- COA or cross-reference letters: If you are using a research grade (not FDA-approved or cleared) reagent as part of the manufacturing process, we recommend that you provide information verifying the source, safety, and performance of the reagent. If the vendor of the reagent has a regulatory file with the FDA, a cross-reference letter from the sponsor may be provided in the IND. If a COA from the reagent manufacturer is used, you may assess whether the testing performed is adequate (see "Qualification Program" below) and provide that information in the IND.

Note to FDA Reviewers: For letters of cross-reference, include the regulatory file number and consider the need for a consultative review to determine whether there are any safety concerns or other outstanding issues.

b. Qualification Program

If the reagent is not FDA-approved or cleared, additional testing may be needed to ensure the safety and quality of the reagent. We recommend that you establish a qualification program that includes safety testing (sterility, endotoxin, mycoplasma, and adventitious agents), functional analysis, purity testing, and assays (e.g., residual solvent testing) to demonstrate absence of potentially harmful substances. The extent of testing will depend on how the specific reagent is used in the manufacturing process.

c. Determination of Removal of Reagents from Final Product

You should test the final product for residual manufacturing reagents with known or potential toxicities and describe the test procedures you use 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

Because some patients may be sensitive to penicillin, we recommend that you do not use beta-lactam antibiotics during the manufacturing of a therapeutic product for humans. If beta-lactam antibiotics are used, we recommend that you provide a rationale for their use and describe precautions to prevent hypersensitivity reactions.

Note to FDA Reviewers: If beta-lactam antibiotics are used during manufacturing, consult with the clinical reviewer concerning appropriate exclusion criteria for the study and proper informed consent to address potential patient sensitivity. Discuss with the sponsor the rationale for using beta-lactam antibiotics and whether an alternative to beta-lactam antibiotics can be used as an appropriate substitute.

4. Excipients

For the purpose of this guidance, an excipient is any component that is intended to be part of the final product, such as human serum albumin or Dimethyl Sulfoxide (DMSO). You must list in your IND all excipients used during manufacture of the product that are intended to be present in the final product (21 CFR 312.23(a)(7)(iv)(*b*)). You should include the concentration and source of the excipients. Also, you must provide information regarding the qualification of all excipients that are present in the final product (21 CFR 211.84(a)). For further information, please see Section III.A.2 above on reagents.

5. Additional Considerations

a. Combination Products

This guidance also applies to combination products containing a human gene therapy biological product in combination with a drug or device as part of the final product.[3] When intended for a different use, the drug or device component may already have FDA marketing approval or clearance (e.g., new drug application (NDA), a premarket approval application (PMA), or a premarket notification (510(k)), or it may be previously unstudied.

If information describing the drug or device component has already been submitted to FDA (e.g., in another IND, IDE, or Master File), you may submit a letter of authorization authorizing FDA to reference the previous submission for CMC or other information related to the drug or device component of your product.

Note to FDA Reviewers: Determine the regulatory status of the drug or device either by contacting the RPM from the center with jurisdiction for the drug or device or the sponsor directly, if necessary. If the drug or device has been approved for any use, confirm and document this in your review. In most cases, request a consultative or collaborative review from the Center for Drug Evaluation and Research (CDER) or the Center for Devices and Radiological Health (CDRH) even if the drugs or device components were previously approved for another use. Confer with your supervisor if it is unclear whether a consultative or collaborative review is needed. Once an adequate letter of authorization is submitted, verify

[3] Regulations on combination products are found in 21 CFR Part 3, which describes how we will determine which component of FDA has primary jurisdiction for the premarket review and regulation of a combination product. Note to FDA Reviewers: If you have any concerns regarding the appropriateness of the jurisdictional assignment or regulatory mechanism, you should contact the Office of Cellular, Tissues, and Gene Therapies' jurisdictional officer.

that the referenced file contains the needed information. Inform the consultative or collaborative reviewer that the information referenced in the letter of authorization should be reviewed as part of the application.

b. Consultative Reviews

Note to FDA Reviewers: Follow the standard operating procedures and policies (SOPP) on the "Intercenter Consultative/Collaborative Review Process" (Ref. 9). Specify the questions the consultative reviewer should address, identify the specific sections of the IND applicable to those questions, and request a date for completion of the consultative review. The requested date should be determined by coordinating with the consulting review center and be based on timeframes mandated by statute, the priority of the consultative review request, and the workload of the designated reviewer. The RPM will request the consultative or collaborative review from the appropriate Center/Division using the form in Appendix 1 of the SOPP. Given the tight IND deadlines, work with the RPM to contact the appropriate Center/Division before sending the consult request to identify the appropriate reviewer and ensure that the review can be completed within the time requirements. Also, as described in the SOPP, the RPM should send to the Office of Combination Products a copy of the consultative/collaborative request for monitoring/tracking purposes. The RPM should follow up with the consultative reviewer to confirm that essential documents are received along with the consultative review request. If problems that affect the timeliness of the consultative review occur during the consult review period, discuss with your supervisor how to share these experiences with the Office of Combination Products, which is responsible for monitoring the efficiency and effectiveness of the intercenter consultative/collaborative review process.

