Guidance for Industry

Guidance for Human Somatic Cell Therapy and Gene Therapy

Comments and suggestions regarding this document may be submitted at anytime to Dano B. Murphy, HFM-17, Center for Biologics Evaluation and Research, Food and Drug Administration, 1401 Rockville Pike, Rockville, MD 20852-1448. For questions regarding this document, contact Suzanne L. Epstein, Ph.D., 301-827-0450, FAX 301-827-0449, e-mail "Epsteins@CBER.FDA.GOV".

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
March 1998

TABLE OF CONTENTS

Note: page numbering may vary for documents distributed electronically.

OVERVIEW (1998) 1

I. INTRODUCTION 3
- A. Definitions of Somatic Cell Therapy and Gene Therapy 3
- B. Types of Therapies 3
- C. Regulatory Considerations 4
- D. General Considerations 5

II. DEVELOPMENT AND CHARACTERIZATION OF CELL POPULATIONS FOR ADMINISTRATION 7
- A. Collection of Cells 7
- B. Cell Culture Procedures 8
- C. Cell Banking Systems Procedures: Generation and Characterization of Master Cell Banks (MCB), Working Cell Banks (WCB), and Producer Cells 9
- D. Materials Used During Manufacturing 11

III. CHARACTERIZATION AND RELEASE TESTING OF CELLULAR GENE THERAPY PRODUCTS 12
- A. Cell Identity 12
- B. Potency 12
- C. Viability 12
- D. Sterility 12
- E. Purity 13
- F. General Safety Test 13
- G. Frozen Cell Banks 13

IV. ADDITIONAL APPLICATIONS: ADDITION OF RADIOISOTOPES OR TOXINS TO CELL PREPARATIONS 13

V. PRODUCTION, CHARACTERIZATION AND RELEASE TESTING OF VECTORS FOR GENE THERAPY 14
- A. Vector Construction and Characterization 14
- B. Vector Production System 14
- C. Master Viral Banks 15
- D. Lot-to-Lot Release Testing and Specifications for Vectors 15

VI. **ISSUES RELATED TO PARTICULAR CLASSES OF VECTORS FOR GENE THERAPY** 18
A. Additional Considerations for the Use of Plasmid Vector Products 18
B. Additional Considerations for the Use of Retroviral Vector Products 18
C. Additional Considerations for the Use of Adenoviral Vectors. 20
D. Other Gene Delivery Systems 22

VII. **MODIFICATIONS IN VECTOR PREPARATIONS** 22

VIII. **PRECLINICAL EVALUATION OF CELLULAR AND GENE THERAPIES** . . . 23
A. General Principles 23
B. Animal Species Selection and Use of Alternative Animal Models 24
C. Somatic Cell and Gene-Modified Cellular Therapies 24
D. Direct Administration of Vectors *In Vivo* 25

IX. **CONCLUSION** 27

GUIDANCE FOR INDUSTRY:[1]
FDA GUIDANCE FOR HUMAN SOMATIC CELL THERAPY AND GENE THERAPY

OVERVIEW (1998)

Since the issuance of the "Points to Consider (PTC) in Human Somatic Cell Therapy and Gene Therapy"in 1991, the range of gene therapy proposals has expanded to include additional classes of vectors and use of vectors *in vivo* via direct vector administration to patients. This guidance document updates and replaces the 1991 PTC with new information intended to provide manufacturers with current information regarding regulatory concerns for production, quality control testing, and administration of recombinant vectors for gene therapy; and of preclinical testing of both cellular therapies and vectors. These guidances are not regulations, but rather represent issues that the Center for Biologics Evaluation and Research (CBER) staff believes should be considered at this time.

Virus or DNA preparations used as preventive vaccines are not covered by this document, though there is some overlap in the issues. Separate guidance on use of plasmid products to prevent infectious diseases is available from the Office of Vaccines Research and Review, CBER (301) 594-2090. One pertinent document is the "Points to Consider on Plasmid DNA Vaccines for Preventive Infectious Disease Indications (December, 1996)", (61 FR 68269). Somatic cell therapies are affected by the evolving criteria for infectious disease testing. For additional guidance refer to the following documents:

---

1 This guidance document represents the Agency's current thinking on the development and regulation of somatic cell therapy and gene therapy products. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of applicable statutes, regulations, or both. For additional copies of this guidance, contact the Office of Communication, Training and Manufacturers Assistance, HFM-40, Center for Biologics Evaluation and Research, Food and Drug Administration, 1401 Rockville Pike, Rockville, MD 20852-1448. Send one self-addressed adhesive label to assist that office in processing your requests. The document may also be obtained by mail by calling the CBER Voice Information System at 1-800-835-4709 or 301-827-1800, or by calling the FAX Information System at 1-888-CBER-FAX or 301-827-3844. Persons with access to the Internet may obtain the document at "http//www.fda.gov/cber/guidelines.htm".

Points to Consider in the Manufacture and Testing of Therapeutic Products for Human Use Derived from Transgenic Animals, (8/22/95), (60 FR 44036);

PHS Guidelines on Infectious Disease Issues in Xenotransplantation, August, 1996, (61 FR 49920) and January, 1997, (62 FR 3563);

Guidance on Applications for Products Comprised of Living Autologous Cells Manipulated *ex vivo* and Intended for Structural Repair or Reconstruction (May, 1996), May 28, 1996, (61 FR 26523).

A Proposed Approach to the Regulation of Cellular and Tissue-based Products, February 28, 1997, (62 FR 9721).

I. INTRODUCTION

A. Definitions of Somatic Cell Therapy and Gene Therapy

Recently, various innovative therapies involving the *ex vivo* manipulation and subsequent reintroduction of somatic cells into humans have been used or proposed. Somatic cell therapy is the administration to humans of autologous, allogeneic, or xenogeneic living cells which have been manipulated or processed *ex vivo*. Manufacture of products for somatic cell therapy involves the *ex vivo* propagation, expansion, selection (see: "A Proposed Approach to the Regulation of Cellular and Tissue-based Products", Feb. 28, 1997, (62 FR 9721)), or pharmacologic treatment of cells, or other alteration of their biological characteristics. Such cellular products might also be used for diagnostic or preventive purposes. Manufacturers should review policy and regulations to determine how a particular somatic cell therapy or gene therapy product is regulated.

Recently, various innovative therapies involving the introduction of somatic cells into humans have been used or proposed. For the purpose of this Guidance, the term somatic cell therapy refers to the administration to humans of autologous, allogeneic, or xenogeneic living non-germline cells, other than transfusable blood products, for therapeutic, diagnostic, or preventive purposes.

