



U.S. Pharmacopeia
The Standard of QualitySM

July 17, 2002

Dockets Management Branch (HFA-305)
Food & Drug Administration
5630 Fishers Lane, Rm. 1061
Rockville, MD 20852

Docket No. 02N-0169

Dear Sir/Madam:

We are submitting two articles to help the FDA address Combination Products Containing Live Cellular Components, Docket No. 02N-0169:

- The general information chapter from *USP 25* "<1046> Cell and Gene Therapy Products"
- "A new United States Pharmacopeia (USP) Chapter 1046: Cell and Gene Therapy Products" (*Cytotherapy* (2000) Vol. 2, No. 1, 45-49)

These articles were referred to in Dr. Seaver's oral presentation at the June 24 hearing. <1046> contains information on these types of products and appropriate requirements for approval. It addresses manufacturing and quality control issues for combination products containing live cells. The *Cytotherapy* article discusses the first draft of this chapter, lists the individuals who were involved with writing this chapter, and has an outline of the chapter which should expedite FDA reviewers search for the sections in <1046> relevant to combination products containing live cells for use in wound healing.

We are also resubmitting Dr. Seaver's oral presentation, which includes the written transcript and a copy of the slides. If you have any questions or would like any further information, please contact Dr. Ian DeVeau at the USP.

Yours truly,

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A new United States Pharmacopeia (USP) Chapter 1046: Cell and Gene Therapy Products

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Abstract

The United States Pharmacopeia (USP) has published the first draft of a new information chapter on cell and gene therapy products in January, 2000. The chapter discusses the manufacturing and testing cell and gene therapy products. It is intended for people working in the field as well as for pharmacists and clinicians who would like more information on this subject. This article outlines the background of

USP and explains how it became involved with cell and gene therapy and what an information chapter is. It details the subjects covered by the chapter and the philosophy behind the chapter. This draft will be revised based on comments received from the public and then published as a revision subject to further comments before it becomes an official chapter in a supplement to USP 24-NF 19.

Introduction

At the meeting of the United States Pharmacopeia (USP) Convention in March, 1995 a resolution, 'Standards and Information for Gene and Cell Therapies', was adopted. It states:

The USP is encouraged to determine, in cooperation with the Food and Drug Administration and in consultation with appropriate international organizations, the feasibility and advisability of developing an information chapter to encompass methodologies describing gene and cell therapies and cell based tissue engineering, standards for reagents and materials used, and information regarding the appropriate use of these technologies.

As an introduction to the USP's efforts in this field this article will describe:

- the USP and its committees
- the different types of information in the USP-NF
- why the USP decided to write an information chapter on cell and gene therapy products
- the contents and philosophy of the chapter
- how the chapter will become official part of USP 24-NF 19.

What is the USP?

The USP is a not-for-profit organization established in 1820. Its mission is to 'promote the public health by establishing and disseminating officially recognized standards of quality and authoritative information for the use of medicines and other health

care technologies by health care professionals, patients and consumers'. The Federal Food, Drug and Cosmetic Act recognizes the USP standards.

The USP holds a meeting of its members every five years. Members include representatives from schools, colleges and state associations of medicine and pharmacy, professional and scientific organizations and designated divisions of the federal government and certain foreign governments and pharmacopeias. At these meetings, USP members elect the members of the USP Committee of Revision (COR) and adopt resolutions to guide the work of COR. COR is responsible for the contents of the USP-NF (United States Pharmacopeia and National Formulary). Members of COR are volunteers and are assigned to specific subcommittees based on each person's scientific and medical expertise, not their affiliation. The USP has a permanent staff of 300 people to assist and implement the work of these volunteers. During the 1995–2000 cycle there have been 20 subcommittees. One of these, the Biotechnology & Gene Therapy Subcommittee, addressed the resolution on cell and gene therapies.

For more information on the USP visit its web site at www.usp.org.

What information does the USP-NF contain?

The USP-NF contains official compendia which are recognized in Food, Drug and Cosmetic Act. Many people refer

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General Chapters

General Information

Add the following:

■ (1046) CELL AND GENE THERAPY PRODUCTS

INTRODUCTION

General Definitions

Recent advances in biotechnology have resulted in the development of two new categories of products—cell therapy products and gene therapy products. Cell therapy products contain living mammalian cells as one of their active ingredients, while gene therapy products contain pieces of nucleic acid, usually deoxyribonucleic acid (DNA), as one of their active ingredients. Some products combine both categories, resulting in a therapy that uses cells that express a new gene product. Both cell and gene therapy products may be combined with synthetic or natural biomaterials to form tissue constructs.

For the purpose of this chapter, cell and gene therapy products include any product that has live cells or pieces of nucleic acid, however formulated. The following are excluded at this time: (1) tissue-based products in which the cells are removed or killed, (2) enhanced reproductive methods such as in vitro fertilization, (3) non-mammalian cell-based products, (4) traditional vaccines such as live attenuated virus, and (5) noncell, nongene products manufactured by using cells or recombinant-DNA (rDNA) technology, which are discussed under *Biotechnology-Derived Articles* (1045).¹

Cell Therapy Products

Cell therapy products are products with live cells that replace, augment, or modify the function of a patient's cells that are diseased, dysfunctional, or missing. Transplantation of bone marrow to replace marrow that has been destroyed by chemotherapy and radiation is an example of a cell-therapy product. These products are also referred to as somatic cell therapy products because nongerm-line cells are used in the product. In addition, cells may be combined with biomaterials. For example, dermal or epidermal cells can be grown on a collagen substrate to produce a sheet of cells for wound and burn therapy. Examples of cell therapy products are shown in *Table 1*.

Table 1. Examples of Cell Therapy Products

Indication	Product
Bone marrow transplantation	Devices and reagents to propagate stem and progenitor cells, to select stem and progenitor cells, or to remove diseased (cancerous) cells

¹ The term *tissue engineering* is not used in this chapter. Definitions and information on tissue engineering are being developed by the ASTM

Indication	Product
Cancer	<i>T</i> cells, dendritic cells, or macrophages exposed to cancer-specific peptides to elicit an immune response Autologous or allogeneic cancer cells injected with a cytokine and irradiated to elicit an immune response
Pain	Cells secreting endorphins or catecholamines (encapsulated in a hollow fiber)
Diabetes	Encapsulated β -islet cells secreting insulin in response to glucose levels
Wound healing	Sheet of autologous keratinocytes or allogeneic dermal fibroblasts on a biocompatible matrix Sheet of allogeneic keratinocytes layered on a sheet of dermal fibroblasts
Tissue repair	
Focal defects in knee cartilage	Autologous chondrocytes
Cartilage-derived structures	Autologous or allogeneic chondrocytes in a biocompatible matrix
Bone repair	Mesenchymal stem cells in a biocompatible matrix
Neurodegenerative diseases	Allogeneic or xenogeneic neuronal cells
Liver assist (temporary; for bridging until liver transplant or recovery)	Allogeneic or xenogeneic hepatocytes in an extracorporeal hollow fiber system
Infectious disease	Activated <i>T</i> cells

There are three sources of donor cells for cell therapy products: (1) the patient's own cells (autologous cell products), (2) the cells from another human being (allogeneic cell products), and (3) the cells derived from animals such as pigs, primates, or cows (xenogeneic cell products). Autologous cells are not rejected by the patient, but they are not available for many treatments because they are missing, dysfunctional, or diseased. In those situations allogeneic and xenogeneic cells can be used. The advantage of allogeneic cells is that they do not trigger a rejection reaction as strong as that caused by xenogeneic cells. Xenogeneic cells are used when human cells with the desired characteristics are not available or the supply of human donors is too limited. Cell therapy products are sometimes encapsulated in a device that prevents the patient's cells and antibodies from killing the xenogeneic cells. However, the use of xenogeneic cells has the potential to cause zoonoses in humans. Much research is focused on identifying and propagating stem cells, regardless of the source, because stem cells can be manipulated to differentiate either during manufacturing or after administration. Cell lines are preferable to freshly isolated cells because they can be tested extensively for viruses, tumorigenicity, and other features. They also ensure a constant and reproducible product by minimizing donor-to-donor variability.

Cell therapy products can be modified by treatment with DNA or another nucleic acid so that the pattern of gene expression is changed. This new product, a combination of gene therapy and cell therapy, is referred to as an *ex vivo* gene therapy product. Typically, cells are taken from the patient and modified outside of the body before they are returned to the patient.

Cell therapy products face several unique manufacturing challenges that are addressed in other sections of this chapter. First, cells cannot be terminally sterilized or filtered so removal or inactivation of microorganisms or viruses without killing the cells is problematic.

Second, every raw material used in manufacturing has the potential of remaining associated with the cells. Qualification and sourcing of all raw materials is critical to producing a safe and effective product. Third, storage of the cell therapy products may present a challenge. Freezing is the main mode of long-term storage, and some cell therapy products cannot be frozen without changing their basic characteristics, especially those for differentiated functions. These types of products may have to be administered to patients within hours, or days at the most, after completion of the manufacturing process. Fourth, there is often an urgent clinical need to administer a product as soon as possible. Fifth, some products consist of a batch size equivalent to one dose, very often in a small volume. For these last three challenges, traditional analytical methods, especially those for sterility, mycoplasma, and potency, are not always applicable because these methods are not rapid or they are not amenable to small volumes. Even when these traditional methods are performed, the results are not available in time for products requiring rapid release. These products are often released on the basis of the results of new, very rapid or small-volume methods. Currently there are no compendial standards for such methods, although, as stated in the *General Notices* and 21 CFR 610.9, alternative methods to compendial tests are permissible, provided they are shown to be equivalent. As such new methodologies become properly validated, they will be included in the compendia.

Gene Therapy Products

Gene therapy products are products in which nucleic acids are used to modify the genetic material of cells. A retroviral vector used to deliver the gene for factor IX to cells of patients with hemophilia B is an example of a gene therapy product. Gene therapy products can be broadly classified on the basis of their delivery system. Means for delivering gene therapy products include viral vectors (viruses with the genes of interest but usually without the mechanism to self-replicate in vivo), nucleic acids in a simple formulation (naked DNA), or nucleic acids formulated with agents, such as liposomes, that enhance their ability to penetrate the cell. Some types of gene therapy block the expression of a gene by the administration of antisense oligonucleotides, which are complementary to a naturally occurring ribonucleic acid (RNA) and block its expression. Most of the initial clinical work has been done using viral vectors. The choice of a gene vector is complex (see *Design Considerations for Gene Vectors under Manufacturing of Gene Therapy Products*). The most common viruses used to date include murine retroviruses, human adenovirus, and human adeno-associated viruses (AAVs). Antisense-oligonucleotide products are in clinical development and on the market. Examples of gene therapy products are shown in Table 2.

Table 2. Examples of Gene Therapy Products

Categories or Strategies	Indication: Administered Product
Gene replacement	
Short term	Cardiovascular disease: growth factor vector on a biocompatible scaffold ¹
Long term	Cystic fibrosis: transmembrane conductance regulatory vector Hemophilia: factor VIII or IX vector
Immunotherapy	Cancer or arthritis: autologous tumor cells or lymphocytes, respectively, transduced with cytokine genes
Conditionally lethal genes ²	Cancer (solid tumor): thymidine kinase (TK) or cytosine deaminase (CD) vector into tumor cells Graft versus host disease (GVHD): TK or CD vector transduced into donor T cells
Antisense	Cancer: anti-oncogene vector
Ribozyme	Cytomegalovirus retinitis: antiviral vector Human immunodeficiency virus (HIV): antiviral ribozyme vector into autologous lymphocytes

Categories or Strategies	Indication: Administered Product
Intrabodies	Cancer or HIV: single-chain antibody to a tumor protein or a viral protein, respectively

¹ This product promotes formation of new blood vessels

² Cells with conditionally lethal genes as well as their neighboring cells are killed after the administration of a second drug in vivo. For TK, the drug is gancyclovir. For CD, the drug is 5-fluorocytosine.

Although manufacturing of vectors or nucleic acids can be analogous to that used for rDNA products or vaccines, there are some unique challenges. Analytical methodologies for vectors (see *Analytical Methodologies*) are still being developed. Methods for quantitating viral vector particles and determining the number of particles that are active (potent) are important areas that are rapidly evolving. Traditional assays for viral dose, such as the plaque assay or the tissue culture infectious dose assay, detect a fraction of the active vector particles. The precision of such assays is about a factor of three (half a log). Further, manufacture of large batches of viral vectors with no, or minimal amounts, of replication-competent viruses (RCVs) is challenging. Detecting a small number of RCV particles in the presence of large amounts of replication-defective vector is difficult. As in cell therapy products, sourcing of raw materials is critical. Removal of adventitious agents or other process contaminants from viral vectors can be impossible. Even defining purity is an issue for enveloped viral vectors, such as retroviruses or herpes viruses, as they incorporate cellular proteins in their envelope when they bud from the cell. This makes it difficult to determine if contaminating extracellular proteins have been adequately removed.

For gene therapy vectors administered directly to patients, there are safety concerns related to the fate of their nucleic acids. For example, alteration of germ-line DNA is undesirable. Integration of gene therapy products into somatic cell DNA carries a theoretical risk of insertional mutation, which could result in modified gene expression and deregulation of the cell. For viral gene therapies, patients may need to be monitored for the presence of RCV. To address the risks associated with specific products, preclinical studies, quality control (QC) testing, and patient monitoring strategies need to be developed in accordance with the applicable regulations and guidance documents. The methodologies used to support these activities, including polymerase chain reaction (PCR) methods, are amenable to compendial standardization.

Chapter Purpose and Organization

Clinical uses for cell and gene therapy products, their manufacturing processes, and analytical schemes for determining identity, dose, potency, purity, and safety are rapidly evolving and are as diverse as the products themselves. This informational chapter summarizes the issues and best current practices in the manufacturing, testing, and administration of cell and gene therapy products. Usually informational chapters focus on materials that are already commercially available. This chapter, however, not only discusses products for commercial applications, but it also addresses the production of clinical trial materials and the other unregulated uses, such as bone marrow transplantation. When different approaches can be used for clinical trial material as compared to those used for commercial product, it is so stated.

The traditional compendial perspective is to develop public standards that can be applied to a particular final product without expressly defining production details. Efforts have been made in this chapter to specify when traditional methodologies or standards can be adapted. Novel methodologies applicable to cell and gene therapies are also highlighted. As these new methodologies become properly validated, they will be included in subsequent publications.

This chapter is extensive because of the diverse nature of the products and the special considerations that they require. Manufacturing has been divided into three sections. The first section, *Manufacturing Overview*, discusses general aspects of manufacturing and process development. The other two manufacturing sections are *Manufacturing of Cell Therapy Products* and *Manufacturing of Gene Therapy Products*. The latter section includes a subsection on designing gene vectors. *On-Site Preparation and Administration* follows the

manufacturing sections because the handling of these products at the clinic often requires facilities and expertise not found in a typical hospital. Storage, shipping, and labeling issues are addressed under *Storage and Shipping* and under *Labeling, Regulations, Standards, and New Methodologies* summarizes existing guidelines and highlights the need for the development and validation of new methodologies to assess product quality. The final sections of this chapter, *Definition of Terms* and *Abbreviations*, list and define the terms and abbreviations referred to in this chapter and those commonly employed in this field.

MANUFACTURING OVERVIEW

Introduction

The manufacturing of cell and gene therapy products has been divided into three sections. This section, *Manufacturing Overview*, discusses five topics that apply to manufacturing of all cell and gene therapy products: (1) raw materials, (2) characterization of banked materials, (3) in-process controls, (4) specifications, and (5) validation considerations. *Manufacturing of Cell Therapy Products* addresses the manufacturing of cell therapy products including cell products into which genetic material has been introduced. *Manufacturing of Gene Therapy Products* addresses the manufacturing of gene therapy vectors, both viral and nonviral, discussing the design of gene vectors in detail.

All the general principles of current good manufacturing practice (CGMP) as outlined by the FDA in 21 CFR 210, 21 CFR 211, 21 CFR 600s (especially 21 CFR 610), 21 CFR 820, and discussed in USP informational chapters, such as *Biotechnology-Derived Articles* (1045), apply to the manufacturing of cell and gene therapy products. The manufacturing facility, equipment and process, raw materials, quality systems, and trained personnel are some of the key elements of CGMP. CGMPs apply throughout the clinical development to both the manufacturing process and facility. The extent of control increases as clinical development progresses, with full CGMP compliance expected by initiation of Phase III of the pivotal clinical trial(s). The facility should be carefully designed, built, and validated to accommodate the unique features of the product's manufacturing process. Equipment should be robust, provide consistent product, and allow periodic calibration. Critical equipment, such as incubators and freezers, needs to be fitted with alarm systems that can remotely signal failure. Quality control (QC) and quality assurance (QA) programs should exert control over the manufacturing facilities, the manufacturing process, the validation efforts, and all testing of the raw materials, in-process material, bulk product, and final formulated product. Training and certification programs are central to maintaining a technically competent manufacturing staff. A documentation program should be implemented to support all manufacturing, training, and quality operations. Changes to processes and procedures should follow a formal program based on well-established CGMP and ISO change control principles.

Raw Materials

TYPES OF RAW MATERIALS

A wide variety of raw materials may be used in manufacturing. They may include relatively simple materials or complex substances, such as cells, tissues, biological fluids, polymeric matrices, mechanical supports, hydrogels, culture media, buffers, growth factors, cytokines, cultivation and processing components, monoclonal antibodies, and cell separation devices. These materials may remain in the final therapeutic product as active substances or as excipients. They may also be used in the manufacturing process as ancillary products. Ancillary products are components or substances that exert an effect on a therapeutic substance (for example, a cytokine may activate a population of cells). However, the mode of action of the ancillary product is limited to the interaction with the therapeutic entity, and the ancillary product is not intended to be present in the final therapeutic product. "Feeder cells," which are used to provide nutrients or growth factors for the product cell, are an example of an ancillary

product. The quality of raw materials used in the production of a cell or gene therapy product can affect the safety, potency, and purity of the product. Therefore, qualification of raw materials is necessary to ensure the consistency and quality of all cell and gene therapy products.

QUALIFICATION

It is the responsibility of the manufacturer of the product to ensure that all raw materials used in manufacturing are appropriately qualified. Qualification is the process of acquiring and evaluating data to establish the source, identity, purity, biological safety, and overall suitability of a specific raw material so as to ensure the quality of all raw materials used in the manufacturing process. The broad natures of the cell and gene therapy products and of the materials used to produce these products make it difficult to recommend specific tests or protocols for a qualification program. Therefore, rational and scientifically sound programs must be developed for each raw material.

Activities involved with raw material qualification will change as products move through various stages from clinical trials to licensure and commercialization. A well-designed qualification program becomes more comprehensive as product development progresses. In the early stages of product development, safety concerns are a focus in a raw material qualification plan. In the later stages, raw material qualification activities should be completely developed and should comply with CGMP. Ultimately, each raw material employed in the manufacture of a cell or gene therapy product should be produced under conditions that are in compliance with CGMP. On rare occasions, complex or unique substances that have been shown to be essential for process control or production may not be available from suppliers that produce them in compliance with CGMP. In these situations, the cell or gene therapy product manufacturer will have to develop a scientifically sound strategy for qualifying the raw material.

A qualification program for raw materials used in cell and gene therapy manufacturing should address each of the following areas: (1) identification and selection, (2) suitability for use in manufacturing, (3) characterization, (4) fetal bovine serum, and (5) quality assurance.

Identification and Selection—In the early stages of product development, important decisions regarding the types of raw materials to be employed in the manufacture must be made. As manufacturing progresses and products mature, certain materials that are deemed necessary at this point may turn out to be impossible or prohibitively expensive to qualify. Attention must be paid to issues such as suitability, toxicity, availability, consistency, contamination, and traceability. Raw materials that could be difficult to qualify may have to be investigated and identified in the early stages of product development.

Every material employed in the manufacturing process should be accounted for. The source and intended use for each material should be established, and the necessary quantity or concentration of each material used should be determined. Primary sources, and when possible secondary sources, for each material should be identified. In all cases, suppliers should provide information regarding the traceability of each material, especially for human- and animal-derived raw materials. For instance, human serum albumin and processed allogeneic non-A,B human serum require donor infectious disease status information prior to use, and a material such as fetal bovine serum (FBS) requires herd qualification and country of origin certification before being used in a manufacturing process (see *Fetal Bovine Serum*).

Suitability—An assessment of the suitability of each raw material used in manufacturing is necessary in order to ascertain the risk that the raw material may pose to the safety, potency, and purity of the final therapeutic product. Knowing the source and the processes employed in the manufacture of each raw material will help determine the relative level of risk for each item. The quantity of the material and its point of introduction in the manufacturing process also affect the risk profile of a raw material. Materials that may have toxic effects or raise biological safety concerns receive special attention. Such materials should be subjected to extensive testing prior to use or should be monitored in the final product. Validation studies that demonstrate that such materials are effectively and consistently removed or rendered inactive in the course of manufacturing will also be necessary for eventual licensure of each product. The biocompatibility of natural or synthetic biomaterials used in cell therapy products may be assessed by subjecting the material to the testing protocols outlined in

the FDA's Blue Book Memorandum (May 1, 1995), which is a modification of the ISO document 10993-1:1997 entitled "Biological Evaluation of Medical Devices—Part 1: Evaluation and Testing". USP chapters *Biological Reactivity Tests, In Vitro* (87) and *Biological Reactivity Tests, In Vivo* (88) should also be consulted.

Characterization—Once the suitability of each raw material employed in manufacture has been assessed, specific QC characterization tests need to be developed or implemented for each material. The test panel for each raw material should assess a variety of quality attributes, including identity, purity, functionality, freedom from adventitious or microbial contaminants, and suitability for intended use. The level of testing for each component is a product of its risk assessment profile and the knowledge gained about each component during development. Test specifications should be developed for each raw material to ensure consistency and performance of the manufacturing process. Acceptance criteria should be established and justified on the basis of the data obtained from lots used in preclinical and early clinical studies, lots used for demonstration of manufacturing consistency, and relevant development data, such as those arising from analytical procedures and stability studies.

Many raw materials, such as cells isolated from blood, serum-derived fluids and extracts, or growth factors, are biological in nature; therefore, they are highly complex and difficult to fully characterize. Tests for sterility, pyrogenicity, mycoplasma, and adventitious or infectious viral agents, including RCV, should be performed on these materials. The specific adventitious viral agent test panel is dependent on the source of the component and how that component is manufactured or prepared. Because these materials exert their effects through complex biological activities—and biochemical testing may not be predictive of process performance—functional or performance testing may be necessary. Performance variability of such materials may have a detrimental impact on the eventual potency of the final therapeutic product. Examples of complex functionality testing for raw materials are growth-promotion testing of individual lots of FBS on the cell line used in manufacturing, performance testing of digestive enzyme preparations, and *in vitro* tissue culture cytotoxicity assays.

Fetal Bovine Serum—One commonly employed animal-derived material in manufacturing is FBS. FBS is often added to the cultivation medium to promote cellular proliferation of a wide variety of cell types, including cell cultures that are derived from primary tissue explants and biopsy specimens. Growth factors, hormones, and other nutritive components present in FBS, many of which are undefined or present in trace quantities, provide the necessary components required by many cells to survive and undergo cellular division *in vitro*. The production of high-titer gene therapy vectors from cell lines can also require rich culture medium that includes FBS at levels between 10% and 15%. Defined, serum-free media have been developed for a number of cell types. Although some cell lines may be gradually adapted to serum-free or low-serum culture conditions, this may not be possible for certain fastidious cells, thereby necessitating FBS use.

Although FBS use may be required, development and assessment of serum-free or reduced-serum culture media should be considered. A number of bovine-derived bacteria, mycoplasma, and viruses are known to be associated with FBS. These organisms could potentially enter the process stream and contaminate the final product. The potential risk of BSE, the bovine form of TSE (transmissible spongiform encephalopathy) transmission with this material has been the subject of international discussion. Although FBS has been categorized as a low-risk material, adequate testing and sourcing of FBS lots must be managed appropriately in a qualification program. Reduction or elimination of FBS in the manufacturing process can reduce the risks associated with contamination by adventitious agents.

