physiological conditions.

   Testing for live and dead bacteria should be part of your stability protocol. We expect that the appropriate acceptance criteria will be different for each bacterial species and we recommend that you establish specifications based on manufacturing and testing experience with your MVGT product.

2. Final Drug Product Stability Testing

   You should include any data that demonstrate that the product is stable between the time of product formulation and patient infusion to aid in establishing an expiration-dating period. We recommend that you conduct the testing at the appropriate temperatures and at time points consistent with predicted storage times. If the product is shipped from the manufacturing site to the clinical site, describe the time and shipping conditions (i.e., packaging, temperature). Your stability protocol should be adequate to demonstrate that product integrity, purity, recoverable sample volume, product aggregation profile, and potency are maintained under the proposed storage and shipping conditions. We recommend that you initiate and complete validation studies using conditions that stress the system by Phase 3.

G. Additional Testing

1. Antibiotic Sensitivity Tests

   Antibiotic sensitivity testing should be performed on the seed stock, MCB and DP, and the results included in the IND, when available. Antibiotic sensitivity tests should contain at least two first line therapy and two second line therapy antibiotics normally used to treat infections with the microbial agent(s) for which the MVGT was derived.

2. Container/Closure

   You should describe the types of containers and closures used and ensure that the containers and closures are compatible with the product (Ref. 7).

3. Tests for Residual Moisture Content

   If using lyophilized/dried bacterial products, please refer to FDA's previous recommendations regarding tests for residual moisture content (Ref. 8).

4. Environmental Assessment

   During clinical development of an MVGT product under an IND, you must submit either a claim for categorical exclusion under 21 CFR 25.31, or an environmental assessment under 21 CFR 25.40 (§ 312.23(a)(7)(iv)(*e*)). Please refer to FDA's guidance for industry entitled, "Determining the Need for and Content of Environmental Assessments for Gene Therapies, Vectored Vaccines, and Related Recombinant Viral or Microbial Products" dated March 2015, for additional information (Ref. 9).

5. Qualification of the Manufacturing Process

   The manufacturing process for MVGT products entails the use of reagents and source materials of differing complexity, variability, and risk for introduction of adventitious agents. You should use the appropriate methods, facilities, and manufacturing controls to ensure that the MVGT manufactured for Phase 1 clinical studies meets appropriate standards of safety, identity, quality, and purity. MVGT products manufactured for Phase 2 and Phase 3 clinical studies, and for marketing under a BLA, must comply with 21 CFR Parts 210 and 211 requirements. FDA has previously issued additional guidance regarding cGMP recommendations for Phase 1 clinical trials (Ref. 10).

IV. PRECLINICAL STUDIES

This section of the guidance describes the considerations for evaluating the activity, safety, and biodistribution of MVGT products in preclinical studies. Preclinical studies are conducted to support the scientific rationale for the use of a particular MVGT product in a target patient population. Adequate information about pharmacological and toxicological studies of the MVGT on the basis of which the sponsor has concluded that it is reasonably safe to conduct the proposed clinical trial, must be submitted in the IND (§ 312.23(a)(8)). FDA has previously issued guidance that provides comprehensive recommendations regarding the selection of appropriate animal species and animal models of disease, as well as the overall design of preclinical proof-of-concept and toxicology studies for investigational products, including MVGT products (Ref. 11). Some additional considerations of preclinical study design for MVGTs are highlighted below.

A. Animal Species and Models

Considerations for selection of biologically relevant animal species and models include the permissiveness/susceptibility of the species to the MVGT vector; the pharmacological response to the MVGT and to the expressed transgene; and the comparative physiology/pathophysiology of the animal species/model to the targeted clinical population. When feasible, we recommend the use of animal models of disease to characterize a dose response/biological activity relationship of the MVGT product. These animal models should also be considered when designing studies to evaluate the toxicology and biodistribution profile of the MVGT product, as disease status may affect the safety data generated from these studies, as well as provide a more relevant estimate of the risk-benefit assessment of the MVGT product. Refer to sections III.A and V.B of FDA guidance document entitled, "Preclinical Assessment of Investigational Cellular and Gene Therapy Products" dated November 2013 (Preclinical Assessment Guidance) (Ref. 11) for a more detailed discussion of this topic.

B. Safety Evaluation

The overall objectives of the preclinical safety studies are to identify, characterize, and quantify any potential local, systemic, acute, or chronic toxicity of the MVGT product. The data generated from these studies will help guide the selection of the starting dose level, dose-escalation scheme, dosing schedule, monitoring parameters, and other elements of the clinical trial. General recommendations for preclinical study designs applicable to MVGT products are provided in sections III and V of the Preclinical Assessment Guidance (Ref. 10). Specific to this product class, parameters to assess include: the potential induction of proinflammatory cytokines and chemokines; and innate and adaptive immune responses mediated by the MVGT or the expressed transgene. A possible association of an immune response with any off-target toxicities resulting from administration of the MVGT product should be explored as this information could affect clinical trial design.