c. Review of Device Components

Note to FDA Reviewers: In the device consultative/collaborative review request, describe the device component, and where to find relevant information in the submission. You should ask the consultative reviewer to identify concerns with how the device component will be used, to determine whether appropriate types of biocompatibility and other device testing were performed adequately, and to assess testing of any hardware and software controlling the hardware. In addition, if the sponsor asserts barrier or performance claims, identify information relative to these claims for the consultative reviewer to assess. You should document in the review basic information concerning the device, such as the device name, vendor or source, purpose, regulatory status, and a brief description of the device. When the consultative/collaborative review is completed, and signed off by the appropriate CDRH supervisory chain, attach it to your review and communicate any outstanding issues to the sponsor.

d. Review of Drug Components

Note to FDA Reviewers: In the drug consultative/collaborative review request, describe the drug component of the combination product and state where to find relevant information on the component in the submission. Ask the consultative/collaborative reviewer to identify any concerns with how the drug will be used and also to evaluate the methods of manufacturing and the adequacy of results from testing of the drug substance and/or drug product. You should document in your review basic information concerning the drug component, such as the drug name, vendor or source, purpose, regulatory status, and a brief description of the use of the drug. When the consultative/collaborative review is completed and signed off by appropriate CDER supervisory chain, attach it to your review and communicate any outstanding issues to the sponsor.

e. Summarize Any Areas of Concern to be Addressed

Note to FDA Reviewers: Summarize any areas of concern identified during the review of the product components. Discuss these concerns with the sponsor and/or communicate them in a letter to the sponsor, as described in Section XI, "Comments to Sponsor Generated by FDA Reviewers".

B. Product Manufacturing – Procedures

You should include a detailed description (list or summary) of all procedures used during the collection, production, and purification of the gene therapy product. We believe that a schematic of the production and purification process, and in-process and final product testing, helps to provide more clearly this information.

Note to FDA Reviewers: If provided by the sponsor, append a copy of the process schematic to the IND review. If a schematic is not included, briefly summarize the processing steps used during product manufacturing. In addition, summarize any areas of concern identified during the review of any submitted product manufacturing procedures. Discuss these concerns with the sponsor and/or communicate to the sponsor in a letter, as described in Section XI, "Comments to Sponsor Generated by FDA Reviewers".

1. Vector Production/Purification

You should include the following in your procedures:

- all purification steps in order of processing, for example: centrifugation, column purification, and density gradients;
- reagents/components used during vector manufacture;
- describe cell lines used during production of vector product including; cell passage number and cell plating density; and
- culturing procedures, and media components used, including serum, growth factors, and antibiotics used during cell propagation.

2. Preparation of ex Vivo Gene-Modified Autologous or Allogeneic Cells

Autologous or allogeneic cells can be modified by using viral or plasmid vectors. We recommend that you describe the following procedures:

a. Method of Cell Collection/Processing/Culture Conditions

You should describe the cells used for ex vivo modification (i.e., cell lines, tumor cells, donor derived cells). If non-banked autologous or allogeneic cells are used, describe the volume and number of cells collected. You should include any mechanical or enzymatic digestion steps used. You should describe the use of any cell selection device or separation device, including density gradients, magnetic beads, or fluorescence activated cell sorting (FACS). You should include a description of culture systems (flasks, bags, etc.), and state whether the system is closed or open. Also, you should describe any in-process testing that will be performed during these procedures.

b. Ex Vivo gene modification

You should describe in detail the modification procedure, such as, transfection, or infection. You should describe in detail the selection of cells (methods, devices, reagents) as well as any other cell modification steps such as irradiation. If the cells are cultured after the genetic modification occurs, you should include the culture conditions used and time in culture.

c. Irradiation of ex vivo gene modified cells

If the autologous or genetically modified allogeneic cell product is irradiated before injection, you should provide data to demonstrate that the genetically modified cells are rendered replication-incompetent, but still maintain their desired characteristics after irradiation. You should describe the documentation of calibration of the cell irradiator source.

d. Final Harvest

You should provide a detailed description of the final harvest. You should inform FDA whether the final cell harvest is centrifuged prior to final formulation, and if so, describe the wash conditions and media used. You should also inform FDA in your IND submission whether the cells are cryopreserved after formulation or formulated immediately and given to the patient. If the final harvest is stored, you should describe the storage conditions and the length of storage, and provide appropriate supporting data (see Section VI below).