FBS must be obtained from herds that are monitored for specific diseases relevant in agricultural settings (for example, tuberculosis or brucellosis) and that are from regions known to be free from BSE. Each lot must meet established guidelines for sterility and endotoxin content as well as freedom from specific bovine viruses. To increase the level of safety assurance, consideration should be given to employing methods, such as irradiation or nanofiltration, that remove or inactivate viral entities known to be associated with FBS.

It should be noted that defined media formulations typically include components, such as albumin and transferrin, that are purified from animal or human plasma. The purification, processing, and extensive testing of such components further minimize, but do not eliminate, the risk of viral or microbial contamination. Apart from the

risks associated with adventitious agents, residual FBS in the final product may trigger an immune response in patients. The level of residual FBS in the final product does not necessarily correlate with the starting amount of FBS, and it can depend upon the nature of the product and the purification process. Even if FBS is not included in the manufacture of the product, residual amounts of other components used in the manufacturing process, including recombinant proteins or other defined media components, may be potentially antigenic.

Quality Assurance—The components of this part of the qualification program are multifaceted and should reflect those found in a typical manufacturing environment for a pharmaceutical product produced in compliance with CGMP. These activities should include the following systems or programs: (1) incoming receipt, segregation, inspection, and release of materials prior to use in manufacturing, (2) vendor auditing and certification, (3) certificate of analysis verification testing, (4) formal procedures and policies for out-of-specification materials, (5) stability testing, and (6) archival sample storage.

Characterization of Cell and Virus Banks

Cell Banks—A cell bank is a collection of vials containing cells stored under defined conditions, with uniform composition, and obtained from pooled cells derived from a single cell clone. The cell bank system usually consists of a master cell bank (MCB) and a working cell bank (WCB), although more tiers are possible. The MCB is produced in accordance with CGMP and preferably obtained from a qualified repository source (source free from adventitious agents) whose history is known and documented. The WCB is produced or derived by expanding one or more vials of the MCB. The WCB, or MCB in early trials, becomes the source of cells for every batch produced for human use. Cell bank systems contribute greatly to consistency of production of clinical or licensed product batches, because the starting cell material is always the same. Mammalian and bacterial cell sources are used for establishing cell bank systems.

Virus Banks—The master virus bank (MVB) is similar to the MCB in that it is derived from a single production run and is uniform in composition. The working virus bank (WVB) is derived directly from the MVB. As with the cell banks, the focus of virus bank usage is to have a consistent source of virus, shown to be free of adventitious agents, to use in production of clinical or product batches. In keeping with CGMP guidelines, testing of the cell bank to be used for production of the virus banks, including quality assurance testing, should be completed prior to the use of this cell bank for production of virus banks.

Qualification—Cell and viral bank characterization is an important step toward obtaining a uniform final product with lot-to-lot consistency and freedom from adventitious agents. Testing to qualify the MCB or MVB is performed once and can be done on an aliquot of the banked material or on cell cultures derived from the cell bank. Specifications for qualification of the MCB or MVB should be established. It is important to document the MCB and MVB history, methods and reagents used to produce the bank, and storage conditions. All the raw materials required for production of the banks, namely, media, sera, trypsin, and the like, must also be tested for adventitious agents.

Qualifying Master Cell Bank—Testing to qualify the MCB includes the following: (1) testing to demonstrate freedom from adventitious agents and endogenous viruses and (2) identity testing. The testing for adventitious agents may include tests for nonhost microbes, mycoplasma, bacteriophage, and viruses. Freedom from adventitious viruses should be demonstrated using both *in vitro* and *in vivo* virus tests, and appropriate species-specific tests such as the mouse antibody production (MAP) test. Identity testing of the cell bank should establish the properties of the cells and the stability of these properties during manufacture. Cell banks should be characterized with respect to cellular isoenzyme expression and cellular phenotype and genotype, which could include expression of a gene insert or presence of a gene-transfer vector. Suitable techniques, including restriction endonuclease mapping or nucleic acid sequencing, should be used to analyze the cell bank for vector copy number and the physical state of the vector (vector integrity and integration). The cell bank should also be characterized for the quality and quantity of the gene product produced.

Qualifying Master Virus Bank—Testing of the MVB is similar to that of the MCB and should include testing for freedom from adventitious agents in general (such as, bacteria, fungi, mycoplasma, or viruses) and for organisms specific to the production cell line, including RCV. Identity testing of the MVB should establish the properties of the virus and the stability of these properties during manufacture.

Qualifying Working Cell or Virus Bank—Characterization of the WCB or WVB is generally less extensive, requiring the following: (1) testing for freedom from adventitious agents that may have been introduced from the culture medium, (2) testing for RCV, if relevant, (3) routine identity tests to check for cell line cross-contamination, and (4) demonstration that aliquots can consistently be used for final product production.

In-Process Control

Manufacturing processes should have well-defined *go-no go* decision point criteria that are applied to key in-process intermediates and are used to pool material that has been processed through a step in several sublots. Quality must be built into the product, rather than tested during batch release. In-process controls are the assays or tests that are performed to ensure that the in-process intermediate is of sufficient quality and quantity to ensure manufacture of a quality final product. Examples of in-process controls are listed in Table 3. The main reason for performing the in-process control is to ensure that the correct product with anticipated quality and yield is obtained. Intermediate in-process material that fails to satisfy the in-process control criteria should not be used for further manufacturing. This material may be reprocessed if there are procedures in place for such activities. The reprocessed material must satisfy the original in-process specifications before it can undergo further manufacturing. If several sublots are to be pooled for further processing, sublots that fail to satisfy the criteria should not be included in the pool, even if the pool containing these failed sublots would pass the in-process assay criteria.

Table 3. Examples of In-Process Control Applications

Type of Product	Attribute to Control
Cell therapy	Quantity and viability of cells after a key processing step
Viral gene therapy	Cell phenotype after a culture step
	Quantity and viability of cells during bioreactor culture
	Quantity of virus after virus culture
Nonviral gene therapy	Specific activity of virus in fractions after column chromatography
	Quantity of host-cell DNA in fractions after column chromatography
	Optical density or change in oxygen consumption during culture
	Amount and form of plasmid prior to culture harvesting
	Amount and form of plasmid after extraction steps
Antisense-oligonucleotide therapy	Amount of pyrogen or endotoxin after extraction steps in plasmid pool
	Purity from strand side-products after key extension steps
	Quantity in fractions after chromatography

During clinical development, assays for product quality and yield should be performed after most processing steps to determine which steps are critical and which assays are most sensitive to deviations in the process. The information from these runs is also used to set the criteria for the selected assays. In-process controls are performed for fully validated processes to ensure that the process continues to be under control. The results of these assays should be trended and actions should be taken to correct problems as they arise.

Specifications

Specifications for cell and gene therapy products should be chosen to confirm the quality of the product by testing to ensure the safety and efficacy of the product. Selected tests should be product-specific and should have appropriate acceptance criteria established to ensure that the product exhibits consistent quality parameters within acceptable levels of biological variation, loss of activity, physicochemical changes, or degradation throughout the product's shelf life. The development and setting of specifications for cell and gene products should follow the principles outlined in the International Conference on Harmonization (ICH) guidance entitled "Q6B Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products".

Establishing specifications for a drug product is part of an overall manufacturing control strategy that includes control of raw materials, excipients, and cell and virus banks; in-process testing; process evaluation and validation; stability testing; and testing for consistency of lots. When combined, these elements provide assurance that the appropriate quality is maintained throughout the manufacture of the product.

Appropriate specifications are established on the basis of thorough characterization of the product during the development phase and an understanding of the process and its capability. Characterization should include measurements of the physicochemical properties, safety, purity, process and product-related impurities, potency, viability, sterility, and quantity. Specifications for each product should be developed from this information by applying appropriate statistical methods. The data should include lots used in preclinical and clinical studies and should also include assay and process validation data that can be correlated to safety and efficacy assessments. Specifications should allow for the inherent variabilities exhibited by the production process and by the assay. The traditional lot-release specifications that apply to biologics may have to be re-examined for these product types. For example, the general safety test stated in 21 CFR 610.11 is a lot-release requirement that has been deleted for cell therapies, because it exhibits little relevance for these products.

Specifications for the product are anchored by an appropriate reference standard for the product. The reference standard for the product ensures that the process, as measured by the release assays, does not change significantly over time. The reference standard is made from a lot that is produced under CGMP and passes all in-process and final release testing. In addition, this reference standard is subjected to an additional level of characterization that includes tests not normally performed for product release. The reference standard need not be stored at the same dose, formulation, or temperature as the product. However, the stability of this reference standard needs to be determined. The reference standard verifies that a test produces acceptable results (passes its system suitability tests). Alternatively, a specific assay standard (working standard) can be used. If so, in the test it should behave similarly to the reference standard. Changing to a new reference standard (lot) should include many tests, all of which are run side by side with the existing reference standard. The impact of any change in the properties of the new reference standard should be carefully evaluated before it is adopted. One option for a reference standard for a cell product with a short shelf life or for a patient-specific application can be a bank of normal donor cells of the appropriate cell type. This cell bank can be used to ensure that the manufacturing process is capable of making a consistent product.

Production of a safe and efficacious product involves establishing not only lot-release specifications but also specifications designed to maintain control of the manufacturing process and the final product. This includes in-process specifications (see *In-Process Controls*), raw material and excipient specifications (see *Raw Materials*), product-release specifications, and shelf-life specifications. Specifications should be established for acceptance of raw materials and excipients used in the final formulation of the product. In addition, tests should be performed at critical decision steps during manufacture or at points where data serve to confirm consistency of the process. In-process release specifications should be established for each control step. Heterogeneity can result from the manufacturing process or storage of the product. Therefore, the manufacturer should define the pattern of heterogeneity within the product and establish limits that will maintain the therapeutic efficacy and safety of the product.

In some cases, specifications may be established for lot release as well as for shelf life. As discussed in ICH guideline Q5C, presented under *Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products* (1049), the use of different speci-

fications should be supported by sufficient data to demonstrate that the clinical performance is not affected. Acceptance criteria should be established and justified on the basis of data obtained from lots used in preclinical and clinical studies and lots used for demonstration of manufacturing consistency and on the basis of relevant development data, such as those arising from validated analytical procedures and stability studies. Acceptance criteria should also be correlated with safety and efficacy assessments.

Once specifications have been established, test results should be trended. Results that are out of specification (OOS), or even those that are out of trend, need to be investigated prior to dispositioning of the material. The purpose of an investigation is to determine the cause of the discordant result. The FDA's *Draft Guidance for Industry: Investigating Out of Specification (OOS) Test Results for Pharmaceutical Production* provides a systematic approach for conducting an investigation. An assay result can be rejected if it can be confirmed that an error, such as an analyst error, calculation error, or equipment failure, has taken place. If the investigation concludes that the product is not within the specification, the lot should be rejected. In unique situations, a product that does not meet all specifications may have to be administered to a patient. However, procedures must be in place to govern the communication of the OOS results to the physician or to the person responsible for making the decision to use the product and to provide instruction for any follow-up testing, patient monitoring, and communication of those results.

Considerations for Validation

The potential for wide biological variation in cell and gene therapy products, particularly for patient-specific treatments, affects the validation effort. Nevertheless, the basic principles of process validation for any biological product, including those recommended by the ICH and FDA guidance documents and recommended under *Validation of Compendial Methods* (1225) and *Validation of Microbial Recovery from Pharmacopeial Articles* (1227), apply to the validation of most cell and gene therapy products. Guidelines for validating viral vaccines can be relevant to gene therapy processes that produce viral vectors. The hold steps in a manufacturing process should be validated to ensure that in-process intermediates are within specification and that the final product can be formulated successfully. Any assay used during the process validation must itself be validated before the process validation is commenced.

Process validation for patient-specific products, such as autologous cell therapy products or custom gene therapy products, presents some unique issues. First, the starting materials for patient-specific products typically arise from patient-derived materials, such as biopsy material or apheresis cell products. The process should be designed to accept a wide range in the quality and quantity of starting material. Sometimes use of alternative procedures with additional steps are required when the starting material is of poor quality or below specified amounts. Validation should confirm that these alternative procedures still result in a final product that satisfies release specifications. Procedures should also be in place to deal with receipt of substantially more of the starting material than normally expected. Such procedures should address the disposition of the extra material. Second, manual processing of cells and tissues will exhibit a degree of inherent variability. It is essential to develop processing steps that will successfully and consistently result in appropriate process components and final product, even if the process is confronted with nonstandard or variable tissue materials, such as a T-cell suspension contaminated with red blood cells or low-weight biopsy material. Process validation should take this variability into consideration and ensure that critical manufacturing and testing endpoints consistently meet specifications. The process validation shows that the procedures can produce a product free of microbial contamination. It should also show that there is no cross-contamination among different patient product lots. If possible, the process should be validated for virus clearance as discussed in ICH Q5A: *Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin*. If this is not possible, cells used for production of the product should be evaluated for their ability to propagate viruses that are known to contaminate these cells or source materials. This should include raw materials used as ancillary products.

As a result of the variability discussed above, the consistency and the robustness of the manufacturing process need to be assessed by testing more than three lots. It is not expected that every manufactur-

ing effort will be successful for patient-specific therapies. However, the success rate should be established and tracked so as to discover any decrease in that rate and to take actions to correct the problem. Well-characterized banked primary cells may be used in the validation of the process if the donors have a range of profiles expected for the patient population to which the therapy will ultimately be directed. Trending of a number of statistically acceptable product administrations can also be appropriate.

MANUFACTURING OF CELL THERAPY PRODUCTS

Introduction

Cell processing for cell therapy applications is a unique form of biologics manufacturing that relies on maintenance of stringent work practices designed to ensure product consistency and prevent contamination by microorganisms or by another patient's cells. Hallmarks of this unique form of manufacturing can include products with limited shelf lives, the need for rigorous control during manual processing steps, a manufacturing environment in which many product lots are simultaneously processed and assembled, raw materials that may or may not be part of the final product, and numerous pieces of processing equipment. By its very nature, cell processing requires a number of operations and manipulations by individuals well trained in aseptic processing techniques. The technical competence of the personnel is particularly crucial to product safety and efficacy with this form of manufacturing. Procedures involving lot segregation, line clearance, and operational discipline must be developed to decrease the chance of mix-up of patient-specific lots.

The degree of control required for cell processing operations is highly dependent upon a number of factors, including the complexity of an aseptic manufacturing process, the primary site of manufacturing, and the mode of administration of the cell product to the patient. Manufacturing processes that involve open manipulation of the cells even in a biological safety cabinet are at greater risk of contamination than the processes done in closed bioreactors or intravenous transfer bag systems that use sterile connection devices and tube-sealing devices. Clean rooms and biological safety cabinets are essential components for processes that involve open manipulations or for patient-specific products. The controlled environment of a carefully designed, constructed, validated, and maintained clean room will minimize the risks of environmental contamination during aseptic processing and decrease the possibility of cross-contamination of patient-specific products. Processes that utilize closed systems do not require clean room environments.

Procurement of Source Material

A variety of human- and animal-derived tissues, which can also include whole organs, serve as sources of cells for cell therapy products. Examples include skin, muscle, cartilage, bone, neural tissue, bone marrow, blood vessels, parenchymal cells from organs such as the liver, pancreas, and adrenal glands, and stem cells from adult and fetal tissues. A few general principles in the sourcing of these tissues are as follows: (1) systems must be developed so as to allow the material to be traced back to the donor; (2) steps must be taken to prevent the transmission of an infectious disease from the donor to the recipient; and (3) adherence to aseptic procedures during procurement and initial processing are necessary to ensure the safety of the final product because terminal sterilization of cells is not possible.

HUMAN TISSUE

Human-derived tissues may be sourced from normal healthy donors, cadaveric donors, or diseased patients, such as those with cancer. Applicable guidelines and standards for the procurement of human tissue are available from the American Association of Tissue Banks (AATB) and the FDA. Additionally, the federal policy in 45 CFR Part 46 is applicable to all federal or federally supported research. This policy requires that a certified institutional review board

review and approve use of any tissue taken from a live human donor. The policy also includes special considerations for research on prisoners, children, and pregnant women or research in other areas involving gestational tissue. In all cases, appropriate written consent must be obtained from the donor or the donor's next of kin, describing which tissue is being procured and for what use it is intended. The donor must meet established guidelines for donor suitability and be tested for the infectious diseases listed in *Table 4*. The medical history of the donor must be reviewed to ensure the absence of signs and symptoms of these diseases and to rule out issues and behaviors that increase the risk of exposure to such diseases.

Human tissue should be obtained under environmental conditions and controls that provide a high degree of assurance for aseptic recovery. Standard hospital operating room practices are applicable for tis-

issues requiring dissection and surgical procurement. The air quality provided in a typical limited-access operating room is adequate for such procedures. Procurement personnel must be appropriately trained in all aspects of tissue recovery, such as surgical scrubbing, gowning, operating room behavior, anatomy, surgical site preparation, and antiseptics. Special care is required when tissue or organ procurement requires extensive manipulation of the bowel and when sharp dissection may result in the inadvertent puncture of the bowel. Tissue that contains microbial flora (for instance, skin) at the time of procurement can be adequately disinfected by using antimicrobial or bactericidal agents and extensive scrubbing.

Table 4. Infectious Diseases Testing for Human Cells and Tissues Used in Cell Therapy Products

Cell Type	Testing					
	HIV 1,2	Hepatitis C	Hepatitis B	HTLV	Cytomegalovirus	Treponema pallidum
Autologous stem cells	R	R	R	R		
Other autologous tissue	R	R	R			
Allogeneic stem cells from family-related donors	X	X	X	X	X	X
Other allogeneic tissue	X	X	X	X	X	X

X—required

R—recommended; the labeling stating “tested negative” or “not tested for biohazards” may be required

* For autologous or allogeneic cord blood donors or fetal tissue, a mother's sample may be used for testing.

HUMAN BLOOD AND BONE MARROW

Hematopoietic progenitor cells represent one of the most extensively used cell sources in the field of human transplantation. These cells can be collected from the bone marrow, peripheral blood, placental umbilical cord blood, or fetal liver. The source of cells is somewhat dependent upon the patient, the disease, and the clinical protocol. Regardless of the cell source, methods for processing the cells are similar.

Human-derived blood cells and bone marrow cells may be sourced from normal, healthy donors or patients with hematological disorders. Applicable guidelines and standards for the collection and processing of these materials have been published by the American Association of Blood Banks (AABB), the Foundation for the Accreditation of Hematopoietic Cell Therapy, the National Marrow Donor Registry (NMDR), and the FDA. Similar issues regarding consent, infectious disease testing, and donor medical history apply in the sourcing of blood- or bone marrow-derived cells for allogeneic transplants. In cases where these cells will be subjected to selection, expansion, genetic manipulation, or other complex processing procedures, the testing outlined in *Table 4* should be followed.

Bone marrow for clinical use is harvested predominantly by percutaneous needle aspiration of the anterior or posterior iliac crests or the sternum. Standard hospital operating room practices are employed by specially trained personnel. Plastic syringes and commercially available aspiration needles are used to draw 3- to 5-mL volumes of marrow from each site of penetration. The material is transferred to a sterile, balanced salt solution or tissue culture medium containing sufficient anticoagulant, such as heparin, to prevent clotting. Removal of bone spicules may be accomplished by passing the material through stainless steel mesh screens or collection kits consisting of sterile, plastic collection bags with in-line filters having about a 200- μ m porosity. The volume of marrow collected is dependent upon the body weights and other characteristics of both the donor and the recipient. The maximum volume to be harvested from a donor is about 10 to 15 mL per kg of body weight.

Circulating hematopoietic, peripheral blood progenitor cells (PBPCs) comprise a small population of peripheral blood mononuclear cells that can be utilized in place of or in addition to bone marrow. PBPCs are collected by apheresis, a procedure by which donor blood is withdrawn from a vein and separated *ex vivo* into some or all of its component parts. One or more of the components are retained as the harvest and the remaining parts are returned to the donor. Conditioning of the donor may enrich the number of circulating PBPCs in the

harvest. Examples of such conditioning include collection during recovery from myelosuppressive chemotherapy and administration of hematopoietic growth factors, such as granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF), or steroids. Collections are also improved by increasing the frequency or volume of apheresis. Apheresis requires one or two large-bore peripheral venous catheters in the upper extremities or a single large-bore, thick-walled, central venous double or triple lumen catheter (Mahurkur type). Two types of apheresis technology are available: the discontinuous-flow cell separators (Haemonetics) and the continuous-flow systems (COBE or Fenwall). Anticoagulation for normal to high flow rates is with a citrate-based material. In a closed system, the risk of contamination is low. The procedure is generally performed by trained, dedicated staff in a blood bank or in a donor center associated with a blood bank.

Placental and umbilical cord blood provides a third source of hematopoietic progenitor cells. Compared to bone marrow and PBPCs, the stem cells of placental and umbilical cord blood have a higher proliferative and self-renewal capacity. Volume of collection and thus cell number are limited and depend upon timing and the presence of a dedicated team of personnel. Collections are made during the third stage of labor. Typically, a closed method of collection is employed and involves cannulation or puncture of the umbilical vein with subsequent collection into plastic syringes or blood collection bags containing citrate-based anticoagulant. The procedure is performed in a controlled-access room away from the site of birth. Cellular content of the collection includes large numbers of erythrocytes, leukocytes, platelets, and target mononuclear cells. An open collection technique, which involves drainage of the blood by gravity from the cut end of the cord into sterile tubes containing anticoagulant, does not afford the same aseptic assurance level as the above-mentioned technique.

A major area of concern with the use of placental and umbilical cord blood relates to potential risks of unknown genetic disorders that may be transmitted to the recipient. Donor suitability is established by the usual infectious disease screening of the mother and the completion of a medical questionnaire. The donation remains anonymous and without any long-term follow-up of the child.

ANIMAL TISSUE

The major area of concern with the use of animal tissue relates to the known and unknown risks of potential infectious disease transmission to humans, and as such, the transplantation of animal cells raises unique public health concerns. Introduction of xenogeneic infectious agents into and propagation through the general human po-

putation is a risk that must be addressed. *Draft Public Health Service (PHS) Guideline on Infectious Disease Issues in Xenotransplantation* (August 1996), and any other related regulatory documents that are generated as this field advances, must be consulted when developing xenotransplant cell therapy products. Developers of such products should understand that the product recipients will be subjected to a high level of scrutiny (for instance, clinical and laboratory surveillance or registry in xenotransplantation databases) because of the above-mentioned public health concerns.

The use of animal tissue in the manufacture of cell therapy products requires that the tissue be sourced in a controlled and documented manner and from animals bred and raised in captivity in countries or geographic regions that have appropriate national health status, disease prevention, and control systems. In addition, the care and use of animals should be approved by a certified institutional animal care and use committee. Donor animals must have documented lineage, be obtained from closed herds or colonies, and be under health maintenance and monitoring programs. The facility for housing these animals should be USDA certified (large vertebrate animals) or Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) certified (small vertebrate animals) and should meet the recommendations stated in the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996), which can be obtained from the AAALAC. Such facility should be staffed with veterinarians and other trained personnel who will ensure animal health and disease prevention. The procedures employed in the facility should be documented and records should be kept. Health maintenance and monitoring programs are based on standard veterinary care for the species and include physical examinations, monitoring, laboratory diagnostic tests, and vaccinations. Use of a stepwise *batch* or *all-in-all-out* method of movement of source animal through the facility, rather than the continuous replacement movement, is recommended. It allows the decontamination of the facility prior to the introduction of the new set of animals, thereby reducing the chance of disease transmission. Feed components should be documented and should exclude, whenever possible, recycled or rendered materials that may have been associated with the transmission of prior-associated diseases.