Gene therapy is a medical intervention based on modification of the genetic material of living cells. Cells may be modified *ex vivo* for subsequent administration to humans, or may be altered *in vivo* by gene therapy given directly to the subject. When the genetic manipulation is performed *ex vivo* on cells which are then administered to the patient, this is also a form of somatic cell therapy. The genetic manipulation may be intended to have a therapeutic or prophylactic effect, or may provide a way of marking cells for later identification. Recombinant DNA materials used to transfer genetic material for such therapy are considered components of gene therapy and as such are subject to regulatory oversight.

This document does not discuss genetic manipulation aimed at the modification of germ cells.

B. Types of Therapies

Examples of somatic cell therapies include implantation of cells as an *in vivo* source of a molecular species such as an enzyme, cytokine or coagulation factor; infusion of activated lymphoid cells such as lymphokine activated killer cells and tumor-infiltrating lymphocytes (addressed in a separate Points to Consider document: see below); and implantation of manipulated cell populations, such as hepatocytes, myoblasts, or pancreatic islet cells, intended to perform a complex biological function.

Initial approaches to gene therapy have involved the alteration and administration of somatic cells. However, additional approaches such as the direct administration to patients of retroviral vectors or other forms of genetic material have been used. The concerns described below apply regardless of the method used, though the applicable tests may be different.

Cells for therapeutic purposes may be delivered in various ways. For example, they may be infused, injected at various sites or surgically implanted in aggregated form or along with solid supports or encapsulating materials. Any matrices, fibers, beads, or other materials which are used in addition to the cells may be categorized as excipients, additional active components, or medical devices.

Because of the complexities of potential interactions with the cells and other constituents, additional components should be considered as part of the final biological product for purposes of preclinical evaluation.

C. Regulatory Considerations

All gene therapy products and most somatic cell therapy products are regulated by the FDA. See "A Proposed Approach to the Regulation of Cellular and Tissue-Based Products," February 28, 1997, (62 FR 9721) as well as subsequent regulations and policy issued in this area.

IND applications for somatic cell and gene therapies should follow the same format and contain the same sections as IND's for any investigational biological product, as described in 21 CFR 312.23. Forms and guidance documents are available from CBER by phone, FAX or E-mail as listed at the end of this document. The particular information required will depend upon the experimental system and the phase of study. For those therapies for which patient entry criteria include results of a genetic test, information should be submitted to the IND documenting and validating the test method.

Biological products are often complex mixtures that cannot be completely defined. Quality control of the manufacturing process as well as the final product is necessary. Poor control of production processes can lead to the introduction of adventitious agents or other contaminants, or to inadvertent changes in the properties or stability of the biological product that may not be detectable in final product testing. For these reasons, the methods and reagents involved in the production process should be defined. Also, cell banks and key intermediates in the production process should be subject to quality control. Lot-to-lot reproducibility of both the final product and of critical materials such as vector-containing supernatants should be examined. Existing general regulations (21 CFR 210, 211, 312, and 600) may be relevant and should be consulted for guidance.

Exploratory phase I trials for somatic cell and gene therapy products should be based on data that assure reasonable safety and rationale. Less data may be submitted to support beginning exploratory trials than may be submitted at later stages of product development, especially in the case of severe or life-threatening diseases. The review of data to support initiation of phase I trials focuses on safety, although some demonstration of rationale should also be provided.

Data from further product testing should be available at later stages of product development. A quantitative potency assay reflective of bioactivity *in vivo* should be developed and product stability should be studied to assure product integrity. In addition to safety, evidence of clinical efficacy is required for licensure.

If product formulation is changed during product development, a comparison of the different formulations should be made by quantitative assays of biological potency and, when appropriate, preclinical safety evaluation. If the product used in later phase trials differs in major ways from that used during earlier trials and if the results of the earlier trials are essential to the final product evaluation, product comparability should be demonstrated or the sponsor should assess whether earlier trials may need to be repeated (see: "FDA Guidance Concerning Demonstration of Comparability of Human Biological Products, Including Therapeutic Biotechnology-derived Products", 4/26/96, (61 FR 10426)).

For vectors intended to be used in a number of different IND's, manufacturing information can be submitted in a master file to simplify the filing process. Neither a master file nor the product it describes is "approved" or "disapproved". Rather the master file contains information and data that supplement the IND. Use of the same product for different patient populations may raise different issues or may indicate different levels of acceptable risk, but use of the master file can help identify common issues and facilitate their efficient resolution. Multiple IND sponsors can be authorized to cross-reference a master file, thus reducing redundant submissions as well as retaining desired confidentiality.

D. General Considerations

Some of the issues regarding cellular and gene therapy products overlap with those discussed in other Points to Consider documents. It is suggested that the most recent versions of Points to Consider or other Guidance documents be reviewed. Other Guidance documents should be consulted as is appropriate, for example:

Application of Current Statutory Authorities to Human Somatic Cell Therapy Products and Gene Therapy Products, October 14, 1993, (58 FR 53248).

Points to Consider in the Production and Testing of New Drugs and Biologicals Produced by Recombinant DNA Technology (1985), and Supplement: Nucleic Acid Characterization and Genetic Stability (1992), July 27, 1992, (57 FR 33201).

Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals (1993), August 12, 1993, (58 FR 42974).

Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use (1997), February 28, 1997, (62 FR 9196).

Points to Consider in the Collection, Processing, and Testing of Ex-Vivo-Activated Mononuclear Leukocytes for Administration to Humans (1989), November 2, 1989, (54 FR 46303).

The following sections indicate areas of concern and questions to be addressed by manufacturers of such products when filing applications. Initial clinical trials should be preceded by submission of data adequate to assure a reasonable degree of safety. A description of the methods used, actual data from appropriate tests, and evidence of assay validation should be included.

It may not be practical or possible to address all of the issues discussed below for a given system. In some instances, tests mentioned will be inapplicable or inappropriate, or alternative procedures may be more appropriate. The methods and procedures mentioned are suggestions: sponsors may propose alternative techniques which will be acceptable if these issues are adequately addressed and supported by data and rationale. In addition, all of the information discussed below may not be necessary before clinical trials are initiated. Sponsors are encouraged to consult with CBER staff for discussion.

The guidance suggested in this document is based in part on an assessment of the available experience with cell and gene therapy products and methods of production. Modifications of procedures will occur with time, and alternate control procedures will be needed. The principles included here can serve as guidance for developing these procedures.