3. Process Timing and Intermediate Storage

Report the approximate time elapsed for each step in vector production including vector purification, intermediate holding steps (i.e., storage of bulk harvest) and cryopreservation of final vector product. In addition, if the final product is ex vivo genetically modified cells, you should report the approximate time elapsed for each step from cell collection, to transduction, to 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. If the final product is cryopreserved before injection into patients, you should include this information along with any stability studies initiated (see Section VI.A.1 below). Also, you should describe the time and conditions of storage prior to patient administration.

Note to FDA Reviewers: Describe and assess the procedures in place to ensure the stability of the bulk harvest while in storage.

4. Final Formulation

You must describe the formulation of the final product, including excipients such as growth factors or human serum albumin (21 CFR 312.23(a)(7)(iv)(*a*)). You should state the source of these components (see Section II.A.3 above). You should identify the vendor and final concentration of these excipients used in the final product. 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. In addition, if the product is shipped, you should provide data on product stability during shipping (see Section VI.A.2 below).

IV. PRODUCT TESTING

We recommend that product testing for gene therapies include, but not be limited to, microbiological testing (including sterility, mycoplasma, and adventitious viral agent testing) to ensure safety and assessments of other product characteristics such as identity, purity (including endotoxin), viability, and potency. We recommend that you perform this testing throughout the manufacturing process, including on the manufacture of cell banks, 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 attributes or variables for the tests described. Specifications should be appropriate to the stage of product development, because release criteria should be refined and tightened as product development progresses toward licensure (see Appendix B). You should submit test results related to lot release, characterization testing, MCB/MVBs, and WCB/WVBs in tabular form including the lot number or identifier, date of manufacture, test, test method, the sensitivity and specificity of test methods and, when appropriate, release criteria.

Product testing is an integral part of ensuring control of the manufacturing process and lot-to-lot consistency. Therefore, it is important to identify critical parameters in the manufacturing process and critical product attributes to ensure the desired clinical effect of the final product. Refer to the "FDA Guidance Concerning Demonstration of Comparability of Human Biological Products, Including Therapeutic Biotechnology-Derived Products" (Ref. 10) for additional information.

Note to FDA Reviewers: Document the testing performed. Assess the appropriateness of that testing and the acceptance criteria, based on any results previously obtained by the sponsor.

A. Microbiological Testing

We recommend that you perform microbiological testing on cell banks, in-process intermediates, and the final product, as appropriate.

1. Sterility Testing (Bacterial and Fungal Testing)

Current practices for sterility testing.

a. Test Method

Suitable sterility tests include the test described in 21 CFR 610.12 and the test described in United States Pharmacopoeia (USP) <71> Sterility Tests (23rd edition, 1995), (Ref. 11). Later versions of the USP<71> are also suitable. If you are using another test method, you should describe its suitability. Note that under 21 CFR 610.9, prior to product licensing, the alternative method must be shown to provide assurances of the safety, purity, potency and effectiveness of the biological product equal to or greater that the assurances provided by the method or process specified in the 21 CFR 610.12 method.

If you use antibiotics in manufacturing, you should provide documentation that the antibiotics were removed prior to sterility testing. If the antibiotics cannot be removed from the final product, we recommend that you assess the validity of the sterility assay using the bacteriostasis and fungistasis testing as described in USP <71> Sterility Tests. Use of this assay is designed to ensure that any residual antibiotic present in the product does not interfere with the results of sterility testing.

Note to FDA Reviewers: If the Sponsor proposes to use an alternative method in place of the traditional sterility methods described in 21 CFR 610.12, then you will need to assess the adequacy of this alternative test method and the data provided to provide assurances of the safety, purity, potency and effectiveness of the biological product equal to or greater than the assurances provided by the method or process specified in the general standard (21 CFR 610.9). The completeness of the validation study for the alternative method used during the

product development should reflect an assessment of risk associated with the application of the method (e.g., stage of the manufacturing process).

b. Test Timing

Sponsors frequently perform in-process sterility testing at critical points during manufacturing, such as during purification, or after ex vivo gene modification or extended culture periods. You should identify when in-process sterility testing is performed during manufacturing and the test method used. The test method that you choose for in-process sterility testing should be adequate to provide assurance of product sterility.

If you freeze the final product before its use, we recommend that you perform testing on the product prior to cryopreservation, so that results will be available before the product is administered to a patient. However, if the product undergoes manipulation (e.g., washing, culturing) after thawing, particularly if procedures are performed in an open system, you may need to repeat sterility testing. You should incorporate the results of in-process sterility testing into your acceptance criteria for final product specifications.