To provide a high degree of assurance of product safety, screening of donors and of tissues derived from these donors should be performed at several stages throughout the process to rule out the presence of microbial agents. These control tests should utilize assays that are sufficiently sensitive and specific to detect bacteria, mycoplasma, fungi, or viruses of interest. Donor animals can be screened for certain diseases prior to donation of tissue by applying a variety of serological monitoring tests. Tissues can be subjected to a panel of tests including, but not limited to, the following:

1. test for sterility;
2. test for mycoplasma;
3. test for cultivable viruses *in vitro*;
4. test for unknown viruses by inoculation of various laboratory animals;
5. tests for xenotropic endogenous retroviruses and other animal retroviruses by *in vitro* cocultivation techniques, biochemical methods (for instance, to detect viral reverse transcriptase), and molecular biology assays (such as PCR assay for viral genomic sequence detection); and
6. direct detection or observation methods such as electron microscopy, detection of specific viral antigens by fluorescent antibody microscopy, or enzyme immunoassay methods.

Most of these tests are addressed under *Analytical Methodologies* or under *Biotechnology-Derived Articles* (1045) and *Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin* (1050). Post-tissue retrieval necropsies, sentinel animal programs, and archival storage of donor organs, tissues, blood, and other specimens are additional components of the overall program to ensure the safety of animal tissue for use in cellular therapeutic applications.

Most of the same aseptic procurement issues apply to animal tissue and to human tissue. Again, the tissue should be obtained under environmental conditions and controls that provide a high degree of assurance of aseptic recovery. Specifically designed procurement facilities, usually closely associated with the animal holding facility, are typically employed. These facilities have specific attributes and design features that may not be available or applicable in the hospital operating-room setting. Such features include the following: (1) staging of various events, such as shaving, sedation, and operating-room preparation, in different rooms that are often separated with air locks

for environmental control; (2) high-efficiency particulate air (HEPA) filtration; (3) adjacent but separate facilities for further tissue processing; and (4) dedicated areas for carcass removal. The issues regarding the training of personnel, bowel manipulation and puncture, and disinfection that are applicable to human tissues apply to the surgical procurement of tissue from animals as well (see *Human Tissue*).

Cell Isolation and Selection

GENERAL CONSIDERATIONS

The general principles for processing human and animal tissues following aseptic procurement are independent of the tissue source. The manufacture of cell products may occur at a clinical site or at a central cell-processing facility. Sites involved in cell processing should ensure reproducibility and safety of the manufactured products through appropriate QC and QA programs.

Regardless of the location, processing should occur in a dedicated area physically separated from the site of procurement. To the greatest extent possible, the facility design and processing procedures should be consistent with those provided by the FDA's *Guidelines on Sterile Drug Products Produced by Aseptic Processing* (June 1987), or provided under *Sterile Drug Products for Home Use* (1206) for processes involving open manipulation. Generally, this requires that properly trained and outfitted processing staff handle blood or tissue samples in a critical zone supplied with class 100 HEPA-filtered air, which is provided by a biological safety cabinet located in a controlled clean room supplied with class 10,000 HEPA-filtered air. The facility and processing areas should be monitored for air quality in a manner that provides a high level of process asepsis. For guidance in this area, see *Microbiological Evaluation of Clean Rooms and Other Controlled Environments* (1116). The material should be packaged in sterile, leak-proof containers and transported from the procurement area to the processing area under controlled conditions that maintain cell viability. The fluid medium in which the specimens are bathed during transportation should be optimized to maintain cell and tissue viability. This transport medium can be supplemented with antibiotics. If so, the antibiotic levels in process buffers are decreased and eventually eliminated during subsequent processing steps, so that antibiotics are not present in the final cellular product. In the case of blood products or tissues containing substantial amounts of blood, the transport media or buffered electrolyte solution should contain an anticoagulant such as heparin or a citrate-based material.

ISOLATION

Solid organs or tissues are usually dissected to expose a desired region. This material may be used as is for transplantation or it may be processed further. If multicellular organoids (for instance, islets of Langerhans) or single-cell suspensions are desired, the tissue may be subjected to mechanical or enzymatic disaggregation. Physical disaggregation may be accomplished through the use of instruments that impart high shear forces on the material (namely, to homogenize) or break the tissue into smaller pieces. Alternatively, the material can be pressed or passed through screens of defined mesh sizes.

Enzymatic digestion of the extracellular connective tissue, which holds cells together within the tissue, is another common method for dissociating solid tissue. Typically, the tissue is minced into small cubes, usually larger than 1 mm³, and incubated in a buffered solution containing a digestive enzyme. Alternatively, the intact organ is infused with a solution to rinse the blood from the tissue followed by the enzymatic solution that aids the digestion. Various enzymes are used to accomplish this. Examples include collagenase, trypsin, elastase, hyaluronidase, papain, and chymotrypsin. Enzymes with nuclease activity, such as deoxyribonuclease, may be added to digest nucleic acids released from damaged cells, preventing excessive cell clumping. At the end of the incubation process, the cell suspension may be subjected to a mild pumping action to further break up multicellular clusters into those of desired size or composition. Enzymatic and physical disaggregation methods are often combined to achieve the desired result.

Because cells isolated from blood and bone marrow products are inherently cell suspensions, mechanical manipulation is limited to plasma removal, which is accomplished by centrifugation and physical removal of clots that occurred during transport via 200- μ m filtration.

SELECTION

Cell suspensions at this stage may be transferred directly to culture vessels as described for *Propagation* under *Cell Propagation and Differentiation*, genetically manipulated as described under *Introduction of Genetic Material into Cells*, or formulated by various techniques as described under *Formulation of Cell Therapy Products*. Cell suspensions often consist of a mixture of cell types that may require further processing to isolate a cell population of interest or to decrease the level of an undesirable cell type such as potentially contaminating tumor cells. Various cell isolation and separation techniques exist that provide high yields of pure cell populations.

Each cell type typically possesses specific size and density; therefore, different cell types will sediment at different rates in a centrifugal field or at unit gravity. Cell populations can be selectively sedimented to yield pure fractions by varying the centrifugation forces and the duration of centrifugation. Separation can also be achieved by isopycnic centrifugation, where the cell suspension is centrifuged in a gradient medium that encompasses all of the densities of cells in the sample. In this procedure, the various cell populations sediment to an equilibrium position at the gradient density equal to the density of the cell population. Specifically designed continuous-flow elutriation centrifuges separate cell populations by subjecting a cell suspension to opposite centrifugal and fluid stream forces in a special chamber within the centrifuge rotor mechanism. Cell populations separate within the rotor on the basis of their various sizes and densities, and they are selectively eluted out of the rotor chamber by increasing the fluid stream force. Finally, methods that do not require centrifugation but instead involve the addition of high-density agents, such as hydroxyethyl starch, to the cell suspension will result in cell separation. The mixture is allowed to settle in a tube at unit gravity, resulting in the separation of different cell types based on buoyant density. Concentration and separation procedures such as these frequently result in cell loss due to clumping and aggregation.

Cell separation can also be achieved by applying techniques that take advantage of unique cytological or biochemical characteristics of different cell populations. Soybean agglutinin binds to and agglutinates cells that bear a particular carbohydrate moiety expressed on mature blood cells, but not stem cells, allowing for purification of the stem cells. Lymphocytes possess the CD2 antigen that acts as a receptor for sheep red blood cells. The lymphocytes form rosettes, which then can be separated via differential centrifugation.

Some applications take advantage of the ability of certain cell populations to adhere to the surface of specific solid substrates such as tissue culture plastic, collagen-coated materials, and natural and synthetic polymeric scaffolds. The specifically bound cell type is selectively recovered onto the surface and removed from the initial cell suspension. When placed under the appropriate culture conditions, these cells will multiply and eventually occupy the available surface or void volume of the substrate.

Monoclonal antibodies directed against specific cell surface antigens or receptors can be used for both positive and negative cell selection. For example, a monoclonal antibody-labeled cell population can be removed from the cell suspension *immunomagnetically*, after exposure to magnetic particles coated with antimono-clonal antibody. The magnetic particles and their bound cells are removed from the cell suspension magnetically. Cells are released from the complex following incubation with reagents, such as specific peptides, that dissociate the monoclonal antibody from the cell. Unlabeled cell suspensions can be poured over or incubated on surfaces such as plastic flasks or microspheres coated with monoclonal antibodies as a means of isolating particular cell populations. In addition, a fluorescence-activated cell sorter (FACS) can be used to separate different cell types by binding antibodies tagged with fluorescent markers to a particular cell type.

Various other techniques purify particular cell populations by destroying unwanted cells present in the mixture. For example, certain cell-bound monoclonal antibodies are able to fix and activate complement, which is added to the cell suspension, resulting in lysis of the cell. Some procedures use cytotoxic agents or mitotic inhibitors to

selectively impede or kill unwanted cells in a cell product. These methods typically target an unwanted cell subpopulation with a high growth rate, such as tumor cells. Finally, an antibody can be conjugated to a toxic moiety, such as ricin, allowing delivery of the cytotoxic agent to the targeted cell population. Most of these procedures require several washing steps after the exposure of the cells to the cytotoxic agents to ensure the removal of the dead cells, cell fragments, and cytotoxic agents from the final cell product.

Cell Propagation and Differentiation

PROPAGATION

A key issue for cell therapy products is the ability to manufacture and deliver a therapeutically relevant dose of the required cell population to the patient. Depending on the application, the product may be a pure, homogeneous cell type or it may be a mixture of different functional cell types. Many target cell populations are present at low level or low purity in complex primary source tissues. In such cases, production of a therapeutic dose may be achieved only by specific enrichment and propagation of the required cells.

Propagation of cells may occur in suspension culture (for example, T cells or hematopoietic stem and progenitor cells), adherent culture (for example, mesenchymal stem cells, embryonic stem cells, neuronal stem cells, or dermal fibroblasts), or a mixture of both (for example, bone marrow stroma expansion). Numerous devices of varying degrees of sophistication and automation exist for cell culture.

In the simplest iteration, cells can be propagated in tissue culture flasks (T flasks), roller bottles, on polymeric scaffolds, or nonrigid, gas-permeable bags inside regular incubator units that are controlled for temperature, humidity, and gas composition. Multilayered plastic cell factories, cell cubes, and multi-bag systems have been developed that enable expansion, harvesting, and formulation to be carried out in a closed system.

Traditional small-scale fermenter units can be used for expansion of cells in suspension culture. It is also possible to expand adherent cells in such units either by providing a surface for attachment (coated beads or disks) or by adapting the cells to propagate in suspension culture. Some culture systems are specifically designed for the propagation of cells for therapeutic applications. These systems tend to be closed systems that use disposable bioreactor cartridges, such as those made of hollow fiber or molded plastic, in automated processing units with direct control of parameters such as temperature, gas composition, and media perfusion rate. These units can provide a completely automated, closed system for expansion and harvesting. In some cases the automated software is set up for patient-donor tracking and will document culture conditions and manipulations for the entire processing run. These features are useful in the design and implementation of QC product-release testing programs and for the QA documentation of processing runs.

In the case of adherent culture, the cells are usually released from the surface upon which they have expanded. Methods of release include physical agitation, enzymatic cleavage with enzymes such as porcine or bovine trypsin, collagenase, or dextranase, chelation of metal ions (for example, with edetate disodium), and competitive inhibition of adhesion or matrix molecules. As described above, consideration must be given to the source, safety, toxicology, and residual testing for any reagent used to release adherent cells during manufacturing.

Some product-specific systems that do not require the release of adherent cells have been developed. In these systems, the cells are expanded upon a synthetic or natural matrix that is then applied topically (for example, in dermal repair products) or the cells are grown inside or outside of fibers for ex vivo perfusion (for example, hepatocytes in hollow-fiber devices to treat liver disease). In these applications, the matrix and device composition must be biocompatible and, in some cases, biodegradable.

In all of the above systems, standard cell culture parameters must be optimized for maximum process efficiency. Such parameters include composition of cellular source material, initial seeding density, media composition, rate of media exchange, temperature, gas composition, and rate of delivery. Depending on the nature of the product, the potential effect of process parameters on the potency and function of the target cells should be defined.

In closed bioreactor systems, it can be difficult to observe or sample cells so as to determine and control the rate of proliferation and thereby the point of harvest. Measurement of traditional fermentation parameters, such as rate of nutrient usage or production of metabolic products, can provide a surrogate method, amenable to validation, with which to evaluate the rate of proliferation and predict when to harvest the cell product. The relationship of such parameters to the viability, potency, and function of the cell product should be well defined. Postexpansion purification and enrichment of target cells by using methods such as those described above may be required.

DIFFERENTIATION

Some cell therapies require lineage or functional differentiation of the source cells. For example, hematopoietic stem cell expansion processes normally result in products containing a mixture of multipotent stem cells, lineage-committed progenitor cells, and lineage-differentiated cells. The composition of these products can be manipulated by using different combinations of growth factors and cytokines during the expansion process. The inverse is true for processes in which mature cells are *de-differentiated* to enable them to then be recommitted to a lineage pathway (for example, chondrocytes in cartilage repair).

Specific examples of *ex vivo* manipulation are the programming of professional antigen-presenting cells, such as dendritic cells and monocytes or macrophages, and the production of antigen-specific T cells to target various specific disease indications. In these applications, the manipulated cells may be engineered to target and attack a specific tumor or tumor cell type, to induce a specific antibody or other cellular response, or to potentially vaccinate a patient. The processes for production of such products can involve one or more exposures of the relevant cells to disease-specific synthetic immunogens (for example, peptides) or natural immunogens (for example, dead tumor cells, viruses, cell membrane fractions, or purified natural molecules) before, after, or during culture expansion. Alternatively, the target cells may be genetically engineered with a specific gene product, such as an HIV-specific receptor. In some applications, relevant cells are cocultured with tumor cells, other diseased cells, or cells producing a transduceable or transfectable gene construct to generate a specifically targeted product.

Again, prior to delivery, the manipulated target cells may require further purification and enrichment by applying the methods described throughout this section. In the case of certain T-cell products, the desired antigen-specific cells can be cloned and then further expanded to provide the therapeutic dose.

Introduction of Genetic Material into Cells

A common extension of cell therapy involves the introduction of genetic material, usually DNA, into cells to alter their pattern of gene expression. For the purpose of this section, it is assumed that the nucleic acid is DNA. Similar scenarios can be applied to RNA or a derivative of DNA, except that the stability and solubility of the particular nucleic acid may dictate modifications of certain steps. This process is often referred to as *ex vivo* gene therapy, because the cells are removed from the patient or donor and the genetic material is introduced while the cells are outside of the body. Genetically modified cells are then administered to the patient. The genetic material introduced can either cause the expression of new genes and products or cause the inhibition of the expression of already expressed genes and products. The latter represents a type of antisense therapy. The genetic material can be introduced by the same range of reagents that are involved with gene therapy: viral vectors, nucleic acids in a simple formulation (naked DNA), or nucleic acids formulated with agents, such as liposomes, that enhance their ability to penetrate the cell. Most of the steps and considerations discussed above also apply to the *ex vivo* introduction of genetic material into cells. However, the main goal of *ex vivo* therapy is to develop robust processes that will work with the majority of patient's or donor's cells. This takes considerably more effort than processes for cell lines.

The method of introduction of new genetic material into cells depends on the biology of the system and the desired stability of gene expression. If a simple retroviral vector such as Molony murine leukemia virus is used for transduction, the cells must be actively dividing because vector DNA is only integrated into the cellular DNA

during replication. This usually leads to long-lasting expression of the desired gene product. Adenoviral vectors, naked DNA, or formulated DNA can be introduced into nondividing cells. However, gene expression will be transient, because the introduced DNA will usually be extrachromosomal.

The main challenge is to achieve efficient transduction or transfection, introducing sufficient DNA into the cell before the DNA degrades. In the case of transduction by retroviral vectors, vectors derived from simple retroviruses, cells are stimulated with reagents that cycle them into the S phase (replication) at the time the vector is applied. Most retroviral vectors are stable in cell culture for a period up to a few hours. Because diffusion is minimal, only a small fraction of viral particles will come into contact with cells over this period. The following techniques can be used to increase the number of viral particles that contact the cell in a given time period:

1. maximization of viral particle concentration and minimization of the media volume during the transduction step
2. multiple applications of the virus
3. centrifugation of virus particles onto the cells
4. placing of cells on a filter and slow pulling of viral media through the filter
5. addition of binding-enhancing polymers to the media

NOTE—Coculturing of the target cells with the viral producer cells is not recommended. This technique increases the chance of a recombinant event occurring and of the production of RCV. Furthermore, any product for which coculturing is used to transduce the human cells would be considered a xenotransplant if the producer cells were not human. The second cell type, whether human or not, may cause inflammation.

Each of the above techniques has its own set of issues that must be addressed in order to develop a robust process. In technique 1, reduction of the volume during transduction results in rapid exhaustion of the medium; therefore, supplemental medium should be added within a few hours. In technique 2, the cells may no longer be in the correct cell cycle phase during later applications or cells may have become refractory because of unproductive transformation during the prior application. Techniques 3 and 4 can work well on a very small scale, but the number of cells that can be transduced may be insufficient to obtain an efficacious dose. In technique 5, polymers may fail to provide a benefit because virus-binding may involve specific receptors whose surface density may prove to be the limiting factor.

Similar issues and techniques can apply with other viruses or DNA preparations. The issue of slow diffusion is even more marked for the use of DNA preparations. Factors such as the cell type in which the viral vector was produced, the media used for vector production, and the purity of the vector can have a dramatic effect on the efficiency of transduction.

While certain methods may not require cells to be actively cycling, in practice, most processes will require that cells be capable of replication because of the following considerations:

1. Safety considerations may dictate that only cells that express the new DNA are returned to the patient, which requires that these cells be selected. As described below, the most common selection method utilizes an antibiotic-resistant gene that is co-introduced with the new genetic material.
2. Further propagation may be required to achieve the therapeutic dose of cells.
3. Economic, biological, or technical reasons may dictate that the DNA introduction step be carried out at a low cell number and that the desired cell population then be expanded to the required dose.

Therefore, conditions that enable the cell or maintain the cell's ability to proliferate must be developed in almost all cases. The biology of the cells, the available technology, and the process economics will determine whether cells are propagated before, after, or during the introduction of new genetic material. Most processes do in fact expand the population after the introduction of the new gene.

Whether cells that do not productively express the gene can be administered to patients depends on the biology of the application, the dose required versus the handling capability of the manufacturing system, and most importantly, the toxicity of the nonproductive cell population. Selection of the genetically modified cell population is commonly carried out using an antibiotic-resistance marker gene, such as neomycin, which is co-introduced into the cell with the new genetic material. For neomycin selection, cells in culture are treated with the antibiotic G418 at a concentration and for a period that have been shown to kill cells with nonproductive expression, while allowing the productively expressing cells to proliferate. In this man-

ner it is presumed that cells that are resistant to the antibiotic will also express the DNA of interest. The expression should be tested as a lot-release requirement or verified in a series of mock runs. Because most antibiotics decrease cellular proliferation, optimization of the culture media composition may be necessary for efficient selection and propagation of the gene-modified cells.

Following the antibiotic selection step, a second phase of antibiotic-free cell propagation may be required in order to achieve the desired dose and to rinse residual G418 out of the system. The selected medium and the total time that the cells are in culture can be critical to maintaining the desired expression of the original differentiated functions. An additional issue associated with the use of selection markers is that they generally are nonhuman genes. The expression of these genes usually elicits an immune response.

Process development is often carried out with cells from healthy donors. Consideration should be given to the fact that for very sick patients, it can be difficult to obtain healthy cells that can be stimulated to undergo efficient, sustained replication.

Formulation of Cell Therapy Products

SUSPENSIONS

Formulations for cell therapy products depend upon the desired length of storage and whether the cells are administered as a suspension or in combination with a matrix. Regardless of the route of administration, cells that will be administered as a suspension can be frozen or not frozen. The most common formulation for cells that are cryopreserved is a 5% to 10% solution of dimethyl sulfoxide (DMSO), with or without hydroxyethyl starch (generally 6%), and a plasma protein, such as 4% to 10% human serum albumin, in a balanced salt solution. DMSO prevents dehydration by altering the increased concentration of nonpenetrating extracellular solutions during ice formation at the time of freezing. The high molecular weight polymeric hydroxyethyl solution protects the cells from dehydration as water is incorporated into the extracellular ice crystals. The use of protein often results in maximum recovery and viability of cells after thawing. Serum (5% to 90%) has been used in place of specific proteins. Some cryopreservation formulations are completely free of protein. If the solution contains a buffer, the pH of the buffer should not be affected by changes in temperature. The optimal concentration of cells for cryopreservation depends on the cell type, but it generally ranges from 10^6 to 10^7 cells per mL. The purity of the cell population can also affect recovery. For instance, granulocytes can be damaged by the cryopreservative and the cell viability can decrease. These effects are dependent upon the concentration of cryopreservative. Both effects subject the patient to an increased level of infusion-related toxicity, although this is related to the volume administered and the final concentration of the cryopreservative.

Formulations for cell suspensions stored without freezing generally contain cell culture media, often without any protein. Because cells continue to metabolize their media even at the reduced temperatures used for storage, the medium supplies the amino acids and other nutrients that help in maintaining cell viability.

PRODUCTS COMBINED WITH BIOCOMPATIBLE MATRICES

Many cell therapy products are administered in combination with a biocompatible matrix. For instance, wound healing or skin substitute products contain cells seeded on a matrix. The biochemical and physical structure of the matrix and the method for combining cells with the matrix are specific to the application. Some common examples include the following:

1. Cells loaded into a semipermeable membrane device—Usually the pore size of the membrane is large enough to allow the cell-secreted therapeutic factors to pass, but it is small enough to stop immunoglobulins and host cells from making contact with, destroying, or having an immune response to the therapeutic cells. The device can be a single hollow fiber or a semipermeable capsule with cells inside that secrete therapeutic compounds, or it can be part of a larger system of pumps and filters, such as hol-

low-fiber modules with hepatocytes for the treatment of liver disease.

2. Cells seeded onto a three-dimensional matrix and allowed to propagate and form a tissue-like structure—In the resulting product, the cells are oriented in a unique manner that is important for the intended use of the product (for example, skin substitutes). In some cases, mechanical force has been used for proper cell orientation.
3. Cells encapsulated in a gel or cross-linkable polymer solution—The resulting implantable structure can serve as a culture vessel, as a means to shield the cells from the host's immune system, or as a way to mold cells into a defined shape. Some of the polymers used include alginate, hyaluronic acid, collagen, chitin, or synthetic polymers. Encapsulated pancreatic β -islet cells have been implanted in patients to treat diabetes. To treat urinary incontinence, chondrocytes have been mixed with alginate to form a structure upon injection.
4. Cells adhered to matrices of defined shape that are then implanted—Some examples include osteogenic precursor cells on matrices of demineralized cadaveric human bone, ceramic hydroxyapatite, ceramic hydroxyapatite-tricalcium phosphate, or biodegradable glass, which can be used in the repair of bone defects.

When manufacturing such products, the primary consideration is the sourcing of a quality matrix material. The matrix material should be biocompatible, should not interfere with cell function, and should not trigger an immune response in the patient. If it is intended that the cells proliferate after loading onto or into the matrix, the matrix and the supporting culture system must allow exchange of nutrients and waste products. Cells may form tissue-like structures under favorable conditions and for those applications where this is required. A thick, impermeable matrix will lead to forming regions of necrotic tissue. Many of these devices are designed so that they can be removed from the patient after a certain period of time.

In all cases where cells are combined with biocompatible matrices, the use of closed systems for the manufacture and the delivery of product is preferable. As cell therapy products of this type can be quite intricate, the manufacturing details for such products are outside the scope of this chapter.

MANUFACTURING OF GENE THERAPY PRODUCTS

Introduction

The principles applicable to the production of pharmaceutical or biological products are also relevant to the production of gene therapy vectors for therapeutic use in humans. The same CGMP requirements can be applied to determine product consistency, process validation, raw material qualification, and compliance of the manufacturing facilities. Manufacturers will face development issues such as scalability, yield, cost efficiency, and product stability.

Most gene therapy vectors have been produced only in relatively small batches necessary to meet the needs of early clinical trials in small numbers of patients. However, areas of rapid progress are large-scale production of vectors, vector purification, and suitable analytical techniques. This section focuses on issues involved with designing vectors for gene therapy and choosing a production technology; it does not focus on specific production technologies.

