⟨1046⟩ CELL-BASED ADVANCED THERAPIES AND TISSUE-BASED PRODUCTS

INTRODUCTION

This general chapter provides a comprehensive overview of considerations for the development of cell-based advanced therapies and tissue-based products. A collection of terms commonly used in this field is provided in *Glossary*. Cell-based advanced therapies and tissue-based therapies are medical products that contain human or animal cells that will be administered to humans to repair, replace, regenerate, or augment a recipient's cells, tissues, or organs that are diseased, dysfunctional, or injured. The source cells or tissues can be harvested for use without manipulation or may be propagated, expanded, pharmacologically treated, or otherwise altered in biological characteristics ex vivo before administration. The diversity of clinical indications and types of cell-based advanced therapies are shown in *Table 1*. Examples of tissue-based products are included in *Table 2*.

Table 1. Examples of Cell-Based Advanced Therapies

Disease/Condition	Example
Hematopoietic stem cell transplantation following ablative therapy	Hematopoietic stem and progenitor cells that have been harvested, propagated, selected, and/or treated for removal of contaminating cells by means of devices and/or reagents
Cancer	T cells, NK cells, dendritic cells, or macrophages targeted to cancer-specific peptides to elicit an anticancer response; autologous or allogeneic cancer cells, genetically or biochemically modified and irradiated to elicit an anticancer response
Diabetes	Encapsulated β-islet cells
Myocardial infarction	Autologous or allogeneic stem/progenitor cells; skeletal myocytes; cardiac-derived stem cells
Graft-versus-host disease	Allogeneic mesenchymal stem cells
Wound healing	Autologous keratinocytes or allogeneic dermal fibroblasts on a biocompatible scaffold
Focal defects in knee cartilage	Autologous or allogeneic chondrocytes with or without a biocompatible scaffold
Bone repair	Mesenchymal stem cells in a biocompatible scaffold
Neurodegenerative diseases	Neuronal progenitor cells derived from embryonic, fetal, or adult source tissues; cells genetically modified to secrete neurotrophic factors, with or without encapsulation
Infectious disease	Activated T cells
Autoimmune disease	Regulatory T cells (T_{reg})
Spinal cord injury	Neuronal progenitor cells
Organ repair or regeneration	Autologous or allogeneic cells on biocompatible biomaterials (gels) or 3-dimensional scaffold structure

Cell therapy products can be modified by treatment with integrating or non-integrating genetic materials (DNA, RNA, siRNA, etc.) so that the pattern of gene expression is changed. Typically, cells are taken from the patient or a healthy donor, and are modified outside of the body before they are given to the patient. Regulatory bodies consider the ex vivo gene-modified cellular product to be a gene therapy product. A great deal of information in this chapter is relevant to processing, characterization, manufacturing, and administration of genetically modified cells. However detailed information about the use of various gene transfer systems, patient monitoring considerations, genetic analysis, and other issues pertinent to gene therapy products are addressed in *Gene Therapy Products* ⟨1047⟩.

Table 2. Examples of Tissue-Based Products

Uses	Example Products
Musculoskeletal	Decellularized, freeze-dried demineralized human bone (cortical or cancellous)
	Decellularized, freeze-dried human bone (cortical or cancellous)
	Decellularized human dermis
	Decellularized bovine dermis
	Decellularized porcine dermis
	Decellularized porcine subintestinal submucosa (SIS)
	Human cancellous bone with adipose-derived stem cells
	Human demineralized cortical bone and cancellous bone with bone marrow-derived stem cells
Neurological	Decellularized human nerve tissue
Ophthalmology	Human amniotic tissue
	Human placental tissue
Wound care	Decellularized human dermis
	Decellularized porcine SIS
	Human amniotic tissue
	Human placental tissue

For tissue-based grafts that contain non-autologous cells, the cells are derived from the same donor; they may be harvested with the tissue, or harvested from a different organ (e.g., bone marrow aspirate, adipose) and recombined with the tissue (e.g., bone).

This chapter describes issues related to the manufacturing, sourcing of components, and characterization of cell-based advanced therapies or tissue-based products to ensure their safety and efficacy. A list of relevant regulatory and guidance documents is presented in the *Appendix*. Manufacturers of cell-based advanced therapies or tissue-based products should consider and apply the controls and procedures outlined in this chapter to ensure the products' safe use in humans. New methodologies are continually being developed and validated and will be included in the United States Pharmacopeia (*USP*) as they become available. *USP* monographs for specific tissue and tissue-based products outline test specifications that should be met throughout a product's time in the market place. The term "cellular therapy product" refers to living human or animal cells or tissues that are subject to approval by the US FDA. This generally means clinical trials are required to demonstrate safety and efficacy prior to commercialization under a biologics license application (BLA), or for a medical device, premarket approval (PMA). Additionally, most cellular therapy products must comply with both good tissue practices (GTPs) and good manufacturing practices (GMPs). A subset of cell-based products, known as combination products, or tissue engineering products, refer to cells combined with a medical device, such as a natural or synthetic scaffold. These products must also meet similar standards for establishing safety and efficacy as well as comply with GTPs and GMPs, or medical device quality system regulations (QSR). Most cell-based advanced therapies and tissue engineering products are likely to fall within the legislation that defines regenerative medicine advanced therapies (RMAT). The majority of commercially available tissue-based products are human derived. Human tissue-based materials are often referred to as HCT/Ps, or human cells, tissues, cellular and tissue-based products. HCT/Ps that meet criteria identified in 21 Code of Federal Regulations (CFR) 1271.3(d)(1) and Section 361 of the Public Health Service (PHS) Act do not require premarket approval by FDA (i.e., BLA or PMA) and only need to comply with GTP regulations. These "361 products" meet criteria for "minimal manipulation", and are utilized in applications consistent with "homologous use". Human tissue products not meeting these criteria may require premarket clearance [510(k)] or PMA or BLA. While less common, some tissue-based materials are animal derived and generally require premarket clearance [510(k)].

Considerations for Incorporating Quality System Concepts Early in Cell-Based Advanced Therapies and Tissue-Based Product Development

Current and future regulatory requirements will continue to challenge developers of cell-based advanced therapies and tissue-based products to incorporate robust quality attributes early in the design phase to ensure a focus on patient safety by means of a high degree of process understanding. Modern quality systems that harmonize current Good Manufacturing Practices (cGMPs) with other non-US pharmaceutical regulatory systems [such as the International Council for Harmonisation (ICH) and the ISO] and the FDA medical device quality system are being recognized for ensuring quality. These new standards include product development concepts such as Quality by Design (QbD) and Process Analytical Technology (PAT). Moreover, these quality systems integrate approaches to continual improvement and risk management that promote adoption of the latest scientific advances and innovative manufacturing technologies.

Employing the principles of Quality Risk Management (QRM) early in product development may identify areas of risk that can be mitigated before they are incorporated into the manufacturing process and affect the safety and efficacy of the product. Developers of cell-based advanced therapies and tissue-based products should employ risk management and assessment techniques as a key component of their quality systems. "Risk management" is defined as a systematic process for the identification, assessment, and control of risks to the quality of the cell-based advanced therapies or tissue-based product across

the product lifecycle. Using QRM techniques can help achieve safe and efficacious products by assessing patient risks, determining design space boundaries, or ranking quality attributes. QRM can also establish and maintain a state of control by using risk management to drive process control. Finally, QRM can be used to facilitate continual improvement by prioritizing opportunities for improvement. The level of effort, formality, and documentation of the risk management process should be commensurate with the level of risk, should be based on scientific and medical knowledge, and ultimately should be linked to patient protection.

The elements of risk management have become an accepted paradigm; these are described in FDA and international regulatory guidance documents, especially ICH Q9. A number of tools have been developed to facilitate this assessment. These tools provide a quantifiable means of prioritizing risk so that higher-risk elements of a process can be identified and corrected.

Depending on the objective of the risk management program, risk analysis can be more or less formalized. A more formalized risk assessment system is necessary for process or product development. This is especially important when limited resources must be prioritized. Formalized systems are predicated on well-established tools that can quantify risk in every phase or step of manufacturing. These systems can also be used in evaluating raw material choices, validation prioritization, facility alterations, equipment changes, and utility deliberations.

Formal risk analysis tools include process mapping, preliminary hazard analysis, Hazard Analysis of Critical Control Points (HACCP), Hazard Operability Analysis (HAZOP), Fault Tree Analysis (FTA), Failure Mode Effects Analysis (FMEA), and Failure Mode Effects and Criticality Analysis (FMECA).

For cell-based advanced therapies and tissue-based products, FMEA has been commonly used to identify, quantify, and prioritize risk. FMEA can assign a numerical rating in one of three categories:

- "Severity", which is the consequence of a failure;
- "Occurrence", which is the likelihood of the failure happening based on past experience or nonconformance; and
- "Detection", based on the ability to detect the failure.

Each category is assigned a numerical rating (typically 1–5 or 1–10) consistent with the severity of the excursion from the operating parameter range, the probability of an excursion, and the likelihood of detecting an excursion before it has an effect on the product. Lower numbers refer to an unlikely probability of detection whereas higher numbers refer to the likelihood of a failure or hazardous effect. The product of the severity, occurrence, and detection values is a Risk Priority Number (RPN). In the risk-evaluation process RPNs are prioritized, and the most immediate remediation can be directed to areas of highest risk.

COMPONENTS USED IN CELL-BASED ADVANCED THERAPIES AND TISSUE-BASED PRODUCT MANUFACTURING

Introduction

Manufacturers of cell-based advanced therapies or tissue-based products must ensure that all components used in manufacturing are appropriately qualified. Examples of components used in the production of cell-based advanced therapies or tissue-based therapies include the source cells and tissues; natural or synthetic biomaterials; ancillary materials required during manufacturing but not intended to be present in the final therapeutic product; and excipients used in the formulation of cell-based advanced therapies or tissue-based therapies. For more information on ancillary materials, see *Ancillary Materials For Cell, Gene, And Tissue-Engineered Products* ⟨1043⟩.

Qualification is the process of acquiring and evaluating data to establish the source, identity, purity, biological safety, and overall suitability of a specific component to ensure quality. The diversity of cell-based advanced therapies and tissue-therapy products and the materials used to produce them makes it difficult to recommend specific tests or protocols for a qualification program. Therefore, rational and scientifically sound programs must be developed for each component.

Material qualification activities will change as products move 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 should be the primary focus of a material qualification plan. In the later stages, material qualification activities should be completely developed and should comply with cGMP.

Qualification of Source Cells and Tissues

Various human- and animal-derived cells and tissues serve as source material for cell and tissue-based products. Three sources of donor cells for cell-therapy products include:

1. The patient's own cells (autologous cell products)
2. Cells from another human being (allogeneic cell products)
3. Cells derived from animals (xenogeneic cell products)

The source of cells used for a particular cell or tissue-based therapy largely depends on compatibility, purity, and availability. Use of autologous cells has the advantage of minimal concerns regarding immune rejection. However, an autologous source is not always available and appropriate if the cell type is dysfunctional, malignant, not readily obtainable, or contaminated.

The alternative is a compatible allogeneic cell source that may be more readily available. Of primary concern with the use of allogeneic cell sources is immune incompatibility, which could lead to rejection of the administered cell- or tissue-based therapy. In immunocompromised recipients, the donor cells may react to the patient's cells, leading to graft-versus-host disease, which can be life threatening. Despite the potential complications of using allogeneic donor cells or tissues, in the absence of other alternatives the risk-to-benefit ratio may be acceptable. A number of approaches successfully circumvent immune barriers for the use of allogeneic sources. Immunosuppressive drugs developed for solid organ transplantation and advances in inducing immune tolerance are increasingly applied to cell transplantation. Certain allogeneic cells elicit minimal immune reactions, even

in human leukocyte antigen (HLA)-mismatched recipients. Examples include mesenchymal stem cells, certain dermal and epidermal cells, and fibroblasts. Cells may be obtained from living or deceased donors.

Despite advances in the derivation of new types of therapeutic cells, particularly stem cells (adult, fetal, embryonic and induced pluripotent cells), the ability to generate certain types of cells or tissues remains elusive. As a result, ongoing efforts use xenogeneic cells and tissues to treat a number of human diseases or conditions. Use of xenogeneic cells and tissues must address concerns about both immune rejection and transmission of animal viruses to humans (see *Animal Sources of Cells and Tissues* below). Both human- and animal-sourced cells and tissues can pose a risk for transmission of adventitious agents.