If your product has a short dating period and must be administered to patients before sterility test results of the final product are available, then you will need to develop an alternate approach to provide sterility assurance. As an alternative approach, we recommend that you perform all of the following tests:

- in-process sterility testing on a sample taken 48 to 72 hours prior to final harvest or after the last re-feeding of the cultures
- a rapid microbial detection test such as a Gram stain or other procedure on the final formulated product
- sterility testing compliant with 21 CFR 610.12 on the final formulated product

Under this alternative approach the release criteria for sterility would be based on a negative result of the Gram stain and a no-growth result from the 48 to 72 hour in-process sterility test. Although in this situation the results of the sterility culture performed on the final product will not be available for product release, this testing will provide useful data. A no-growth result will provide assurance that an aseptic technique was maintained. A positive result will provide information for the medical management of the subject, and trigger an investigation of the cause of the sterility failure. The sterility culture on the final formulated product and when possible the in-process culture should be continued to obtain the full 14 day sterility test result even after the product has been given to the patient.

In all cases where product release is prior to obtaining results from a full 14 day sterility test, the investigational plan should address the actions to be taken in the

event that the 14 day sterility test is determined to be positive after the product is administered to a subject. You should report the sterility failure, results of investigation of cause and any corrective actions, in an information amendment submitted to your IND in a timely manner, within 30 calendar days after initial receipt of the positive culture test result (21 CFR 312.31).

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

2. Mycoplasma

There are several potential sources of mycoplasma contamination. Two major sources include animal serum products used in culture and the culture facility environment, particularly with open culture systems. We recommend that you perform mycoplasma testing on the product at the manufacturing stage when the test is most likely to detect contamination, such as after pooling of cultures for harvest but prior to cell washing. Testing should be conducted on both cells and supernatant. We recommend that you inform FDA whether there is in-process testing for mycoplasma during extended culture procedures. Due to the limited dating period of many ex vivo genetically modified cellular products, it is frequently not feasible for a sponsor to perform the recommended culture-based assay (see Ref. 4) for release testing. In those cases, we recommend the use of polymerase chain reaction (PCR)-based mycoplasma assays or another rapid detection assay during product development. As part of your BLA, you should submit appropriate data to demonstrate that the PCR or alternative test has adequate sensitivity and specificity.

3. Adventitious Agent Testing

As appropriate, you should perform and describe in your IND adventitious agent testing as set out below. For more information on adventitious agent testing, refer to "Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals" and ICH guidance Q5A: "Guidance on Viral Safety Evaluation of Biotechnology Products Derived From Cell Lines of Human or Animal Origin" (Refs. 4 and 12).

a. In Vitro Viral Testing

When cell lines are used, you should describe the cell lines and perform in vitro viral testing. In vitro viral testing should be performed on the MCB, WCB, MVB,

WVB, and final vector product, and as a one time test on the EOP. Testing should be conducted by inoculating the test sample (MCB, WCB, MVB, WVB, final vector product) onto various susceptible indicator cell lines such as the human cell line MRC-5 or Vero cells which are primate in origin. The choice of cells used would depend on the species of origin of the product to be tested. An appropriate test should include monolayer cultures of the same species and tissue as that used for production of the product, as well as a human and a non-human primate cell line susceptible to human viruses. In addition, the test would include a measure of both cytopathic and hemadsorbing viruses.

In the event that the product is a cytolytic virus, it will need to be neutralized using specific antibodies prior to in vitro adventitious testing of the MVB, WVB and final product. If neutralizing antibodies are not available, then only in vivo adventitious agent testing needs to be performed on the MVB, WVB and final product until appropriate neutralizing antibodies are developed.

b. In Vivo Viral Testing

When cell lines are used, we recommend that you perform and submit data on in vivo viral assays carried out by inoculating the test sample (MCB, MVB) into animals such as adult and suckling mice and embryonated hen eggs. You should consider whether to include additional testing of guinea pigs, rabbits, or monkeys. Such studies would assess the test animals for any indication of illness. If such additional testing is appropriate, you should describe and explain the suitability of the animals used.

c. Selected Testing for Adventitious Viruses

We recommend that you test your MCB and MVB for appropriate, species-specific viruses. You should describe the testing that is performed, the different stages of manufacturing where those tests are performed (e.g., cell banks, viral banks, final product), and the test methods used.

1) Species-Specific Viruses

You should describe all species-specific virus testing performed on the MCB and MVB. We believe that all rodent cell lines used during product manufacturing should be tested for rodent specific viruses. These viruses are usually detected by antibody production tests, murine antibody production (MAP), rat antibody production (RAP), or hamster antibody production (HAP). If human cell lines are used in the therapeutic product, we recommend that you perform testing for human pathogens (CMV, HIV-1 & 2, HTLV-1 & 2, EBV, HBV, HCV, and B19) and other human viral agents, as appropriate. We recommend such testing because these cells are manipulated (cultured for extended time periods) and human pathogens can be introduced or propagated

during the extended culture periods. Human viral agents may be tested using a PCR-based test system.

When the gene therapy product is produced in a human cell line, e.g., an adenoviral vector produced in human 293 cells, you should test for the presence of additional human viruses such as adenovirus and adeno-associated virus (AAV), and describe those tests in your IND.