Design Considerations for Gene Vectors

TYPES OF VECTORS

A typical gene therapy vector is composed of (1) the vector backbone, viral or plasmid, (2) a promoter, (3) the therapeutic gene of interest, including introns, and (4) a polyadenylation signal. Murine and human retroviruses, adenoviruses, parvoviruses such as adeno-associated virus (AAV), herpes viruses, poxviruses, toga viruses, nonviral plasmid therapy systems, and synthetic antisense-oligonucleotide therapy systems are being developed for gene therapy applications. The properties of these vectors (see Table 5) differ greatly in terms

of their capacity to deliver genes to cells. Some viral vectors preferentially target dividing cells while others are capable of transducing both dividing and nondividing cells. There are significant variations in transgene capacity, meaning that there are limitations on the size of the foreign DNA fragment that can be incorporated into the recombinant genome. The *ideal gene therapy vector* has often been described as one capable of efficient transduction, targeted delivery, and controlled gene expression. The level, timing, and duration of gene ex-

pression required will depend on the clinical indication. Low-level, long-term gene expression is thought to be required for some diseases including adenosine deaminase (ADA) deficiency or type A and type B hemophilia. High-level, short-term expression may be more appropriate for cancer when genes that induce apoptosis are used, or for cardiovascular disease where preventing hyperproliferation of smooth-muscle cells may impede restenosis of saphenous vein grafts.

Table 5. Types of Gene Vectors

FAMILY Example Species	RETROVIRIDAE		ADENOVIRI- DAE	VIRAL PARVOVIRIDAE	HERPES-VIRI- DAE	TOGAVIRIDAE	POXVIRIDAE	NONVIRAL
	Murine Leukemia Virus	HIV	Adenovirus	AAV	Herpes Simplex Virus	Sindbis	Poxvirus (Vaccinia)	Plasmid derived
	<i>Vector Characteristics</i>							
Insert size limit	8 kb	8 kb	4.3 to 34 kb	4 to 5 kb	40 to 150 kb	5 kb	25 to 50 kb	12 kb
Chromosome integration	Yes	Yes	No; episomal	Can be integrated or episomal	Can be integrated or episomal	No	No	Yes, but at very low frequency
Therapeutic protein expression	Stable	Stable	Stable or transient	Stable	Stable or transient	Transient	Transient	Stable or transient
Vector localization	Nucleus	Nucleus	Nucleus	Nucleus	Nucleus	Cytoplasm	Cytoplasm	Nucleus
Types of cells transduced	Dividing only	Dividing and quiescent	Dividing and quiescent	Dividing and quiescent	Dividing and quiescent	Dividing and quiescent	Dividing and quiescent	Dividing and quiescent
Efficiency of gene transfer	High	High	High	High	High	High	High	Low
Expression of viral proteins	No	No	Yes, unless viral genes deleted	No	Yes	Yes	Yes	No
Other	Tropism can be altered by pseudotyping					Can be used as a plasmid therapy system		

VECTOR DESIGN CRITERIA

Vectors are designed and selected for disease states on the basis of the following criteria:

1. capacity to accommodate the DNA for the therapeutic gene and its transcription cassette
2. host-vector interactions, both cellular and humoral
3. capacity to target intended cells
4. control of therapeutic gene expression
5. vector replication status
6. capacity for integration into chromosomes of target cells

Selection of the route of administration and manipulation of the total dose of vector are strategies that can be used to compensate for some features of specific vector systems. The design and selection of a vector system include the evaluation of the disease of interest.

Additionally, there are advantages and disadvantages for the manufacture of each of the different vector systems. Production consistency favors those systems with well-defined fermentation or culture systems, such as plasmid, retroviral, or adenoviral vectors, or chemically defined systems, such as synthetic antisense-oligonucleotide systems. For those viral vector systems that require helper functions (see below), a rationally engineered cell line can overcome the scalability and consistency limitations of cotransfections. Engineered cell lines can also eliminate the possibility of replication-competent recombinant virus appearing in viral culture. Use of a cell line that is adapted to suspension culture can affect scalability and cost efficiency.

TARGETING TRANSDUCTION

To be effective, a vector must first find and transduce its target cell. Viruses have a natural host range that is strongly influenced by the expression levels of specific cell-surface receptors in target tissues, the cell cycle status of the target cells, and the route of administration. Integrins are a class of cell-adhesion receptors known to interact with either the penton base or the fiber protein of adenoviruses. The Coxsackie and adenovirus receptor (CAR) is also known to interact with adenoviruses. However, the expression levels of integrins and of CAR vary according to tissue type, affecting the transduction efficiency of adenoviral vectors. Amphotropic retroviruses infect cells via a sodium-dependent phosphate transporter molecule that is expressed at a detectable level in every human cell type.

The host and tissue range can be modified or targeted by a variety of approaches. Retroviruses, and lentiviruses, in particular, encode an envelope protein that mediates virus binding and entry via a specific host-cell receptor. Envelope proteins from one retrovirus may be interchanged with a protein from another retrovirus or a protein, such as the vesicular stomatitis virus glycoprotein, from an entirely different virus. This process is referred to as pseudotyping. Viral protein coats may be modified in several ways. By engineering the fiber and knob of adenovirus, it is possible to change the intrinsic integrin specificity. Similarly, viral coat proteins can be chemically modified for ligand-mediated receptor targeting. It is feasible to create ligand-plasmid fusion molecules for receptor-mediated targeting of nonviral vectors. Some lipid formulations for nonviral vectors incorporate antibody Fab fragments or ligands to target plasmid delivery.

With respect to cell cycling, adenoviruses easily infect both quiescent and rapidly dividing cells, while murine leukemia virus-based retroviral vectors are efficient only when transducing rapidly dividing cells. Lentiviral vectors can infect quiescent cells, including cells of neuronal origin. In general, nonviral vectors have lower transduction efficiencies than viral vectors. Transduction efficiencies of nonviral vectors are strongly influenced by the formulation used and route of administration.

IMPACT OF HUMORAL IMMUNE SYSTEM

Regardless of the route of administration, the intended target cell, and the dose, the vector is likely to encounter some component of the immune system as it moves toward the target cell. For viral vectors, the humoral (antibody-based) immune system cannot readily distinguish between wild-type viral infections and recombinant viral vectors because the humoral response is directed against proteins contained in the viral coat or package. Protein-containing formula-

tions of nonviral vectors can also elicit a humoral immune response. Either specific or cross-reacting humoral responses may pre-exist or they may be elicited during dosing, and the antibody response may vary in its capacity to diminish gene transduction in individual patients. It is possible to compensate for the neutralizing activity of the antibodies by increasing the vector dose or by altering the dosing interval to coincide with periods of low antibody titer. Because neutralizing capacity is frequently enhanced upon multiple dosing, effective dosing by repeated administration may be problematic. This issue is generally avoided by the use of nonviral systems. Alternatively, viral vectors can be engineered to evade the immune system. For adenoviral vectors, one approach involves increasing expression of specific viral genes that allow the virus to evade the host's humoral response.

IMPACT OF CELLULAR IMMUNE RESPONSES

Once protein expression is under way, cellular immune responses can lead to a rapid removal of both viral and nonviral vector-transduced cells from the body and a decrease in therapeutic effectiveness. Although protein synthesis is not required for cellular immune responses to viral vector envelope proteins, de novo synthesis of viral genes can exacerbate host-cellular responses. To reduce potential cellular responses, viral vectors have been designed with specific backbone deletions to eliminate the expression of viral structural genes. Examples of such vectors include the E1- and E4-deleted adenoviruses, the adenoviruses and herpesviruses in which all viral genes have been deleted (*gutless*) or are helper dependent, and the recombinant adeno-associated viral vectors. Certain plasmid sequences especially those with the C_pG motif, can elicit a strong cellular immune response.

ANTIGENICITY OF GENE PRODUCT

The therapeutic gene product may also be antigenic. When proteins that are retained in the target cell are used, cellular responses may eliminate the target cell. In some cases this is undesired therapeutic effect, particularly in the antigen-based immunotherapy for cancer or a viral disease. However, if sustained protein expression is required, the cellular immune response may decrease the effectiveness of the therapy or eliminate it entirely. The antigenicity of the therapeutic gene may reflect a variety of experimental conditions. If a gene such as the cystic fibrosis transmembrane conductance regulator (CFTR) is truncated to fit within a chosen vector, this modification may result in creation of a distinct antigen. By using the gene that encodes thymidine kinase derived from the herpes simplex virus (HSV), a foreign protein is introduced into a human subject and thus it can function as an antigen. Any patient with a monogenic deficiency disorder is at risk for lack of tolerance to the normal protein that is defective or absent in the disease state (for example, dystrophin in Duchenne muscular dystrophy).

COMPLEMENT INACTIVATION

Retroviral vectors are also subject to another host-defense mechanism—the complement component of the immune system. Retroviral vectors are reported to be rapidly inactivated by complement in sera from primates, but not from lower mammals. In considering the replication cycle of retroviruses, it is known that glycosylation epitopes are derived from the host cell during the budding process. Because many retroviral vectors used in gene therapy are murine in origin and have been grown in mouse packaging cell lines, they will have envelopes containing mouse glycoproteins. When retroviral vectors are made in human cells, they are substantially more resistant to human complement. It is reasonable to assume that the mechanism of resistance involves incorporation of natural human cell-membrane complement control proteins that have been incorporated into the vector envelope during the budding stage of particle assembly.

VECTOR LOCALIZATION WITHIN THE TARGET CELL

Once the vector reaches the target cell, several factors can affect the level and duration of therapeutic gene expression, and these factors dictate the choice of an appropriate vector system for a specific clinical indication. The localization of the vector genome within the cell, the strength of the gene expression control elements, the stability of the message, and the stability of the translated protein will all affect the therapeutic impact. Alphavirus-based vectors, such as those derived from Sindbis or Semliki Forest virus, reside in the cytoplasm and typically exhibit a very high level of gene expression. Retroviral, adenoviral, and other viral vectors have advantages in gene delivery with their natural mechanisms for nuclear delivery of the therapeutic gene and reasonable levels of gene expression from viral or other promoters. Nonviral plasmid vectors are episomal and are often susceptible to DNA degradation when they are shunted into cell endosomes. However, some nonviral systems incorporate nuclear targeting signals as a means of increasing therapeutic gene-transcription efficiency.

TISSUE-SPECIFIC PROMOTERS

Another means of controlling gene expression is to incorporate tissue-specific promoters to stimulate or to restrict expression of the therapeutic gene. Drug-responsive promoters are being used to control gene expression. Rapamycin, mifepristone, or the tetracycline *on* systems have been used to repress gene expression. This type of regulation may be required for certain proteins, such as erythropoietin, where constitutive expression may produce toxicity.

IMPACT OF REPLICATION STATUS OF VECTOR

Replication status is another important consideration for vector design and selection. Viral vectors are most frequently constructed to be incompetent or replication-defective in order to limit uncontrolled vector spread and pathogenicity. However, when effective therapy requires infection of virtually all the target cells, replication can be engineered to be conditional when specific viral gene interactions are matched with intracellular pathway targets. When these targets are defective or missing, such as in cancer cells, the virus can replicate, but when the target cell is functioning normally, viral replication is repressed. One of the risks inherent in the use of conditionally replicating viral vectors is that the growth of the virus is not absolutely restricted to a single cell type, that is, the system may be *leaky*. As compensation, the susceptible target cells may be efficiently transduced at a dose that is significantly lower than that necessary for non-target cells.

Nonviral vectors are normally designed as nonreplicating systems, but some groups are experimenting with replicating nonviral plasmids to increase gene expression levels given the low transduction efficiency of most nonviral systems and to increase the duration of gene expression. Additional preclinical studies are needed to establish the safety of these systems. Artificial chromosomes have also been designed to take advantage of normal mechanisms for retaining gene expression in rapidly dividing target cells.

VECTOR INTEGRATION

The duration of gene expression is also a function of the stability of the vector genome. Retroviral vectors can stably integrate into the host-cell genome. Adenoviruses do not integrate because their DNA remains episomal. Recombinant AAV vectors integrate, but because the *rep* genes responsible for site-specific integration are normally excluded from the construct in order to increase the vector-packaging capacity, integration is not site-specific as it is for wild-type AAV. Nonviral plasmid DNA does not integrate efficiently. However, stable episomes have been observed in certain cell types, such as muscle cells. Site-specific integration can be a desirable feature for vectors intended to correct genetic disorders. Although it is not currently possible, the control of the site of integration is desirable in order to prevent insertional mutagenesis. Insertional mutagenesis

has the potential to kill a cell, if a critically functioning gene is inactivated, or to predispose a cell to malignant transformation, if a tumor-suppressor gene is inactivated.

The success of any gene therapy product is dependent on the relationship between the vector-delivery system and the requirements of the disease application in terms of the site, level, and duration of therapeutic gene expression. It is unlikely that there will ever be a universal vector, and the challenge is in fitting the vector to the disease.

Manufacturing and Purification Strategies

VECTOR CONSTRUCTION

Viral and nonviral gene-transfer vectors are constructed by using standard molecular biology protocols. For viral vectors, the vector backbone consists of viral RNA or DNA sequences from which the regions encoding viral structural genes or the regions required for replication have been deleted. The deleted region of the vector is usually modified with specific restriction endonuclease sites used to allow insertion of the gene of interest. For nonviral vectors, the plasmid DNA backbone contains multiple restriction sites for cloning and the bacterial elements necessary for plasmid production. Vector backbones can accommodate single or multiple gene inserts depending on the maximum amount of sequence they can carry. The promoter that facilitates transcription of the gene insert can be a related viral promoter, such as murine leukemia virus long terminal repeat (MuLV LTR), or a heterologous promoter that is either tissue-specific, such as alpha crystalline promoter (of the eye), or constitutive, such as cytomegalovirus (CMV). For example, in a retroviral vector construct containing two gene inserts, transcription of one is regulated from the 5'-LTR-promoter sequence, while a second gene insert can be linked to an internal heterologous promoter from Simian virus 40 (SV40). The complementary DNA (cDNA) containing the therapeutic gene of interest, including its introns, is excised from its source by using restriction enzymes and is inserted at the multiple cloning site of the gene-transfer vector. The polyadenylation signal can be derived from multiple sources such as the SV40 virus or human growth hormone. Characterization and testing of gene therapy vectors are described under *Analytical Methodologies*.

HELPER FUNCTION SYSTEMS

Recombinant viral vectors are most often modified to be replication-defective, a condition created by deletion or modification of the viral genes needed for replication and production of infectious virus. As a result, viral vectors require help to produce infectious vector particles. Helper functions are often provided by packaging cell lines to deliver the necessary viral element from a source outside of the gene of interest (*in trans*). Packaging cell lines should be designed to minimize the risk of production of RCV through recombination between the vector and the packaging elements.

Plasmids encoding the necessary elements are introduced into the packaging cell by standard methods such as calcium phosphate-mediated transfection or electroporation. If multiple trans-acting elements are needed, these elements are introduced on separate plasmids in order to increase the number of recombination events needed to form a wild-type viral genome, thus decreasing the frequency of the event. An additional approach to eliminate production of RCV is the elimination of common sequences between the packaging cell plasmids and the gene therapy vector.

Stable packaging cell lines should be selected and clonal MCBs prepared. In retroviral vector production systems, typically the proviral form of the retroviral vector is stably incorporated into the packaging cell, resulting in what is referred to as the producer cell line. A stable, banked packaging cell-producer line will lead to consistency in production and control of adventitious agent contamination. Alternatively, the system can be transient, with the packaging plasmids transfected along with the gene therapy vector for each round of vector production. However, a transient transfection system is less efficient and limited in scalability.

Typical helper function systems are as follows:

1. **Retroviral Vector Systems**—The murine fibroblast cell line NIH 3T3 has been the basis for several packaging cell lines.

The *gag*, *pol*, and *env* functions can be co-located on a single plasmid (PA317) or placed on individual plasmids (psi-CRIP). This increases the number of recombination events required to produce an RCV. The human embryonic kidney cell line 293 has been modified to be a packaging cell line for retroviruses, because use of a human cell line allows production of a retroviral vector that is not affected by the human complement system.

2. **Adenoviral Vector Systems**—HEK 293 cells are widely used to supply the E1 function necessary for efficient adenoviral replication that is deleted from first-generation adenoviral vectors. Other complementing cell lines, such as E1-modified A549 cells (human lung carcinoma) and the PER.C6 cell line (human embryonic retinoblast), have also been created to supply E1 or other missing functions. The latter contains the E1 region under the control of a phosphoglycerate kinase (PGK) promoter and has no flanking adenoviral sequences in order to eliminate production of replication-competent adenovirus (RCA).
3. **AAV Vector Systems**—These systems classically use adenovirus-infected human 293 cell lines transiently transfected with AAV helper plasmid containing the *rep* and *cap* genes, which are required for AAV replication and capsid formation, respectively, and which are deleted from the AAV vector. The HeLa cell line (from human uterine cervical carcinoma) has also been used as a transient production system. More recently, both of these cell lines have been used to establish stably transfected packaging cell lines that express *rep* and *cap* genes and in some cases express the adenoviral functions needed for AAV replication when *rep* and *cap* are present (E1a, E1b, E2a, E4, and VA RNA).
4. **Gutless Adenoviruses**—The manufacturing systems for gutless adenoviruses are similar to classical AAV vector manufacturing systems in that human 293 cells are transiently transfected with helper plasmid containing required adenoviral functions.

VIRAL GENE THERAPY VECTORS

Retrovirus and adenovirus have classically been produced on the laboratory scale by using traditional cultivation methods for anchorage- and serum-dependent cell lines, employing flasks, trays, and roller bottles. Initially, gene therapy vectors were produced by using these exact methods because large volumes of product were not required for early clinical studies. Cell bank systems are used as the source of cells and virus banks as the source of virus for clinical production. In most cases, supernatant is collected, clarified, and stored frozen in bags at -70° . In many early clinical trials unpurified supernatant has been used for ex vivo gene transfer.

More recently, larger-scale upstream production methods have been reported including suspension, bioreactor, and fixed-bed or microcarrier culture methods. Some groups have reported adapting their process cells to serum-free culture conditions. Cells are harvested and lysed or supernatant collected. The harvest is clarified and purified to remove host-cell debris, host-cell DNA, and other process-derived contaminants.

Traditionally, viruses are purified by gradient ultracentrifugation, but this is time-consuming and unsuitable for larger-scale production purposes. The selection of downstream process steps and their sequence is determined by the nature of the virus itself and the upstream process used for manufacturing the virus. As processes are being developed for the manufacture of gene therapy vectors, many different purification steps have been reported. These include ion-exchange and sulfonated-cellulose chromatography, zinc ion affinity chromatography, size-exclusion chromatography, and DNase or other nuclease treatments. AAV production and lentiviral production are complicated by a need for transient transfection or cotransfection of plasmid or helper virus. These processes have so far required anchorage-dependent cell lines that are difficult to scale up. The development of stably transfected cell lines would allow large-scale production.

PLASMID VECTORS

Plasmids are double-stranded, circular DNA molecules that exist in bacteria as extrachromosomal, self-replicating molecules. They have been modified to serve as cloning systems, to contain multiple restriction endonuclease recognition sites for insertion of the cloned transgene, and to contain selectable genetic markers for identification of

cells that carry the recombinant vector. Plasmid-based nonviral vectors are frequently used as gene delivery systems for both in vivo and ex vivo gene therapies. They are in the form of naked DNA or complexed with lipids or other agents that facilitate transfer across the cell membrane and delivery to the cell nucleus without degradation. An advantage of a plasmid-vector system is the efficient production of large quantities of the vector that is easily characterized and involves no risk of contamination with the RCV.

Nonviral vectors are typically produced by using an *Escherichia coli* bacterial system. Plasmids are transfected into *Escherichia coli*, and an appropriate single bacterial colony is selected and expanded to create an MCB. After reselection of a colony from a bacterial plate inoculated from the MCB, plasmid DNA is isolated from cultures that can range in size from 1 liter on a laboratory scale to hundreds of liters in bacterial fermenters. Plasmid DNA can be purified by several methods including affinity or ion-exchange chromatography and cesium chloride-ethidium bromide density gradients. Cesium chloride-ethidium bromide density gradients are not recommended for production of clinical-grade material.

OLIGONUCLEOTIDE VECTORS

Antisense oligonucleotides are manufactured by synthetic chemistry procedures. Currently, the method of choice is solid-phase phosphoramidite chemistry. Synthesis is linear, rather than convergent, and a high level of efficiency must be maintained at each synthesis step. This is accomplished by using molar excesses of highly pure raw materials to drive the reaction kinetics towards completion. Synthetic oligonucleotide manufacturing may require metric-ton quantities of nucleoside phosphoramidites and other compounds such as activator and sulfur-transfer reagents for commercial-scale manufacturing. An issue for oligonucleotide manufacturing is that during the preparation of raw materials and the synthesis of oligonucleotides, precaution must be taken regarding moisture, because moisture is detrimental to both yield and purity. Purification of the single-strand oligonucleotide product requires removal of residual solvents and synthetic strand by-products. Nevertheless, current oligonucleotide-manufacturing technology is readily scalable and cost-efficient, and it results in products with purity levels similar to those of classical small-molecule pharmaceuticals.

FORMULATION OF GENE THERAPY PRODUCTS

Final formulations for vector products are still in early development. So far, mannitol, sucrose, lipids, polymers, and serum albumin have been utilized as stabilizers. Aseptic filling of large numbers of vials by using classical manufacturing processes may be problematic. For example, some viral vectors are thermally sensitive and storage at ultra-low temperatures is often required. Progress is being made for both viral and nonviral vector lyophilization and in the use of stabilizers for liquid formulations.

ON-SITE PREPARATION AND ADMINISTRATION

General Considerations

One or more product modifications or preparative steps may be required prior to administration of the cell or gene therapy product to the patient. These modifications or steps are frequently performed close to the time of administration; and, therefore, they are performed under conditions not under control of the original manufacturing facility. The nature of these modifications is largely dictated by characteristics of the product in relationship to the particular application. These include thawing, washing, or filtration to remove unwanted cells or substances accumulated during storage, transfer to an infusible solution, or compounding with a vehicle or structural material. In addition, patient considerations, such as the need to dose the product according to patient weight or blood volume, may influence these steps.

All product modifications performed between the time of initial product manufacture and final administration to the patient should be viewed as a part of the overall manufacturing process. The practical implications of this concept are that the process controls must be established for all product storage intervals, transport steps, and modifications, starting with a clear definition of critical control points. Operational requirements for performing any of these steps after initial product manufacture include defined physical space with appropriate environmental controls, trained personnel, detailed standard operating procedures, and a comprehensive quality program.

The unique and irreplaceable nature of many cell and gene therapy products, many of which have originated from an autologous or a selected allogeneic tissue source, creates special considerations for product manufacture, release, and administration. It is critical to both anticipate the need for and to establish policies and procedures to guide product modification or administration steps in cases where predefined release criteria cannot be met. These procedures should include a mechanism to obtain medical consultation to assess risk and benefit considerations for the patient and to provide complete documentation of any decision to modify predefined product specifications.

On-Site Preparation

PRODUCT MANIPULATIONS

Prior to administration, on-site preparation of the cell or gene therapy product may involve one or more manipulations. These manipulations include the following:

1. **Change in Final Container**—The manufactured product may have been stored or transported in one container but may require transfer to a different container for administration.
2. **Change in Physical State or Temperature**—A product may require thawing from the frozen state or warming from the refrigerated state.
3. **Change in Solution or Suspension**—A product may have to be dissolved, diluted, or suspended in a liquid.
4. **Addition to Biocompatible Structural Material**—A cell or gene therapy product may need to be combined with living, natural, or synthetic structural tissue or matrix. Examples of matrix material include hollow fibers, fibrous sheets, gels, plugs, capsules, sponges, or granules.
5. **Admixture or Compounding with Other Nonstructural Materials**—A product may require mixing or compounding with drugs, cytokines, biologics, or other nonstructural materials.
6. **Filtration or Washing**—Unwanted materials in the manufactured product, such as particulates, cellular debris, metabolites, or compounds remaining from previous manipulations may require washing or filtration steps.
7. **Sampling**—Sampling of the final product immediately prior to administration may be required for certain clinical protocols.