Some general principles in the sourcing of tissues include: 1) systems must allow the material to be traced back to the donor, while adhering to privacy legislation; 2) steps must be taken to prevent the transmission of infectious diseases from the donor to the recipient; and 3) while terminal sterilization is employed for some decellularized tissue-based materials, aseptic procurement and processing must ensure the safety of the final product because terminal sterilization of products containing living cells and tissues is not possible. The FDA has promulgated a specific set of regulations, referred to as GTPs, that specifically address the need to procure and process tissues in a manner that avoids transmission of a communicable disease. GTPs and/or GMPs must be followed for cell-based advanced therapies or tissue-based therapy products, depending on cell source.

DONOR ELIGIBILITY

FDA has enacted a comprehensive set of regulations governing human tissues and human cells that are intended for implantation, transplantation, infusion, or transfer into a human recipient. These materials are referred to as HCT/Ps. Paramount for procurement of HCT/Ps for medical use is adherence to donor eligibility requirements. These dictate that a donor's relevant medical records must be reviewed to evaluate risk factors and clinical evidence of communicable disease agents. This includes obtaining a health history and could include performing a physical assessment on a donor to screen for communicable diseases. In addition, donors must also undergo appropriate laboratory testing using FDA-cleared or -approved test kits for specific relevant communicable disease agents and diseases (RCDADs). Required disease testing will expand as new RCDADs are identified and FDA-cleared or -approved tests or test kits become available. Sources for information about communicable disease testing are FDA's *Guidance on Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps)*, American Association of Tissue Banking (AATB) Standards, and subsequent recommendations for emerging RCDADs such as West Nile Virus and Zika Virus are available from the FDA website (https://www.fda.gov/regulatory-information/search-fda-guidance-documents/eligibility-determination-donors-human-cells-tissues-and-cellular-and-tissue-based-products).

HUMAN CELLS, TISSUES, OR CELL-BASED ADVANCED THERAPIES OR TISSUE-BASED PRODUCTS

HCT/Ps may be sourced from normal healthy donors, cadaveric donors, or patients with diseases such as cancer. The suitability of tissue sourced from patients with cancer and other diseases should be assessed before collection to ensure adequate safety and function of the final cell therapy product. Additionally, the regulations in 45 CFR Part 46 apply to all federally supported human subject research. These regulations require that an Institutional Review Board review and approve the use of any tissue taken from a human donor. The regulations also include special considerations for research on prisoners, children, pregnant women, or gestational tissue. In all cases appropriate written consent must be obtained from the donor or the donor's next of kin describing the tissue that is being procured and its intended use.

The risk of disease transmission to the manufacturing operator should be minimized by appropriate training for handling potentially infectious materials and by the use of protective equipment and clothing. Tissues should be obtained under environmental conditions and controls that provide a high degree of assurance for aseptic recovery.

Hematopoietic progenitor cells (HPCs) are one of the most extensively used cell sources for human transplantation. These cells can be collected from the bone marrow, peripheral blood, or umbilical cord blood. The source of cells depends on the patient, the disease, and the clinical protocol. Regardless of the cell source, methods for processing the cells are similar. HPCs can be sourced from healthy donors or patients with hematological disorders. In addition to FDA's HCT/Ps regulations, 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, and the National Marrow Donor Program (NMDP).

For cell or tissue sources obtained from surgical specimens or cadaveric donors, standard hospital operating room practices are applicable. The air quality 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 aseptic technique. Special care is required when tissue or organ procurement requires extensive manipulation of the bowel, which may result in the inadvertent puncture of the bowel. Consideration should be given to sampling incoming tissues for microbial bioburden. Though it is recognized that results of such bioburden testing would likely not be available until after the tissue has been processed, in the event of a subsequently observed microbial contamination during downstream processing or positive sterility result, the bioburden results from the incoming tissue could aid in the investigation of the source of the contamination.

ANIMAL SOURCES OF CELLS AND TISSUES

Ideally, cellular therapy products would consist of human cells manufactured with minimal exposure to animal-based materials. However, at present important unmet medical needs may potentially be addressed by cellular therapy products from animal cells or tissues. One example is pancreatic islets intended to treat diabetes. Currently human sources of pancreatic islets are available only from pancreas donated at the time of death. The quality of donor organ islets is variable, and the available supply is inadequate to meet potential demand. One approach is procurement of pancreatic islets from appropriately qualified animal sources for subsequent use in humans (xenotransplantation).

Developers who intend to use viable animal cells or tissues in a cell-based advanced therapy or tissue-based product must adequately address public health concerns and must develop approaches to mitigate the potential risk of introduction and propagation of zoonotic infectious agents into the general human population. The *PHS Guideline on Infectious Disease Issues in Xenotransplantation* (January 2001) describes potential risks. The FDA guidance *Source Animal, Product, Preclinical, and Clinical Issues Concerning the Use of Xenotransplantation Products in Humans* (April 2016) reflects updated approaches and expectations to minimize risks of xenogeneic cellular products (https://www.fda.gov/regulatory-information/search-fda-guidance-documents/source-animal-product-preclinical-and-clinical-issues-concerning-use-xenotransplantation-products). In most cases, decellularized (i.e., nonviable) xenograft tissue-based products are not subject to the PHS guideline and FDA guidance mentioned above and are generally regulated in the US as medical devices and must follow the applicable regulatory pathway [e.g., 510(k)] and all related requirements.

The use of viable animal tissue in the manufacture of cell-based advanced therapies requires that the tissue be sourced in a controlled and documented manner from designated pathogen-free animals bred and raised in captivity in countries or geographic regions that have appropriate 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 animal housing facility 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 current version of the *Guide for the Care and Use of Laboratory Animals* (National Research Council), which can be obtained from AAALAC (www.aaalac.org). Such facilities should be staffed with veterinarians and other trained personnel who ensure animal health and disease prevention. The facility's procedures should be documented, and records should be kept. Health maintenance and monitoring programs should be based on standard veterinary care for the species, including physical examinations, monitoring, laboratory diagnostic tests, and vaccinations. A stepwise "batch" or "all-in–all-out" method of source animal movement through the facility can minimize the potential for transmission of infectious agents.

Feed components should be documented and should exclude recycled or rendered materials in order to reduce the risk of prion-associated diseases.

To provide a high degree of assurance of product safety, animal donors and tissues should be screened 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 should be screened for relevant diseases before tissue procurement. Post-tissue-retrieval necropsies, sentinel animal programs, and archival storage of donor organs, tissues, blood, and other specimens also ensure the safety of animal tissue for use in cellular therapeutic applications.

In general, similar aseptic procurement issues apply to animal and human tissues. 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, should be employed. Recommended design features and attributes of the animal tissue procurement facility should include the following: 1) staging of events such as shaving, sedation, and operating room preparation in separate rooms with appropriate environmental controls; 2) high-efficiency particulate air (HEPA) filtration; 3) adjacent but separate facilities for further tissue processing; and 4) dedicated areas for carcass removal. Issues relating to personnel training, bowel manipulation and puncture, and disinfection apply to the surgical procurement of both human and animal tissues (see *Human Cells, Tissues, or Cell-Based Advanced Therapies or Tissue-Based Products* above). When researchers establish animal cell lines for use in production, cell banks should be created, tested, and characterized as described in the next section.

CELL BANK SYSTEM

A cell bank is a collection of cells obtained from pooled cells or derived from a single cell clone or donor tissue that is stored in bags or vials under defined conditions that maintain genotypic and phenotypic stability. The cell bank system usually consists of a master cell bank (MCB) and a working cell bank (WCB), although alternative approaches are possible. The MCB is produced in accordance with cGMP and preferably is obtained from a qualified source with known and documented history. Human cells and tissues should be obtained by means of a licensed tissue acquisition vendor with a donor qualification program in accordance to 21 CFR 1271. The WCB is produced or derived by expanding one or more vials of the MCB. The WCB or MCB becomes the source of cells for every batch produced for human use. Cell bank systems contribute greatly to production batch consistency because the starting cell material is always the same. However, it may not be possible or feasible to create a cell bank, so appropriately tested and qualified primary cells may be used in lieu of creation of cell banks. The MCB and WCB should at a minimum be tested for identity, sterility, purity, viability, and the presence of viruses and mycoplasma.

CELL BANK QUALIFICATION

Cell bank safety testing and characterization are important steps toward obtaining a uniform final product with lot-to-lot consistency and freedom from adventitious agents. ICH Q5A, *Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin*, gives specific recommendations for testing cell banks for viral agents. While this guideline is not specifically intended to cover cell- or tissue-based products, the same tests are generally applicable. Additional virus testing may be needed depending on the prevalence of viral diseases endemic in the donor population. Testing to qualify the MCB 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 should be prospectively established. It is important to document the MCB history, the methods and reagents used to produce the bank, and the storage conditions. All the ancillary materials required for production of the banks, such as media, sera, cytokines, growth factors, and enzymes, should also be qualified, documented, and appropriately tested.

SAFETY TESTING OF MCB AND WCB

Master cell bank: Safety testing to qualify the MCB includes testing to demonstrate freedom from adventitious agents and endogenous viruses. The testing for adventitious agents should include tests for bacteria, fungi, mycoplasma, and viruses. Freedom from adventitious viruses should be demonstrated using both in vitro and/or in vivo test systems and appropriate species-specific tests, demonstrated using both in vitro and/or in vivo test systems, such as appropriate species-specific tests, massive parallel sequencing (MPS) methods, degenerate polymerase chain reaction (PCR), hybridization to oligonucleotide arrays, or mass spectrometry, as allowed by regulatory authorities.
Working cell bank: Safety testing of the WCB is less extensive and generally focuses on the potential for introduction of adventitious viruses or activation of latent virus during the additional culture required to create the WCB. End-of-production (EOP) safety testing should also be performed to ensure that the cells can be expanded a known maximum number of generations while still producing an acceptable product. For information about which types of adventitious virus testing should be performed on the MCB, WCB, and EOP cells, consult *Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin* ⟨1050⟩.

CHARACTERIZATION OF MCB AND WCB

Characterization of the MCB and WCB includes identity testing to establish species origin, e.g., isoenzyme analyses to confirm the human origin of the cells. However, cell bank characterization should encompass additional assessments such as the following:

- Growth kinetics and population doubling time
- Morphological assessment
- Percent confluence at passage
- Cell counts
- Viability (pre- and postcryopreservation)
- Phenotypic expression of desired and undesired cell types (pre- and postcryopreservation)
- Monitoring of unique biochemical markers (pre- and postcryopreservation)
- Assessments of functional activity (pre- and postcryopreservation)
- Gene and protein expression analysis (pre- and postcryopreservation)
- Expression of immune histocompatibility antigens (HLA/MHC)
- Molecular fingerprinting
- Chromosomal stability

Biocompatible Scaffold Materials

As previously described, most natural or synthetic scaffold materials are regulated as medical devices, although scaffolds derived from human tissues that meet minimal manipulation and homologous use criteria (e.g., demineralized cortical bone particles, or cancellous bone matrix granules) are regulated as "361 tissue" HCT/Ps. When suitable, use scaffolds that have previously been approved for other clinical uses because these materials should have already undergone extensive safety and quality testing. For applications in cell or tissue-based products, the scaffold material should allow cells to attach, proliferate, and migrate, and high porosity is often desired to facilitate cell seeding within the material. The scaffold must provide adequate diffusion of nutrients for cell health and release of cell-excreted products. Depending on the intended use, the material should have adequate mechanical strength and be amenable to manipulation, chemical modification, and manufacture. The scaffold material should be biocompatible, relatively inert, and immunologically benign.

Scaffolds can generally be classified as hard or soft. Hard scaffolds are used in applications where a specific shape is required, such as forming a blood vessel or a bladder. Soft scaffolds are used in applications where the product needs to conform flexibly to an existing shape in the body.

Scaffold materials can be synthetic or natural polymers, biodegradable or permanent. Biodegradation allows the scaffold to be resorbed or removed from the body without manipulation and for the defect to be filled with viable tissue. The scaffold degradation rate must coincide with the rate of formation or regeneration of the tissue. The natural scaffold structure must replace the degrading scaffold in such a way that it maintains the structural integrity of the tissue or organ being regenerated. For example, a newly formed blood vessel must withstand both the internal blood pressure as well as external mechanical forces.