C. Attenuation Evaluation

The majority of the clinical population receiving an MVGT may be immuno-compromised or immunosuppressed to some extent. Therefore, the degree of product attenuation and the stability of the attenuated phenotype should be assessed. For example, if MVGT product replication is dependent on special nutrient requirement(s) or a specific microenvironment, then in vitro and/or in vivo data demonstrating the rigorousness of this dependence and the lack of product replication in the absence of these factors should be submitted in the IND application. As applicable, the effect of abrogation of virulence factors or the introduction of new virulence factors in the MVGT product on the host cells/tissues should also be evaluated. For MVGT products capable of forming spores, the conditions under which the spores form or replicate, including the target site(s), should be well characterized.

D. Biodistribution/ Shedding

In many clinical trials, administration of the MVGT product is not via its natural route(s) of infection or transmission. Thus, the biodistribution/shedding profile of the MVGT and the expressed transgene (if applicable) following administration via the planned clinical route of administration in animals is an important safety endpoint. We recommend that you collect biodistribution/shedding data from target and non-target tissues/biological fluids at multiple time points following administration of the MVGT product. The tissues/samples should be analyzed using well characterized methods that use appropriate positive and negative controls, and the number of live and dead microorganisms in the MVGT product should be determined. FDA has previously provided recommendations for general principles regarding study design (Ref. 2). You may also refer to the FDA guidance entitled, "Design and Analysis of Shedding Studies for Virus or Bacteria-Based Gene Therapy and Oncolytic Products" dated August 2015 (Shedding Studies Guidance) (Ref. 12), for additional information on the design and analysis of shedding studies.

E. Antibiotic Use

The in vivo biological activity, safety, and biodistribution/persistence profiles of the MVGT product in the presence and absence of various antibiotics should be evaluated (see section III.G.1 of this guidance for recommendations on the types of antibiotics to include). Data obtained from these studies can help guide the selection of effective antibiotics, as well as appropriate timing of antibiotic administration in the clinical trial.

V. CLINICAL STUDIES

This section of the guidance focuses on clinical development and early clinical trial design issues that are unique to the study of MVGT products. General trial issues are discussed in the ICH guideline E8 General Considerations for Clinical Trials (Ref. 13). Clinical considerations that are particularly relevant to early-phase clinical trials of MVGT products are described below.

A. General Considerations

Administration of MVGT products in clinical trials may present substantial safety concerns. Such concerns derive from the infectious nature of the product, the risks of necessary concomitant medications, and the risks of the study procedures. The purpose of this section is to discuss general elements of risk mitigation that may guide the design and conduct of an early-phase trial of an MVGT product.

Specific MVGT products (e.g., live bacterial product) and specific study populations (e.g., immunocompromised patients) entail a risk of clinically significant sepsis and/or disease. Prophylactic, concomitant, or post treatment antibiotics may be indicated to decrease this risk of infection. Antibiotic sensitivity assays (see section III.G.1) and

preclinical studies with antibiotics (see section III.E of this guidance) may support the choice of first- and second-line antibiotics for the clinical trial. Relevant clinical or preclinical data should also support the dosing schedules and duration of administration of these antibiotics.

In vivo survival of an MVGT product may depend on concomitant administration of immunosuppressive drugs. However, such immunosuppressive drugs may substantially increase the risk of serious infection for the study subjects. Therefore, the administration of immunosuppressive drugs must be justified and incorporated into the study with appropriate precautions (e.g., careful dose selection and monitoring for infectious adverse events).

MVGT products may be studied in combination with other products (e.g., antibiotics or immunosuppressive drugs, as described above), administered through invasive procedures (e.g., surgery), or delivered systemically. In such cases, the rationale and data to justify the starting dose, dosing schedule, and dose escalation should consider the expected risks from both the product(s) and the study procedures. The study protocol should include a plan to monitor for these expected risks.

In early-phase clinical trials, a pre-specified plan for management of risks reduces the variability and severity of the risk outcomes due to differences in interventions. Thus, a pre-specified risk management plan may improve the interpretability of the safety data. Risk assessments and managements for clinical trials of MVGT products will continue to evolve as new data emerge.

B. Prior Human Experience

The specific investigational MVGT product, or a related product, may have been previously administered to humans. If so, then relevant clinical safety and activity data may be available to inform the design of the proposed study. To assess the relevance of the available data to the specific investigational MVGT product, adequate characterization of the previously administered MVGT product(s) is necessary (please refer to section III of this guidance). Thus, the characterization of these previously administered MVGT products determines the extent to which the available data can inform the design of a trial for a proposed MVGT product.

In situations where previous human data exist, and depending on the relevance of those data, the conduct of additional preclinical studies may not be necessary to support the starting dose and/or the dose-escalation scheme. FDA recommends that you contact CBER to discuss these dosing issues prior to conducting additional preclinical studies. You should provide comprehensive activity and safety data from the previous human experience to support the safety of the proposed dosing of the MVGT product.