FACILITY REQUIREMENTS

Facility requirements for performing on-site preparative steps or administration of cell and gene therapy products depend on the nature of the products, their applications, and the manipulations required. The most important determinant of facility features is the level of risk for microbial contamination associated with each step. Definition of low-risk and high-risk conditions can be made according to a framework similar to that defined for *Low-Risk* and *High-Risk* in the *Risk Levels* section under *Sterile Drug Products for Home Use* (1206).

RELEASE OF FINAL PRODUCT

Cell and gene therapy products that undergo on-site preparative steps or manipulations must be subjected to appropriate checks or tests to ensure that all quality specifications are met prior to release for patient administration. The nature and extent of manipulations

will determine whether release requirements or critical specifications must be added to those required immediately after initial manufacture. Pre-release requirements usually include the following:

1. physical inspection of the product, which typically includes measures to ensure appropriate product appearance with regard to color, turbidity, particulates or foreign matter, container integrity; product temperature; and accuracy and convenience of labeling;
2. review of process records; and
3. for patient-specific products, clerical checking of product labeling or records in relationship to identity of the intended recipient.

In addition, products considered to be high-risk products according to the description under *Sterile Drug Products for Home Use* (1206) should undergo additional product testing. For all high-risk products, quality assays for the identity, potency, and purity of the active ingredients should be defined and performed. For high-risk products in Category II, sterility and endotoxin testing should be performed.

Administration to Patients

PRE-ADMINISTRATION REQUIREMENTS

Depending on the specific cell or gene therapy application, steps may need to be taken by trained patient-care staff to prepare the patient for product administration. These steps are aimed at ensuring that the product will provide the intended therapeutic outcome and aimed at minimizing the risk of adverse effects.

In cases where autologous, selected allogeneic, or xenogeneic tissue is the source of the cell or gene therapy product, determination of the patient suitability for the therapy, including the evaluation of histocompatibility between the donor and the recipient, typically occurs prior to the product preparation. However, because of the possibility of changes in clinical status of the patient after the time of tissue collection, such as fever, infection, recurrence or spread of tumors, or organ dysfunction, a thorough re-evaluation of the patient's general condition and suitability for therapy must be performed in close proximity to product administration. This evaluation usually includes a patient history, physical examination, and laboratory studies such as blood counts and chemistries. In addition, baseline physical or functional measurements, laboratory tests, or imaging studies relevant to the specific application may be obtained. Examples include pulmonary function tests for a therapy aimed at improving lung function, measurement of blood levels of an enzyme that is the gene product in a gene therapy application, and nuclear imaging of organs prior to anticancer therapies.

A variety of patient interventions related to route of product administration may be required before product administration. For cellular therapies requiring intravenous administration, patients with poor peripheral venous access may require placement of a central venous catheter. In applications where cells or matrices combined with cells are implanted into the patient, the site of implantation may require preparation in the operating room. This may involve surgically opening the site, removing the degenerated or damaged tissue, trimming of the adjacent tissue to accommodate the implant, and excising the tissue from a second site to be used as an anchor or support for the implant. For instance, in the case of cell products for wound healing, it is critical that the site for grafting be free from infection and demonstrate a well-prepared wound bed. In cells intended to repair cartilage defects, the site of damage needs to be prepared so that the cells can be applied to a watertight compartment. For applications involving direct administration of the product into an organ system (for example, bronchioalveolar system) or vascular network (for example, coronary arteries), the patient may require endoscopic or surgical access to these sites.

In all cases, the need for adequate anesthesia and premedication must be carefully evaluated in conjunction with these steps prior to product administration. For example, if it is anticipated that DMSO will remain in a thawed, cryopreserved cellular product, the patient is given an antihistamine prior to administration of the cells to block adverse effects associated with histamine release induced by the DMSO. Pre-administration patient evaluation must also include assessment of concurrent therapies that may interact with the cell or gene therapy product to modify its effects. Some therapies may be considered adjunctive to the cell or gene therapy, such as cytokines

that promote proliferation or differentiation of the infused or implanted tissue. Other commonly used drugs such as antibiotics, anti-neoplastics, anticoagulants, and anti-inflammatory agents must be evaluated for possible effects on the efficacy of the cell or gene therapy product.

PATIENT TREATMENT

Some cell or gene therapy products are patient-specific, in that they are manufactured from a selected tissue source, such as autologous, selected allogeneic, or xenogeneic tissue. Certain patient-specific products have a defined potential for benefit or adverse immunoreactivity. Systems must be in place to prevent administration of such a product to the wrong patient. Recommended systems include procedures similar to those used for administration of human blood products, with special attention given to the correct identification of the patient and patient-specific product by at least two people immediately prior to administration.

Cell and gene therapy products can be administered by a variety of routes. These include the intravenous route, the parenteral routes (subcutaneous, intramuscular, and intra-arterial), and the respiratory or gastrointestinal tract route. Other possibilities include direct application of cell or gene therapy products into regional vasculature, organs, tissues, or body cavities by means of needles or catheters or following surgical exposure of the tissue. While parenteral administration can be accomplished in routine outpatient or inpatient facilities, the other means of administration may require specialized facilities, such as an aseptic operating theater or endoscopic suite. In all cases, standard operating procedures and a quality program must be in place to ensure that the product is administered in the intended manner.

POSTADMINISTRATION MONITORING OF PATIENT

There should be written policies and procedures for monitoring patient outcomes and managing reports of adverse events. Patient-outcome assessment should include indicators that are likely to detect errors or problems related to the entire manufacturing process, with special attention given to manipulations, storage, or transportation after the initial manufacture of the product. Management of adverse reactions should include procedures for ensuring prompt medical evaluation and treatment of patients with suspected adverse effects and a system for reporting and evaluating adverse effects that may point to a potential defect in the administered product. Reporting procedures include providing details required for federal, state, or USP adverse-event reporting programs.

Follow-up and monitoring procedures should be implemented for patients who have received gene therapy vectors or ex vivo gene therapies. To the extent that it is relevant and that it can be assessed, vector or modified cell biodistribution and persistence in vivo should be monitored. With direct administration of vectors, localization to the germ line may be an issue. Although preclinical studies can be used to address this issue, useful information may be gained through patient monitoring. In the case where a retroviral vector has been administered, patients should be monitored for replication-competent retrovirus (RCR) according to the FDA's *Draft Guidance for Indus-*

try: 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 (October 2000). This involves active monitoring during the first year and archiving of patient samples thereafter if RCR is not detected initially.

Database systems to collate and track patient-monitoring results are essential to management of this information. National registries or publication of data should be considered for establishing the collective safety of gene therapy.

ANALYTICAL METHODOLOGIES

General Considerations

The complexity and scope of cell and gene therapy products is reflected in the wide range of analytical methods that are used to assess product quality. Approved cell and gene therapy products must comply with applicable sections of 21 CFR 211 and 21 CFR 610 to ensure their identity, dose, potency, purity, and safety. Specific guidance for the identification, development, and validation of analytical methodologies to support cell and virus bank characterization, final-product release, and stability studies is currently provided in the Center for Biologics Evaluation and Research (CBER) *Points to Consider for Human and Somatic Cell and Gene Therapy* (April 1998); under *Validation of Compendial Methods* (1225); and in the ICH guidelines entitled "Q2A Validation of Analytical Procedures", "Q2B Validation of Analytical Procedures: Methodology"; and "Q6B Specification, Tests and Procedures for Biotechnological/Biological Products". Most product-specific analytical methods for cell and gene therapy products have not been standardized. Even well-defined tests such as those described under *Sterility Tests* (71) may not be directly applicable to certain cell and gene therapy products. For some cell and gene therapy products, large quantities of clinical material may not be available during early clinical development. Some required tests (e.g., sterility) may have to be modified. Consultation with the regulatory authorities is advised.

Table 6 provides an overview of the product-specific testing parameters for the biological component and general methods or approaches being used to satisfy the testing requirements for cell therapy products and for nonviral, viral, and antisense-oligonucleotide gene therapy products. The analysis of cell and gene therapy products relies heavily on biological assays but it also utilizes methodologies developed for biotechnology-derived products (see *Biotechnology-Derived Articles* (1045)). Antisense-oligonucleotide products are regulated by the FDA Center for Drug Evaluation and Research (CDER) and rely heavily on physicochemical methods. The intent of this section is to outline the types of methods and their specific applications with regard to product characterization, stability, and release testing. Process validation may alleviate the need for certain specific lot-release tests. Development of appropriate reference materials and standards for cell therapy and viral and nonviral gene therapy products should be a part of product development. Reference materials should be fully characterized in order to provide continuity between standards over time. In the case of cellular products the reference material may be a surrogate tissue or simulated product.

Table 6. Analytical Tests for Cell and Gene Therapy Biological Products

Test	Cell therapy Products	Gene Therapy Products	
		Viral	Nonviral and Antisense-Oligonucleotide
Identity of Biological Substance	Surface marker determination	Restriction enzyme map	Restriction enzyme map
	Species	PCR	PCR
Dose	Morphology	Immunoassay for expressed gene	Immunoassay for expressed gene
	Bioassay	Sequencing	Sequencing
	Biochemical marker		
	Viable cell number	Particle number	Plasmid-DNA weight
	Enumeration of specific cell population	Transducing units (DNA hybridization assay)	Formulated-complex weight
Potency	Total DNA	Total protein	HPLC or capillary electrophoresis assay using authenticated reference standard
	Total protein	HPLC assay using authenticated reference standard	
	Viable cell number (cells intended for structural repair)	Function of expressed gene (induction of secondary effect and other bioassays)	Function of expressed gene (induction of secondary effect and other bioassays)
Purity	Bioassays:		
	Colony-formation assay		
Safety	Function of expressed gene		
	Induction of secondary effect (e.g., human leukocyte antigen (HLA) induction, secretion of cytokines, and up-regulation of surface marker)		
	Percentage of viable cells	Residual host-cell DNA	Percentage of specific physical form (e.g., percentage supercoiled)
	Percentage of transduced cells	Process contaminants (e.g., serum and cesium chloride)	Residual host-cell DNA
	Percentage of cells with specific surface marker	Residual helper virus	Residual RNA
	Process contaminants (e.g., serum)	Optical density ratio	Residual host-cell proteins
		Residual host-cell proteins	Residual solvents
		Viral protein profile (HPLC assay for defective or immature particles)	Optical density ratio
		Residual RNA	Process contaminants (e.g., cesium chloride and synthetic oligonucleotide by-products)
			Pyrogen and endotoxins
Safety	Mycoplasma	General safety	Sterility
	Sterility	Mycoplasma	
	Pyrogen and endotoxins	Sterility	
	Adventitious viruses	Pyrogen and endotoxins	
	Residual virus (for transfected cells)	Adventitious viruses	
	Replication-competent vector virus (transfected cells)	RCV	

Cell therapy products may require a *rapid-release* approach if they have a limited shelf life. The rapid-release approach is not usually applied to viral and nonviral gene therapy products because these products are sufficiently stable for completion of testing prior to release. Some formulated nonviral gene therapy products also have limited shelf lives. In these cases, the individual components are tested prior to release and the formulated complex is not tested. The formation and stability of the formulated nonviral gene therapy complex is established through validation studies during product development.

As specified in the CFR, product samples must be retained after product-release testing is completed. Additional samples may need to be retained if rapid-release strategies are employed, so that the product quality can be reassessed by alternative or traditional test methodologies if necessary.

New Methodologies and Compendial Perspective

Although the application of compendial methods is encouraged, there are many instances where the analytical method that best addresses the issue is a new method not found among the compendial methods. USP encourages the development of appropriate methods and submission of these methods, once validated, to the USP for addition to the compendia.

One such new methodology is the PCR-based assay. PCR-based assays are utilized in a variety of applications for both cell and gene therapy products. PCR assays can be a viable substitute for long-term bioassays and should be considered when a rapid-release strategy is used. Other applications include the use of PCR-based assays to assess adventitious viral agents in product or in cell and virus banks.

PCR might also be used in an identity test of a gene therapy product. In all cases, validation of the new method and assay equipment and qualification of analytical personnel are required.

PCR assays are based on amplification of specific target DNA by using PCR technology. Traditionally, a pair of DNA oligonucleotide primers is used in combination with nucleotides and the *Taq* polymerase to increase the amount of a specific-size oligomer in a series of alternating cycles of permissive and nonpermissive polymerase temperature conditions. The reaction mix is resolved by gel electrophoresis and visualized by staining with ethidium bromide in order to detect the amplified target (amplicon). RT-PCR involves the use of a reverse transcriptase to create cDNA from RNA prior to performing the PCR reaction, so that the RNA can be detected. PCR and RT-PCR methods can be used qualitatively (positive or negative readout) or quantitatively.

Currently there are two common approaches to quantitation using PCR: (1) competitive PCR that involves use of a spiked mimic and (2) *real-time* or kinetic PCR that is based on the 5' nuclease activity of the *Taq* polymerase. In competitive PCR, quantitation is based on the ratio of the amplified mimic to the amplified target. In *real-time* PCR, the degradation of a dual-labeled, fluorescent, target-specific oligonucleotide probe is monitored in real time, as PCR amplification is occurring. The probe is labeled with a reporter fluorescent dye at the 5' end and a quencher fluorescent dye at the 3' end. When the probe is intact, the fluorescence emission of the reporter is quenched due to the physical proximity of the two dyes. The probe sequence is selected so that it hybridizes to the target between the two primer sites. During the extension phase of the PCR cycle, the probe is cleaved by the 5' nuclease activity of the *Taq* polymerase, while the reporter dye signal is increased by the release of reporter dye from the probe. Additional reporter dye molecules are cleaved during each cycle, resulting in an increase in the fluorescence intensity of the reporter dye

proportional to the amount of amplicon produced. The resulting relative increase in reporter fluorescent dye emission is detected in real time during PCR amplification and it allows the threshold cycle number to be related to the target copy number. The threshold cycle number is defined as the PCR cycle number where the increase in reporter fluorescence is detected above the background fluorescence in the assay system. A greater quantity of input DNA or messenger RNA (mRNA) results in a lower threshold cycle, as a result of requiring fewer PCR cycles for reporter fluorescence-emission intensity to reach the threshold. Typically, assays can be designed to detect 1 to 10 copies of the target per reaction.

Like all analytical methods used to release product, PCR assays must be validated. Validation should include the rationale for the selection of primer and probe sequences and a demonstration of the specificity and efficiency of the primers and, for real-time quantitative PCR assays, of the probe. Because primers and probes are the main components of a nucleic acid-based detection system, the performance of the assay is highly dependent on the quality of these reagents. Specificity is generally demonstrated by assessing the resulting PCR product by gel electrophoresis to show that the amplicon is the expected size. For quantitative assays, the design and nature of the quantitation standards must also be addressed.

Assay validation must also address linearity, accuracy, ruggedness, and reproducibility with regard to both the assay itself and the sample preparation, that is, extraction of the sample DNA for PCR or sample RNA for RT-PCR. Validation should include a demonstration of the specific limit of detection in the sample type employed, because some sample types contain inhibitors of PCR. Validation should also address the reproducibility of the sampling scheme and the efficiency of nucleic acid extraction and purification procedures to produce the starting material (DNA or RNA). Well-designed spiking studies where samples are spiked both prior to and after extraction can address these issues.

PCR assays are occasionally subject to false-positive results because of the contamination of equipment or samples during handling in preparation for the assay. The most abundant source of contaminating target nucleic acid is the previously generated amplicon. However, the PCR reaction can be modified so that the resultant amplicon is sensitive to uracyl-*N*-glycosylase digestion and can therefore be eliminated. In addition, isolation of sample preparation areas from other phases of the assay and the use of dedicated equipment for each assay phase are generally necessary to prevent amplicon contamination of test samples and, hence, false-positive signals. Assay protocols that include appropriate controls, such as nontarget sequence and nucleic acid-free controls, can aid in determining the source and point of contamination if it occurs. Validation should address the procedures implemented to prevent contamination.

Sampling Issues

Sampling for lot-release testing should be based on the potential distribution for the parameter tested. See *Stability-Protocol Development under Stability* for additional considerations. Samples from each lot need to be retained in case there is a safety or quality issue with the lot. Even if the product has a very short shelf life, these retained samples can be used to detect impurities and other substances. The need for proper design of the sampling scheme is highlighted in safety testing for adventitious agents for cell or viral gene therapy products or in assessment of RCV for viral gene therapy products. In such cases, process validation will assist in determining the appropriate statistically based sampling design.

Safety

GENERAL CONSIDERATIONS

Safety testing for cell and gene therapy products focuses on three issues: (1) preventing the unwitting use of contaminated cells, tissues, or gene therapy agents with the potential for transmitting infectious diseases, (2) preventing the use of improperly handled or processed, and consequently contaminated, products, and (3) ensuring safety when cellular and gene therapies are adapted for use other than in their normal functions or setting.

The primary means of assessing safety are the performance of biological assays to measure adventitious agents directly. Molecular biology-based assays that measure adventitious agent DNA or RNA are also used.

CELL THERAPY PRODUCTS

Direct transmission of infectious disease is a major concern for cell therapy products. The degree of risk is dependent upon various factors such as whether the cells or tissues are to be used in a person different from the one they were obtained from; whether they are banked, shipped, or processed in a facility that handles cells and tissues from multiple donors; and how extensively they are processed. Improper handling can alter the integrity and function of cell therapy products by introducing microorganisms or by contaminating the therapeutic cell products with other donor or patient cells during collection, processing, or storage.

In addition to transmittable-disease screening and testing of allogeneic donors of all viable and nonviable tissues intended for use as cell therapy products, appropriate labeling and tracking are required. These requirements are not only based on the potential risk of disease transmission from donor to recipient but also on the following: (1) the unusual, but documented, possibility of product-to-product transmission (for example, viral contamination may occur among disrupted bags in the liquid phase of a liquid nitrogen freezer) or (2) the possibility of erroneous administration of a product to the wrong recipient. Specific donor screening and testing requirements for allogeneic cell products are based on those currently required for human blood products. These include (1) specific donor testing for HIV Type 1, HIV Type 2, hepatitis B, hepatitis C, and syphilis and (2) medical history screening for high risk for HIV, hepatitis B, Creutzfeldt-Jakob disease, and tuberculosis. Some of these tests and screening measures are also recommended, but not required, for autologous tissues.

The risk of cross-species infectivity during xenotransplantation is still unknown. Assessing the risk of infection from a new transmissible agent is difficult. In vitro coculture assays involving sensitive human indicator cell lines for the donor species should be developed. In particular, assay of endogenous retrovirus (ERV) present in the xenogeneic cell or tissue is required. In the case of porcine cells and tissues, both PCR and RT-PCR assays for porcine endogenous retrovirus (PERV) have been described and are applied to donor cells and tissues. These tests are also being used for patient monitoring. Assays for PERV antibody have also been developed for patient monitoring. Published studies indicate that the risk of PERV transmission to patients may be low.

Often the shelf life of cell therapy products is shorter than the time required to test for sterility and adventitious agents using traditional cell-based methods. However, as already discussed, development of validated rapid PCR-based methods allows both assessment and timely release. Presence of mycoplasma and a range of specific adventitious viruses and bacteria can be tested by using PCR or DNA- or RNA-hybridization dot blot analysis. Fourteen-day sterility testing is not always a viable alternative for final release of cell therapy products; in these cases automated methods that rely on colorimetric detection or on continuous monitoring may be acceptable if they are validated. Facility and process validation are necessary adjuncts to ensure safety with regard to sterility and mycoplasma, particularly when rapid-release strategies are employed.

Additional testing for safety may be required when cell therapy products are used in the patient for a purpose other than that which the cells or tissue fulfills in its native state or when placed in a location of the body where such structural function does not normally occur. Testing should be designed to predict product behavior under these settings and should be designed based on the context of use. For example, in the case of a cell therapy product for cancer patients, where the cells are activated by culture on a feeder layer of cells during processing, it may be necessary to test the product cells for the presence of feeder cells. Residual feeder cells in the final product may cause an inflammatory response. In addition, products using non-human feeder cells are considered xenogeneic products. Cell therapies are exempted from general safety testing.

If the cells were modified by a viral gene vector during manufacturing, presence of RCV must be tested. Typically RCV testing (see *Viral Gene Therapy Products under Safety*) is limited when rapid release is required by shelf life. Again, molecular biology-based meth-

ods such as PCR can be used in rapid screening situations. In these cases, during product development, testing that employs cell-based assays (for example, detection of cytopathic effect on indicator cell lines) is performed after release to validate the molecular biology-based test result.

VIRAL GENE THERAPY PRODUCTS

One of the primary safety concerns associated with viral vectors used for gene therapy is RCV. Regardless of the virus, these concerns are based on the potential lack of predictability for the pathogenicity of a contaminating virus for a specific route of administration, particularly if it is not the normal route of infection or if humans are not a natural host for the virus.

The pathogenesis of a wild-type adenovirus infection is known but may not be predictive for the routes of administration employed with recombinant adenoviral vectors. For adenoviral vectors, a limit of one RCA per dose is currently considered acceptable; other limits have been established for dose levels greater than 1×10^9 particles, specific indications, and routes of administration based on preclinical safety studies and patient-monitoring studies during clinical development. Limits as high as several thousand RCAs per dose have been reported. Typically, RCA levels are determined by using a cell-based assay that allows amplification of the RCA while preventing replication of the product. The cell line recommended for amplification and detection of RCA is the A549 cell line. However, some recombinant adenoviral vectors express therapeutic genes that interfere with analysis on A549 cells. In these cases, a bioassay utilizing two cell lines is used, with the first cell line chosen on the basis of resistance to the effects of expression of the therapeutic gene of interest and with subsequent passage of cell lysate onto A549 cells for amplification and detection of the RCA. RCA is most often detected by visual observation of the cytopathic effect but it may also be detected in the A549 cell culture by using immuno- or PCR-based methods. Quantitation of the RCA level is based on the quantity of sample tested and the detection limit of the assay. Typically, RCA bioassays are validated as being able to detect 1 plaque-forming unit or infectious unit of RCA in the test sample over a wide range of test-sample sizes. Test-sample sizes can range from 1×10^8 to 1×10^{12} particles but they are typically based on clinical-dose size. To verify detection limits, spike controls should be included as part of the test, even with validated assays. For recombinant adenoviruses produced using 293 cells, RCA detection by PCR methods can be confounded by detection of residual 293 host-cell DNA (detection of the E1 region). PCR assays, however, can be designed to specifically quantitate host cell DNA contamination and can be made specific to particular forms of slow-growing RCA. Quantitative PCR assays can be used in conjunction with a cell-based method for precise quantitation of RCA levels. When a tested sample is found to be positive, the identity of the RCA is usually confirmed by conducting PCR analysis. This rules out the possibility that contamination of the assay by exogenous wild-type adenovirus or other adventitious agents is responsible for the positive result.

For retroviral vectors, testing for RCR is required for cell banks, viral vector production lots, and any resultant ex vivo product lots (see the FDA's *Draft 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*, October 2000). Standard assays have been designed to detect replication-competent murine leukemia virus (MLV). The pathogenesis and potential long-term toxicity of low-level amphotropic MLV in human beings is not known. Methods commonly used to detect RCR include an amplification of virus titer by application of product to a replication-permissive cell line such as *Mus dunni*. Because infection is limited by the ability of a virus to reach the cells through Brownian motion, procedures (e.g., centrifugation and filtration) that physically bring the virus into contact with the cells may be used to enhance detection. However, high-titer recombinant vector can interfere with the detection of low-level RCR and this interference may be enhanced through such methods. Infected cells are passaged several times to allow viral replication. Culture medium is harvested at the end of the culture period and RCR detected by using an indicator cell line. If the product is an amphotropic MLV, RCR may be detected by using a feline cell-based PG4 S+L- assay, a mink cell-based MiCl S+L- assay, or a marker rescue assay. In S+L- assays, the RCR expresses proteins that

lead to transformation and subsequent plaque formation on the monolayer. In a marker rescue assay, RCR infects a cell line that expresses a retroviral vector encoding a marker gene such as β -galactosidase, drug resistance, or a fluorescent protein. The vector is packaged by the proteins supplied to it in trans by the RCR. The potentially vector-laden supernatant is transferred to naive target cells that are then screened for expression of the marker vector.