The most commonly used synthetic biodegradable polymer is polyglycolic acid (PGA). Polylactic acid (PLA) is also widely used, sometimes in combination with PGA. These polymers degrade within the body, are readily removed before degradation, and have a long history of use in suture materials. Polycaprolacton (PCL), which exhibits a slower rate of degradation than PLA or PGA, is used in applications that require a long presence in the body.

Extracellular matrix (ECM) and its derivatives are natural materials used for scaffolds in the manufacture of cell–biomaterial combination products. Example sources of ECM include dermis, subintestinal submucosa (SIS), pericardium, and other predominantly collagen containing organs. Proteins such as collagen or fibrin and polysaccharides such as chitosan or glycosaminoglycans (GAGs) have also been used in growing cells to make combination products. Collagen is by far the most popular substrate for cells and has been molded into scaffolds for a variety of products, mainly in tissue-engineered skin applications. Cross-linking agents such as glutaraldehyde and water-soluble carbodiimides have been used to enhance the strength of natural scaffolds. Depending on the source of the material, and the extent of decellularization, natural scaffolds can be immunogenic.

The safety and biocompatibility of the scaffold and product-contact materials must be established. A full battery of tests should be performed as recommended by *Biological Reactivity Tests, In Vitro* ⟨87⟩, *Biological Reactivity Tests, In Vivo* ⟨88⟩, ISO

10993-1, or FDA Guidance: Use of International Standard ISO 10993-1. Process residuals and degradation products from the preparation of the scaffold should be quantified and limits should be established. The stability and storage conditions of scaffold materials should be established.

Qualification of Ancillary Materials

Ancillary products include a wide variety of raw materials and components used in manufacturing, but are not intended to be present in the final therapeutic product. They include substances such as culture media, buffers, growth factors, cytokines, cultivation and processing components, monoclonal antibodies, and components of cell-separation systems.

Residual ancillary materials may be antigenic, so their removal from the final product should be assessed, and appropriate limits should be established when necessary.

Ancillary material quality can profoundly affect the safety, potency, and purity of the cellular or tissue-based product. Ideally, each ancillary material should be produced under conditions that are in compliance with cGMP. However, some complex or unique substances may not be available from cGMP–compliant suppliers. In these situations, the cellular or tissue-therapy product manufacturer should develop a scientifically sound strategy for qualifying the ancillary material. Such a qualification program should address each of the following areas: 1) identification and selection, 2) suitability for use in manufacturing, 3) characterization and acceptance criteria, 4) vendor qualification, and 5) quality assurance (QA). Lot history files should be constructed for each ancillary material.

Conformance to specifications should be compared to the certificate of analysis data. Traceability is essential, and lot numbers for each ancillary material used should be noted in the productions records of the cell-based product. Chapter ⟨1043⟩ contains specific information about implementing an appropriate qualification program for these materials. Other *USP* chapters provide considerations about the qualification of specific ancillary materials (e.g., *Bovine Serum* ⟨1024⟩, *Fetal Bovine Serum—Quality Attributes and Functionality Tests* ⟨90⟩, and *Growth Factors and Cytokines Used in Cell Therapy Manufacturing* ⟨92⟩).

Qualification of Excipients

During the final steps in the manufacturing process, excipients or substances that increase stability and functionality of the therapeutic cells may be included. Examples of excipients include culture media, saline TS or other electrolyte solutions approved for injection, exogenous proteins such as human serum albumin, or cryoprotectants such as dimethyl sulfoxide (DMSO). Excipients are not intended to exert a direct therapeutic effect upon the patient; rather they are intended to contribute to the maintenance of the quality attributes of the final cellular product. Because excipients will be administered to the patient along with the cells, particular attention must be paid to their qualification. In general, excipients that are already FDA-approved for human use should be used whenever possible. If non-approved excipients are used, a complete safety assessment should be done. For novel excipients such as cryopreservation solutions, appropriately designed preclinical safety studies may be needed. In addition, preclinical studies should include product formulated the same way as intended for clinical use.

MANUFACTURING OF CELL-BASED ADVANCED THERAPIES OR TISSUE-BASED PRODUCTS

Introduction

The manufacturing of cell-based advanced therapies or tissue-based products requires a number of operations and manipulations by individuals who are well trained in aseptic processing techniques. The technical competence of the personnel is particularly crucial to product safety and efficacy.

Cell Isolation and Selection

Regardless of the source, there exist several generally-accepted principles for processing human or animal cells and tissues. The source cell or tissue material should be packaged in sterile, leak-proof containers and transported from the procurement area to the processing area under controlled conditions optimized to maintain quality. The transport medium may contain antibiotics, but care should be taken during processing so that antibiotics are not present in the final cellular product. In the case of blood-derived materials, or tissues containing substantial amounts of blood, the transport medium should contain an anticoagulant.

ISOLATION

Solid organs or tissues are usually dissected to expose a desired region. If multicellular organoids (for instance, islets of Langerhans) or single-cell suspensions are desired, the tissue may be subjected to mechanical or enzymatic disaggregation. In order to prevent excessive cell clumping, enzymes such as deoxyribonuclease may be added to digest nucleic acids released from damaged cells.

Cell and tissue isolations involving open manipulation steps should be carried out in an ISO 5 (Class 100) biological safety cabinet; the environment surrounding the biological safety cabinet should maintain aseptic processing operations. For minimally manipulated HCT/Ps in closed systems, these environments may be controlled but unclassified. However, for cell and tissue-based therapies that are manipulated and manufactured under cGMPs, the environment surrounding the biological safety cabinet should be controlled and classified, usually as an ISO 7 (Class 10,000) clean room. In all cases, proper in-process labeling, line clearance, and lot segregation should be employed in order to avoid product cross-contamination.

SELECTION

Cell mixtures may require further processing in order to enrich a desired cell population or remove an undesirable cell type.

Cell populations can be enriched by varying the force and duration of centrifugation, often in conjunction with a variable density gradient medium. Continuous-flow elutriation centrifuges separate cell populations by subjecting them to opposing centrifugal and fluid stream forces within a special rotor chamber.

Cell separation can also be achieved by taking advantage of cytological or biochemical characteristics unique to different cell populations. Soybean agglutinin aggregates cells that bear a particular carbohydrate moiety expressed on mature blood cells, but not stem cells, allowing enrichment of the stem cells. Certain cell populations adhere to specific solid substrates such as tissue-culture plastic, collagen-coated materials, or polymeric scaffolds; this allows the surface-bound cell type to be selectively removed from the initial cell suspension.

Monoclonal antibodies directed against specific cell-surface proteins can be used for positive or negative cell selection. For example, a monoclonal antibody-bound cell population can be removed from the cell suspension after incubation with antibody-specific magnetic nanoparticles, followed by magnetic collection. Target cell populations can be isolated from unlabeled cell suspensions by incubating the suspension with antibody-coated plastic surfaces or microspheres. A fluorescence-activated cell sorter (FACS) can be used to separate different cell types bound to fluorescently-labeled antibodies.

Cell populations may also be enriched by destroying unwanted cells, e.g., via antibody binding and exogenous complement fixation, or by incubation with cytotoxic agents or mitotic inhibitors. Such destructive procedures require thorough processing to the remove dead cells, cell fragments, and cytotoxic agents from the final cell product.

Cell Ex Vivo Expansion and Differentiation

EX VIVO EXPANSION

A key issue for manufacturers of cell-based advanced therapies and tissue-based products is the ability to produce 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 a low level or low purity in complex primary source tissues. In such cases, production of a therapeutic dose are typically achieved by specific enrichment and ex vivo expansion of the required cells.

Ex vivo expansion of cells may occur in suspension culture (e.g., T cells or hematopoietic stem and progenitor cells), adherent culture (e.g., mesenchymal stromal cells, embryonic stem cells, induced pluripotent stem cells, neuronal stem cells, or dermal fibroblasts), or a mixture of both (e.g., bone marrow stroma expansion). Numerous technologies exist for cell culture. Cells can be propagated in tissue-culture flasks (T flasks), in roller bottles, on polymeric scaffolds, or in nonrigid, gas-permeable bags, usually inside incubator units controlled for temperature, humidity, and gas composition. Multilayered, high-capacity cell culture systems composed of tissue culture plastic, multibag systems, and bioreactors using microcarriers 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 (microcarriers, 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 tend to be closed systems that use disposable bioreactor cartridges in automated processing units with direct control of temperature, gas composition, and media perfusion rate. In some cases automated software allows patient–donor tracking and documentation of culture conditions and manipulations. These features are useful in the design and implementation of quality control (QC) product release testing programs and for the QA documentation of processing runs.

In adherent culture, the cells are usually harvested from the surface upon which they have expanded. Methods of release include physical agitation, enzymatic cleavage, and chelation of metal ions 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 do not require the release of adherent cells. Cells are expanded on a biocompatible synthetic or natural scaffold that is then applied topically (for example, engineered skin substitutes), 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 all cases standard cell culture parameters should be optimized for maximum process efficiency and consistency. Such parameters include composition of cellular source material, initial seeding density, media composition, rate of media exchange, temperature, gas composition, pH, 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.

Bioreactors: Specialized bioreactors and devices may be needed for manufacturing certain three-dimensional combination products. These bioreactors hold the biocompatible scaffold/matrices for the manufacture of the construct. Although the bioreactor can provide a closed system for construct manufacturing, it creates a challenge in providing access to the scaffold for seeding cells and sampling for product release testing while maintaining sterility. Bioreactors are often single-use devices that ensure that no cross-contamination occurs between products. Preferably the product will not be repackaged for transport and delivery. For example, bioreactors may also serve as the final container for product shipment.

Container–closure testing must be performed for all final container–closure systems. Compatibility for sterilization of the bioreactor and the scaffold should be verified, and the sterilization process must be validated for each product configuration. Leachables and extractables from product-contact materials such as bioreactors and packaging components should be quantified, and limits should be established.

In closed bioreactor systems it can be difficult to observe or sample cells. Measurement of metabolic parameters can provide a surrogate method that is 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 that contain a mixture of multipotent stem cells, lineage-committed progenitor cells, and lineage-differentiated cells. The composition of these products can be manipulated by 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 production of antigen-specific T cells to target various specific disease indications or derivation of therapeutic cell types from embryonic stem cells. Before release for clinical use, the resulting differentiated target cells should be appropriately characterized. Assessing the potential for de-differentiation of multipotent cells that have undergone differentiation may be necessary to ensure the safety of the product. Where the cells have been expanded and subsequently differentiated, karyotype analysis or in vitro transformation assays may be performed to demonstrate the cells are acceptable for clinical use.

EX VIVO GENETIC MANIPULATION

Genetic modification of cells ex vivo is a common processing procedure that is used to alter the pattern of gene expression in a defined population. The introduction of integrating or nonintegrating genetic materials (DNA, RNA, siRNA, virus) is performed in order to induce the expression of new genes and products or to change endogenous gene expression. Ex vivo genetic modification in autologous transplantation settings involves the manipulation of a harvested or expanded cell population from a patient and subsequent re-administration of the cells to the donor. In a typical allogeneic transplant setting, a stable, genetically modified cell population that has been characterized and banked is administered to a broad patient population. In order to control graft-versus-host disease in allogeneic bone marrow transplants, selected donor T cells have been treated with lethal genes such as thymidine kinase that make the cells susceptible to gancyclovir treatment after transplant. Examples of autologous genetically modified cell therapy products include chimeric antigen receptor T-cells (CAR-T), the transduction of tumor cells with cytokine or other immunomodulatory genes, lymphocytes transduced with receptors for tumor antigens, and the introduction into harvested lymphocytes of an antiviral ribozyme vector as a strategy to treat human immunodeficiency virus infection. Allogeneic cell therapy product examples include CAR-T, genetically modified and irradiated tumor cell lines used as tumor vaccines, and encapsulated cells transfected with a gene to express a neurotrophic factor for localized therapeutic protein delivery in the central nervous system.

Ex vivo genetically modified cells are considered gene therapy. Issues associated with gene therapy products are addressed in detail in ⟨1047⟩, especially the production of the vector or genetic material used to accomplish gene transfer, analytical testing strategies, patient safety, and monitoring. The manufacturing, cell processing, and process control methodologies addressed above are applicable in the procedures used for genetic manipulation. Frequently cell populations that are genetically modified are isolated and/or expanded or selected before the introduction of the genetic material. Specialized equipment and processes for introduction of genetic material must be validated and monitored. Issues associated with cell banking and stability apply to cell lines used in cell therapy product manufacturing. Finally, issues associated with analysis and administration of the genetically modified cell population are discussed later in this chapter.