II. DEVELOPMENT AND CHARACTERIZATION OF CELL POPULATIONS FOR ADMINISTRATION

A. Collection of Cells

The following information should be provided:

1. Cell types: The type(s) of cell to be used should be classified as autologous, allogeneic, or xenogeneic in origin. The tissue source and other relevant identifying information should be provided.

2. Donor selection criteria: Any relevant characteristics of the donor(s) should be specified, including age and sex. As stated in the "Points to Consider in the Collection, Processing, and Testing of Ex-Vivo-Activated Mononuclear Leukocytes for Administration to Humans," as a minimum, allogeneic donors should meet the standards for blood donors (21 CFR 640.3), the testing and acceptance procedures should be described, and any deviations should be justified. Where applicable, additional Public Health Service recommendations regarding organ and tissue donors should be incorporated. Exclusion criteria should focus on the presence or likelihood of infection by HIV-1 and HIV-2, hepatitis B and C viruses, HTLV-1, and other infectious agents. Serological, diagnostic, and clinical history data to be obtained from donors should be specified. Provision for follow-up of donors will be appropriate in some cases and methods of obtaining donor data and record keeping should be thoroughly described.

If autologous cells are used, please refer to "A Proposed Approach to the Regulation of Cellular and Tissue-based Products", February 28, 1997, (62 FR 9721) for additional guidance on adventitious agent testing and labeling. If animal species other than humans are used, a description should be provided of the origin, relevant genetic traits, husbandry, and health status of the herd or colony (see also "PHS Guidelines on Infectious Disease Issues in Xenotransplantation", August, 1996, 61 FR 49920 and January, 1997, (62 FR 3563).

3. Tissue typing: If allogeneic donors are to be used, typing for polymorphisms such as blood type should be included when appropriate. The importance of matching for histocompatibility antigens (HLA class I and/or II, and perhaps minor antigens in some cases) between donor and recipient should be addressed, and typing procedures and acceptance criteria provided.

Should it be indicated or necessary to use mixtures of cells from multiple donors, special attention should be paid to possible cell interactions that could result in

immune responses or other changes that might alter the performance of the cells. Characterization of multiple-donor cell mixtures may be problematic. Multiple-donor cell mixture products would not meet the criteria set forth in the "Proposed Approach to the Regulation of Cellular and Tissue-based Products", February 28, 1997, (62 FR 9721) for regulation as human cellular or tissue-based products under section 361 of the Public Health Service Act (the PHS Act). Such products would be subject to regulation under the Federal Food, Drug, and Cosmetic Act and section 351 of the PHS Act.

4. Procedures: The procedures for the collection of cells, including the location of the facility, and any devices or materials used, should be submitted.

B. Cell Culture Procedures

1. Quality control procedures: In general, cell culture operations should be carefully managed in terms of quality of materials, manufacturing controls, and equipment validation and monitoring. See I, C, General Considerations.

2. Culture media: Acceptance criteria should be established for all media and components, including validation of serum additives and growth factors, as well as verification of freedom from adventitious agents. Records should be kept detailing the components used in the culture media, including their sources and lot numbers. Medium components which have the potential to cause sensitization, for example certain animal sera, selected proteins, and blood group substances, should be avoided. For growth factors, measures of identity, purity, and potency should be established to assure the reproducibility of cell culture characteristics. More detailed discussions of specifications for medium components and biologicals added to cultures are presented in the "Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals (1993)" and the "Points to Consider in the Collection, Processing, and Testing of Ex-Vivo-activated Mononuclear Leukocytes for Administration to Humans (1989)."

As stated in the "Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals," it is recommended that penicillin and other beta-lactam antibiotics be avoided during production, due to the risk of serious hypersensitivity reactions in patients.

3. Adventitious agents in cell cultures: Documentation should be provided that cells are handled, propagated, and subjected to laboratory procedures under conditions designed to minimize contamination with adventitious agents. During long term culturing, cells should be tested periodically for contamination. Testing should ensure that cells are free of bacteria, yeast, mold, mycoplasma, and adventitious viruses. For a discussion of adventitious agent testing and details

regarding virus testing and mycoplasma testing, the "Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals" (1993), should be consulted.

4. Monitoring of cell identity and heterogeneity: Both manufacturing and testing procedures should be implemented which ensure the control of cell cultures with regard to identity and heterogeneity.

Cell culturing practices and facilities should be designed to avoid contamination of one cell culture with another.

During cell culturing, extensive drift in the properties of a cell population, or overgrowth by a different cell type originally present in low numbers, may occur. To detect such changes, cell identity should be assessed quantitatively, for example, by monitoring cell surface antigens or biochemical markers. The method of identification chosen should also be able to detect contamination or replacement by other cells in use in the facility. Acceptable limits for culture composition should be defined. Quantitative assays of functional potency may sometimes provide a method for population phenotyping. The desired function should be monitored when the cells are subjected to manipulation, and the tests carried out periodically to assure that the desired trait is retained. Identity testing should in some cases include verification of donor-recipient matching and immunological phenotyping.

5. Characterization of therapeutic entity: If the intended therapeutic effect is based on a particular molecular species synthesized by the cells, enough structural and biological information should be provided to show that an appropriate and biologically active form is present.

6. Culture longevity: The essential characteristics of the cultured cell population (phenotypic markers such as cell surface antigens, functional properties, activity in bioassays, as appropriate) should be defined, and the stability of these characteristics established with respect to time in culture. This profile should be used to define the limits of the culture period.

C. Cell Banking System Procedures: Generation and Characterization of Master Cell Banks (MCB), Working Cell Banks (WCB), and Producer Cells

Cell banking systems are appropriate for use with some somatic cell therapy products that are made repeatedly from the same cell source, and with packaging or producer cells used to make gene therapy vectors, for example, bacterial cells producing a plasmid or mammalian cells producing a recombinant viral vector. These cell stocks should be handled by a formal cell banking system (often a two-tiered system). Specific guidance

for the establishment of Master Cell Banks and Working Cell Banks is provided in the "Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals (1993)", 58 FR 42974. In addition, 21 CFR 610.18 may be applicable. The cell bank system used should be described as follows:

1. Origin and history of cells: A description should be provided.

2. Procedures: The procedure for freezing and for recovering the cells should be described. Components used (such as DMSO or glycerol) should be specified. The number of vials preserved in a single lot and the storage conditions should be specified.