Testing for RCR is performed by cocultivation of the cell line or amplification of vector supernatant with an RCR replication-permissive cell line, typically *Mus dunni*, for several passages. Culture medium is harvested at the end of this cocultivation process and applied to an appropriate indicator cell line as described above. It is important to note that artifacts may be generated during the cocultivation assay by expression of an endogenous virus in the permissive cell line or through fusion if the vector-producing cell line is cultured directly with a marker rescue cell line. In addition, cocultivation may not be possible for ex vivo cell products that have specific culture requirements or limited culture life spans.

Methodologies for testing the presence of RCR in crude, purified bulk or final vector products are not specified. The FDA has deposited a reference standard of an amphotropic hybrid MLV with the ATCC. This viral stock has been assigned a label titer and should be used in assay validation. Method validation should demonstrate the ability to reproducibly detect a single RCR particle in individual product types because the product and its related impurities can interfere with the detection of RCR. Currently there are no acceptable limits for RCR contamination in products. Any product lot found to contain RCR cannot be used for human use.

Reference standards for assessing RCV in other viral vectors including ecotropic, xenotropic, or pseudotyped MLV, adenovirus, and lentivirus have not been developed. Amplification and detection of replication-competent HIV, especially its pseudotyped variants, may warrant special containment and handling procedures.

Additional safety testing usually focuses on methods similar to those described under *Biotechnology-Derived Articles* (1045), for *Safety Tests—Biologicals* under *Biological Reactivity Tests, In Vivo* (88), and under *Sterility Tests* (71). For viral gene therapies produced using a human cell line, performance of the in vitro adventitious agent bioassays using 3 cell lines is recommended on either the bulk or final product. For adenoviral vectors, specific tests for adeno-associated virus are also recommended on either the bulk or final product. For adeno-associated virus, specific tests for adenovirus and herpesvirus are recommended on either the bulk or final product.

NONVIRAL GENE THERAPY PRODUCTS

Safety testing usually focuses on methods similar to those described under *Biotechnology-Derived Articles* (1045), for *Safety Tests—Biologicals* under *Biological Reactivity Tests, In Vivo* (88), and under *Sterility Tests* (71). However, the General Safety test is not required for therapeutic DNA plasmid products (even if formulated). Safety testing should be performed on nonviral formulated material. If the shelf life of the formulated nonviral therapy is very short, then the components should be tested individually.

Dose-Defining Assays

GENERAL CONSIDERATIONS

An assay that precisely measures the amount of the product is referred to as a dose-defining assay, and it is selected on the basis of its precision and accuracy. An assay that measures therapeutic activity of the product is referred to as a potency assay and it is designed to measure product function. The design of the assay is dependent upon the type of product. In the case of drugs, the assays measuring the amount of active ingredient (dose) are referred to as strength assays.

Product dose can be defined as the concentration or amount of the drug product administered to the patient and it is typically measured as product mass. For cell and gene therapy products, attributes such as viable cell number, milligrams of plasmid or antisense oligonucleotide, or the viral particle number are often used to define the dose of the product.

CELL THERAPY PRODUCTS

Cell therapy products may be dosed on the basis of enumeration of one or more cell populations. For *ex vivo* gene therapy, dose may be based on cell number as well as level of expression of the gene product. For products in the form of a homogeneous, single-cell suspension, viable cell number is the most frequently used assay. Such assays may include enumeration of all cells, total nucleated cells, or another subset of cells. Viability assays are usually based on a cell's ability to exclude a supravital dye, such as trypan blue. Results are expressed as the number of cells that exclude the dye and are therefore considered viable. The compound 7-AAD, a red-fluorescing compound that binds to nuclear proteins and is also excluded by viable cells, may be incorporated into flow-cytometric methods for simultaneous determination of viability and cell-identity markers.

Cell counting may be performed rapidly by manual or automated methods. Manual cell counting by visual enumeration of cells in a hemacytometer chamber is a readily available technique with acceptable accuracy, but a lower degree of precision than most automated methods. Typical instruments for automated cell counting provide reproducible enumeration of nonnucleated cells (e.g., erythrocytes and platelets) and nucleated cells and differential counting of the nucleated cells into mononuclear and polymorphonuclear leukocyte populations. Further discrimination of specific cell populations usually requires cell-surface phenotype analysis by flow-cytometric or other methods (see *Cell Therapy Products* under *Identity*). The proportion of a specific subpopulation of cells may be determined by FACS analysis or by flow cytometry.

An example of a cell enumeration assay is the enumeration of CD34-positive (CD34+) hematopoietic progenitor cells, the number being expressed as the number of cells per recipient's body weight. In numerous studies this measurement has been shown to predict hematologic reconstitution following myelosuppressive or ablative therapy in autologous or allogeneic hematopoietic transplantation.

For products that contain cells in a nonhomogeneous suspension, such as cells that form a two- or three-dimensional structure, alternative measures for cell enumeration, such as total area of a cell sheet, wet weight, total protein, and total DNA, have been used. If such measures are used to determine product dose, then supplemental tests must be performed to demonstrate therapeutic activity.

VIRAL AND NONVIRAL GENE THERAPY PRODUCTS

Particle concentration is a commonly used measure for viral vector product dose. Particle concentration may be measured by physical, biophysical, or *in vitro* cell-based assays. For example, quantitation of purified adenoviral particles may be determined by using the optical density of a solution of virus in 0.1% (w/v) sodium dodecyl sulfate (SDS) solution, at 260 nm, because a relationship between absorption and particle concentration has been published for adenovirus. The particle number concentration is equivalent to the product of the absorbance at 260 nm in a 1-cm cell, the dilution factor, and 1.1×10^{12} particles.² Other methods to determine particle concentration include particle counting by electron microscopy and integration of viral peak area against an authenticated reference standard in an anion-exchange resin-based high-pressure liquid chromatographic (HPLC) assay.

Virus concentration can also be assessed through the measurement of selected structural proteins with known molecular masses and known copy numbers within the virion. For this method, the virus has to be lysed, and the structural proteins have to be separated by using an appropriate, high-recovery chromatographic procedure (e.g., reverse-phase HPLC). The chromatographic separation and the identity and the purity of the selected structural protein has to be verified during assay validation by methods such as SDS polyacrylamide gel electrophoresis (SDS-PAGE), peptide sequencing, and mass spectroscopy. The selected structural proteins have to be quantified, for example, by integrating chromatographic peaks at 214 nm and comparing the area to an authenticated reference standard. The virus concentration can then be calculated based on the molecular mass, the copy number, and the measured mass of the protein. Very importantly, the virus concentration can be estimated simultaneously

for multiple structural proteins, allowing the use of this assay in relatively impure virus preparations. This method has been applied to adenovirus and should be applicable to other viral vector types.

Biophysical methods of determining particle number include direct quantitation of vector nucleic acid by radiolabeled-probe hybridization and indirect quantitation by amplification of template nucleic acid (e.g., PCR and RT-PCR) or by signal amplification (e.g., branched-chain DNA using multiple-probe hybridization).

In cases where biophysical methods are not available, bioassays that measure gene-vector titer have been used. These involve infection, transfection, or transduction of a susceptible cell line *in vitro*, followed by some measure of the product uptake. Methods for quantitation or estimation of the number of infection, transfection, or transduction events include plaque-forming unit assays, tissue culture infectious dose, 50% (TCID₅₀) assays based on cytopathic effect or immunofluorescent detection of an expressed vector protein, or a quantitative DNA-hybridization assay. Examples are as follows.

For retroviral or lentiviral gene therapy products or AAVs that carry a selectable marker (e.g., that for neomycin resistance) or a reporter gene (e.g., β -galactosidase) in addition to the therapeutic gene, the infectious titer is commonly determined by measuring the number of transduced or infected cells expressing these nontherapeutic proteins. Vector titer is typically reported as the number of colony-forming units (cfu) per mL for cells transduced with viral vectors containing drug-resistance markers and selected for growth in drug-containing medium. Titer based on β -galactosidase can be expressed in terms of blue (cfu) per mL after staining and counting the cells that convert the β -galactosidase substrate X-Gal into a blue chromophore. For vectors without a marker gene, quantitation of transduction has been measured precisely by using quantitative PCR.

Most nonviral gene therapy products contain plasmid DNA and their usual measure of dose is the DNA mass. The DNA mass may be determined in the formulated state, and, if recombinant protein is included in the formulation, the total combined mass of all formulation components based on a specific ratio can be used. DNA concentrations greater than 500 ng per mL are most simply determined by using optical density measurement at 260 nm. This method is not generally applicable to lipid-formulated DNA. Because RNA and proteins also have significant absorbances at 260 nm, other analyses must be performed to demonstrate that there is minimal contamination with RNA, protein, or residual host-cell chromosomal DNA. Dyes that specifically bind to double-stranded DNA allow the DNA concentrations of less than 500 ng per mL to be measured accurately when calculated against an authenticated DNA standard curve. PicoGreen is one such fluorescent dye and it is minimally affected by single-stranded DNA, RNA, proteins, salts, and detergents. The fluorescent dye Hoechst 33258 also binds to both double-stranded and single-stranded DNA and it can be used to determine DNA concentrations as low as 0.3 ng per mL. The Hoechst 33258 does not bind to protein or RNA and it can accurately determine the DNA concentrations in crude samples.

Methods, such as capillary electrophoresis, employing an authenticated reference material can also be used to determine the strength of nonviral and antisense-oligonucleotide products.

Potency

GENERAL CONSIDERATIONS

Potency is defined as the therapeutic activity of the drug product. Together with dose, potency defines the biological activity of each lot (see *General Considerations* under *Dose-Defining Assays*). Potency may be assessed through *in vitro* or *in vivo* bioassays. It is not uncommon for these assays to have coefficients of variation between 30% and 50%. These assays require a well-defined, representative reference material that can be used as a positive control for the assay. The positive control serves to qualify the performance of an individual assay. Potency assay development should focus on characterizing and controlling variability. The high-precision assays are more effective tools in monitoring product quality. Information about potency-assay variability should be incorporated into the stability study design and the proposed statistical approach to assignment of expiration date (see *Stability*).

² From CBER's *Points to Consider for Human and Somatic Cell and Gene Therapy*, April 1998.

CELL THERAPY PRODUCTS

Functional assays that can be performed on cellular products are application-related and include viable cell number and a wide range of colony-forming assays, proliferative assays, cell-to-target killing assays, and assays that quantitate gene expression following gene transduction. For hematopoietic progenitor cells prepared from marrow, peripheral blood, or cord blood, traditional colony-forming assay quantitates committed progenitor cells such as colony-forming unit-granulocyte-macrophage (CFU-GM); this assay has been correlated with clinical engraftment outcomes in some studies. More recently, process-monitoring programs incorporate assays such as the long-term culture-initiating cell (LTCIC) assay or the *in vivo* animal models such as competitive repopulation in immunodeficient mice to monitor the activity of the most primitive hematopoietic stem cells. In the case of cells intended for structural repair, proliferation under a set of defined *ex vivo* conditions may be used as the potency assay. If the cells release an enzyme or active molecule, a potency assay could be based on the units of enzymatic activity or on the total of active molecules released. For instance, the production of insulin in response to changes in glucose levels could be the basis of a potency assay for cells intended to treat diabetes.

Patient-specific products, such as autologous cancer vaccines that elicit an *in vivo* immune response, present a challenge in demonstrating therapeutic activity in an *in vitro* or *in vivo* assay system. Assessment of potency in these circumstances is currently the subject of public-policy debate. Novel approaches to measuring potency, such as the correlation of clinical outcome to other characterization tests such as identity tests, may be appropriate and should be discussed with regulatory authorities early in development. For example, the ability to determine specific cell-surface identity markers by employing flow-cytometric techniques or vital stains may be an acceptable measurement of potency if properly validated and correlated with clinical outcome.

VIRAL AND NONVIRAL GENE THERAPY PRODUCTS

Bioassays employed to measure the potency of viral and nonviral gene therapy products generally involve infection, transfection, or transduction of a susceptible cell line *in vitro*, followed by some functional measure of the expressed gene of interest. Functional assays for the therapeutic gene (e.g., those measuring enzyme activity and cytokine activity) should generally be used instead of analytical methods such as enzyme-linked immunosorbent assay (ELISA), HPLC, or FACS, which provide information about the level of expression but only infer function. In addition, for viral vectors, infectious titer measurements by themselves are generally not considered an adequate measure of product potency. The design and ultimate suitability of an assay system for determining product potency will depend on the relationship between the intended human target cell *in vivo* and the following: (1) the transduction or transfection efficiency of the cell line used *in vitro*; (2) the protein expression levels, and (3) the duration of expression required for the therapeutic effect.

In vivo tests may also be used to measure vector-product potency. Readouts may be based on a response per animal (e.g., blood levels of therapeutic protein 24 hours after treatment) or a group response rate (e.g., percentage of animals that elicited an immune response or survived virus challenge). The availability of an appropriate *in vivo* test system will depend on the vector-host range (for viral vectors), the pharmacokinetics and biodistribution of the vector and its resultant gene product relative to its human counterpart, and the time frame required to observe the therapeutic effect or surrogate. Issues of cost, facilities, validation, and ethics will determine the practicality of an *in vivo* potency test.

Purity

GENERAL CONSIDERATIONS

Analytical methods that separate, isolate, and specifically quantify the intended active product components determine product purity. Impurities are either product- or process-related components that can be carried through to the final product. The manufacturing and

purification process should be optimized to consistently remove impurities while retaining product activity. The requirement to test for a particular impurity for product lot release will depend on the following: (1) the demonstrated capability of the manufacture and purification process to remove or inactivate the impurity through process validation, and (2) the toxicity potential associated with the impurity.

Examples of process-related impurities associated with cell and gene therapy products include residual production-medium components (e.g., FBS, antibiotics, cytokines, and *Escherichia coli* chromosomal DNA in a plasmid product), ancillary products used in downstream processing (e.g., nucleases such as DNase I), and residual moisture for lyophilized vector products. Impurities may be bioactive (e.g., cytokines and hormones) or immunogenic (e.g., product aggregates, degradation products, plasmid-selection markers, and nonhuman-derived proteins) or they may have other deleterious effects (e.g., they may compete with the product) if administered at a dose equal to that of the product. Product-related impurities are specific to each product type. Examples include differentiated cells in a stem-cell therapy product, nicked plasmid forms in nonviral products, and defective or immature virus particles in retroviral or adenoviral vector products. Analytical methodologists to assess purity require quantitation or physical separation of intended product from its impurities. Common sense should drive the need to quantify specific impurities. It may be possible to validate the manufacturing process to the extent that specific lot-release testing for impurities will be very limited. An emphasis may be placed on demonstrating the consistency of the product-impurity profile.

Testing for impurities is often extensive during product characterization and process validation when the consistency of the manufacturing and purification process is being demonstrated. Testing for impurities as part of lot-release testing should reflect the safety risks associated with the impurity and the ability of the process to consistently remove that impurity.

CELL THERAPY PRODUCTS

One measure of purity is the percentage of viable cells in the total cell population. Another measure is the percentage of transduced cells or the percentage of cells with a specific marker. If an entire population of cells is the therapeutic agent, methods should determine the relative amounts of each subpopulation. Limits should be defined for each cell subpopulation. These assays may be based on immunological methods utilizing flow cytometry or DNA-hybridization dot blot analysis. Additional tests for process contaminants are performed depending on the specifics of the manufacturing process. For example, a quantitative ELISA for residual serum proteins may be required. If the cells are genetically modified during manufacture, then testing for residual vector may need to be performed.

For gene-modified cell therapies, determining the purity of the cell therapy product depends on the availability of reagents and methods to distinguish therapeutic cells from the other cells present in the product. As in the case of gene therapy products, gene-modified cells can be distinguished from the unmodified cells on the basis of expression of the transgene. FACS using an antibody that detects the therapeutic protein or fluorescent probes that detect expressed RNA allow the separation and quantitation of the transgene expressing and nonexpressing cells. By adding an antibody to a cell-specific phenotypic marker and by using a double-sorting technique, FACS can be used to further identify the subpopulation of cells that are modified.

Endotoxin testing is also required. Biomaterials used with cell therapy products should also be tested for their biocompatibility.

VIRAL GENE THERAPY PRODUCTS

Product-related impurities for viral vectors include aggregates and defective and immature particles that may be produced during the manufacture or purification of the recombinant vector. Aggregates of vector may form if the product is highly concentrated, stored under certain conditions (e.g., under certain pH or temperature), or reconstituted after lyophilization. Assays to detect aggregates include particle size analysis by laser light-scattering and the use of nonreducing, nondenaturing PAGE, followed by staining of the gel or transfer and detection of viral proteins by Western blot analysis. Sedimentation

rate analysis also allows separation of aggregates from monomers based on size. Optical density analyses of light-scattering are also used to assess vector aggregation.

Defective particles are viral particles that do not contain the appropriate recombinant genome, that is, they contain some other nucleic acid or contain no genome at all, or the vector has some missing, defective, or otherwise altered structural component that impairs its ability to transduce a cell. For viral vector systems that have capsomeric symmetry, which requires the appropriate nucleic acid incorporation for configuration, empty particles may be readily distinguished from those carrying genomes. For enveloped viruses, empty particles may not be as readily separated from those with encapsidated nucleic acid.

For some viral vector products, active viral particles may be separated from defective particles by using analytical HPLC. Anion-exchange resins have been used to separate active adenovirus from defective virus particles. However, this method might not be useful for an adenoviral vector purified by anion-exchange chromatography unless the resin for the assay is different from that used during manufacture. Depending on the nature of the viral vector and its nonactive or defective forms, other methods of separation, such as equilibrium centrifugation in a cesium chloride density gradient, may need to precede the quantitation of the active particle. Ideally, the method of separation will allow quantitation.

Defective particles that carry a noncell-derived oncogenic gene or other undesirable genes may pose a special concern. For example, in murine-based retroviral packaging cell lines, small viral elements called VL30 sequences can be packaged in about one third of all particles. Assays may need to be developed to quantify specific defective particles if they are known to be present in quantities sufficient to pose a safety concern.

Virus quality and the comparability of preparations can also be assessed by measuring selected structural proteins with known molecular masses and known copy numbers within the virion. For this method, the virus is lysed, and the structural proteins are separated by using reverse-phase HPLC or some other high-recovery chromatographic procedure. The chromatographic separation should be validated and the identity of the selected structural proteins verified by methods such as SDS-PAGE, peptide sequencing, or mass spectroscopy. One can *fingerprint* the batch based on quantification of the selected structural proteins and comparison to a reference standard. When the method incorporates the use of mass spectroscopy, impurities such as structural variants can also be identified. For adenovirus preparations, some precursor and most mature virion proteins can be monitored, thus allowing monitoring of the product and of the immature virion forms.

Host cell-derived proteins may be considered impurities for some viral vector products and may be separated and quantified by PAGE or HPLC or detected by amino acid analysis, Western blot, or immunoassay-based methods. However, for enveloped viruses such as retroviruses, host cell-derived membrane proteins are an integral part of the product. In those vector systems, it may be difficult to determine the presence of contaminating exogenous host-derived proteins.

Presence of specific process-related impurities depends on the manufacture and purification process of each vector or product type. However, most products will need to be tested for residual endotoxin (see *Bacterial Endotoxins Test* (85)). Acceptable limits of endotoxins have been determined and can be directly applied to viral vector products.

Although genomic DNA derived from continuous cell substrates used to manufacture biological product has been considered historically as potentially tumorigenic, recent studies suggest that the risks are very low. However, every attempt should be made during process development to reduce contaminating DNA levels. The need to test for residual DNA as part of product lot release should be evaluated on a case-by-case basis and may be dependent upon the size distribution of the DNA, its association with the product or its formulation components, and the route of administration of the product. Quantitative PCR assays have been developed to analyze the amount of residual host-cell DNA by using primers designed to amplify evolutionarily conserved and abundant target sequences, such as 18S for 293 cells.

Quantitation of residual serum components such as bovine serum albumin (BSA) can be achieved by using ELISA and a BSA reference standard. Specific functional or immunological methods may need to be developed for other ancillary products including other culture media or purification process components such as cytokines or enzymes (e.g., nucleases such as DNase I or benzonuclease).

NONVIRAL GENE THERAPY PRODUCTS

Testing is usually performed on the individual components, the plasmid DNA, the lipid or lipoplex components and (recombinant) protein components if any are present in the formulation. Plasmid DNA is characterized for a variety of impurities including residual host-cell DNA, residual RNA, and residual protein. Residual protein testing is frequently included in lot-release testing. Optical density ratios, usually the ratio of the measurement at 260 nm to that at 280 nm, are frequently used in purity specifications for plasmid DNA.

In addition, the plasmid DNA should also be characterized with regard to its form. Plasmid DNA forms include monomeric supercoiled, relaxed monomer, and linear forms. The profile of forms needs to be monitored for product consistency. Additionally, it may be possible to correlate form with *in vivo* transfection behavior. While monomeric supercoiled plasmid has been shown to be more efficient than relaxed monomer, linear, or multimeric forms of the plasmid in transfecting cell lines *in vitro*, *in vitro* transfection has been shown to not always predict *in vivo* behavior. Formulation, delivery method, and route may impact *in vivo* transfection. Agarose gel electrophoresis can resolve these forms of plasmid, which are then detected by UV after ethidium bromide staining. This method provides information about the relative levels of the plasmid forms, but it is not highly quantitative for the individual species. Analytical anion-exchange HPLC can be used as a quantitative assay for monomeric supercoil and percentage of other forms, including concatamers. Other sophisticated methods, such as capillary zone electrophoresis, linear-flow dichroism, and atomic-force microscopy have been proposed as replacements for agarose gel analysis. Until they are validated, these analytical methods may be more appropriate for characterization studies in support of process development and validation rather than for lot-release testing. The appropriate methods for lot release will depend on what effect these alternate plasmid forms have on the product potency. Many of these methods, such as HPLC, are also applicable to the assessment of the purity of antisense-oligonucleotide products and the determination of the level of by-products.

Tests for process-related impurities, such as cesium chloride, must also be conducted. In the case of antisense-oligonucleotide products, residual solvents must be quantified. Lipid and lipoplex formulation components must also be tested for their chemical purity. Testing for specific chemical impurities is commonly performed by using gas chromatography-mass spectroscopy (GC-MS), high-pressure liquid chromatography (HPLC), or thin-layer chromatography (TLC) methods.

If protein is part of the formulated complex, then the protein must also be tested for purity. The methods outlined under *Biotechnology-Derived Articles* (1045) or under *Biotechnology-Derived Articles—Tests* (1047) are relevant.

Bacterial protein, DNA, RNA, and endotoxins are the major types of host-derived process contaminants. Standard protein assays (e.g., Lowry, Bradford, or Coomassie), PAGE followed by silver staining or Western blot analysis, or ELISA can be used to detect residual host protein in the nanogram range. Host chromosomal DNA may be detected by slot blot hybridization (detection in picogram range) or by PCR using highly conserved target sequences (e.g., 18S for *Escherichia coli*). However, low background may be unavoidable in PCR-based assays because the recombinant polymerases used for the amplification of target also contain residual bacterial DNA. PAGE or agarose gel electrophoresis followed by fluorescent dye staining may be used to detect residual RNA. Quantitation may not be required given the labile nature of RNA and the low-level toxicity associated with it.

Certain antibiotics, such as kanamycin, that may be used during the fermentation process must be removed during the process, and validation of the process or lot-release testing must be performed to confirm removal during the purification of the plasmid. HPLC is one method that can be used to detect low-level residual antibiotic.

LYOPHILIZED VIRAL AND NONVIRAL VECTOR PRODUCTS

Residual moisture may affect the stability of a lyophilized vector product. The FDA's *Guideline for the Determination of Residual Moisture in Dry Biological Products* (January 1990) recommends a 1% residual moisture level, although data indicating no adverse effects on product stability at higher levels will be considered accepta-

ble. Residual moisture levels can be determined by using a standard method (see *Water Determination* (921)) that is compatible with the formulated product.