Formulation of Cell and Tissue-Based Products

Approaches for formulating cell and tissue-based products depend largely on the planned storage time for the cells before delivery to the patient. For some cell-based products, the time between completion of manufacturing and delivery to the intended recipient can be measured on the order of hours to days. Other cell-based products may be cryopreserved in order to extend their shelf life. A different approach for formulating cell and tissue-based products may involve the addition of a natural or synthetic scaffold that can facilitate handling, protecting the cells from immunological responses, and creating a specific shape that contributes to the therapeutic effect. Considerations for formulating each of these types of cell and tissue-based products are discussed below.

Decellularized Tissue-Based Products

The cells from decellularized tissue materials are killed, lysed, and removed from tissue using one or more of a variety of methods. Protocols may include physical methods (e.g., exposure to freezing temperature, force and pressure, or electrical disruption) and/or exposure to enzymes or chemicals (e.g., acids, bases, or detergents). These step(s) are typically followed by thorough rinsing and soaking in buffer to remove reagents and all cellular debris, as well as to neutralize reagents as needed. Tissues may then be further processed, such as demineralization (bone), before packaging, freeze-drying, and/or other final processing steps.

NONCRYOPRESERVED CELL-BASED PRODUCTS

Products consisting of suspensions of cells for delivery to patients within hours after the completion of manufacturing frequently are formulated in sterile, buffered solutions suitable for direct administration. For other noncryopreserved cell-based products extension of shelf life from hours to days may be possible by use of solutions that contain appropriate nutrients and antioxidants. In most cases, these excipients are not intended for direct administration into patients. Consequently, the excipients may require removal before delivery to the patient (see *Clinical Site Preparation and Administration*). If an unapproved formulation buffer will be administered to patients, preclinical toxicology testing should be performed.

CRYOPRESERVED CELL-BASED PRODUCTS

Most cell cryopreservation medium formulations are supplemented with 5%–10% DMSO with or without hydroxyethyl starch (generally 6%) and a plasma protein such as 4%–10% human serum albumin in a balanced salt solution. DMSO prevents dehydration by altering the increased concentration of nonpenetrating extracellular solutions during ice formation. The high molecular weight polymeric hydroxyethyl solution protects the cells from dehydration as water is incorporated into extracellular ice crystals. The use of protein often results in maximum recovery and viability of cells after thawing. Some cryopreservation formulations are completely free of protein.

The optimal concentration of cells for cryopreservation depends on the cell type and should be determined empirically, but it generally ranges from 10^6 to 10^7 cells/mL. The homogeneity and viability of the cell population being cryopreserved can also differ after thawing and should be carefully assessed. In situations where the final cell-based product is intended to be thawed and administered immediately, the presence of DMSO in the formulation buffer does 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. Refer to *Clinical Site Preparation and Administration* for additional considerations.

CELLS COMBINED WITH BIOCOMPATIBLE SCAFFOLDS

Many cell and tissue-therapy products are administered in combination with a biocompatible scaffold (also referred to as tissue engineering). Some wound healing or skin substitute products contain cells seeded on a scaffold. The biochemical and physical structure of the scaffold and the method for combining cells with the scaffold are specific to the product. The biocompatible scaffold (e.g., demineralized cortical bone particles, cancellous granules) may also be human tissue derived from the same donor as the allogeneic cells.

Cells can be loaded into a semipermeable membrane device for delivery. 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 mounting 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 hollow-fiber modules with hepatocytes for the treatment of liver disease.

Cells can be seeded onto a three-dimensional scaffold 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 (e.g., skin substitutes).

Cells can be encapsulated in a gel or cross-linkable polymer solution, and 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.

Cells can be adhered to scaffolds of defined shape that are then implanted. Some examples include osteogenic precursor cells on scaffolds of demineralized cadaveric human bone, ceramic hydroxyapatite, ceramic hydroxyapatite–tricalcium phosphate, or biodegradable glass, which can be used in the repair of bone defects.

Change to read:

ANALYTICAL METHODS

General Considerations

The complexity and scope of cell-based therapies are reflected in the wide range of analytical methods that are used to establish in-process controls and final product release criteria. Quality specifications for cell-based advanced therapies and tissue products should be chosen to confirm the product's quality, safety, and potency. Selected tests should be product specific and should have appropriate acceptance criteria 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 tissue products should follow the principles outlined in ICH Q6B, *Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products*.

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. Manufacturers should develop specifications for each product developed from this information by application of 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 stability, safety, and efficacy assessments.

In-process controls and specifications for the product should ideally be anchored by use of an appropriate reference standard or reference material. An autologous product may rely on a reference standard generated from processing cells or tissue from a healthy donor or from a source that supplies cells and tissues to research institutions. The reference standard ensures that the process, as measured by the release assays, does not change significantly over time, and it verifies that a test produces acceptable results, i.e., system suitability requirements are met. The reference standard is made from a lot that is produced under controlled conditions 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 final product. However, the stability of this reference standard must be determined.

Alternatively, a working standard can be used. If so, in the test it should behave like the reference standard. Changing to a new reference standard 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 an autologous or 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.

In-Process Controls

Manufacturing processes should have well-defined go–no go decision criteria that are established for key in-process manufacturing steps. In-process control tests ensure that the in-process material is of sufficient quality and quantity to ensure manufacture of an acceptable final product. Examples of in-process controls include:

- Cell enumeration and viability
- Microbiological (sterility, endotoxin, mycoplasma)
- Expression of phenotypic or genotypic markers
- Verification of morphology against visual reference standards
- Production of a desired bioactive substance
- Determination of population doublings, passage number, age of culture
- Assays for process impurities
- Monitoring of culture system parameters [% carbon dioxide (CO_2), % relative humidity, pH, glucose, lactate, etc.]
- Functional tests such as colony forming units (cfu) and expression of cell-specific proteins
- Content uniformity
- Quantitation of particulate matter
- Visual inspection for cellular aggregates and other impurities

A primary reason for in-process control tests is to reliably obtain adequate product quality and yield. A secondary reason is to gather process and product characterization data that can be useful in assessing the impact of process changes or excursions. Refer to ▲*Quality Systems*▲ (ERR 1-Nov-2020) for discussion on critical process parameters (CPP). In-process sampling must always be balanced against adequate final product yield. Therefore, only truly informative and timely in-process testing should be implemented. Intermediate process material that fails to satisfy in-process control criteria should not be used for further manufacturing. Non-conforming material may be reprocessed if there are procedures in place. The effect of reprocessing on other quality attributes such as stability must be defined before the material can undergo further manufacturing. The reprocessed material must satisfy the original in-process specifications. If several sub-lots (e.g., cells harvested from different culture vessels) will be pooled for further processing, non-conforming sub-lots should not be included even if the larger pool containing these failed sub-lots would pass the in-process assay criteria. For manufacturing processes that yield multiple containers of cell suspensions, in-process visual inspections are highly recommended. For example, fibers, polymer fragments and other visible particulate matter can accumulate and co-mingle with cells presenting a patient safety concern. In-process visual inspection after the final container filling, but prior to the final product labeling or cryopreservation (if frozen), allows the culling of nonconforming containers before final product sampling and testing. Timely in-process visual inspection may be challenging, particularly when attempting to limit cell exposure to cryopreservatives such as DMSO.[1]

During process development, assays for product quality and yield should be performed after most processing steps to determine which steps are quality-critical, stability-indicating, or sensitive to process deviations. Statistical process controls and critical product quality parameters, along with statistically valid sampling, should be used to establish limits for process validations and manufacturing investigations. In-process controls should be performed even for fully validated manufacturing processes to ensure proper process control. Assay results should be tracked and trended over time, and actions should be taken to correct problems as they arise.

Final Product Release Specifications

Cell-based therapies regulated as biological products must comply with applicable sections of 21 CFR 211 and 21 CFR 610 to ensure they meet essential quality attributes such as identity, purity, potency, and microbiological safety.

Because terminal sterilization is not possible for a living cell-based product, essentially all cell-based products are required to meet acceptance criteria for product tests such as sterility, mycoplasma (not needed if cells are cultured less than 24 h, or if tissue has been decellularized), and endotoxin—typically, negative or no growth demonstrates sterility and the absence of mycoplasma, and products must demonstrate <5 endotoxin units (EU) per kilogram of patient body weight. In the case of intrathecal injection, the specified endotoxin limit is more stringent: ≤0.2 EU/kg of patient body weight. Adventitious virus testing is rarely performed on the final cell-based therapy product because the source cells or cell banks and ancillary materials of biological origin have been screened and tested for viral agents of concern before manufacturing.

For almost all other final product release criteria, such as those for identity, purity, and potency, the analytical procedures with methods and acceptance criteria are specific to the individual cell-based product. *Table 3* provides an overview of the expected final product release tests for cell-based therapies and lists examples of approaches that are used to satisfy the testing requirements.

[1] Clarke D, Jean S, Powers D, Karnieli O, Nahum S, Abraham E, et al. Managing particulates in cell therapy: guidance for best practice. *Cytotherapy*. 2016;18(9):1063–1076.

Table 3. Overview of Final Product Release Testing

Release Test	Examples	Criteria
Sterility	*Sterility Tests* ⟨71⟩	Negative
Mycoplasma	Direct and indirect culture method (FDA *Points to Consider*)	Negative; not detected. Testing not usually required if cells are cultured less than 24 h.
Endotoxin	*Bacterial Endotoxins Test* ⟨85⟩	<5 EU/kg (<0.2 EU/kg intrathecal)
Identity	• Surface marker determination • Isoenzyme analysis • Genetic fingerprint • Morphology • Bioassay • Biochemical marker	Product specific
Purity	• Percentage of viable cells • Percentage of cells expressing specific marker(s) • Limits on undesired cell types • Limits on process contaminants (e.g., serum) • Scaffold composition	Product specific
Potency	• Viable cell number • Colony-formation assay • Change in expression of specific genes • Secretion of desired macromolecule • Expression of cell-surface molecules HLA • Target specific cell killing • Evidence of metabolic activity • Evidence of cell function	Product specific
Dose	• Viable cell number • Enumeration of specific cell population • Total DNA • Total protein	Product specific
Appearance	*Injections* ⟨1⟩, *Visible Particulates in Injections* ⟨790⟩	Product specific. Cell suspensions should be free of unexpected visible particulate matter.
Others	• Morphology • Size • Tensile or burst strength	Product specific

STERILITY

Cell and tissue-based products are required to comply with final product release testing requirements, including sterility. Sterility testing is also frequently performed in-process to establish microbial purity for cells that require extended culturing. A suitable sterility test includes the test described in *Sterility Tests* ⟨71⟩. These culture-based test methods require 14 days and thus are suitable only for cell-based therapy products that have extended shelf lives (e.g., following cryopreservation). Many cell-based therapies have short shelf lives and must be delivered to patients before the 14-day test results are available. In such situations, the FDA has identified an approach that will allow the administration of the cell-based product to patients in this setting [see *Guidance for FDA Reviewers and Sponsors: Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Somatic Cell Therapy Investigational New Drug Applications (INDs)*]:

- In-process sterility testing on a sample taken 48–72 h before final harvest or after the last refeeding of the cultures
- A rapid microbial detection test such as a Gram stain or other procedure on the final formulated product
- Sterility testing compliant with 21 CFR 610.12 on the final formulated product

Under this alternative approach, the release criteria for sterility would be based on a negative result of the Gram stain and a no-growth result from the 48- to 72-h in-process sterility test. In the event that the 14-day sterility test is determined to be positive after the product is administered to the subject, the manufacturer is required to report the sterility failure, results of investigation of the cause, and any corrective actions as an amendment to the IND within 30 calendar days after initial receipt of the positive culture test result.

Because of concerns regarding the sensitivity of a Gram stain and the inability to obtain full sterility results for 14 days after administration to the patient, there is widespread interest in the use of rapid microbiological methods as an alternative to the 14-day culture method. This is discussed in *Alternative Test Methodologies*.

MYCOPLASMA

Testing for mycoplasma is recommended for all raw materials derived from a human or animal source; it is required as a lot-release assay for cell-and tissue-based products if they are cultured for longer than 24 h. The FDA has published a document (*Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals*) describing in detail the accepted methods for the cultivation and isolation of mycoplasma. Methods for mycoplasma detection are also described in *Mycoplasma Tests* ⟨63⟩.