3. Characterization: The identity of the cells should be confirmed by appropriate genotypic and/or phenotypic markers, and the fraction of the cell population having such identity markers measured as an indication of purity. In the case of transduced or vector-producing cells, vector retention and identity should be confirmed by restriction mapping or assay of the bioactivity of protein expressed by an inserted gene.

4. Testing for contaminating organisms: MCB's should be shown to be free of contaminating biological agents, including fungi, viruses other than a vector, mycoplasma, bacteria other than an intended bacterial host strain, and replication-competent viruses related to vector in certain cases (see section VI, B and C).

In the case of MCB's consisting of bacteria carrying plasmids of interest, testing for bacteriophage is not required but the possible presence of bacteriophage should be considered, since it could adversely affect stability and yield.

5. Expiration dating: Product development plans should include accumulation of data demonstrating how long and under what conditions the cells can remain frozen and still be acceptably active when thawed.

6. Tests on thawed cells: Tests of viability, cell identity, and function should be repeated after thawing and/or expansion. The yield of viable cells and of quantitative functional equivalents should be compared to those values before freezing. Sterility should be confirmed using aliquots of the frozen cells.

Working Cell Banks, if used, should undergo limited testing for identity by phenotypic or genotypic markers. Vector retention and identity should be confirmed as in MCB's by restriction mapping or assay of secreted protein activity. They should also be shown to be free of microbial and viral contamination.

For producer cells, extended culture of end-of-production cells should be performed on a one-time-basis to evaluate whether new contaminants are induced by growth conditions or if vector integrity is compromised (See "Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use," 1997 revision, 62 FR 9196). Sponsors should propose a schedule of testing at steps which will be most informative and sensitive.

It may not be feasible to use cell banking practices with cell therapies made differently for each patient, for example autologous cells for treatment of individual patients. However, consideration should be given to testing of the final cellular product for crucial characteristics.

D. Materials Used During Manufacturing

Materials used during in vitro manipulation procedures, for example antibodies, cytokines, serum, protein A, toxins, antibiotics, other chemicals, or solid supports such as beads can effect the safety, purity, and potency of the final therapeutic product. These components should be clearly identified and a qualification program with set specifications should be established for each component to determine its acceptability for use during the manufacturing process. When using reagent grade material, the qualification program should include testing for safety, purity, and potency of the component where appropriate. Abbreviated testing may be appropriate for use of clinical grade components. Materials of animal origin will in some cases need to be tested for adventitious agents. The country of origin should be certified when there is risk of transmissible agents causing spongiform encephalopathy

Limits should be established for the concentrations of all production components that may persist in the final product. The methods used to remove them and the results of quantitative testing (including a description of methods and sensitivity) to show the effectiveness of their removal should be provided. Some added components, by virtue of binding or uptake, may be present in measurable amounts when the cells are administered. In such cases, consideration should be given to assessing toxicity of these components in animals or other appropriate systems.

III. CHARACTERIZATION AND RELEASE TESTING OF CELLULAR GENE THERAPY PRODUCTS

Applies to cellular products including *ex vivo* transduced cells for gene therapy.

The final biological product to be administered, as well as the production process and materials used, should be subjected to quality control testing. The specifications to be applied to the final product and to other elements of the production process, along with the range of acceptable values for each, should be specified.

One lot of a biological product is considered to be a quantity of material that has been thoroughly mixed in a single vessel. This concept can be applied to somatic cell and gene therapy for purposes of planning lot testing procedures. This means that each cell population, vector preparation, or other product for such therapies prepared as a unique final mixture should be subjected to appropriate lot release testing. Preparations intended solely for individual recipients differ from products prepared as large batches, and appropriate lot release criteria should be chosen to fit the practical constraints of each protocol. Lot-to-lot variation provides a measure of the reproducibility of the procedures.

A. Cell Identity

Quantitative testing by phenotypic and/or biochemical assays should be used to confirm cell identity and assess heterogeneity (21 CFR 610.14).

B. Potency

The relevant function of the cells, if known, and/or relevant products biosynthesized by the cells should be defined and quantitated as a measure of potency (21.CFR 610.10).

C. Viability

The viability of the cells should be quantitated and a lower limit for acceptability established.

D. Adventitious Agent Testing

Tests should demonstrate that the cells are not contaminated with adventitious agents such as bacteria, fungi, (21 CFR 610.12), and mycoplasma, (Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals, (1993), Attachment #2, (58 FR 42974) and viruses. The agency is considering proposed rulemaking to allow for validation of a mycoplasma free manufacturing process in cases where the final cell therapy product is too short lived to complete adequately sensitive testing prior to administration to patients.

E. Purity

Purity (21 CFR 610.13) or validation of endotoxin testing by LAL or other acceptable assays should be established. The suitability and appropriateness of methods of endotoxin testing should be considered on a case-by-case basis. The test used should be validated to show that the cell preparation does not interfere with endotoxin detection. See "Guideline on validation of the limulus amebocyte lysate test as an end-product endotoxin test for human and animal parenteral drugs, biological products, and medical devices", December, 1987.

F. General Safety Test

The general safety test (21 CFR 610.11) must be performed on the final product. When appropriate, modified procedures may be developed according to 21 CFR 610.9. Please note that the agency is considering proposed rulemaking to amend the GST rules and scope of applicability especially for cell therapy products.

G. Frozen Cell Banks

When cell populations frozen for subsequent administration are thawed, expanded, and then administered to patients, lot release testing on the thawed cells is needed, and can be adapted from Part II, C on cell banking practices.

IV. ADDITIONAL APPLICATIONS: ADDITION OF RADIOISOTOPES OR TOXINS TO CELL PREPARATIONS

Therapeutic or diagnostic applications may be proposed involving cells which are modified by radiolabeling or pre-loading with bioactive materials such as toxins. Thus, the cell implant may be used as a delivery system not only for its own products and functions but also for other products. Novel safety concerns may arise related to the site of cell implantation and localization of the radionuclide or toxin, or due to metabolic properties of the cells. These should be anticipated and addressed where possible.

Similar special issues have been raised in the past by use of radiolabeled or toxin-conjugated antibodies, and are addressed in the "Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use" (1997). Although the application to somatic cell therapies may differ, that document should be consulted.

V. PRODUCTION, CHARACTERIZATION AND RELEASE TESTING OF VECTORS FOR GENE THERAPY

The information requested below may be difficult to acquire in some systems. Sponsors may present alternative methods and data to CBER staff for review. The types of information which will assure adequate safety depend in part on the nature of the proposed clinical trial, such as: route and frequency of administration, and the intended patient population.