2) Testing for Retroviruses

When the MCB and MVB are used for production of vectors other than retroviral vectors, you should test the MCB and MVB for retroviral contamination using Reverse Transcriptase (RT) assays and electron microscopic analysis, and include a description of those tests in your IND.

We recommend that you perform testing for replication competent retrovirus (RCR) in the production of retroviral vectors at multiple points in production, including MVB, WVB, vector supernatants, end of production cells, and ex vivo modified cells. For further information on retroviral testing refer to the "Guidance for Industry: Supplemental Guidance on Testing for Replication Competent Retrovirus in Retroviral Vector Based Gene Therapy Products and During Follow-up of Patients in Clinical Trials Using Retroviral Vectors" (Ref. 13).

For cells that produce vectors containing amphotropic murine leukemia virus envelope, we recommend that you test for RCR using a permissive cell line such as *Mus dunni* and describe that testing in the IND. If an ecotropic packaging cell line is utilized during retroviral vector production, you should conduct and describe an ecotropic retroviral assay for the detection of low-level viral contamination in the MCB. Murine ecotropic viral contamination can be detected using either XC or D56 plaque assay methods.

You should describe how vector supernatant is tested. An appropriate test of vector supernatant lots would be by amplification on a permissive cell line such as *Mus dunni*, followed by detection in an appropriate indicator cell assay such as PG-4 S+L-. An appropriate test of the pooled End of Production (EOP) cells would be by co-culture with a permissive cell line such as *Mus dunni* for amphotropic virus, and the amplified material assayed in an appropriate indicator cell assay. We have determined that detection of RCR in ex vivo modified cells requires cells to be cultured for at least 4 days post transduction. Therefore, ex vivo gene modified cells that are cultured for $\geq$ 4 days should use RCR testing as a release assay. If ex vivo gene modified cells are cultured for < 4 days, archiving cells would be appropriate in place of active RCR testing. If it is not possible to have results from the RCR assay prior to treatment, we recommend that you initiate the culture assay and perform an alternative method (such as PCR) for product release.

3) Adenoviruses

For studies using adenoviral vectors, we recommend that you conduct tests for replication competent adenovirus (RCA) on MVB as well as the final vector. We believe that an appropriate maximum level of RCA contamination would be < 1 in 3×10^{10} viral particles, and that the adenoviral particle to infectious unit (iu) ratio would be ≤ 30 to 1.

4) Adeno-Associated Virus (AAV)

For studies using AAV, we recommend that you conduct tests to determine the amount of replication competent AAV present in the final vector product and report the results in your IND.

B. Identity

We recommend that you verify the identity of the MCB/MVB, WCB/WVB, and the final product by assays that will identify the product and distinguish it from other products being processed in the same facility. For the final product, identity testing is important to ensure that the contents of the vial are labeled appropriately. For additional information on labeling, refer to Section VII.B below.

If the final product is an ex vivo genetically modified cell product, we recommend that you test the final cell product for identity. Testing should include an assay to measure the presence of vector (i.e., expression assay, restriction digest) and an assay specific for the cellular component of the final product (i.e., cell surface markers).

C. Purity

Product purity is defined as relative freedom from extraneous material in the finished product, whether or not harmful to the recipient or deleterious to the product (21 CFR 610.3(r)). Purity testing includes assays for pyrogenicity/endotoxin (see below), residual proteins or reagents/components used during vector manufacture, such as cytokines, growth factors, antibodies, and serum, and in the case of ex vivo modified cells any unintended cellular populations or cell debris.

1. Residual Contaminants

The appropriate purity testing should include assays for residual proteins, DNA, RNA, and solvents used during production and purification, and reagents used during manufacture such as cytokines, growth factors, antibodies, and serum. If the product is a genetically modified ex vivo cell therapy product, appropriate purity testing should include a measurement of contaminating cell types or cell debris. For

further information, refer to ICH Q3 on "Impurities" (Ref. 14). You should describe in your IND the purity testing you conduct and your specifications for release.

2. Pyrogenicity/Endotoxin

The rabbit pyrogen test method is the currently required method for testing biological products for pyrogenic substances (21 CFR 610.13). Although the pyrogenicity test is required, there may be specific cases where this test method can not be performed for release due to properties of the final product (i.e., short product shelf life, toxicity of product in rabbits). Under these circumstances, a test method such as the Limulus Amebocyte Lysate test method (LAL) may be used as an alternative method, but prior to licensure must be shown to provide equal or greater assurances of safety, purity, and potency (see 21 CFR 610.9). The 1987 FDA Guideline on Validation of the Limulus Amebocyte Lysate (LAL) Test as End-Product Endotoxin Test for Human and Animal Parenteral Drugs, Biological Products, and Medical Devices (Ref. 15) sets forth acceptable conditions for use of LAL.