C. Patient Population

The clinical development of MVGT products may involve initial testing in a wide spectrum of medical conditions. These range from relatively benign conditions that are easily treated to advanced stages of fatal or disabling diseases with only limited treatment options, and also include rare genetic diseases without available treatments. In general, the eligibility criteria for early-phase trials should define an appropriate disease setting that is relevant to the treatment indication being pursued. You should consider selection of a population with relatively reduced risks from the intrinsic or allergenic properties of the MVGT product. For example:

- Anaerobic bacterial spore-based products may produce spores that could proliferate in necrotic tissues. To reduce the risk of proliferation of spores, consider excluding patients with the presence of, or propensity to develop, necrotic tissue (e.g., patients with brain abscess, diverticulitis, or recent radiation).
- MVGT products with organ-specific tropism may have inherent risks to those organs. Consider selection of subjects to minimize these potential inherent risks. For example, in a trial of an MVGT product with infectious tropism to the liver, excluding patients with underlying liver disease may reduce the risk from hepatotoxicity.

D. Starting Dose, Dose Escalation, and Dosing Schedule

The dose selection and dosing schedule in early-phase clinical trials may substantially impact the risks associated with the MVGT product. These potential risks have been discussed in the CMC and preclinical sections of this guidance. To minimize the risks to human subjects, the results of preclinical proof-of-concept and safety studies can help guide the selection of the initial clinical dose level, dose-escalation scheme, and dosing schedule. Sponsors are encouraged to actively engage with the FDA early in product development to discuss the preclinical data needed to support early-phase trials for MVGT products.

The starting dose, dose-escalation scheme, and dosing schedule may also be supported by previous clinical data, if the rationale and justification provided for the dose-escalation plan are adequate. Appropriate definitions for dose-limiting toxicities (DLTs) and maximum tolerated dose (MTD) also minimize risks associated with dose escalation. Consider including specific MVGT-related risks in these definitions. For example:

- Clinically symptomatic septicemia may be a criterion for study DLTs.
- Fever and positive blood culture, even without hospitalization, after administration of an MVGT product may be defined as serious and unexpected adverse reactions for expedited reporting to the FDA and incorporated into DLTs and/or stopping rules (Ref. 14).

- MVGT products administered systemically may have expected risks such as fever or positive blood cultures immediately following administration. Prolonged duration or delayed occurrence of these expected events may serve as a DLT and/or stopping criterion.

E. Treatment Modifications

Study stopping rules and "off-treatment" criteria for individual subjects enhance the safety of an early-phase clinical trial. An example of an MVGT-related risk reduction procedure includes study stopping rules based on excessive rates or severity of expected infectious risks (e.g., abscess formation in studies of anaerobic MVGT products; septicemia).

F. Monitoring

Safety monitoring plans should consider the potential risks associated with the specific MVGT product and the transgene product as identified in the previous preclinical and clinical experience. Examples of specific monitoring plans include the following:

- Long-term safety monitoring for MVGT products with the potential to germinate, re-germinate, or re-seed.
- Monitoring plans to perform blood cultures and imaging studies at onset of non-specific symptoms to mitigate potential risks for abscess formation due to anaerobic microbial products.
- Consider long-term safety monitoring for MVGT products with anticipated or known delayed adverse events or cumulative toxicities. Safety data, particularly the nature and timing of adverse events, from prior human experience with related MVGT products may help determine an appropriate duration for long-term monitoring. Assessment of the relevance of the prior clinical experience with related MVGT products should include consideration of the dose level, route of administration, duration of exposure, and number of subjects exposed and evaluated.

For early-phase clinical trials, monitoring for shedding of MVGT products is recommended. Monitoring for shedding should be continued until after adequate successive measurements do not demonstrate the presence of the MVGT product. The choice of samples (e.g., bodily secretions and/or excretions) may depend on the specific MVGT product and the proposed route of administration. Please refer to FDA's Shedding Studies Guidance for additional information (Ref. 12).

VI. REFERENCES

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Appendix 1

Examples of suggested testing for MVGT products (Table-1)

	Seed Stock	Master Cell Bank	Working Cell Bank	In Process Testing	Drug Substance	Drug Product
Identity	+	+	+			+
Purity	+	+	+		(+)	+
Monoculture Test	+	+	+		(+)	+[#]
Sterility						+[***]
Tests for Plasmid(s)	+	+	(+)	(+)	+	(+)
Antibiotic Sensitivity Profile	+	+	(+)		(+)	+
Host Genetic Makeup	+	+			(+)	+
Potency		+			(+)	+
Gene Expression		+			(+)	+
Confirmation of Attenuation*	(+)	+			(+)	(+)
Viability	+	+	+		+	+
Cell Number		+	+		+	+
Live: Dead Cell Ratio						+
Residual Moisture**						+
Residual Antibiotics					(+)	+
Residual Growth Medium Components					(+)	+
Presence of Aggregates					(+)	+
Stability Testing		+	+	+		+

+ Recommended testing.
(+) May be needed, depending on the manufacturing process.
* IND should include information confirming any attenuating point mutations/insertions/deletions by sequencing of relevant gene/regions in the vector.
** For lyophilized or dried final product.
*** For killed MVGT final product.
[#] For live MVGT DP.