A. Vector Construction and Characterization

Vector source materials should be characterized and documented thoroughly. Viral vectors or plasmids should be generated from cloned and characterized constructs, and subjected to confirmatory identity tests. Information supplied should include vector derivation, including descriptions of any vectors, helper viruses, and producer cell lines used for preparation of the final construct. Known regulatory elements such as promoters or enhancers contained within the construct should be identified.

Early in product development, vector characterization consisting of sequence data of appropriate portions of vectors and/or restriction mapping supplemented by protein characterization is acceptable. For later phases of product development and licensure, more extensive sequencing information should be provided. When sequencing of the entire vector is not feasible due to the size of the construct, it may be sufficient to sequence the genetic insert plus flanking regions and any significant modifications to the vector backbone or sites known to be vulnerable to alteration during the molecular manipulations. Vector sequences which modulate vector-host interactions should be described if known, and stability of the host cell/vector system considered.

B. Vector Production System

The vector production system is composed of the host cell, final gene construct or when appropriate vector intermediate, used to produce the vector (for example, retroviral producer cell). The procedure for selection of the final gene construct, method of transfer of the gene construct into the host cell and selection and characterization of the recombinant host cell clone including vector copy number, and the physical state of the final vector construct inside the host cell (i.e. integrated or extra chromosomal), should be described in detail. In addition, a detailed description of procedures for the propagation and expansion of the recombinant host cell clone, establishment of the seed stock and qualification of the seed stock should be provided. For information on cell banking procedures refer to Section II, C.

C. Master Viral Banks

When a virus, with or without a therapeutic gene, is used as a seed in the manufacture of a therapeutic vector, it is recommended that a Master Viral Bank be created and characterized. This would include vectors derived from adenovirus, adeno-associated virus, herpes virus, poxviruses, and other lytic and non-lytic viruses. The sponsor should describe the source materials, (i.e. plasmids, vectors, oligomers, etc.) and molecular methods used to produce the source or seed vector. The genetic integrity and stability (i.e. identity) of the seed vector should be confirmed and bioactivity of the vector seed should be demonstrated. In the absence of bioactivity data, expression of the gene should be assessed.

Master seed stocks should also be demonstrated to be free of adventitious agents, including virus, bacteria, fungi, and mycoplasma. In the case of replication-defective or replication-selective vectors, Master Viral Banks should be demonstrated to be free of replication-competent viruses, which may arise as a result of contamination or recombination during the generation of the MVB. Testing for other inappropriate viruses will depend upon the vector and feasibility of assays in the presence of vector virus. The "Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals (1993)" (58 FR 42974), should be considered as background information.

D. Lot-to-Lot Release Testing and Specifications for Vectors

General testing recommendations are discussed in the sections that follow. Not all tests listed will be applicable to every vector class. Sponsors should choose appropriate testing protocols, and consult CBER if there are questions as to the applicability of a specific test. Note that if drug substance (defined as bulk product not necessarily in final formulation) and drug product (defined as product in its final formulation) are the same, then only a single set of tests is necessary.

Any standard assays for the properties listed below can be used if they are quantitative, and are of adequate specificity and sensitivity. Assay methods should be validated by testing of known amounts of reference lots, spiked samples, or other appropriate measures, and data documenting assay performance submitted to the IND.

1. Tests of drug substance (bulk product not necessarily in final formulation):

    a. Purity (21 CFR 610.13)

        i. Test for total DNA or RNA content if appropriate to vector composition, e.g. A_{260}/A_{280}.
        ii. Test for homogeneity of size and structure, supercoiled vs. linear, e.g. agarose gel electrophoresis.

iii. Test for contamination with RNA or with host DNA, e.g. gel electrophoresis, including test with bacterial host-specific probe.
iv. Test for proteins if present as a contaminant, e.g. silver stained gel.
v. Test for non-infectious virus in cases in which that would be a contaminant, such as empty capsids. See: Section VI C.
vi. Tests for toxic materials involved in production.

b. Identity (21 CFR 610.14)

Test for vector identity by methods such as restriction enzyme mapping with multiple enzymes or PCR should be performed on the drug substance (see 21 CFR 610.14). In the case of a facility making multiple constructs, it should be verified that the identity testing is capable of distinguishing the constructs and detecting cross-contamination.

c. Adventitious agents

As methods of testing for adventitious agents become increasingly sensitive and specific over time, sponsors are encouraged to accumulate data validating testing methods other than those indicated, to permit future updating of this policy. In cases in which a vector product interferes with appropriate assays, for example, a lytic viral vector that kills indicator cells in an assay for adventitious virus, some information may be obtained by parallel mock cultures using the same media and other reagents to allow outgrowth of a contaminant, or by assays in the presence of neutralizing antibody. The following tests should be performed:

i. Sterility test (21 CFR 610.12), for aerobic and anaerobic bacteria and fungi.
ii. Mycoplasma testing, as specified in the "Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals (1993)", Attachment #2, (58 FR 42974), which specifies the procedures for detecting mycoplasma contamination.
iii. Testing for adventitious viruses, in some cases, source materials or cell lines used in vector production introduce the risk of contamination with adventitious viruses. In other cases, adventitious virus can be introduced during product manufacture. Testing for an appropriate range of possible contaminating viruses is recommended, as discussed extensively in the "Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals (1993)" 58 FR 42974,

and the ICH draft guideline Q5A, "Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin," Step 4, approved by ICH, 3/5/97. Testing for replication-competent retrovirus and adenovirus is discussed below.

d. Potency (21 CFR 610.10)

Potency assays should be validated during the product development process. Expression of the inserted gene can be determined by transfection of appropriate cells and demonstration of active gene product by an appropriate assay, characterized as to its sensitivity and specificity. Whenever possible, a potency assay should measure the biological activity of the expressed gene product, not merely its presence. For example, if enzymatic activity is the basis of the proposed therapy, an enzyme activity assay detecting conversion of substrate to product would be preferred over an immunological assay detecting epitopes on the enzyme. If no quantitative potency assay is available, then a qualitative potency test should be performed.

2. Tests of drug product (product in its final formulation): The vector product in final container form should be tested for the properties listed below by quantitative, validated assays. Tests for endotoxin and general safety if performed on a drug product (final product) need not be performed on drug substance (bulk product).

a. Sterility (21 CFR 610.12), identity (21 CFR 610.14), and potency (21 CFR 610.10).

b. Purity (21 CFR 610.13) or validation of endotoxin testing by LAL or other acceptable assay (see "Guideline on validation of the limulus amebocyte lysate test as an end-product endotoxin test for human and animal parenteral drugs, biological products, and medical devices, December, 1987).

c. General safety, as per 21 CFR 610.11. Note that this test is not needed for therapeutic DNA plasmid products because they are among the specified biotechnology products (Federal Register notice, Vol. 61, No. 94, May 14, 1996), even if liposomes are added.