Because the classical assay takes 1 month to complete, alternative methods are being developed and validated for the rapid detection of mycoplasma; this is discussed in *Alternative Test Methodologies*.

ENDOTOXINS

Endotoxins exert numerous biological effects: they can affect secretion and cytokine production, induce fever in recipients, or serve as powerful mitogens. Because of these wide-ranging impacts, endotoxin assessment must be included in raw material and manufacturing process qualifications for cell and tissue-based therapies. *Bacterial Endotoxins Test* ⟨85⟩ describes methods for measuring endotoxins, all based on the Limulus amoebocyte lysate assay. This assay can be validated for a wide range of biopharmaceutical products; one important feature is the ability to rapidly conduct the assay before the release of short-shelf-life products.

IDENTITY

Lot-release testing for cell-based products must include an identity test to unequivocally identify the product. The complexity of the identity test depends on the nature of the specific product and the array of products being manufactured, e.g., in a single-product vs. a multi-product facility.

Identity tests for cell-based products must be relevant to the cell type and manipulations applied during processing. Differential surface markers are frequently used to ascertain product identity, as described in *Table 3*. Flow-cytometric immunoassay methods are the most common means of detecting and quantifying these markers. Isoenzyme analyses can confirm species of origin, which is especially useful for xenogeneic cell products. Cell morphology may be used to distinguish specific cell types. Genetic fingerprint technologies such as short tandem repeats can also establish cell line identity.

PURITY

Purity methods specifically quantify the intended active product components, or unwanted impurities (product- or process-related residual contaminants), in the final product. *Table 3* describes examples of several impurity methods. The requirement to test for a particular impurity for product lot release depends on: 1) the capability of the manufacturing process to remove or inactivate the impurity, as demonstrated by process validation, and 2) the impact of the impurity, such as toxicity or altered product function.

Examples of process-related impurities associated with cell-based therapy products include residual production-medium components (e.g., serum, antibiotics, or exogenous cytokines), ancillary materials used in downstream processing (e.g., nucleases or proteases), and leachables (e.g., plasticizers from tubing or culture plastic). Impurities may be bioactive (e.g., cytokines or hormones) or immunogenic (e.g., aggregates, degradation products, or animal-derived proteins). Impurities may have other deleterious effects, depending on the dose of the product.

Product-related impurities are specific to each product type. Impurity examples include cell debris; cells that are dead, non-functional, altered, or otherwise unwanted. Analytical methods should quantitate both product and impurities, and should allow the manufacturer to assess the lot-to-lot consistency of the product-impurity profile. It may be possible to validate the manufacturing process to the extent that specific lot-release testing for impurities can be limited.

Testing for impurities is often extensive during product characterization and process validation, when the consistency of the manufacturing and purification process is being assessed. In-process testing for impurities can provide valuable information regarding the consistency of manufacturing and materials. *In-Process Controls* describes the role that in-process testing can play in monitoring and limiting impurities, both during process development and manufacturing. Lot-release impurity testing should reflect the safety risks associated with the impurity, and the ability of the process to consistently remove that impurity.

POTENCY

Potency is defined under 21 CFR 600.3(s) as "the specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, to effect a given result." Together with dose, potency defines the biological activity of each lot (see *Dose-Defining Assays*, below). The relationship between product potency measurements and clinical safety and efficacy is key to their use in batch release. Potency may be assessed by cell enumeration, in vitro or in vivo bioassays, or a combination of these. Because it is not uncommon for potency assays to have large coefficients of variation, these assays often require well-defined and fully characterized representative reference material. In part because of their inherent variability, patient-specific products such as autologous immunotherapies present a particular challenge in demonstrating potency. Potency assay development should focus on characterizing and controlling the sources of assay variability. Information about potency assay and product variability should inform the product stability study design, as well as the proposed statistical approach to expiration date assignment (see *Stability*, below).

Assays measuring the potency of cell-based therapy products vary widely, and depend on the products' unique characteristics and shelf life. For some cell-based products, product potency may correlate with clinical efficacy. For example, a colony-forming assay or enumeration of committed progenitor cells may correlate with clinical engraftment outcomes in clinical studies. Product potency may also correlate with responses measured in animal or cellular disease models. If the cell-based product releases a bioactive macromolecule, a potency assay could be based on the units of activity released. For example, the production of insulin in response to changes in glucose levels could be the basis of a potency assay for a cell-based therapy product intended to treat diabetes.

Approaches to measuring product potency should be discussed with regulatory authorities early in development. The FDA has issued guidance that discusses using a matrix of biological and nonbiological assays, including both qualitative and quantitative assays, to establish product potency. Information in this guidance is particularly relevant for cell-based products that have a short shelf life, a complex mechanism of action, or multiple biological activities (see the FDA's *Guidance for Industry: Potency Tests for Cellular and Gene Therapy Products*).

A validated potency assay is typically required before regulatory approval, which means that a well-defined product potency assay (or assays) should be in place before the initiation of pivotal clinical trials. Therefore, implementation of one or more candidate potency assays early in clinical development is strongly encouraged. Data from these candidate potency assays can also be particularly useful for assessing proposed manufacturing changes, during technology transfer, and in determining product stability.

DOSE-DEFINING ASSAYS

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 accuracy and precision.

Cell-based therapy products may be dosed on the basis of enumeration of one or more cell populations. For products consisting 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. Fluorescent compounds that bind to nuclear proteins and are 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 blood 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. Other automated cell counters ▲rely▲ (ERR 1-Nov-2020) on computer algorithms for visual field analysis of stained cell populations. Beyond cell counting, further identification and enumeration of specific cell populations usually requires cell-surface phenotype analysis by flow cytometric or other methods (see *Identity* above). The proportion of a specific subpopulation of cells may be determined by FACS analysis or by flow cytometry. An example of a specific cell enumeration assay that incorporates viability assessment is described in *Flow Cytometric Enumeration of CD34+ Cells* ⟨127⟩.

For products that contain cells in a nonhomogeneous suspension, such as cells that are combined with a biomaterial (e.g., a scaffold), alternative cell enumeration measures have been used, including total area of a cell sheet, wet weight, total protein, and total DNA. If such measures are used to determine product dose, then supplemental tests should be performed to establish relevance.

Considerations for Release Testing of Cell–Biomaterial Constructs

For some cell-based therapy products such as cells combined with biomaterials to form combination products, it may not be feasible to directly test the cell–biomaterial construct. This is frequently the case when autologous cells are involved and the cell–biomaterial construct consists of a single unit and sampling of the construct is not feasible. In such cases, the individual components are tested before they are combined, and the final construct is not subjected to direct testing. Indirect measures such as sampling of the culture media can be employed to address regulatory requirements. The quality and stability of the formulated cell–biomaterial construct and relevance of indirect measures must be established by validation studies during product development.

Sampling Issues

GMP samples from each lot must be retained after the completion of final product release testing, in case safety or quality issues arise later. Even if the product has a very short shelf life, retained samples can be used to detect impurities and other unwanted substances. If rapid-release strategies are employed, manufacturers may need to retain additional or alternatively stored (such as frozen or fixed) samples, so that product quality can be reassessed if necessary. In all cases, sample handling and storage must be validated against relevant testing methods and stability requirements. The FDA (in 21 CFR 820.250) requires product and material sampling plans to be written, specific, and justified on the basis of statistics, science, and risk. Sampling considerations are especially important for products dispensed into multiple containers. Sampling should be based on the appropriateness, stability, and expected distribution for each parameter tested. Proper sampling plan design should include considerations for samples (e.g., uniformity, size, handling, storage) and statistics (e.g., frequency, redundancy, confidence limits, replicates). Process development studies generally help determine the appropriate parameters and limits for the statistical sampling plan design; process validations must confirm or support those statistical sampling plan parameters and limits. See *Stability Protocol Development* (below) for additional considerations, and ⟨71⟩ or *Visible Particulates in Injections* ⟨790⟩ for examples of statistical sampling approaches.

Alternative Test Methodologies

As described in *Final Product Release Specifications* (above), cell-based therapies must undergo testing for sterility, mycoplasma, and endotoxin. Additional acceptance criteria for tests relating to identity, purity, potency, dose, and other relevant characteristics must be met before clinical use. With the exception of sterility, mycoplasma, and endotoxin, most of the test procedures and their underlying methods used to ensure that the final product meets release acceptance criteria are unique to the product and can be adapted to meet the specific characteristics and applications of the cell-based therapy product. In general, test methods should be developed based on the best available science and should be suitable for use in a GMP manufacturing environment. The assays should be robust, reliable, and capable of being validated and should provide results before release for clinical use. *Validation of Compendial Procedures* ⟨1225⟩ provides basic considerations for methods validation.

For some cell-based therapies, the sample size and volume of material required for testing or the length of time necessary to obtain test results can consume significant amounts of the final product, or the time required for obtaining results may exceed the product's shelf life—or both. This creates problems with the available supply of product to treat patients and in other situations precludes the possibility of obtaining results before administration to patients. This is a particular problem for the compendial sterility test as well as the FDA-recommended broth–agar culture method for mycoplasma. Consequently, both industry and regulatory authorities have shown considerable interest in facilitating the development of alternative test methods for both sterility and mycoplasma.

FDA regulations for biological products specifically address the use of equivalent methods provided they ensure that the safety, purity, potency, and effectiveness of the biological product is equal to or greater than the assurances provided by the specified method (21 CFR 610.9). Some of the available alternative test methods for sterility and mycoplasma are described below.

The range of available technologies is broad and continues to be developed by assay designers for use in the cell therapy industry. Attributes that should be included in any review of proposed technology include accuracy for the intended purpose, speed in productivity, cost, acceptability by the scientific community and regulatory agencies, simplicity of operation, training requirements and reagents, the reputation of the vendor, technical services provided by the vendor, and, finally, utility and space requirements.

Validation of these test methods and demonstration of equivalence as described in 21 CFR 610.9 are required at the time of BLA or a PMA submission.

STERILITY

Detection platforms for alternative microbiological methods have been described in *Rapid Microbial Tests for Release of Sterile Short-Life Products: A Risk-Based Approach* ⟨1071⟩. The primary advantage of these systems is the automated nature of the test results and recovery of microorganisms for failure investigations and other microbial characterization methodologies. Principles of validation of alternative microbiological methods are also described in *Validation of Alternative Microbiological Methods* ⟨1223⟩.

MYCOPLASMA

Compendial testing methodologies for mycoplasma are growth based in agar and broth cultures and require at least 28 days to monitor appropriately the presence of mycoplasma contamination. Because of this limitation, a number of rapid mycoplasma testing technologies have been developed based on nucleic acid amplification techniques such as PCR, as well as nonamplified nucleic acid hybridization assays, enzyme-linked immunosorbent assays (ELISA), and enzyme-based assays.

QUALITY SYSTEMS

Quality systems weave together all aspects of manufacturing. As with biopharmaceutical and pharmaceutical products, the FDA requires manufacturers of cell-based therapies and tissue-based products to employ QC and QA programs to oversee manufacturing facilities, processes, validation efforts, document and change management systems, labeling, employees training, and the testing of all raw materials, in-process samples, bulk products, and final formulated products. Human cells and tissue must be handled in accordance with GTP as described in 21 CFR 1271. In addition, cGMP as outlined by the FDA in 21 CFR 210, 211, 600s (especially 21 CFR 610), and 820 apply to the manufacturing of cell-based therapies and tissue-based products that are subject to PMA. The extent of quality oversight increases as clinical development progresses, and full cGMP compliance is expected by the time Phase III clinical trial(s) begin.

Data obtained from in-process and final product release testing should be recorded and monitored. Results that are out of specification (OOS), or even those that are out of trend, must be investigated before disposition of the material. The FDA's *Guidance for Industry: Investigating Out-of-Specification (OOS) Test Results for Pharmaceutical Production* (October 2006) provides a systematic approach for conducting an investigation.

An effective risk-management approach at the earliest stages of cell-based product development helps ensure the highest product quality by proactively understanding, identifying, and mitigating potential quality issues. Risk management can also be used to establish meaningful specifications and CPPs to ensure that quality attributes are met.