For any parenteral drug, except those administered intrathecally, we recommend that the upper limit of acceptance criterion for endotoxin be 5 EU/kg body weight/hour. For intrathecally-administered drugs, we recommend an upper limit of acceptance criterion of 0.2 EU/kg body weight/hour. You should describe in your IND the pyrogenicity/endotoxin testing you conduct, and your acceptance criterion for release.

Note to FDA Reviewers: Document in your review the specification for pyrogenicity/endotoxin testing and verify that testing is on the final product and that results are available prior to lot release.

D. Potency

You should describe and justify all assays you will use to measure potency. We recommend that these assays be quantitative, but in addition, they may include a qualitative biological assay. For a Phase 1/Phase 2 study, we recommend that the assay quantify the expression of a gene therapy vector product. For a Phase 3 study, we recommend that the potency assay consist of in vivo or in vitro tests that measure an appropriate biological activity. If development of a quantitative biological assay is not possible, then a quantitative physical assay which correlates with and is used in conjunction with a qualitative biological assay can be used. Note that potency assays are part of lot release and should be validated prior to licensure.

E. Other

1. General Safety Testing

Testing for general safety is required for licensure of all gene therapy vector products, unless the product is exempt under 21 CFR 610.11(g). General safety testing is performed on biological products intended for administration to humans and specific tests are described in 21 CFR 610.11. You should inform FDA whether general safety testing is being performed during product development.

Note to FDA Reviewers: Advise the sponsor regarding the applicability of the general safety test for the product under review. (Revisions to the General Safety Requirements for Biological Products (68 FR 10157; March 4, 2003) (Ref. 16)).

2. Viability

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

3. Cell Number/Dose

If your final product is a genetically modified cell therapy, you should develop specifications for the minimum number of viable and functional cells as part of product testing and release. We recommend that you inform FDA whether a maximum number/dose of cells to be administered has been established, and the basis for that level. For administration of a gene vector, you should describe your dose as the concentration of plasmid DNA, viral particle number, or titer.

V. FINAL PRODUCT RELEASE CRITERIA TESTING

The final product is the final formulated product used for administration to human subjects. Final product release criteria testing should be performed on each lot of product manufactured. In some situations, each dose could be considered a single lot, depending on the manufacturing process. The results from final product release criteria testing should be available prior to administration to a human subject. If results from final product testing will not be available prior to release, we recommend that you clearly indicate this in the IND, together with your specifications, and include a description of the reporting notification process if the acceptance criteria are not met. We recommend that you provide, in table format, all of your proposed specifications (tests for safety, purity, potency, and identity as described in Section IV, test methods, and acceptance criteria), including test sensitivity and specificity, where appropriate,

for the final product. (Note that, before the product may be licensed, these parameters must be validated (21 CFR 211.165(e)).

VI. PRODUCT STABILITY

Stability testing must be performed during early phases of the clinical trial to establish that the product is sufficiently stable for the time period required by the study (21 CFR 312.23(a)(7)(ii)). Data supporting a final formulation and dating period will be necessary for licensure. You should describe the stability measures you will use to support your studies. For further information, refer to ICH Q5C: "Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products," (Ref. 17), ICH Guideline Q1A(R): "Stability Testing of New Drugs and Products" (revised guideline) (Ref. 18), ICH Guideline Q1E: "Evaluation of Stability Data" (Ref. 19).

Note to FDA Reviewers: Assess the product development plan in the IND review to determine how much stability data are needed for the current phase of investigation and whether sufficient data are included in the submission. If submitted, include preliminary data in your review. Assess whether proposed expiration dating is appropriate.

Stability Testing

Under 21 CFR 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. If a very short term clinical investigation is proposed, the stability data submitted may be correspondingly limited. You should submit a stability protocol and data for both in-process material and the final gene product. A proposed stability protocol should include a measure of product sterility, 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 those parameters for the duration of storage required by the clinical protocol. We recommend sterility testing be performed at zero-time, end of stability study, and at an intermediate point during the study.

Note to FDA Reviewers: If the sponsor plans to use the product past the duration of the clinical trial (e.g., for a separate trial being conducted after the initial trial), verify that testing establishes stability throughout the relevant time period.

1) In-Process Stability Testing

You should describe the stability protocol that will be used to ensure that the final product is stable during the period of cryopreservation, measuring the parameters described in Section VI.A, as appropriate. A comparison is often made of analyses carried out pre-freeze and post-thaw. You should describe any stability

testing performed on the product during the holding steps, such as cryopreservation of final product and storage of bulk product.

2) Final Product Stability Testing

You should include any data that demonstrate that the product is stable between the time of product formulation and infusion to subjects 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 (e.g., packaging, temperature). Your stability protocol should be adequate to demonstrate that product integrity, sterility, and potency are maintained under the proposed shipping conditions. We further recommend that validation studies using conditions that stress the system be initiated by Phase 3 and completed prior to submission of a BLA.