VI. ISSUES RELATED TO PARTICULAR CLASSES OF VECTORS FOR GENE THERAPY

A. Additional Considerations for the Use of Plasmid Vector Products

Many product and quality control considerations covered in the general sections above are appropriate to plasmid DNA products. The "Points to Consider on Plasmid DNA Vaccines for Preventive Infectious Disease Indications (1996)", 61 FR 68269, may also be a useful reference. In general, complete sequencing of the plasmid should be performed. Plasmids should be characterized and specifications set with regard to the presence of RNA, protein, and bacterial host DNA contaminants, quantities of linear and supercoiled DNA in the preparation, and presence of toxic chemicals. Toxic chemicals such as ethidium bromide should be avoided during production.

As stated in the "Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals," it is recommended that penicillin and other beta-lactam antibiotics be avoided during production, due to the risk of serious hypersensitivity reactions in patients. If antibiotic selection is used during production, it is preferable not to use selection markers which confer resistance to antibiotics in significant clinical use, in order to avoid unnecessary risk of spread of antibiotic resistance traits to environmental microbes. Also, residual antibiotic in the final product should be quantitated when possible, and the potential for allergy considered. See 21 CFR 610.61(m) concerning labeling requirements for approved biological products if antibiotics are used during manufacture. Concerning environmental impact and the use of drug resistance traits, consult the NIH Guidelines for Research Involving Recombinant DNA Molecules, Section III-A-1-a (59 FR 34496, amended 61 FR 59732). Non-antibiotic selection systems can also be used.

Plasmid vectors may be administered in conjunction with lipid preparations, local anesthetics, or other chemicals intended to facilitate DNA uptake. If such a facilitating agent is added during formulation, a specification for its amount and identity in the final product should be established. If toxic organic solvents such as chloroform are used in producing a lipid component, then processing should remove them and lot release specifications should include testing for residual solvent.

B. Additional Considerations for the Use of Retroviral Vector Products

1. Testing for replication competent retrovirus: The following testing scheme for detection of replication competent retroviruses (RCR) summarizes current recommendations based on information available at this time. This scheme is currently being reevaluated, and modified guidance will be made public when available. Testing at multiple stages in production is recommended due to the limited knowledge of the risks of retrovirus exposure and the possibility of

generation of recombinant RCR at any point in the production process. Alternative assays (e.g. marker rescue) are acceptable if sensitivity is comparable to the PG4 S^+L^- assay. Testing should be complete prior to patient administration, particularly if cells can be cryopreserved; otherwise testing should be performed concurrently. In order to gain biological information about events during production, molecular characterization of any RCR detected in clinical lots is also recommended.

a. Master Cell Bank of vector-producing cells (one time testing):

   i. Supernatant testing, 5% of the total supernatant from culture of cells for a master cell bank should be tested by amplification on a permissive cell line (e.g., *Mus dunni*) including several blind passages followed by the PG4 S^+L^- or alternative assay.
   ii. Producer cell testing, 1% of pooled producer cells or 10^8 cells, whichever is fewer, should be cocultured with a permissive cell line (e.g., *Mus dunni*) including several blind passages. Supernatant from the coculture should be tested by PG4 S^+L^- or alternative assay.

b. Working cell bank (one time testing): Either supernatant testing <u>or</u> cocultivation of cells is recommended, using conditions described for master cell bank testing.

c. Lot testing of vector products:

   i. Clinical grade supernatant, 5% of the supernatant should be tested by amplification on a permissive cell line (e.g., *Mus dunni*) including several blind passages, followed by the PG4 S^+L^- or alternative assay.
   ii. Testing of end of production cells, 1% of total pooled end of production cells or 10^8 cells, whichever is fewer, should be cocultured with a permissive cell line (e.g., *Mus dunni*), and then amplified by several blind passages. Supernatant from the coculture should be tested by S^+L^- or alternative assay.

d. Lot testing of *ex vivo* transduced cells:

   i. 1% of pooled transduced cells or 10^8 cells, whichever is fewer, should be cocultured with a permissive cell line (e.g., *Mus dunni*) including several blind passages. Supernatant from the coculture should be tested by PG4 S^+L^- or alternative assay.

ii. 5% of supernatant from the transduced cells should be tested by amplification on a permissive cell line (e.g., *Mus dunni*) including several blind passages. Supernatant from the coculture should be tested by PG4 S^+L^- assay.

2. Patient monitoring: Patients given retrovirus-related products should be monitored for RCR exposure. Please consult CBER for guidance.

C. Additional Considerations for the Use of Adenoviral Vectors.

1. Measurement of particles vs. infectious units: Patient doses of adenovirus-based gene therapy vectors are presently based upon some method of enumerating viral particles, plaque forming units (PFU), or infectious units (IU) measured in cell lines complementing the replication defect (not to be confused with measurement of RCA; see Section 2 below). Given the potential toxicity of the adenoviral particles themselves, CBER recommends that patient dosing be based on particle number. This recommendation also reflects that particle number can be readily and reproducibly measured. However, since it is quite possible that some outcomes are a function of the number of infectious units administered, it is important for investigators or sponsors to develop *in vitro* infectivity assays which are reproducible and informative, and to set appropriately tight specifications on the ratio of infectious particles to total viral particles.

Adenovirus particle measurement is commonly based on genomic DNA quantitation. Using the absorbance at 260 nm in the presence of sodium dodecyl sulfate or other virus lysing agents, the maximum number of adenoviral particles can be calculated from OD_{260}. The presence of non-adenovirus nucleic acid may yield inaccurate particle numbers and should be minimized during the manufacturing and viral purification process. Electron microscope particle count has also been used for viral particle enumeration.

Presently the titer of an adenovirus vector preparation usually refers to the infectious titer. The cell used for determining the titer is often the producer cell line. Differences in viral vectors may lead to changes in growth properties and kinetics. The plaque method is efficient for adenoviral vectors with an easily complemented replication defect. Vectors with multiple replication defects may be more readily titered by alternative methods such as use of fluorescent antibodies. Both assays require optimization for time of adsorption, need for deaggregation of virus, stability, etc. Inclusion of a standard wild type virus control may facilitate the comparison of titers between different vectors and laboratories.