Risk analysis and mitigation systems use experience and process knowledge to define risk categories. As an example, Risk Levels 1–4 are defined below and can be adopted as one means of conducting a preliminary risk assessment:

Risk Level 1: *Technicality*—Poses no risk to the patient and does not impact the safety and effectiveness of the product. Example: A missing signature on a batch record.

Risk Level 2: *Alert*—May pose a safety risk to the patient or may have a potential impact on the safety and efficacy of the product. Compliance must be re-established with appropriate justification to proceed after QA review and approval. Example: Digestion time for biopsy processing falls outside a defined range.

Risk Level 3: *Do not ship/reject lot*—May pose a safety risk to the patient or may impair the efficacy of the product even after corrective action. Shipment is not permitted. Example: Cultures fail to demonstrate adequate cell growth.

Risk Level 4: *Post-Distribution Event*—May pose a safety risk to the patient or may impair the safety, potency, or purity of the product. The defect is identified after product distribution. Example: Failed sterility test occurred after distribution of product.

FACILITY DESIGN AND OPERATION CONSIDERATIONS

Like biopharmaceutical operations, cell-based advanced therapy and tissue-based product manufacturing facilities must be carefully designed to maintain cGMP-compliant aseptic processing operations while also accommodating unique product

requirements. Cells or tissue starting materials may need to be received and processed in a segregated area under quarantine to avoid cross-contaminating the main facility. Specially designed equipment and spaces may be required for tissue or cell processing. The facility may need to store, process, and dispose of specialized chemicals or infectious waste. All of these issues may constrain the design of the facility, especially air-handling systems and clean room environments.

While protecting the cell-based advanced therapy or tissue-based product from inadvertent contamination is a primary concern, minimizing risk to the manufacturing operators must also be ensured through appropriate training, protective clothing (gowns, gloves, sleeves, surgical masks, eye protection, and head coverings) and engineering controls (biological safety cabinets, appropriate air quality, etc.).

The degree of control required for cell and tissue processing operations depends on a number of factors, including the complexity of the aseptic manufacturing process, the primary site of manufacturing, and the final product shelf life. ISO 7 (Class 10,000) clean rooms and ISO 5 (Class 100) biological safety cabinets are typical components for cell therapy manufacturing processes, especially those that involve open manipulations.

The differential pressures between classified manufacturing should comply with the FDA's September 2004 document, *Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing—Current Good Manufacturing Practice*. The facility and processing areas should be monitored for air quality in a manner that provides a high level of aseptic assurances. For guidance in this area, see *Microbiological Control and Monitoring of Aseptic Processing Environments* ⟨1116⟩.

Facility cleaning, component and product segregation, sanitization procedures, and environmental monitoring systems must be in place to avoid microbial contamination and cross-contamination between production lots. Techniques such as bar-coding and radio frequency (RF) tags can be used for product tracking and segregation. For guidance in this area, see 21 CFR 211.42, 211.113, 1271, and the FDA's September 2004 *Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing—Current Good Manufacturing Practice*.

Unlike facilities designed for allogeneic products which rely on volume scale-up, facilities for autologous products typically utilize multiple small-unit scale out, which must be considered in the design and operation of the facility. Automation can be used to effectively manage repetitive manual cell manipulations and multiple simultaneous production lots.

Process equipment and software that controls or monitors critical parameters must be qualified and validated; such equipment should also be fitted with alarm systems and emergency back-up power.

CONSIDERATIONS FOR VALIDATION AND QUALIFICATION

The principles of validation recommended by ICH and FDA guidance documents and ⟨1225⟩ and *Validation of Microbial Recovery from Pharmacopeial Articles* ⟨1227⟩ apply to cell or tissue-based products. Analytical and manufacturing equipment and methods should be validated following the principles described in ⟨1225⟩, in addition to guidance documents issued by ICH [see Q2 (R1)].

Cell-based advanced therapies and tissue-based products present some unique validation challenges. First, the cellular or tissue starting materials can be quite variable, with a wide range in the quality and quantity. Second, manufacturing process steps (especially manual manipulations) can be complex and variable. Manufacturers should develop and validate raw material acceptance criteria, robust manufacturing processes, and analytical methods that consistently result in acceptable final products, even if the process relies on nonstandard or variable tissue materials.

Process validation should take this variability into consideration and should ensure that critical manufacturing and testing endpoints consistently meet specifications.

Aseptic process validations should be performed using microbial growth media in place of the usual process materials, in order to show that the manufacturing staff can execute the procedures and produce a product free of microbial contamination. Procedures intended to maintain segregation during manufacturing should be challenged to verify that there is minimal opportunity for cross-contamination or inadvertent exchange among different patient product lots. Personnel training plans should be established, and proficiency qualifications should be performed periodically. At facilities where raw materials are procured or products are administered, personnel may need to be trained and qualified in order to minimize variability.

Depending on the variability in the source cells or tissues and the complexity of the manufacturing process, it may be necessary to manufacture more than three qualification lots to verify the consistency and the robustness of the manufacturing process. Manufacturing data should be tracked and trended to enable manufacturers to discover and correct problems. Annual product reviews should summarize such factors as in-process and final product testing results, process failures, deviations, OOS, and effectiveness of corrective and preventive action (CAPA) over a 12-month period.

Equipment and facility cleaning validations should be performed to evaluate cell and DNA carry-over, and to demonstrate the efficacy of cleaning and sanitizing agents on both standard microbial and fungal contaminants and environmental isolates from the manufacturing facility. Measurement of residual cleaning agents should be addressed in equipment cleaning validations.

Shipping validations, including environmental challenges such as extremes of temperature and vibration, should be performed for the cellular or tissue starting materials, as well as the final product. Validations should also be performed on software that tracks and controls labeling, shipping, and inventory systems.

CLINICAL SITE PREPARATION AND ADMINISTRATION

Before administration of some cell or tissue-based products, one or more product modifications or preparative steps may be required. These modifications or steps are frequently performed close to the time of administration, and, therefore, they may not be under the direct control of the original manufacturer. The nature of these modifications is largely dictated by characteristics of the product.

At the clinical site, written procedures and process controls must be established for all product storage intervals, transport steps, and modifications, starting with a clear definition of critical control points. The unique and irreplaceable nature of many cell or tissue-based products heightens the need for well-established procedures for clinical site preparation and administration.

Product Manipulations

Typical manipulations include the following:

- **Change in final container:** The manufactured product may have been stored or transported in one container and may require transfer to a different container for administration.
- **Change in physical state or temperature:** A product may require thawing or warming.
- **Change in solution or suspension:** A product may have to be dissolved, diluted, or suspended in a liquid.
- **Combination with a biomaterial:** Therapeutic cells may require combination with a scaffold material such as decellularized extracellular matrix sheets, gels, plugs, capsules, sponges, particles, or granules. In other cases, cells can be added to an existing medical device such as a hollow-fiber filtration unit before use.
- **Admixture or compounding:** For some cell products, mixing or compounding at the clinical site may be necessary.
- **Filtration or washing:** The presence of unwanted materials in the manufactured product, such as particulates, cellular debris, metabolites, or compounds remaining from previous manipulations, may require washing or filtration steps.
- **Sampling:** Sampling of the final product before administration may be required to test the final formulation.

Thawing Cell-Based Products

Thawing is performed rapidly. If a small number of cells will 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 must be removed or cells must be concentrated for administration, the thawed cell suspension is generally serially diluted (to avoid osmotic shock) and resuspended in a protein-containing medium. Cell viability and potency may be monitored after thawing, but the information is frequently intended only to gather information rather than as a specification that must be met for clinical use of the cellular product.

Additional Release Testing of Clinical Site-Manipulated Cell Products

Cell-based therapy products that undergo preparative steps or manipulations at clinical sites must be subjected to appropriate checks or tests to ensure that all quality specifications are met before release for patient administration. The nature and extent of manipulations determines whether additional release requirements or critical specifications must be added to those required immediately after initial manufacture.

Prerelease steps usually include the following:

- Physical inspection of the product, including product appearance (color, turbidity, particulates, or foreign matter), container integrity, temperature, and accuracy and convenience of labeling
- Review of process records and/or certificate of analysis
- For patient-specific products, verification of product labeling and records related to identity of the intended recipient

Administration to Patients

In all cases, adequate anesthesia and premedication must be carefully evaluated. For example, if the DMSO will remain in a thawed, cryopreserved cellular product, the patient may be given an antihistamine before administration. Pre-administration evaluation must also include assessment of concurrent therapies that may interact with the cell-based advanced therapy or tissue-based product to modify its effects. Some therapies may be adjunctive to the cell-based advanced therapy or tissue-based, such as cytokines that promote proliferation or differentiation of the infused or implanted tissue. Other commonly used drugs such as antibiotics, antineoplastics, anticoagulants, and anti-inflammatory agents must be evaluated for possible effects.

DELIVERY OF CELL-BASED THERAPY TO PATIENTS

Some cell-based advanced therapy or tissue-based products are patient specific because they are manufactured from a selected autologous or allogeneic tissue source, cells, or 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, and at least two people should verify the identity of the patient and patient-specific product immediately before administration.

A variety of delivery systems such as catheters, syringes, and intravenous (IV) lines are frequently used to administer cells to patients. Before clinical use manufacturers should ensure that these medical device components are compatible with the cells and formulation solutions. 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.

STABILITY

General Considerations

Stability studies for cell-based therapies and tissue-based products should be based on a comprehensive understanding of the final therapeutic product and its intended use. Manufacturers should assess the stability of the final product, in-process hold steps, cell banks, critical raw materials, and reference standards.

Where feasible, stability testing should be carried out in accordance with the principles described in ICH guideline Q5C, presented in *Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products* ⟨1049⟩. Stability data should also be collected for bulk and other in-process materials that are stored before final processing and filling.

For some cell-based advanced therapies and tissue-based products, final lots may have small volumes and/or shelf lives. In such cases, stability protocols should be based on materials from multiple donors. Because it is difficult or unethical to obtain sufficient stability study cells or tissues from patients, cells or tissues from several sources such as normal donors, research tissue repositories, cadaveric sources, or well-characterized banked primary cells may be used in stability studies to validate storage, shipping, and expiration dating. These results must be interpreted with caution until data confirm that the actual product cells and "surrogate" source materials exhibit similar stability profiles.

For combination products that include cells and biomaterials, the stability of all components when combined must be considered. When biodegradable scaffold materials are present, scaffold degradation should be considered in determining the stability and shelf life of the combination product.

Stability Protocol Development

Formal stability studies to support licensure and 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 date.

Stability studies must verify that the storage conditions maintain the quality attributes of the product so that it complies with stability specifications. These specifications may differ from release specifications, but they must address product potency.

Initial studies to establish a provisional expiration date must be conducted before administration to the first patient. Such initial studies are useful for determining which assays are stability indicating, that is, the best indicators of product degradation.

Shipping validations are a special type of stability study. Typically, the product is packaged and shipped (or subjected to simulated shipping) under normal and extreme conditions, and the material is tested before and after shipping to ensure that it still meets the product release requirements. As described in *Storage and Shipping* (below), special attention must be given to the specific thermal, mechanical, and radiological stresses that cell-based therapies and tissue-based products will likely encounter.

Stability Challenge Conditions

Based on risk assessment, stability studies for cell-based advanced therapies and tissue-based products should include challenge conditions outside of the specified storage ranges, 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 caused by shipping to hot or cold climates, hypobaric conditions 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 one just outside of an established limit may be equally detrimental. 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 light-reactive components that may give rise to cytotoxic by-products.

Accelerated aging studies are useful to characterize how the cell-based advanced therapy or tissue-based product degrades, and which assays are stability indicating. 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

Storage conditions are chosen to preserve the quality of the cell-based advanced therapy or tissue-based product, so that product specifications are maintained throughout storage, shipping, and handling at the clinic. Before clinical trial use, initial studies must be conducted to determine acceptable storage, shipping, handling, and expiration dating. Once stability-indicating methods are developed and the final container–closure, storage, and shipping conditions are chosen, these conditions must be validated, as discussed in *Stability* (above).

The product in its final container closure should be placed in a lightproof, leakproof shipping container with adequate physical support to ensure stability and prevent leakage during shipment. Special consideration should be given to the ability of gas to permeate the shipping container, especially if the cell-based therapy product is stored or shipped on dry ice or liquid nitrogen.