VII. OTHER ISSUES

A. Product Tracking

For all products, you should establish a product tracking and segregation system. An adequate system should allow identification of the therapeutic product from collection to administration of the product and should include procedures to ensure that the product is segregated from other products in incubators, hoods, and cryopreservation units. You must establish and maintain a system of tracking that enables the tracking of all products from the donor to the consignee or final disposition, and from consignee or final disposition to donor (21 CFR 1271.290(b)).

B. Labeling

You should describe the product labeling used throughout the manufacturing process and on the final product container. As described in 21 CFR 312.6(a), the label for an investigational product must contain the following statement: "Caution: New Drug – Limited by Federal law to Investigational Use." To minimize the potential for mix-ups for products manufactured in multi-use facilities and for patient-specific products, we recommend that the product label contain the date of product manufacture, storage conditions, expiration date and time (if appropriate), product name, and two non-personal patient identifiers. As HCT/Ps, labeling for ex vivo genetically modified cell therapy products must meet the requirements in 21 CFR 1271.250. For autologous donors and other situations described in 21 CFR 1271.90(a) for which a donor eligibility determination is not required, you must include the applicable required labeling in 21 CFR 1271.90(b). For example, for autologous cells intended for autologous use you must label the product "FOR AUTOLOGOUS USE ONLY" (21 CFR 1271.90(b)(1)), and "NOT EVALUATED FOR INFECTIOUS SUBSTANCES" if donor testing and

screening is not performed (21 CFR 1271.90(b)(2)). For more information, refer to Ref. 3.

C. Container/Closure

You should describe the types of container and closure used, and that you have determined that the containers and closures are compatible with the product. For more information, refer to "Guidance for Industry: Container Closure Systems for Packaging Human Drugs and Biologics; Chemistry, Manufacturing, and Controls Documentation" (Ref. 20) and "Guidance for Industry: Container Closure Systems for Packaging Human Drugs and Biologics, Questions and Answers" (Ref. 21).

D. Environmental Impact

Under 21 CFR 312.23(a)(7)(iv)(*e*), you must submit either a claim for categorical exclusion under 21 CFR 25.30 or 25.31, or an environmental assessment under 21 CFR 25.40. Such categorical exclusion is ordinarily granted, absent extraordinary circumstances indicating that the specific proposed action might significantly affect the quality of the human environment. Extraordinary circumstances are described in 40 CFR 1508.27 and may include actions that create a potential for serious harm to the environment and actions that adversely affect a species or the critical habitat of a species determined to be endangered, threatened, or entitled to special protection (21 CFR 25.21). See the "Guidance for Industry: Environmental Assessment of Human Drug and Biologics Applications" (Ref. 22) for additional information.

Note to FDA Reviewers: Document in your review whether the sponsor has claimed a categorical exclusion or evaluate the sponsor's assessment of any extraordinary circumstances using this product.

E. Qualification of the Manufacturing Process

The manufacturing process for gene therapy products entails the use of reagents and source materials of differing complexity, variability and risk for introduction of adventitious agents. Qualification of reagents and source materials, as well as ensuring that appropriate controls are in place for monitoring manufacturing consistency and product quality, are key elements in ensuring that subjects receive a product that is consistently safe, pure, and potent. We recommend that, prior to production of clinical lots and initiation of clinical studies, you establish and implement written procedures to ensure proper manufacturing oversight. This includes the responsibilities and procedures applicable to the quality control unit. We recommend that you establish a quality control (QC) plan and document that plan in writing. For example, a sound QC plan must provide for the following functions:

- Responsibility for examining the various components used in the production of a product (e.g., containers, closures, in-process materials) to ensure that they are appropriate and meet defined, relevant quality standards (21 CFR 211.84(a));

- Responsibility for review and approval of production procedures, testing procedures and acceptance criteria (21 CFR 211.22(c));
- Responsibility for releasing or rejecting each clinical batch based on a cumulative review of completed production records and other relevant information (21 CFR 211.22(a), 21 CFR 211.165, 21 CFR 211.192); and
- Responsibility for investigating and initiating corrective actions if unexpected results or errors occur during production (21 CFR 211.22(a), 21 CFR 211.192).

You should summarize the QC plan that is in place to prevent, detect, and correct deficiencies that may compromise product integrity or function, or that may lead to the possible transmission of adventitious infectious agents. We recommend that QC responsibilities be performed independently from production responsibilities by dedicated QC personnel who are familiar with QC principles. You should conduct internal audits at planned intervals to evaluate effective implementation of the quality plan and to determine if processes and products meet established parameters. You should develop and document audit procedures to ensure that the planned audit schedule takes into account the relative risk of the various QC activities, the results of previous audits and corrective actions, and the need to audit the entire operation at least annually.

Note to FDA Reviewers: Document that you have reviewed the summary of the QC plan. Note each individual who has authority over the QC unit and the individual's assigned duties. Document the date of the most recent internal audits of the manufacturing operations and those of contract manufacturers, vendors, or other parties.