Identity

GENERAL CONSIDERATIONS

Lot-release testing for cell and gene therapy products must include an identity test. This test serves to specifically identify the product. The complexity of the identity test will depend on the nature of the specific product and the array of products being manufactured. For example, more extensive and rigorous testing may be performed for an autologous gene-modified cell therapy product at a facility where multiple patient products are manufactured than for a viral vector product produced at a site that manufactures a single vector product.

CELL THERAPY PRODUCTS

Cell therapy identity tests must be relevant to the cell type and manipulations applied during processing. Differential surface markers (for instance, CD3, CD4, CD34, and CD45) are frequently used to ascertain product identity. Flow-cytometric immunoassay methods are the most common means of detecting and quantifying these markers. In this type of assay, a sample of the cells is stained with fluorescently labeled antibodies directed against specific identity markers and then passed as a single cell suspension in front of a laser source. Identification and quantitation of particular cell subsets is accomplished by multiparameter analysis, usually of size and granularity (measured by forward and side light-scattering) and of one or more identity markers (measured by emitted fluorescence). Simultaneous quantitation of cell viability can be performed by adding 7-amino-actinomycin D (7-AAD) to cell suspensions marked with antibodies conjugated to green (e.g., FITC) or orange (e.g., phycoerythrin) fluorescent compounds.

Analyses, such as isoenzyme analyses, employing biochemical markers are also used. For example, isoenzyme analyses are used to confirm species in the case of xenotransplants. Cell morphology can be used if it can distinguish specific cell types or unique function. Morphology can be combined with doubling-time parameters to better distinguish different cell types.

VIRAL GENE THERAPY PRODUCTS

Restriction enzyme mapping and sequencing of the transcription unit DNA are the most commonly used approaches to establishing the identity of viral vectors for characterization purposes. PCR-based methods, restriction enzyme mapping, and transgene expression-based immunoassays are most commonly used to confirm the identity during lot-release testing.

NONVIRAL GENE THERAPY PRODUCTS

Restriction enzyme mapping is the most common identity method for plasmid-DNA and antisense-oligonucleotide products. The number of enzymes used to create the vector fingerprint will vary with the complexity of the DNA and the degree of similarity between multiple products. If lipids, lipoplex agents, or proteins are used to formulate the DNA, then their identity must also be tested. Lipids and lipoplex chemicals may be identified by procedures used for traditional pharmaceuticals, such as GC-MS, TLC, and the like. Protein components of the formulation may be identified by peptide mapping or other means outlined under *Biotechnology-Derived Articles—Tests* (1047).

STABILITY

General Considerations

The shelf lives of cell and gene therapy products will vary widely, depending on the nature of the product, its intended clinical use, its specific attributes, and the recommended storage, packaging, and shipping conditions. Therefore, it is difficult to draft uniform guidelines regarding stability-study duration and testing frequency applicable to all gene and cell therapy products. In all cases, the stability study should be designed on the basis of scientifically sound principles and approaches and a comprehensive understanding of the final therapeutic product and its intended use. Stability of in-process hold steps, cell and virus banks, critical raw materials, and reference standards also needs to be assessed. A well-designed and executed stability program will provide a high degree of assurance that the product is stable within the specified shelf life.

For viral and nonviral vector gene therapy products and cell therapy products that are not patient-specific, the selection of batches to support license application and final-product labeling should be carried out in accordance with the principles of stability testing, such as those described in ICH guideline Q5C, presented under *Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products* (1049). Stability data should also be collected for bulk material and other in-process points if material is stored before final processing and filling.

Nonviral DNA plasmid vectors are often formulated with specific mixtures of lipids, proteins, or lipoconjugates to form liposomes or encapsulated complexes. Depending on the formulation used, a shelf life of hours to years can be attained. Where a product has a short shelf life, the final formulation may need to be prepared at the clinic just before administration. Instability is frequently observed as aggregation and precipitation. Formation and stability of the formulated complex must be established through validation studies during product development. Stability data should also be collected for major components of the formulated complex, such as the lipids, the liposomes, and the DNA itself.

For many patient-specific cell products including transduced products, each product is unique and often only one lot is prepared for a single patient. In general, the lots tend to be of small volumes, sometimes less than 10 mL, and they may involve products that cannot be frozen and hence have short shelf lives (between 24 and 72 hours) as the cells continue to metabolize their medium. Protocols to establish stability of patient-specific therapy should use materials from multiple donors and at least three lots. Well-characterized banked primary cells may be used in the validation of storage, shipping, and expiration dating if the donors have a range of profiles expected for the patient population to which the therapy will ultimately be directed. The stability of the product under the holding conditions at the medical center should be validated.

Stability-Protocol Development

Formal stability studies to support licensure as well as early phase product stability information gathering should be detailed in a written plan that describes how stability data will be collected and analyzed to support the expiration period of the product. Protocols should follow the format recommended in existing guidelines and include the scope, storage conditions, number of lots to be tested, test schedule, assays to be used, data analysis, and product specifications. Any assay used in a formal stability study for licensure must be validated before the study begins. The specific study design should take into account the reasonably expected possibilities the product may encounter (see *Accelerated and Most Appropriate Challenge Conditions*) and it should incorporate the latest knowledge in the biological sciences while addressing existing regulatory requirements. For instance, if the final formulation of the product is performed at the clinical site, stability studies on this final formulation should be done to establish the time and conditions under which the product can be held.

Stability studies must verify that the storage conditions maintain the purity and potency of the product, so that the product administered to the patient is still capable of satisfying the stability specifications. These specifications may differ from the release specifications. However, stability specifications must be verified with clinical data.

Stability assessment should include assessment of product functionality (potency). The potency assay often has a high degree of inherent variability. Measuring and calculating the decay of product activity by employing the standard statistical methodologies may require multiple, frequent sampling intervals over an extended period of time and may require analysis of more than three production lots to compensate for the variability of the assays. Initial studies to establish a provisional expiration date must be conducted prior to administration to the first patient. Initial studies are also useful for determining which assays are stability-indicating, that is, the best indicators of product degradation. Because existing compendial methods do not address the unique characteristics of cell and gene therapy products, the development of assays that would address these unique characteristics is encouraged.

Accelerated and Most Appropriate Challenge Conditions

The stability-indicating profile of a cell or gene therapy product may vary with time under the influence of a wide variety of environmental conditions, including temperature, extremes in physiological storage conditions, and light. Multifactorial degradation pathways must be considered in the development of a program investigating the effects of these parameters on the stability of the products. Studies should include conditions that are outside of the specified storage ranges, that is, challenge conditions such as those encountered during periods of abnormal storage, shipping, or handling. Examples include brief incubator malfunctions, incubator or cold storage failure, periods of extreme temperature fluctuation due to shipping to hot or cold climates, hypobaric conditions experienced in the cargo hold of a commercial airliner, or temperatures likely to be encountered in the surgical suite. A short exposure to an environmental condition well outside of an established limit and a long exposure to an environmental condition just outside of an established acceptable range may be equally detrimental. The slow and constant rate of product degradation at a specified temperature may increase if a different set of storage conditions is applied. The effect of light on the stability-indicating profile should be investigated if it is scientifically warranted. Special attention should be given to products stored in fluids containing light-sensitive or reactive components that may give rise to cytotoxic by-products.

Studies analogous to accelerated aging studies typically used in pharmaceutical stability-monitoring programs are also useful to determine how the product degrades and which assays are stability-indicating. These studies can be the same as some of those mentioned in the preceding paragraph. Other studies include placing a product at 37° or at 18°, while its normal storage temperature is 25 ± 2°, or placing a lyophilized product in a high-humidity environment. Such studies should be performed before formal stability studies begin, so that the formal studies incorporate the validated stability-indicating assays into the protocol.

STORAGE AND SHIPPING

General Considerations

The storage conditions are chosen to preserve the purity and potency of the product so that the specifications for the product are maintained throughout storage, shipping, and handling at the clinic. Initial studies must be conducted prior to patient administration to determine acceptable storage, shipping, and handling conditions. The initial storage and shipping conditions need not be those envisioned for the commercial product. They should ensure that the product specifications are maintained beyond the initially proposed expiration dating. For products with short shelf lives, storage and shipping conditions, even within a medical center, need to be considered together because shipping constitutes the bulk of storage time after manufacturing. Special consideration should be given to the ability of gas to permeate the shipping container, especially if the cell or gene therapy product is stored or shipped on dry ice. Once stability-indicating methods are developed and the final storage and shipping conditions are chosen, these conditions are validated as discussed under *Stability*.

Most products with limited shelf lives will be shipped by using reliable overnight courier systems. In some cases, some critical products are hand-carried onto commercial aircraft. Special permission must be obtained by commercial carriers if scanning by airport X-ray equipment is to be avoided. Cargo shipping studies should be designed during the development of packaging systems to identify stresses to which biological products may be subjected. Bracing and insulating materials should then be chosen and validated to provide a packaging system that will alleviate the extreme conditions of shipping.

Cell Therapy Products

Cryopreservation—Cryopreservation is the main mode used for the long-term storage of cells, that is, storage of cells for periods longer than 1 year. (See also *Suspensions* under *Formulation of Cell Therapy Products*.) The rate of cooling for the cell solutions is important because of the mechanical and dehydration injuries resulting from the formation and growth of ice crystals. The ideal temperatures are dependent upon the type of cells being cryopreserved and the concentration of the cryopreservative. The optimal cooling rate for most cells is between 1° and 3° per minute. Controlled-rate freezers, which can reproducibly produce this optimal cooling rate, are critical when large numbers of vials or large volumes of cells in bags are being frozen. Once cooled to below freezing, cells need to be stored at temperatures below –130°. This can be achieved with electric freezers or with liquid nitrogen. Storage of cells in the vapor phase of a liquid nitrogen freezer reduces the risk of cross-contamination with other material in the freezer. However, a map of freezer temperatures needs to be generated so that cells are not stored so far from the liquid nitrogen that they are subjected to temperatures above –130° as the liquid nitrogen evaporates or during the opening of the freezer. Some cells can be stored at –80° if the cells are to be used within a few weeks.

Thawing—Thawing is performed fairly rapidly. If a small amount of cells is to be reinfused or transplanted, DMSO does not need to be removed from the suspension, because most cell preparations can be concentrated adequately to keep the DMSO concentration within tolerable limits. DMSO use has two effects on cells after thawing: cells may clump if damaged, and DMSO reduces cell viability in minutes. If the DMSO needs to be removed or cells need to be concentrated for administration, the defrosted cell suspension is generally serially diluted to avoid osmotic shock and resuspended in a protein-containing medium. Cell viability and potency are generally determined after thawing, but the information may not influence the clinical use of the material.

Frozen Products—Frozen cell therapy products are shipped to the medical center on dry ice or in liquid nitrogen dry shippers. Dry shippers may be preferable because temperature is more readily maintained during shipping. Dry ice and liquid nitrogen are both considered hazardous materials during shipping. Storage conditions at the clinic need to be defined. Most pharmacies do not have access to liquid nitrogen freezers. At best, they have mechanical freezers capable of maintaining the temperature at –70°. Clinics that have cell-processing centers or are involved with bone marrow transplantation have liquid nitrogen freezers. If further processing, for example, defrosting and administering, of the cell product is performed at the clinic, the storage conditions and expiration date for the product should be specified. Often the laboratory that is handling the cells and the clinic that will administer the product have to closely collaborate because cells in a concentrated suspension survive for only a few hours.

Unfrozen Products—Other cell therapy products are stored unfrozen. Because cells continue to metabolize during storage, their expiration period is typically between 24 and 96 hours. The expiration date can be extended to several weeks by increasing the volume of storage medium, by reducing the storage temperature, or by attaching a series of bags or compartments that allow the medium to be exchanged without breaching the sterility of the system. These products are shipped in insulating containers with refrigerant packs to maintain a defined temperature range. To stabilize the excursions from these defined and validated temperature ranges, well-designed container configurations with dense foam insulation, which protects the product from shifts in external environmental temperatures, are used. The product purity and potency should not be affected over practical shipment intervals, that is, intervals of 24 to 96 hours, either

at higher or lower temperatures. However, if potency may be affected, the shipping box configuration must be re-engineered to maintain optimal potency for the longest possible shipping periods. The product itself should be placed in a lightproof, leakproof container with adequate physical support to ensure stability and prevention of leakage during typical conditions of shipment.

Gene Therapy Products

Most gene therapy products can be lyophilized or formulated by means similar to those employed for many recombinant protein products or cell therapy products. These storage formulations typically have expiration periods longer than one year and no unusual shipping requirements. Nonviral gene therapy products, which may be unstable in their final formulation, can have similar expiration periods if they are stored in a multiple-vial kit with the nucleic acid in one vial and a carrier, such as lipids, in the other. The final formulation is performed at the medical center just before administration.

LABELING

Product labeling is regulated by the FDA and compliance with existing regulations is required. Labeling of cell and gene therapy products as regulated biologics will be subject to these regulations. For biologics and devices (21 CFR 610 Subpart G and 21 CFR 801, respectively), the labeling requirements are separated into container labeling and package labeling requirements. Both the container label and the package label must include the expiration date. If the container is packaged, then the recommended storage conditions are included on the package label. If the container is not packaged, the recommended storage conditions and all other requirements of a package label must appear on the container label. Labeling must also comply with relevant national and international requirements.

If antibiotics are used in the cell processing and therefore may be present in the final product, the labeling should reflect this. For cell therapy products that must be applied to the patient in a particular physical orientation, labeling that indicates the correct orientation should be apparent even after the package is opened. Similarly, if a device should be only grafted in a defined area, that area should be made readily apparent by labeling that is evident once the package is opened. Unless the product has been screened for pathogenic or microbial contaminants prior to release, appropriate biohazard labeling may be required. For products with very short shelf lives, expiration dating will require adjustment and correction for time zones to provide the user an accurate assessment of shelf life. Clinical procedures will be scheduled around these crucial time frames. For patient-specific products, the patient's full name, initials, or a combination of these will need to appear on the labeling, in addition to lot designation, to ensure that the product will be administered to the appropriate patient.

REGULATIONS, STANDARDS, AND NEW METHODOLOGIES

Summary of Regulations and Standards

The technologies involved in cell and gene therapy products have been widely documented in the literature and are rapidly evolving. These products can be regulated as drugs, biologics, or devices, or not regulated at all, depending on how they are manufactured and used. The novel approaches permitted by these technologies may make it difficult to determine which FDA centers will be involved in their regulation and the FDA has advised manufacturers to seek clarification in the early stages of development. Regulation is the same as that for biotechnology-derived products. The general requirements are described primarily in the 21 CFR. The federal government has issued many general guidelines as *Points to Consider or Guidance* documents (see www.fda.gov). ICH guidance documents for many of the quality-related areas are directly relevant to qualifying cell and gene therapy products (see www.ifpma.org/ich1.html) and some of these documents are reproduced in *USP 25* as general infor-

mational chapters. National Institutes of Health (NIH) has published *Guidelines for Research Involving Recombinant DNA Molecules* (see <http://www4.od.nih.gov/oba/guidelines.html> for text of the document and its amendments) that require NIH review of research, including clinical research or trials, conducted or sponsored by institutions receiving NIH funding. These guidelines apply to many gene therapy products. AATB has developed guidelines for sourcing allogeneic tissue. Public Health Service (PHS), with input from the NIH, FDA, the Centers for Disease Control and Prevention (CDC), and Health Research Services Administration (HRSA), has developed guidelines for the use of xenogeneic-derived products (*Draft Public Health Service (PHS) Guideline on Infectious Disease Issues in Xenotransplantation*, August 1996). In addition, ASTM is also developing standards for tissue-engineered medical products.

Need for New Methodologies

Cost-effective commercialization of cell and gene therapy products requires the development and validation of new methodologies to assess product quality. USP will adopt such new methodologies when they have been properly validated. Similarly, if reference standards or reference materials are needed and available, they could be included in the USP inventory to allow comparative analysis among various clinical trials or to serve as points of reference by manufacturers of these products for raw materials, process components, and process impurities.

DEFINITION OF TERMS

ADENOVIRUS—Virus belonging to the family *Adenoviridae* of DNA viruses having a nonenveloped virion with 252 capsomeres and a diameter between 70 and 90 nm; a single linear molecule of double-stranded DNA (36 to 38 kb); at least 10 structural ether-resistant and acid-stable proteins; virions are released by cell destruction.

ADENOVIRUS-ASSOCIATED VIRUS (AAV)—Human parvovirus contains a single-stranded DNA genome and depends on helper viruses (adenovirus, herpesvirus, or vaccinia-virus) for replication. Without coinfection, the wild-type virions integrate at a specific site on chromosome 19 and remain latent.

ADVENTITIOUS AGENT—A foreign substitute that is introduced accidentally or inadvertently; not natural or hereditary (as in microbial, chemical, or biochemical contamination of a purified substance).

ALLOGENEIC—From an unrelated member of the same species; from the same species, but with a different genotype.

AMPHOTROPIC VIRUS—A virus that infects and replicates in cells from multiple species.

ANCILLARY PRODUCTS—Components used during manufacturing that should not be present in the final product. Examples: growth factors, cytokines, monoclonal antibodies, cell separation devices, media, and media components.

ANTISENSE THERAPY—The use of antisense oligonucleotides (a complementary segment to RNA) to control or inhibit gene expression.

APHERESIS—Procedure of withdrawing blood from a donor, removing select components (e.g., platelets or leukocytes), and retransfusing the remainder into the donor.

AUTOLOGOUS—From one's own body.

BASE PAIR—Two nucleotide bases on different strands of the nucleic acid molecule that bond together.

BIOASSAY—Measurement of the effectiveness of a compound by its effect on animals or cells in comparison with a standard preparation. (See also *Potency*.)

BIOLOGICAL PRODUCT—Any virus, therapeutic serum, toxin, antitoxin, or analogous product applicable to the prevention, treatment, or cure of diseases or injuries in humans. (The term *analogous product* has been interpreted to include essentially all biotechnology-derived products and procedures including gene therapy, transgenics, and somatic cell therapy.)

BIOTECHNOLOGY—Any technique that uses living organisms (or parts of organisms) to make or modify products, to improve plants or animals, or to develop microorganisms for specific uses. The newer definition refers to the industrial and pharmaceutical use of rDNA, cell fusion, novel bioprocessing techniques, and gene therapy.

B LYMPHOCYTES (B cells)—A class of lymphocytes that produce antibodies and are derived from the bone marrow.

BONE MARROW CELLS—A variety of undifferentiated cells (stem cells) and differentiated cells (lymphocytes, granulocytes, erythrocytes, and platelets) found in the internal cavities of bones or bone marrow.

BONE MARROW TRANSPLANTATION—Transplantation of bone marrow cells that are capable of maintaining the hematological functions indefinitely. Technique used in the treatment of immunological disorders (severe combined immune deficiencies such as ADA deficiency), hematological disorders (anemia), metabolic disorders (Gaucher's disease), and malignant diseases (leukemia, lymphoma, or solid tumor).

CD34—Cluster of Differentiation cell-surface marker 34. CD34 is a protein that distinguishes stem and progenitor cells from more mature blood cells.

CELL LINES—Cells that are derived from primary culture embryos, tissue, or organs. Such cell lines may have a finite life span or be immortalized (made to replicate indefinitely).

CELL THERAPY—Therapy that uses whole cells to treat a disease, condition, or injury.

CGMP—Current good manufacturing practice. The FDA outlines CGMP in the 21 CFR and in the *Federal Register* and its *Points to Consider*.

CHONDROCYTES—Cells that produce the components of cartilage.

CLONAL—Genes, cells, or entire organisms derived from and genetically identical to a single common ancestor gene, cell, or organism.

CLONOGENIC ASSAY—Procedure based on the ability to give rise to a clone of cells.

COMPLEMENTARY DNA (cDNA)—DNA synthesized from an mRNA rather than a DNA template. It is used for cloning or as a DNA probe for locating specific genes.

CYTOKINE—Any factor that acts on cells; usually a protein that promotes growth.

CYTOPLASM—Cellular material that is within the cell membrane and surrounds the nucleus.

CYTOTOXIC—Able to cause cell death.

DENDRITIC CELL—Cells that sensitize T cells to antigens.

DIFFERENTIATION—A process of biochemical and structural changes by which cells become specialized in form and function.

DIPLOID CELL—A cell with two complete sets of chromosomes (see *Haploid Cell*).

ECOTROPIC VIRUS—A virus that infects and replicates in cells from only the original host species.

ELECTROPORATION—Physical means of gene transfer (using a brief electrical field), involving creation of temporary pores in cell membrane to introduce DNA.

ELISA—Enzyme-linked immunosorbent assay. An immunoassay that utilizes an enzyme-labeled antigen or antibody to detect the binding of a molecule to a solid matrix.

ENDOTHELIAL CELLS—Epithelial cells of mesodermal origin that line the internal cavities of the body, such as heart and blood and lymph vessels.

ENGRAFTMENT—Process whereby cells, tissues, or organs are implanted or transplanted into another organism. Refers both to the mechanical and the biological processes necessary to have a fully functional graft.

ENVELOPED VIRUSES—Viruses containing a lipoprotein bilayer surrounding the capsid and acquired by budding through the cell membrane of the host cells.

EPIDERMAL—Pertaining to the outermost and nonvascular layer of the skin derived from embryonic ectoderm.

EPISOMAL—Pertaining to any accessory extrachromosomal genetic material.

EPITHELIAL CELLS—Cells from the linings of various organs. Examples: respiratory, intestinal, or vascular epithelial cells.

EXTRACORPOREAL—Situating or performed outside of the body.

EX VIVO—Procedure performed outside of the living organism.

FIBROBLASTS—Connective tissue cells that have the capacity to produce collagen.

FLUORESCENCE-ACTIVATED CELL SORTER (FACS)—A machine that sorts cells based on fluorescent markers attached to them.

FORMULATED—Prepared in accordance with a prescribed method or conditions.

FUSION—Joining of the membrane of two cells, creating a daughter that contains some of the same properties from each parent cell. It is used in making hybridoma cells in which antibody-producing cells are fused to mouse myeloma cells.

G-418—The antibiotic used to select and isolate cells that contain neomycin-resistance gene.

GENE CONSTRUCT—Expression vector that contains the coding sequence of the protein and the necessary elements for its expression.

GENE THERAPY—Therapy that uses DNA to treat a disease or condition. FDA defines gene therapy products as products containing genetic material administered to modify or manipulate the expression of genetic material to alter the biological properties of living cells.

GENOME—Total hereditary material of a cell.

GERM CELL—Reproductive cell (sperm or egg), gamete, or sex cell.

GRAFT VERSUS HOST DISEASE (GVHD)—Rejection of the transplanted tissue by the host. It is the leading cause of patient death when mismatched allogeneic tissue is used.

GRAFT VERSUS LEUKEMIA (GVL)—Rejection of host leukemia cells by donor T cells.

GRANULOCYTE—One of three types of white blood cells. These cells digest bacteria and other parasites.

GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR (GM-CSF)—A natural hormone that stimulates white blood cell production, particularly that of granulocytes and monocytes.

GROWTH FACTORS—Factors responsible for regulatory cell proliferation, function, and differentiation.

HAPLOID—A cell with half the usual number of chromosomes or only one chromosome set. Germ cells are haploid.

HELPER VIRUS—Aids the development of a defective virus by supplying or restoring the activity of a viral gene or by enabling the defective virus to form a functional envelope.

HEMACYTOMETER—A device used to manually count cells.

HEMATOPOIETIC—Pertaining to or affecting the formation of blood cells.

HEPATOCTES—The predominant cell type in the liver that has an important role in metabolism and is a source of serum proteins. These cells are generally not dividing, but when injured they can divide and regenerate until the injured cells are replaced.

HERPES SIMPLEX VIRUS (HSV)—A DNA virus that is a member of the family *Herpesviridae*. It can infect both warm- and cold-blooded vertebrates by contact between moist mucosal surfaces.