Storage

For each type of cell-based therapy product, the manufacturer should establish product storage specifications and acceptable storage conditions, including temperature range or liquid nitrogen level. Storage unit monitoring and alarm systems should

immediately notify manufacturing of unacceptable storage conditions. The stability of the product during routine storage should be monitored and documented via a stability program (see *Stability*, above).

Cryopreservation is the main mode of long-term cell storage. Cell-based therapy products are most often cryopreserved using controlled-rate freezing, or equivalent procedures known to maintain viability. Freezer equipment should be validated and temperature mapped so that cells are stored at an appropriately low temperature. Product stability should be validated under the holding conditions at both the manufacturing facility and clinical site.

Many cell-based products cannot be cryopreserved. Because cells continue to metabolize during storage, their expiration period is short—on the order of hours or days—though the expiration date may be extended by increasing the volume of the storage medium, or by adjusting the storage temperature.

Shipping

Shipping containers and procedures for cell-based therapies and tissue-based products must ensure acceptable temperatures are maintained under conditions of actual use. These conditions include extremes of temperature inside and outside the shipping container, and other shipping challenges such as X-rays or mechanical vibration. Shipping studies should be conducted during product development in order to identify which stresses affect the product. Bracing and insulating materials should then be chosen and validated to provide a packaging system that will protect the product against extreme temperatures and mechanical stresses and maintain the final container closure integrity. Shipping validations must ensure that the product meets quality specifications (including potency) once it reaches its final destination.

Most cell and tissue-based products are shipped by commercial shippers or courier systems. Commercial aircraft shipments must obtain special permission in order to bypass scanning by airport X-ray equipment. Special attention should be paid to shipping container labels because both biohazard and patient-specific information may be required in specific areas of the packaging. Shipping validations must be conducted under predefined protocols with predetermined acceptance criteria to ensure that the product meets quality specifications (including potency) once it reaches its final destination.

Cryopreserved cell-based therapy products are typically shipped to medical centers on dry ice or in liquid nitrogen dry shippers. Dry shippers may be preferable because temperature is more readily maintained and monitored. Dry shippers also allow continuous monitoring of the shipper's temperature, which can be collected and logged for up to 14 days. Dry ice and liquid nitrogen are both considered hazardous materials during shipping and must be labeled accordingly.

LABELING

Labeling of cell therapy products is regulated by the FDA under 21 CFR 201, 601, 610, and 1271. For biologics, 21 CFR 610 Subpart G outlines the requirements for container and package labeling. When possible, a full label should be affixed to the product container. When partial labels are used, the container must be placed in a package that contains a label bearing all the items required for the package label. For containers that cannot accommodate any label, the container must be placed in a package that bears all the information required for a package label. When affixed to the container the label should not impede inspection of the contents. For products with very short shelf lives, expiration dating requires adjustment and correction for time zones to provide the user an accurate assessment of shelf life.

Regulations in 21 CFR 610.62 refer to the position and prominence of the proper or United States Adopted Names (USAN) name in relation to a trade name.

Additional labeling requirements in 21 CFR 1271.90 apply because some cell-based therapy products are also considered HCT/Ps. For autologous cell therapies, the manufacturer is exempt from the requirements of determining donor eligibility. However, if the recommended testing for pathogenic or microbial contaminants is not performed before release, the label must contain the statement "FOR AUTOLOGOUS USE ONLY" or "NOT EVALUATED FOR INFECTIOUS SUBSTANCES." The label must also contain the Biohazard legend shown in 21 CFR 1271.3(h) with the statement "WARNING: Advise patient of communicable disease risks." For patient-specific products, the patient's full name, initials, or a combination of these must appear on the labeling to ensure that the product will be administered to the appropriate patient. For materials containing human tissue, per AATB standards, transport package labels should also include prominent identification of contents as "DONATED HUMAN TISSUE" as well as prescribed storage conditions and transport expiration date (if applicable).

Regulations in 21 CFR 201.56 and 201.57 govern the content and format of the package insert.

Several groups have designed a standard for uniform labeling of cellular therapy products, ISBT 128, which defines the data structures and the placement of bar codes and their corresponding eye-readable text that appears beneath the bar code. In addition, ISBT 128 provides class names for different types of cellular products, and various other texts that must go on the labels. Although this voluntary ISBT 128 standard meets different organizations' requirements for labeling cellular products, it does not currently meet FDA regulatory requirements. Consequently, labels that comply with ISBT 128 must be supplemented with additional information required by FDA.

CONSIDERATIONS FOR TECHNOLOGY TRANSFER

Transfer of the skills, knowledge, technologies, and methods of manufacturing necessary to create a cell or tissue-based product is essential to ensure that scientific and technological developments are accessible and transferable to others. Some general considerations for technology transfer activities are summarized below.

The process of developing a cell-based therapy or tissue-based product is complex and often involves several rounds of technology transfer throughout the product's life cycle. Some examples of technology transfer activities include: from bench research to translational research; transfer from research and development to GMP-compliant manufacturing; and change in manufacturing facility (for example, from in-house manufacturing to a contract manufacturer).

Manufacturers should anticipate the need for technology transfer during the research and development stage of a cell or tissue-therapy process. This should result in good documentation practices for product research and development, including testing procedures. Data and results should be retained in the format of development reports or technical reports to provide historical information that can be referenced and used in regulatory filings. Critical raw materials, procedures, and equipment should also be identified during technology transfer. Product and process development progress should be monitored against milestones established as part of risk assessment and gap identification in the technology transfer plan. *Table 4* provides an overview of the steps involved in technology transfer.

Table 4. Technology Transfer—Fundamental Steps

Preparation	• Define the scope, strategy, and risks associated with the project that will be transferred • Identify overall gaps and process transferability • Assess availability of documentation such as manufacturing and testing procedures, sampling plans, in-process and final product data and specifications, material specifications (including source, testing requirements, and quantities required for a manufacturing procedure or test procedure), equipment specifications, specialized training requirements, facility requirements, and infrastructure requirements • Establish a governance body consisting of leads, experts, and mentors from both the sending and receiving sides; determine responsibilities for each group and individual • Define communication and reporting channels • Identify performance measurements, milestones, and timelines
Development and Implementation	• Establish a risk management plan • Establish a technology transfer master plan • Develop a training plan • Establish documents at the receiving site (specifications, SOPs, batch records, and standard test methods) • Train operations, quality, and support personnel for sustainability • Qualify materials and vendors • Establish and execute equipment comparability/suitability protocols • Calibrate equipment at receiving site • Qualify personnel, equipment, and facility at receiving site (includes execution of aseptic process validations, sterile media fills, and cleaning validations) • Establish and execute methods/assay qualifications • Establish a product stability program • Perform engineering and consistency/qualification runs • Assess need to establish comparability and prepare regulatory filings • Establish and execute shipping qualifications
Maintenance	• Collect and trend process/product data • Monitor product stability • Manage change control • Train and requalify personnel • Recalibrate and requalify equipment • Update regulatory filings

The ultimate goal of technology transfer is for the recipient to consistently reproduce a process in order to make a comparable product in compliance with regulations. It is not atypical for manufacturers to develop and implement process improvements during early stages of technology transfer to support scale-up and manufacturing for Phase I/II clinical trials. However, for technology transfer during Phase III studies, pivotal trials, or commercial manufacturing, changes to the process or product should be avoided because they could require additional clinical studies and adversely affect time to market.

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REGULATIONS AND STANDARDS

The Federal Food, Drug, and Cosmetic Act (FD&C Act) and the Public Health Service Act (PHS Act) provide the legal framework for FDA regulation of biological products, including cell-based therapy products. A list of frequently used terms in regulation of cellular-therapy products is presented in *Table 5*. In 1993 FDA provided notice that it intended to regulate cellular and gene-therapy products as biological products (*Federal Register* 1993;58:53248–53251). The FDA defined somatic cellular-therapy products as autologous (i.e., self), allogeneic (i.e., intraspecies), or xenogeneic (i.e., interspecies) cells that have been propagated, expanded, selected, pharmacologically treated, or otherwise altered ex vivo for administration to humans for the prevention, treatment, cure, diagnosis, or mitigation of disease or injuries. For other biological products and drugs, clinical trials involving somatic cellular therapy products must be initiated under an investigational new drug (IND) application. After a sponsor submits sufficient evidence of product safety and clinical effectiveness, FDA approval can be obtained for marketing in the form of a BLA or PMA.

As defined by the FDA, cellular therapy products are considered to be drugs, biological products but also HCT/Ps that are regulated under Section 351 and/or Section 361 of the PHS Act. This means that cell-based therapies are subject to cGMP (21 CFR 210 and 211), Biologics Product regulations (21 CFR 610), and HCT/P regulations (21 CFR 1271) including cGTP.

In recent years, the FDA has issued a number of regulations and guidance documents for human cell and tissue products (see *Appendix* and https://www.fda.gov/vaccines-blood-biologics/guidance-compliance-regulatory-information-biologics/biologics-guidances). Of particular importance are the regulations at 21 CFR 1271 that establish a tiered, risk-based approach for HCT/Ps. In this regulatory framework, many conventional human cells or tissues are not subject to premarket approval and have only to comply with GTPs, including donor eligibility. This lower tier of regulatory oversight is intended to prevent the

introduction, transmission, or spread of communicable disease. When human cells or tissue are the starting material for the creation of a novel cell-based product, additional regulatory requirements are applicable. This higher tier of regulatory oversight includes compliance with GMPs, biological product standards, and premarket approval (see 21 CFR 1271.10). In almost all cases, the cell-based products described in this chapter should comply with the higher tier of regulatory oversight.

In addition to cellular therapy-specific regulations and guidance, many general guidelines such as those related to aseptic processing, GMP expectations during development, process validation, and others are relevant and applicable (see www.fda.gov). Additionally, ICH has issued guidance documents for qualifying cell-based therapy and tissue-based products (see *Appendix* and www.ich.org). Some of the guidelines and concepts in these documents are reproduced in *USP–NF*.

The regulatory pathway for cell-based therapy products parallels that of pharmaceuticals, and as the product moves from early research through pivotal trials and finally marketing approval, the degree of manufacturing control becomes increasingly stringent. This has implications for the manufacturing unit and may dictate that the site be moved. Standards-setting organizations encourage the use of a fully functional quality unit to oversee manufacturing progress. Information is available on the FDA website, along with references to groups charged with guiding the medical community and the manufacturing unit during development.

In addition to *USP* general chapters and monographs for cell-based therapies and tissue-based products, a number of professional standards-setting organizations (see *Table 6* and *Appendix*) have worked closely with regulatory authorities to develop standards and practices. These organizations ensure that standards are current and comply with governmental regulations. Such standards are a supplemental source of knowledge in identification of donors, donor screening and testing, product collections, processing of cellular products, administration, adverse event reporting, and follow-up after treatment. The AATB has developed guidelines for sourcing allogeneic tissue. Over the years various organizations have tried to harmonize standards, including the development of common information circulars that can be compared with package inserts. At present, however, compliance with one organization's standards does not ensure compliance with those of any other organization.

Many benefits accrue to manufacturing facilities that participate in voluntary standards programs. Professional standards-setting organizations participate in educational workshops and disseminate information about operational issues. They also maintain close surveillance of FDA activity and training of inspectors. Further, the FDA relies on accreditation by voluntary standards program, and the FDA's unannounced inspections have led to an increasingly high level of compliance in laboratory and clinical settings and has also undoubtedly increased patient safety. Third-party payors and hospital-ranking services have begun to use accreditation reports in their evaluation of quality programs.