It is currently recommended that a ratio in the product of viral particles to biologically active virus of less than 100:1 be employed in phase I studies. The purpose of this specification is to ensure the consistent manufacture of recombinant viruses and the highest bioactivity/particle/ patient dose and to limit possible toxicity due to viral structural proteins. As new assays are developed and validated, their comparison to old ones and their use in product characterization is encouraged.

2. Detection of replication-competent adenovirus: The presence of RCA in clinical lots of adenovirus vector raises a variety of safety concerns, including the possibility of adenovirus infection, unintended vector replication due to the presence of wild-type helper function, and exacerbation of host inflammatory responses. The safety risks entailed by these events and other potential adverse events will differ depending on the indication and the patient population. Preclinical safety studies are inherently limited in assessment of RCA-related risks since there are no animal models that support extensive replication of human wild-type or replication competent recombinant adenovirus.

Therefore, adenovirus vectors intended to be replication-defective should be examined for the presence of replication-competent adenovirus (RCA). RCA may arise at multiple steps during the manufacturing process, through recombination with host sequences or by contamination. The amount of RCA generated during manufacture will be influenced by the overall design of the vector. Use of replication-selective adenovirus vectors raises additional considerations and may call for additional or different testing strategies. Such cases should be discussed with CBER.

Detection of RCA in final vector product by a cell culture/cytopathic effect method is preferred at this time. Validation of assay sensitivity by spiking decreasing numbers of wild type adenovirus particles into the test inoculum is recommended. Input multiplicity of infection (MOI) should be carefully chosen because of toxicity of higher doses of virus inoculum unrelated to the presence of RCA. It should also be noted that too high an input MOI may lead to suppression of RCA outgrowth by the vector. One or two blind passages on cells permissive for growth of the RCA, for example A549 cells, may be performed to amplify RCA before reading out on an indicator cell line. In addition, it is recommended that the assay be quantitative, that is, able to determine the number of RCA present in any patient dose.

Previous recommendations from the FDA have been that patient doses should contain no more than 1 pfu of RCA or equivalent in patients in whom adenovirus infection would be considered a potential risk. However, the agency recognizes that current production techniques in combination with proposed dosing schemes

may make this recommendation prohibitively burdensome. Therefore, if sponsors wish to propose a different specification, data should be provided demonstrating that the level of RCA present represents an acceptable risk for the intended patient population, route of administration, and dose. In order to gain biological information about events during production, molecular characterization of any RCA present in clinical lots is also recommended at this time, and should be as thorough as is practical until more is known about the types of recombination that are occurring.

3. Adeno-associated virus: Because of the association of AAV with adenovirus, testing for AAV is currently recommended in the Master Cell Bank, the Master Virus Seed Stock, and the final product.

D. Other Gene Delivery Systems

Other gene delivery systems including additional types of viral or nucleic acid vectors are currently under development. Sponsors using new systems are encouraged to contact CBER early in product development, to facilitate a safe and efficient development process.

VII. MODIFICATIONS IN VECTOR PREPARATIONS

In the past, CBER has considered any change in a vector to result in a new product, and has requested submission of a new IND. There is now an accumulating body of scientific evidence that will permit flexibility in this policy. CBER's goal is to facilitate progress towards effective therapies by abbreviating testing and reducing documentation, whenever this can be done while preserving patient safety.

Two aspects of IND submission are affected by consideration of a product as a modified vector: the decision as to whether a new IND should be submitted, and the decision as to what data should be submitted for review. Certain changes, for example minor modifications in the genetic insert or changes in the antibiotic resistance gene, do not necessarily call for a new IND or for full product retesting. In all cases, derivation of a new vector should be described and the vector should meet the specifications for release testing. The other data which should be collected will vary with the degree and nature of the modifications to the vector. The need for additional preclinical testing is determined by the likelihood of altered vector biology, not just the number of nucleotide changes. In some cases, tissue localization, germ line alteration, and animal pharmacology/ toxicology studies may be optional. Instead, the relevant safety studies could focus on specific safety concerns related to changes in the vector.

When a number of related vectors involving minor modifications are studied, they may be considered members of a panel, analogous to panels of monoclonal antibodies described in the

"Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use," 1997 revision, 62 FR 9196. As stated, such panels could be studied under a single IND and submitted for approval in a single license application. Phase 3 clinical trials should include some experience with all panel members, and efficacy established for the overall panel.

Vector modifications should be discussed with CBER case by case. If a sponsor wishes to abbreviate testing and IND submission for a product or product series, the sponsor should verify with CBER the adequacy of the proposed abbreviated testing scheme prior to initiating clinical trials. Data forthcoming from sponsors can help establish whether particular changes in vectors alter their behavior when they are compared *in vivo*, and therefore whether complete testing should continue to be performed.

VIII. PRECLINICAL EVALUATION OF CELLULAR AND GENE THERAPIES

A. General Principles

Preclinical studies are intended to define the pharmacologic and toxicologic effects predictive of the human response, not only prior to initiation of clinical trials, but also throughout drug development. The goals of these studies include the following: to define safe starting doses and escalation schemes for clinical trials, to identify target organs for toxicity and parameters to monitor in patients receiving these therapies, and to determine populations which may be at greater risk for toxicities of a given cellular or gene therapeutic.

Design of preclinical studies should take into consideration: 1) the population of cells to be administered or the class of vector used, 2) the animal species and physiologic state most relevant for the clinical indication and product class, and 3) the intended doses, route of administration, and treatment regimens. Parameters which should be studied will be discussed below.

Due to the unique and diverse nature of the products employed in cellular and gene therapies, conventional pharmacology and toxicity testing may not always be appropriate to determine the safety and biologic activity of these agents. Issues such as species specificity of the transduced gene, permissiveness for infection by viral vectors, and comparative physiology should be considered in the design of these studies. Available animal models mimicking the disease indication may be useful in obtaining both sufficient safety and efficacy data prior to entry of these agents into clinical trials.

The ICH Draft Guideline S6, "Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals," (Step 4, approved by ICH, 7/16/97) discusses in Section 3.1 the flexible application of Good Laboratory Practices in testing of biotechnology products. Although pivotal safety studies in support of marketing (e.g. carcinogenicity, reproductive

toxicology) are expected to be conducted in compliance with the regulations as outlined in 21 CFR part 58, it is recognized that studies in support of entry into clinical trials may not always strictly adhere to GLP. In these cases, the principles of the regulation should be followed as closely as possible, and where deviations occur, they should be evaluated for impact on the expected clinical application, and discussed in the report submitted to the agency.