HUMAN LEUKOCYTE ANTIGEN (HLA)—Proteins controlled by the major histocompatibility complex. These proteins play a key role in determining transplant compatibility.

HUMORAL—Pertaining to elements found in body fluids (for example, humoral immunity and neutralizing antibodies).

HYBRIDIZATION DOT BLOT (DNA or RNA)—A technique for detecting, analyzing, and identifying protein; similar to the Western blot but without electrophoretic separation of proteins.

IMMUNOASSAY—Technique for identifying substances based on the use of antibodies.

IMMUNOFLUORESCENCE—Technique for identifying a fluorescent label.

IMMUNOGEN—Substance capable of inducing an immune response; a form of antigen that induces immune response, as opposed to a *tolerogen* that induces tolerance.

IMPLANTATION VS TRANSPLANTATION—Implantation is the insertion or grafting of a biological, living, inert, or radioactive material into the body. Transplantation is the grafting of tissues from the patient's own body or from another person's body.

INSERTIONAL MUTAGENESIS—A type of mutation that is caused by the insertion of a foreign gene into a host-cell chromosome. There are multiple negative consequences of such an event, including death of a cell if an essential gene is inactivated or predisposition to cancer if a tumor suppressor gene is inactivated.

INTEGRATION—Assimilation of genetic material (DNA) into the chromosome of a recipient cell.

INTERLEUKIN (IL)—Lymphokine that regulates the growth and development of white blood cells. More than 12 have been identified.

INTRABODIES—Intracellular antibodies that are not secreted and that are designed to bind and inactivate target molecules inside cells.

IN VIVO—Procedure performed in the living organism.

IN VITRO—Procedure performed outside of the living organism. It may involve cells or tissues derived from the organisms.

ISLET CELLS— β -islet cells of the pancreas that secrete insulin.

ISOGENIC—Of the same genotype.

KERATINOCYTES—Differentiated epidermal cells that constitute the top layer of cells in the skin.

LEUKEMIA—Malignant neoplasm of the blood-forming tissues.

LINEAGE (COMMITTED PROGENITOR CELLS, DIFFERENTIATED CELLS)—Specific path of cell differentiation that can be traced to a single cell of origin.

LIPOPLEX—A formulation of lipids and polymers and/or proteins.

LIPOSOME—A spherical lipid bilayer enclosing an aqueous compartment.

LYMPHOKINE—Class of soluble proteins produced by white blood cells that play a role in the immune response.

LYMPHOMA—Form of cancer that affects the lymphatic tissue.

MICROINJECTION—Physical means of gene transfer involving a direct injection of the cell with a syringe and a needle.

MACROPHAGE—Any of many forms of mononuclear phagocytes that are found in tissues and arise from hematopoietic stem cells in the bone marrow.

MOCK RUN—A test run that deliberately omits some critical reagents.

MONOCLONAL ANTIBODIES—Antibodies that are derived from a single cell clone.

MONOCYTES—One of the three types of white blood cells. They are precursors to macrophages.

MYELOSUPPRESSION—Inhibition of bone marrow activity resulting in depletion of red cells, white cells, and platelets.

MYOCYTES—Fundamental cell units in the muscle. Target cells for insertion of genes that encode secretory proteins.

NEOMYCIN—Antibiotic derived from *Streptomyces fradiae*.

NAKED DNA—Isolated, purified, and uncomplexed DNA (no protein or lipid).

OLIGONUCLEOTIDE—A polymer consisting of a small number of nucleotides, usually 5 to 30.

ONCOGENES—Genes associated with neoplastic proliferation (cancer) following a mutation or perturbation in their expressions.

ONCOGENIC—Cancer-causing.

OSTEOGENIC CELLS—Derived from or involved in the growth or repair of bone.

PACKAGING CELL LINE—Cell line that produces all of the proteins required for packaging and production of viral vectors in an active form, but does not produce replication-competent virus.

p53 GENE—Gene whose mutation is the most common alteration observed in human cancers. It is not required for normal development, but the lack of this gene highly increases the potential risk of cancer.

PARVOVIRUS—DNA viruses of the family *Parvoviridae*. Host range includes many vertebrate species.

PERCUTANEOUS—Performed through the skin. An example of a percutaneous procedure is the injection of an agent or removal of a tissue (sample for biopsy) with a needle.

PERITONEAL MESOTHELIUM—Lining of the peritoneal cavity consisting of a single sheet of cells covering a broad surface. It has abundant lymphatic drainage and permits diffusion of macromolecules.

PLASMID—A small circular form of DNA that carries certain genes and is capable of replicating independently in a host cell.

POLYCLONAL—Derived from a population of cells consisting of many clonal types.

PROCESS VALIDATION—Means for providing documentation that the manufacturing process is controlled, reproducible, and capable of consistently producing a product that meets predetermined specifications.

PRODUCER CELL LINE—An established cell line used to produce virus vectors, often at a large scale.

POLYMERASE CHAIN REACTION (PCR)—Technique to amplify a target DNA or RNA sequence of nucleotides by several hundred thousand-fold.

POTENCY—A quantitative measure of biological activity based on the attribute of the product linked to the relevant biological properties.

PROGENITOR CELL—Parent or ancestral cell, usually one that is already committed to differentiate into a specific type or lineage of cells.

PROMOTER—DNA sequence that is located at the front of a gene and controls gene expression. It is required for binding of RNA polymerase to initiate transcription.

RADIOIMMUNOASSAY (RIA)—Technique for quantifying a substance by measuring the reactivity of radioactively labeled forms of the substance.

RECOMBINANT-DNA—DNA produced by joining fragments DNA from different sources by *in vitro* manipulations.

REPLICATION-COMPETENT VIRUS—A virus that can complete entire replication cycle without a need for a helper virus; an autonomously replicating virus.

RESTRICTION ENDONUCLEASE—An endonuclease that recognizes specific sequence of bases within double-stranded DNA.

RETROVIRUS—A virus that contains the reverse transcriptase which converts viral RNA into DNA that then integrates into the host cell in a form called a provirus.

SERUM-FREE—Refers to cell growth medium that lacks a serum component.

SOMATIC CELLS—Cells other than the germ cells.

S PHASE—Part of the cell cycle during which DNA replication occurs.

STEM CELL—Immortal cell that is capable of proliferating and differentiating into different types of specialized cells. Each major tissue system is thought to have its own putative stem cell.

STROMAL—Refers to cellular support elements that contain essential nutrients or growth factors.

SUPRAVITAL DYE—A dye that stains only living cells.

SUSPENSION CULTURE—Cells capable of growth in suspension, no requiring substrate (attachment) on which to grow.

T CELLS—Lymphocytes that acquire functional repertoires and the concept of self in the thymus and are responsible for cell-mediated immunity. There are several subsets of T cells (helper T cells, suppressor T cells, and cytotoxic T cells).

TCID₅₀ ASSAY—Tissue Culture Infectious Dose, 50% Assay. An assay measuring the amount of product at which 50% of culture cells in the assay are killed (cytopathic effect) or are expressing a vector protein.

TRANSDUCTION—Transfer and expression of genetic material into a cell by means of a virus or phage vector.

TRANSFECTION—Transfer of DNA into cells by physical means such as by calcium phosphate coprecipitation.

TRANSGENE—Refers to the foreign or therapeutic DNA that is part of a vector construct.

TUMORGENICITY—Having the properties of inducing a malignant neoplasm.

VECTOR—The agent (plasmid, virus, or liposome-protein or DNA-protein complex) used to introduce DNA into a cell.

VIABILITY—State of being alive and functional.

VIRION—An elementary viral particle consisting of genetic material (nucleocapsid) and a protein covering.

VIRUS—Submicroscopic organism that contains genetic information necessary for reproduction. It is an obligate intracellular parasite.

WESTERN BLOT—An electroblotting method in which proteins are transferred from a gel to a thin, rigid support (e.g., nitrocellulose membrane) and detected by binding radioactively labeled antibody or antibody coupled to an enzyme, allowing use of a precipitating chromogenic or chemiluminescent substrate.

XENOGENEIC—From a different species.

XENOTRANSPLANTATION—Transplantation of organs from one species to another (e.g., from pigs to humans).

ZOONOSIS—The disease of animals transmitted to humans via routine exposure to or consumption of the source material.

ABBREVIATIONS

AABB	American Association of Blood Banks
7-AAD	7-amino-actinomycin D
AATB	American Association of Tissue Banks
AAV	adeno-associated virus
ADA	amino deaminase
BSE	bovine spongiform encephalopathy (mad cow disease)
CBER	FDA Center for Biologics Evaluation and Research
CDC	The Centers for Disease Control and Prevention
CDER	FDA Center for Drug Evaluation and Research
CDRH	Center for Devices and Radiological Health
cfu	colony-forming unit
CGMP	current good manufacturing practice
CSF	colony-stimulating factor
cDNA	complementary DNA

DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
FBS	fetal bovine serum
FACS	fluorescence-activated cell sorter
GVHD	graft versus host disease
GM-CSF	granulocyte-macrophage colony-stimulating factor
HSV	herpes simplex virus
HRSA	Health Research Services Administration
HLA	human leukocyte antigen
kb	kilobase
NIH	National Institutes of Health
NMDR	National Marrow Donor Registry
PBPC	peripheral blood progenitor cell
PCR	polymerase chain reaction
QC-QA	quality control-quality assurance
RCA	replication-competent adenovirus
RCR	replication-competent retrovirus
RCV	replication-competent virus
rDNA	recombinant DNA
RNA	ribonucleic acid
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
TCID ₅₀	tissue culture infectious dose, 50% ¹

Add the following:

■ (1146) PACKAGING PRACTICE— REPACKAGING A SINGLE SOLID ORAL DRUG PRODUCT INTO A UNIT-DOSE CONTAINER

INTRODUCTION

Repackaging of solid oral drug products, such as tablets and capsules, into unit-dose configurations is common practice both for the pharmacy that is dispensing drugs pursuant to a prescription and for the pharmaceutical repackaging firm. This general chapter contains minimum standards to be used as a guideline for repackaging practices. This guideline is not intended to replace or supplant the requirements of regulatory agencies.

Repackaging preparations into unit-dose configurations is an important aspect of pharmaceutical care and of optimization of patient compliance. For purposes of this chapter, there are two types of repackaging: the first involves pharmacies that dispense prescription drugs; the second concerns commercial pharmaceutical repackaging firms.

NOMENCLATURE AND DEFINITIONS

DISPENSER—A dispenser is a licensed or registered practitioner who is legally responsible for providing a preparation for patient use, with a specific patient label, pursuant to a prescription or a medication order. In addition, dispensers may prepare limited quantities in anticipation of a prescription or medication order from a physician. Dispensers are governed by the board of pharmacy of the individual state.

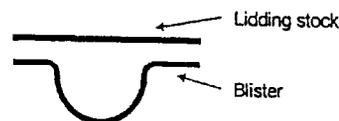
PHARMACY—A pharmacy is an establishment that is legally responsible for providing the drug preparation for patient use, with a specific patient label, pursuant to a prescription or a medication order. The terms dispenser and pharmacy are used interchangeably.

REPACKAGING—Repackaging is the act of removing a preparation from its original primary container and placing it into another primary container, usually of smaller size.

REPACKAGER—A repackager is an establishment that repackages drugs and sends them to a second location in anticipation of a need. Repackaging firms repackage preparations for distribution (e.g., for resale to distributors, hospitals, or other pharmacies), a function that is beyond the regular practice of a pharmacy. Distribution is not patient specific in that there are no prescriptions. Unlike dispensers, repackaging firms are required to register with the FDA and to comply with the Current Good Manufacturing Practice regulations in 21 CFR 210 and 211.

MATERIALS

Blister packages offer a wide array of designs of both functionality and appearance. Various packaging materials are utilized to create blisters that are tailored to provide optimum performance. The blister container consists of two components: the blister, which is the formed cavity that holds the product, and the lid stock, which is the material that seals to the blister, as shown below. Because of the variety of blister films available, film selection should be based upon the degree of protection required. The choice of lid stock depends on how the blister is to be used, but generally the lid stock is made of aluminum foil. The material used to form the cavity is typically a plastic, which can be designed to protect the dosage form from moisture. There are widely varying degrees of moisture protection now available. For purposes of this general chapter, they are referred to as nominal, medium, high, and extreme moisture barrier properties.



Schematic Presentation of a Typical Blister Pack

Polyvinyl Chloride—The most commonly used blister material is polyvinyl chloride (PVC). This material, which provides a nominal or zero barrier to moisture, is used when the product does not require effective moisture protection. PVC is available in a range of gauges and can be made opaque or can be tinted with pigments to block out specific light wavelengths.

The thickness of the PVC used is determined by the depth and size of the cavity to be formed. Because the plastic thins during the blister-forming process, care should be taken to ensure that the finished blister provides sufficient protection from light (if required) and that it is strong enough to adequately protect the dosage form. Common gauges of PVC used in the pharmaceutical industry range from 7.5 to 15 mil (0.0075 to 0.015 inch).

Barrier Films—Many drug preparations are extremely sensitive to moisture and therefore require high barrier films. Several materials may be used to provide moisture protection. Barrier films commonly used in the pharmaceutical industry are described below.

PVC/PCTFE Laminations—Polychlorotrifluoroethylene (PCTFE) film¹ is a thermoplastic film made from polychlorotrifluoroethylene fluoropolymer. The PCTFE film is laminated to the PVC by an adhesive layer between the PVC and the PCTFE film (duplex structure) or by a layer of polyethylene between the PVC-adhesive and the PCTFE-adhesive layers (triplex structure). By using various gauges of the PCTFE film, *medium* to *extreme* moisture barriers can be obtained.

¹ PCTFE film is available from Allied Signal (as Aclar) and from other sources.

I would like to thank the Office of Ombudsman at the FDA for this opportunity to speak at this hearing. I am speaking today in my role as Chair of the United States Pharmacopeia's Expert Committee on Gene Therapy, Cell Therapy and Tissue Engineering. This presentation is based on this committee's past work which culminated in the information chapter <1046> Cell and Gene Therapy Products, its current work on an information chapter on Ancillary Products for Cell and Gene Therapy Products and its work with companies to write monographs for wound healing products that contain live cells.

It is not the committee intention today to testify on which center in the FDA should have jurisdiction over these products or what the primary mechanism of action of these products. Our work has always assumed that reproducibly manufacturing a safe product with live, functioning cells was the goal. I intend to focus today on our work to provide information for cell and gene therapy products in general and monographs and reference standards for wound healing products with live cells, in particular.

The forerunner of this expert committee was an advisory group to the subcommittee on Biotechnology and Gene Therapy, which was formed in December 1997. This group was composed of scientists and clinicians from academic medical centers, the biopharmaceutical industry, both large and small, and the government, including the FDA. The members have experience with cell based products for wound healing, bone marrow transplantation, xenogeneic cell therapies, patient specific cell therapies, viral and non-viral gene therapies as well as traditional biotechnology derived products. At that December meeting we decided that we would write an informational chapter on cell and gene therapies after which we could focus work on the issue of ancillary products for these products. The goal of this first chapter, <1046> Cell and Gene Therapy Products, was "to summarize the issues and best current practices in the manufacturing, testing and administration of cell and gene therapy products." In other words, we wanted the chapter

- to contain all of the information the committee would have liked to have known if they were starting out in these fields today,
- to cite examples that are directly relevant to those making cell and gene therapy products,
- to look forward towards the standards and practices for approved products but
- to present also general information on the development, manufacturing and testing of these products.

Since this is a new field, we considered the relevance of all regulatory guidances whether or not they specifically were devised for these products. These included the numerous CBER Points to Consider and Guidances, the ICH guidances, especially those for biotechnology derived products, 21CFR210, 211, 600s and 820, the Quality Systems Regulations (QSR) and ISO guidances. Aspects from all of these sources influenced us. In fact an overriding theme of this chapter is that the ICH guidelines, especially those for biotechnology derived products, are useful in that principles of these guidelines can be applied to cell and gene therapy products even if the guidelines specifically state that they are not applicable to cell and gene therapy. To help apply the important principles of these guidances and regulations the panel tried to provide as many useful examples that are specific to cell and gene therapy as are practical. The goal with these examples is to go beyond the FDA definitions and guidances to make these guidances relevant to the field of cell and gene therapy.

A complete outline of <1046> is attached. I will highlight only those sections that are relevant for wound healing products containing live cells. We divided manufacturing into

multiple sections. The first section is a manufacturing overview. It discusses raw materials sourcing and qualification, characterization of cell and virus banks, in process controls, specifications and considerations for validation. This section was strongly influenced by the risk assessment and design approaches of QSR and by the numerous CBER points to consider and guidances on testing these products for adventitious agents. The manufacturing of cell therapy product section contains much information on directly relevant to these type of wound healing products including a concise list of the desired qualities for the supporting matrix. We felt that if any preparation of the final product is done at the clinical site that it should be viewed as an extension of manufacturing and should be supported by appropriate SOPs and facilities and by people trained in the processing. We organized the analytical methodology section so that safety was the first item detailed, not the last. In addition, these products need assays for defining the dose, the potency, purity and identity of the product. The Stability section points out that a brief excursion in temperature to outside the stated limits such as may occur in an airplane hold or in a surgical suite may be as damaging as a long term exposure to conditions just outside storage specifications. The Storage and Shipping section discusses the issue both with storing a product frozen as well with shipping it in an unfrozen form. The chapter concludes with brief sections on Labeling these products, on Regulations, Standards and New Methodologies for these products and a list of definitions and abbreviations.

An initial draft of <1046> was published in Pharmacopeia Forum (PF) January, 2000 for comments. A revised version was published in PF in January, 2001. Based on a few additional comments, a final version of <1046> was approved by the now Expert Committee and published as part of the first supplement to USP 25/ NF 20, which became effective April 1, 2002.

In 2000 the Expert Committee also started work on a second information chapter that discusses sourcing and qualifying ancillary products for use in the manufacturing of cell and gene therapy products. Ancillary products are those materials used in the manufacturing of the therapeutic product that are not intended to be in the final product. Again the committee has found CBER's extensive guidances on adventitious agents and handling of biotechnology derived products to be important. We also find that CDRH's QSR approach extremely helpful. The QSR spell out a more comprehensive approach to quality than CBER's specification for the quality function. QSR start right at the conceptualization, design and development phases (design controls) and directly address risk assessment for products and raw materials. Addressing these issues up front is important for developing a manufacturing process to produce safe cell or gene therapy products, which consistently demonstrate the expected activity.

More recently the Expert Committee has work with three companies on monographs for wound healing products containing live cells. A draft monograph for one of these products was published in Pharmacopeia Forum in November, 2001. The other two monographs are still in an early stage. Each monograph contains sections that describe (a) the product configuration and adventitious agent and other non-USP specified testing, (b) packaging and storage, (c) labeling, (d) USP reference standard and (e) tests to identify the product. The committee would like to emphasize that in the USP tradition these tests are not intended to be routine release tests for these products but are a set of tests that can be used to distinguish these cellular products from each other and from other wound healing products. These three wound-healing products are all clearly different.

After working on its first monograph, the committee decided that monographs for wound healing products containing live cells should include the following for types of tests.

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1. Detailed histology of the product which clearly demonstrates the organization of the different cell types relative to the matrix as well as the gross structural properties of the matrix.
2. Test(s) to identify the different cell types and the matrix.
3. Test(s) that show the cells to be viable or to have the expected metabolic activity in the final product.
4. Test(s) for other unique properties, if relevant. For instance, in the draft monograph published last fall there is an assay that demonstrates that the top layer of that particular wound healing product with live cells was cornified.

The reference standard for that monograph are a series of photomicrographs of the histology of the product that depict product that passes these specifications as well as product that fails these specifications. These photomicrographs are in the process of being reviewed by independent pathologists for their acceptability as a reference standard.

The committee urges that all wound healing products with live cells be reviewed by the same center so there is consistency of review. They urge that center to be versed in both the issues with live cell products as well as the issues with biomaterials and their sourcing and with ancillary products needed to make these cell products. They also feel that if the jurisdiction changes, there should be no undue regulatory burden placed on the wound healing products already on the market as it is the committee's perception that there have been no major safety issues with these approved products. Finally, the committee would like to thank both CBER and CDRH for the good relationships it has with them and looks forward to working with whatever center regulates wound healing products with live cellular components.

Wound Healing Products with Live Cells

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<1046> Cell and Gene Therapy Products

I. Introduction

General Definitions
Cell Therapy Products
Gene Therapy Products
Chapter Purpose and Organization

II. Manufacturing Overview

Introduction

Raw Materials

Types of Raw Materials

Qualification

Identification and Selection

Suitability

Characterization

Fetal Bovine Serum

Quality Assurance Characterization of Cell and Virus Banks

Cell Banks

Virus Banks

Qualification

Qualifying Master Cell Bank

Qualifying Master Viral Bank

Qualifying Working Cell or Viral Bank

In-Process Control

Specifications

Considerations for Validation

III. Manufacturing of Cell Therapy Products

Introduction

Procurement of Source Material

Human Tissue

Human Blood and Bone Marrow

Animal Tissue

Cell Isolation and Selection

General Considerations

Isolation

Selection

Cell Propagation and Differentiation

Propagation

Differentiation

Introduction of Genetic Material into Cells

Formulation of Cell Therapy Products

Suspensions

Products Combined with Biocompatible Matrices

IV. Manufacturing of Gene Therapy Products

Introduction

Design Considerations for Gene Vectors

Types of Vectors

Vector Design Criteria

Targeting Transduction

Impact of Humoral Immune System

Impact of Cellular Immune Responses

Antigenicity of Gene Product

Complement Inactivation

Tissue Specific Promoters

Impact of Replication Status of Vector

Vector Integration

Manufacturing and Purification Strategies

Vector Construction

Helper Function Systems

Viral Gene Therapy Vectors

Plasmid Vectors

Oligonucleotide Vectors

Formulation of Gene Therapy Products

V. On-Site Preparation and Administration

General Considerations

On-Site Preparation

Product Manipulations

Facility Requirements

Release of Final Product

Administration to Patients

Pre-administration Requirements

Patient Treatment

Postadministration Monitoring of Patient

V. Analytical Methodologies

- General Considerations

- New Methodologies and Compendial Perspective

- Sampling Issues

- Safety

 - General Considerations

 - Cell Therapy Products

 - Viral Gene Therapy Products

 - Nonviral Gene Therapy Products

- Dose-Defining Assays

 - General Considerations

 - Cell Therapy Products

 - Viral and Nonviral Gene Therapy Products

- Potency

 - General Considerations

 - Cell Therapy Products

 - Viral and Nonviral Gene Therapy Products

V. Analytical Methodologies (cont.)

- Purity

 - General Considerations

 - Cell Therapy Products

 - Viral Gene Therapy Products

 - Nonviral Gene Therapy Products

 - Lyophilized Viral and Nonviral Vector Products

- Identity

 - General Considerations

 - Cell Therapy Products

 - Viral Gene Therapy Products

 - Nonviral Gene Therapy Products Dose-Defining Assays

VI. Stability

- General Considerations

- Stability-Protocol Development

- Accelerated and Most Appropriate Challenge Conditions

VII. Storage and Shipping

General Considerations

Cell Therapy Products

 Cryopreservation

 Thawing

 Frozen Products

 Unfrozen Products

Gene Therapy Products

VIII. Labeling

IX. Regulations, Standards, and New Methodologies

 Summary of Regulations and Standards

 Need for New Methodologies

X. Definitions of Terms

XI. Abbreviations

Monograph for Wound Healing Products with Live Cells

- Description, General Characteristics & Adventitious Agent Testing
- Packaging and Storage
- Labeling
- USP Reference Standard
- Tests
 - Histological characterization
 - Cell and matrix identity
 - Cell viability/metabolic function
 - Other unique characteristics



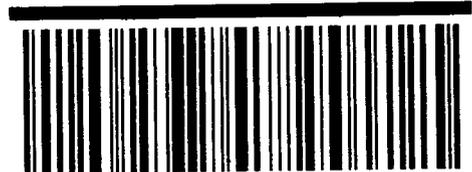
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Label 314, July 2000