Table 5. Frequently Used Terms in Regulation of Cellular-Therapy Products

Term	Definition
351 products	Regulated under Section 351 of the PHS Act
361 products	Regulated under 21 CFR 1271, Human Cells, Tissues, and Cellular and Tissue-Based Products
BLA	Biologics License Application
CAR-T	Chimeric Antigen Receptor T-cells
CBER	Center for Biologics Evaluation and Research
CDRH	Center for Devices and Radiologic Health
cGMPs	current Good manufacturing practices
GTP	Good tissue practices, ▲21▲ (ERR 1-Nov-2020) CFR 1271, Human Cells, Tissues, and Cellular and Tissue-Based Products
HCT/Ps	Human cells, tissues, and cellular and tissue-based products
IDE	Investigational Device Exemption. An IDE allows the investigational device to be used in a clinical study in order to collect safety and effectiveness data required to support a Premarket Approval (PMA) application or a Premarket Notification [510(k)] submission to the FDA.
IND	Investigational New Drug. An IND is a request for FDA authorization to administer an investigational drug to humans. IND regulations are contained in 21 CFR 312.
MCB	Master cell bank
PMA	Premarket approval
WCB	Working cell bank

Table 6. Cellular Therapy Product Standards-Setting Organizations

AABB	AABB, formerly known as the American Association of Blood Banks, is an international association representing individuals and institutions involved in activities related to transfusion and cellular therapies, including transplantation medicine.	www.aabb.org/

Table 6. Cellular Therapy Product Standards-Setting Organizations *(continued)*

AATB	The American Association of Tissue Banks is an educational and scientific, tax-exempt organization that facilitates the provision of transplantable tissues of uniformly high quality to meet national needs. AATB publishes standards to ensure that the conduct of tissue banking meets acceptable norms of technical and ethical performance. AATB conducts an accreditation program for establishments that retrieve, process, store, or distribute human tissue for transplant. A certification program is administered for tissue-bank personnel to ensure that tissue-banking activities are performed in a professional manner consistent with the standards of the association.	www.aatb.org/
ASTM	ASTM International (ASTM), originally known as the American Society for Testing and Materials, is one of the largest voluntary standards-development organizations in the world and provides technical standards for materials, products, systems, and services. ASTM International standards are used in the information infrastructure that guides design, manufacturing, and trade in the global economy.	www.astm.org/
FACT	The Foundation for the Accreditation of Cellular Therapy is a nonprofit corporation co-founded by the International Society for Cellular Therapy (ISCT) and the American Society of Blood and Marrow Transplantation (ASBMT) for voluntary inspection and accreditation in the field of cellular therapy.	www.factwebsite.org/
NMDP	The National Marrow Donor Program is a nonprofit organization that operates the federally funded registry of volunteer hematopoietic cell donors and umbilical cord blood units in the United States.	www.nmdp.org/
ICCBBA	The International Council for Commonality in Blood Banking Automation was established and given the responsibility for implementation and management of the ISBT 128 standard, a system for identification, labeling, and processing of human blood, tissue, and cellular-therapy products using an internationally standardized system.	www.iccbba.org/

GLOSSARY

Adventitious agent: A foreign material that is introduced 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 but with a different genotype.

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

Apheresis: Procedure of withdrawing blood from a donor, removing select components (e.g., platelets or leukocytes), and transfusing the remainder into the donor.

Autologous: From one's own body.

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 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 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).

Cellular therapy: Therapy that uses whole cells to treat a disease, condition, or injury.

cGMP: Current good manufacturing practice.

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.

Combination products: Therapeutic products that combine drugs, devices, and/or biological products.

Culture medium: The liquid that covers cells in a culture vessel and contains ingredients to nourish and support the cells. Culture medium may also include growth factors added to produce desired changes in the cells.

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 cells: Cells that sensitize T cells to antigens.

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

Enzyme-linked immunosorbent assay (ELISA)**:** An immunoassay that uses an enzyme-labeled antigen or antibody to detect the binding of a molecule to a solid matrix.

Embryonic stem cell, human (hESC)**:** Stem cell derived from the inner cell mass of the blastocyst.

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 to both the mechanical and the biological processes necessary to have a fully functional graft.

Epidermal: Pertaining to the outermost and nonvascular layer of the skin derived from embryonic ectoderm.

Epithelial cells: Cells from the linings of various organs, e.g., respiratory, intestinal, or vascular epithelial cells.

Ex vivo: Outside of the living body. Refers to a medical procedure in which an organ, cells, or tissue are taken from a living body for a treatment or procedure, and then returned to the living body.

Feeder cells: Cells used in co-culture to maintain pluripotent stem cells. For hESC, typical feeder layers include mouse embryonic fibroblasts or human embryonic fibroblasts that have been treated to prevent them from dividing.

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.

Graft-versus-host disease: Rejection of the transplanted tissue by the host. It is the leading cause of patient death when mismatched allogeneic tissue is used.

Granulocyte: One of three types of white blood cells. These cells digest bacteria and 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.

Hemacytometer: A device used to manually count cells.

Hematopoietic: Pertaining to or affecting the formation of blood cells.

Hematopoietic stem cells: Stem cells that give rise to all red and white blood cells and platelets.

Hepatocytes: 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.

Human leukocyte antigen (HLA)**:** Proteins controlled by the major histocompatibility complex. These proteins play a key role in determining transplant compatibility.

Immunoassay: Technique for identifying substances based on the use of antibodies.

Immunofluorescence: Technique that combines an antibody detection strategy with a fluorescent label for visualization often used in combination with microscopy or fluorescence activated cell sorting.

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

In vitro: In the laboratory (outside the body). The opposite of in vivo (in the body).

In vivo: Procedure performed in the living organism.

Islet cells: β-islet cells of the pancreas that secrete insulin.

Keratinocytes: Differentiated epidermal cells that constitute the top layer of cells in the skin.

Lineage (committed progenitor cells, differentiated cells)**:** Specific path of cell differentiation that can be traced to a single cell of origin.

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

Mesenchymal stem cells: Multipotent stem cells that can differentiate into a variety of cell types.

Monoclonal antibodies: Antibodies that are derived from a single cell clone.

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

Natural killer cells (or NK cells)**:** Cytotoxic lymphocytes that constitute a major component of the innate immune system.

Neuronal stem cells: Stem cells found in neural tissue that can give rise to neurons and glial cells.

Osteogenic cells: Derived from or involved in the growth or repair of bone.

Passage: The process in which cells are disassociated, washed, and seeded into new cultures after a round of cell growth and proliferation. The number of passages is a good indication of the age of the cultures and expected stability.

Process validation: Means for providing documentation that the manufacturing process is controlled, reproducible, and capable of consistently producing a product that meets predetermined specifications.

Polymerase chain reaction (PCR)**:** Technique to amplify a target DNA or RNA sequence of nucleotides by cycles of polymerase-based copying, resulting in geometric increases in copy number.

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.

Regenerative medicine: An emerging interdisciplinary field of research and clinical applications focused on the repair, replacement or regeneration of cells, tissues or organs to restore impaired function using a combination of approaches including, but not limited to, the use of soluble molecules, gene therapy, stem cell transplantation, tissue engineering, and the reprogramming of cell and tissue types.

Serum-free: Refers to cell growth medium that lacks a serum component.

SIS: Subintestinal submucosa.

Somatic cells: Cells other than the germ cells.

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.

Supravital dye: A dye that stains only living cells.

Suspension culture: Growth, in suspension, of cells not requiring attachment to substrate in order to undergo cell division.

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).

Umbilical cord blood stem cells: Stem cells derived from the blood that remains in the placenta and in the attached umbilical cord after childbirth.

Undifferentiated cells: Cells that have not yet developed into a specialized cell type or tissue.

Working standard: In-house or secondary standard that is qualified against and used in place of a reference standard.

Xenogeneic: From a different species.

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

APPENDIX

Lists of Relevant Regulatory References

Cellular therapies and cell-therapy components are regulated by FDA as biological products. The general requirements are listed in national laws and international guidance. In the US, national requirements are codified in different sections of 21 CFR, and additional recommendations are available in FDA guidance documents. International guidance documents are available from the ICH, the European Agency of Medicines (EMA), and the World Health Organization (WHO). Although guidance documents from the ICH are well referenced in this chapter, those from WHO and European Medicines Evaluation Agency (EMEA) are not, and manufacturers of cellular or tissue-based products intended for markets outside the United States are advised to refer to relevant guidances from relevant nations. Beyond *USP* chapters referenced in this chapter, the following lists include regulatory documents as well as best practices in product and process development, manufacturing, quality control, and quality assurance.

Code of Federal Regulations (CFR)

Regulations	Scope
21 CFR 201	Labeling
21 CFR 210	cGMP for drugs
21 CFR 211	cGMPs for final products
21 CFR 600.3	Definitions of important terms for biological products
21 CFR 601	Requirements for marketing authorization of biologics under BLA
21 CFR 610	Identity, purity, potency, and microbial safety testing requirements for biological products
21 CFR 610.12	Sterility
21 CFR 610.60–68	Product and container labeling requirements for biological products
21 CFR 820	Quality systems for products regulated as medical devices
21 CFR 1271	Good tissue practices (GTPs)
45 CFR Part 46	Department of Health and Human Services, Part 46, Protection of Human Subjects

FDA Guidance Documents

- *Guidance for FDA Reviewers and Sponsors: Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Somatic Cell Therapy Investigational New Drug Applications (INDs)*, April 2008. https://www.fda.gov/downloads/biologicsbloodvaccines/guidancecomplianceregulatoryinformation/guidances/xenotransplantation/ucm092705.pdf.
- *Guidance for Industry: Potency Tests for Cellular and Gene Therapy Products*, January 2011. https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM243392.pdf.
- *Guidance for Industry: Biologics License Applications for Minimally Manipulated, Unrelated Allogeneic Placental/Umbilical Cord Blood Intended for Hematopoietic and Immunologic Reconstitution in Patients with Disorders Affecting the Hematopoietic System*, March 2014. http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM357135.pdf.
- *Guidance for Industry: Considerations for Allogeneic Pancreatic Islet Cell Products*, September 2009. https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM182441.pdf.
- *Guidance for Industry: Current Good Tissue Practice (cGTP) and Additional Requirements for Manufacturers of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps)*, December 2011. https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Tissue/UCM285223.pdf.
- *Guidance for Industry: Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps)*, February 2007. https://www.fda.gov/downloads/biologicsbloodvaccines/guidancecomplianceregulatoryinformation/guidances/tissue/ucm091345.pdf.

- *Guidance for Industry: Donor Screening Recommendations to Reduce the Risk of Transmission of Zika Virus by Human Cells, Tissues, and Cellular and Tissue-Based Products*, March 2016. https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Tissue/UCM488582.pdf.
- *Guidance for Industry: Use of Nucleic Acid Tests to Reduce the Risk of Transmission of West Nile Virus from Living Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps)*, May 2017. http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Tissue/UCM372084.pdf.
- *Guidance for Industry: Use of Nucleic Acid Tests to Reduce the Risk of Transmission of Hepatitis B Virus from Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products*, August 2016. http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/UCM516650.pdf.
- *Guidance for Industry: Use of Donor Screening Tests to Test Donors of Human Cells, Tissues and Cellular and Tissue-Based Products for Infection with Treponema pallidum (Syphilis)*, September 2015. http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Tissue/UCM373311.pdf.
- *Guidance for Industry: Source Animal, Product, Preclinical, and Clinical Issues Concerning the Use of Xenotransplantation Products in Humans*, December 2016. https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Xenotransplantation/UCM533036.pdf.
- *PHS Guideline on Infectious Disease Issues in Xenotransplantation*, January 2001. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/phs-guideline-infectious-disease-issues-xenotransplantation.
- *Guidance for Industry: Investigating Out-of-Specification (OOS) Test Results for Pharmaceutical Production*, October 2006. www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070287.pdf.
- *Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing—Current Good Manufacturing Practice*, September 2004. https://www.gmp-compliance.org/guidelines/gmp-guideline/fda-guidance-for-industry-sterile-drug-products-produced-by-aseptic-processing-current-good-manufacturing-practice-september-200.
- *Guidance for Industry and Food and Drug Administration Staff, Use of International Standard ISO 10993-1, "Biological evaluation of medical devices–Part 1: Evaluation and testing within a risk management process"*. https://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/UCM348890.pdf.

National and International Regulatory Documents

- The United States Consensus Standard for the Uniform Labeling of Cellular Therapy Products using ISBT 128. Available at: http://www.iccbba.org/.
- ISO 10993-1:2018, Biological evaluation of medical devices—Part 1: Evaluation and testing within a risk management process. Available at: http://www.iso.org.
- ICH Q2(R1): Validation of Analytical Procedures: Text and Methodology. Available at: http://www.ich.org.
- ICH Q5A(R1): Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin. Available at: http://www.ich.org.
- ICH Q5C: Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products. Available at: http://www.ich.org.
- ICH Q6B: Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products. Available at: http://www.ich.org.
- ICH Q9: Quality Risk Management. Available at: http://www.ich.org.
- USAN Naming Guidelines for Cellular and Non-Cellular Therapies. Available at: http://www.ama-assn.org/.
- Guide for the Care and Use of Laboratory Animals (National Research Council, 1996). Available at: http://www.nap.edu/.