If a product is comparable to agents for which there is wide previous clinical experience, or for which the insertion of a different expression cassette is not expected to influence the toxicity or the dissemination of the vector, less extensive preclinical testing may suffice (see Section VII, above).

It is recommended that plans for preclinical studies be discussed with representatives from CBER prior to their initiation. Clinical plans requiring rapid enrollment of patients should be anticipated and preceded by adequate preclinical testing.

B. Animal Species Selection and Use of Alternative Animal Models

It is recognized that animal models of disease may not be available for every cellular or gene therapy system. Preclinical pharmacologic and safety testing of these agents should employ the most appropriate, pharmacologically relevant animal model available. A relevant animal species would be one in which the biological response to the therapy would be expected to mimic the human response. For example, a vector expressing a human cytokine would best be tested in an animal species in which that cytokine binds to the corresponding cytokine receptor with affinity comparable to that seen with human receptors, and initiates a pharmacologic response comparable to that expected in humans.

C. Somatic Cell and Gene-Modified Cellular Therapies

1. *In vivo* biological/pharmacological activity: The transduction procedure, dose of expanded or genetically modified cells, and route of administration planned for the clinical trial should be evaluated preclinically. Pharmacologic studies in animals may provide useful information regarding the *in vivo* function, survival time and appropriate trafficking of the modified cells.

2. Toxicologic testing: Safety testing of expanded, activated, or genetically modified somatic cells should be conducted in an appropriate animal model. Data on distribution, trafficking, and persistence of these cells *in vivo* mentioned above should be evaluated for safety implications as well. At a minimum, treated animals should be monitored for general health status, serum biochemistry, and hematologic profiles. Target tissues should be examined microscopically for histopathological changes.

D. Direct Administration of Vectors *In Vivo*

A number of different vectors are currently in development for direct administration to human subjects. Direct administration of any of these vectors presents a number of safety concerns that will be addressed here. All toxicity and localization studies, including studies of gonadal tissue described below, should use the final formulated product, since added materials such as liposomes, or changes in pH or salt content, may alter the toxicity or distribution pattern.

Specific concerns for each vector subclass will generally be handled on a case-by-case basis, and discussion with CBER is encouraged.

1. Route of administration: The route of administration of vectors can influence toxicity *in vivo*. Safety evaluation in the preclinical studies should be conducted by the identical route and method of administration as in the clinical trial whenever possible. When this is difficult to achieve in a small animal species, a method of administration similar to that planned for use in the clinic is advised. For example, intrapulmonary instillation of adenoviral vectors by intranasal administration in cotton rats or mice is an acceptable alternative to direct intrapulmonary administration through a bronchoscope.

2. Selection of animal species: The species of animal chosen for preclinical toxicity evaluations should be selected for its sensitivity to infection and pathologic sequelae induced by the wild-type virus related to a vector, as well as its utility as a model of biologic activity of the vector construct. Rodent models, rather than non-human primates may be useful if they are susceptible to pathology induced by the virus class. When evaluating the activity of a vector in an animal model of the clinical indication, safety data can be gathered from the same model to assess the contribution of disease-related changes in physiology or underlying pathology to the response to the vector.

3. Selection of dose to be employed: The doses of vectors studied preclinically should be selected based on preliminary activity data from studies both *in vitro* and *in vivo*. A no-effect level dose, an overtly toxic dose, and several intermediate doses should be determined, and appropriate controls, such as naive or vehicle-treated animals, should be included. For products in limited supply or with inherently low toxicity, a maximum feasible dose may be administered as the highest level tested in the preclinical studies. Preclinical safety evaluations should include at least one dose equivalent to and at least one dose escalation level exceeding those proposed for the clinical trial; the multiples of the human dose required to determine adequate safety margins may vary with each class of vector employed and the relevance of the animal model to humans. Scaling of doses based on either body weight or total body surface area as appropriate facilitates

comparisons across species. Information generated can be used to determine the margin of safety of the vector for use in the clinical trial, as well as to gauge an acceptable dose-escalation scheme.

4. Toxicologic testing: Treated animals should be monitored for general health status, serum biochemistry, and hematology, and tissues should be examined for pathological changes in histology.

5. Distribution of vector out of the site of administration: Localization studies, designed to determine the distribution of the vector after administration to its proposed site, are also recommended. Whenever possible, the intended route of administration should be employed. Additional groups of animals may be treated intravenously, as a "worst-case" scenario representing the effects of widespread vector dissemination. Transfer of the gene to normal, surrounding, and distal tissues as well as the target site should be evaluated using the most sensitive detection methods possible, and should include evaluation of gene persistence. Dose levels selected should follow those used in toxicity testing. When aberrant or unexpected localization is observed, studies should be conducted to determine whether the gene is expressed and whether its presence is associated with pathologic effects.

a. Expression of gene product and induction of immune responses

Expression of the therapeutic gene product in intended or unintended tissues may result in unexpected toxicities, which should therefore be addressed in preclinical studies. Inflammatory, immune, or autoimmune responses induced by the gene product may be of concern. Animal studies should be conducted over a sufficient duration of time to allow development of such responses. Host immune responses against viral or transgene proteins may limit their usefulness for repeated administration in the clinic.

b. Vector localization to reproductive organs

With vectors for direct administration, the risk of vector transfer to germ cells should be considered. Animal testicular or ovarian samples should be analyzed for vector sequences by the most sensitive method possible. If a signal is detected in the gonads, further studies should be conducted to determine if the sequences are present in germ cells as opposed to stromal tissues, using techniques that may include but are not limited to cell separations or *in situ* PCR, or other techniques. Semen samples for analysis can be collected from mature animals including mice (G.D. Snell, et. al., *Anat. Rec.*, 90:243-253, 1944; J.C. Kile, Jr., *Anat. Rec.*, 109:109-117, 1951), for determination of vector incorporation into germ cells.

6. Host immune status and effects on gene therapy vectors: Immune status of the intended recipients of a gene therapy should be considered in the risk-benefit analysis of a product, particularly for viral vectors. If exclusion of immunocompromised patients would unduly restrict a clinical protocol, immune suppressed, genetically immunodeficient, or newborn animals may be used in preclinical studies, to evaluate any potential safety risks.

IX. CONCLUSION

As new classes of gene therapies and somatic cell therapies are developed, concerns and methods of testing will most likely change. The above guidance outlines the types of issues that should be examined and provides a framework for analysis of new technologies as they emerge. CBER encourages comments and suggestions from the academic and commercial communities and other interested parties during the evolution of policy in this area.