You should describe the changeover procedures that are followed to ensure that no cross-contamination occurs among ex vivo genetically modified cells intended for an individual subject and other products stored or produced in the same facility. You should describe the use of PCR assays for detecting area clearance, cleaning and decontamination reagents and segregation of activities, and the qualification of aseptic processing steps. Because most ex vivo genetically modified cell therapy products are not subject to final sterile filtration prior to infusion, you should manufacture these products under aseptic conditions. A media fill is an appropriate method to qualify that the process produces a sterile product. Refer to the "Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice" (Ref. 23) for further information.

Note to FDA Reviewers: Obtain consultative reviews from the Division of Manufacturing and Product Quality, CBER, to assess any data submitted by the sponsor on facilities and environmental issues such as decontamination and cleaning validation.

F. Biostatistics

Note to FDA Reviewers: Obtain consultative reviews for relevant portions of the CMC section from the Division of Biostatistics to ensure the adequacy of proposed experimental designs and analytic plans. There are many significant design and analysis issues in the areas of assay validation, establishing specifications, evaluation of product

potency, and evaluation of product stability. Proper statistical design and analysis of such studies are essential to ensure reliable documentation of the safety, purity, and potency of the product. If applicable, document in your review recommendations from the Biostatistics consult.

VIII. PRECLINICAL STUDIES TO BE DOCUMENTED BY FDA REVIEWERS

A. Summary of Concept Studies

Note to FDA Reviewers: Document information provided by the sponsor to support the scientific rationale underlying the proposal. Include a brief summary of preclinical data that was generated using in vivo animal studies and/or in vitro studies to assess the product's activity, efficacy, and any safety issues observed during the study. Some issues specific to gene therapy that should be documented include localization or trafficking of vectors, and level and persistence of gene expression.

B. Gonadal Distribution

Note to FDA Reviewers: For gene therapy vectors used for direct in vivo administration, work in consultation with the pharmacology/toxicology reviewer to document data that demonstrate the extent to which a vector is able to disseminate out of the administration site and distribute to the gonads. In most cases, this information is not available at the beginning of the Phase 1 study but would become available in the course of product development. Such data are usually obtained by using a PCR assay. In cases where a novel vector, route of administration, indication, or vector delivery system is proposed, preclinical studies to assess vector dissemination may be appropriate prior to initiation of the Phase 1 study. Document in your review the sensitivity of this assay (amount vector/μg cellular DNA), including assay controls (positive, negative and spiked controls). We believe that the PCR assay sensitivity should be less than 50 copies of vector genome per μg cellular DNA.

IX. CLINICAL STUDIES TO BE DOCUMENTED BY FDA REVIEWERS

Note to FDA Reviewers: Provide a brief description of the following in the CMC review:

A. Protocol Title

Name of the protocol.

B. Subject Population

Define the population; e.g., size, gender, race, age, etc.

C. Route of Administration

Define; e.g., oral, intravenous, intramuscular, etc.

D. Dose

Include the dosing regimen and whether there is a dose escalation. Document the dosing range and number of subjects enrolled in each dose. Describe the plans for dose escalation and what time interval/data evaluations occur between dose increases.

E. Frequency

Include the frequency of dose injections per treatment cycle and the number of proposed cycles.

F. Genetic, Biochemical, and Immunological Testing

Assess, in conjunction with the clinical reviewer, whether all genetic and/or product-specific biochemical and immunological testing of subjects is appropriate for the stage of clinical investigation. Evaluate the sensitivity and specificity of the test methods used to demonstrate biological activity (e.g., immunological assay, PCR) and document this information in your review. In conjunction with the clinical reviewer, verify and document that serum from a patient on a retroviral gene therapy protocol is analyzed for the presence of RCR at 3, 6, and 12 months after treatment. If all post-treatment assays are negative during the first year, then yearly patient samples may be archived (Ref. 13).

G. Informed Consent

If the informed consent document is submitted for your review, verify that the product is described accurately and completely.

H. Recombinant DNA Advisory Committee (RAC) Review

If the sponsor or the sponsor's institution receives NIH funding for DNA recombinant studies or if any clinical sites used in the study receive NIH funding for Recombinant DNA studies, you should remind the sponsor that under the NIH Recombinant DNA Guidelines, NIH will not allow the protocol to be initiated until the Recombinant DNA Advisory Committee (RAC) review has occurred. Confirm that the sponsor has submitted the protocol to RAC for review. For additional information refer to the "NIH Guidelines for Research Involving Recombinant DNA Molecules" (Ref. 24).

X. RECOMMENDATION TO BE DETERMINDED BY FDA REVIEWERS

Note to FDA Reviewers: Describe any information that is missing or incomplete, and issues that require additional clarification. Provide an overall assessment from the CMC perspective of