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Stem cells are among the most complex biologics to date. Food and Drug Administration (FDA) recommendations for stem cell–based product manufacture and characterization incorporate the tremendous experience gained in many cell therapy clinical trials and the experience of the entire field of stem cell research. In advising sponsors about their stem cell development program, the FDA recognizes the need for flexibility in its recommendations and will consider many factors, including the intended target population, the seriousness of the disease under study, and the potential benefits and risks from the investigational product. The agency continually updates and reassesses recommendations for stem cell production and testing based on the growing experience and on feedback from a variety of sources. This chapter describes development of FDA recommendations and summarizes current recommendations applicable to stem cell–based cellular therapies.

Introduction

The medical use of stem cells promises profound impact on our search for therapies to improve public health. Stem cells are endowed with seeming unlimited ability to form any cell type. This ability could potentially allow application of stem cells to treatment of a wide variety of medical conditions. Successful management of previously untreatable disease seems feasible. The challenges of translating the promise of stem cells from successful basic scientific research into clinical use are great, but the FDA is prepared to work with the scientific community to bring safe and effective stem-cell therapies into the medical armamentarium. The molecular mechanisms that regulate stem cell self-renewal and commitment to a variety of cell fates pose vexing problems in developmental biology. Nonetheless, FDA regulatory oversight of stem cell–based therapy must address these complexities. Although many of the scientific questions of gene regulation, cell–cell interaction, and mechanisms controlling differentiation are unsolved, the regulatory approach used in the Division of Cellular and Gene Therapies and developed in many parts of the FDA is designed to allow progress in clinical research while ensuring, to the best of our ability, patient safety and protection of patient rights.

The FDA regulatory approach requires multidisciplinary expertise to evaluate proposed clinical trials and includes reviewers with knowledge of medicine, pharmacology/toxicology, cellular therapies, and cell biology. Accordingly, the Office of Cellular, Tissue, and Gene Therapies (OCTGT) employs scientists and clinicians involved in full-time review and some who, in addition to their review responsibilities, also pursue laboratory research programs in areas such as developmental biology. This approach ensures that all aspects of product, preclinical, and clinical development are overseen by staff possessing state-of-the-art expertise and scientific judgment. Moreover, substantial experience with the regulation of other emergent technologies such as use of recombinant proteins, gene transfer, and cellular therapies has resulted in a regulatory and policy framework that can meet the challenge confronting medical use of stem cells. Finally, oversight of this novel therapeutic approach benefits from input provided by external expert panels such as the FDA’s Biological Response Modifiers Advisory Committee (BRMAC). For example, the BRMAC convened to consider challenges posed by stem cell–based therapies and has assisted tremendously in development of a regulatory framework for oversight of this field.1

Stem cell–based therapies will likely include a variety of approaches including administration of undifferentiated stem cells or transplantation of differentiated cells derived from stem cells. Consequently, there is a need for increased understanding of the mechanisms regulating stem cell growth, cell fate determination, and differentiation. An important goal of this understanding is to eliminate the risk of inappropriate cell differentiation or transformation while ensuring that long-term benefits include prolonged function and viability of the therapeutic stem cells or their cellular descendents, in the absence of adverse events.

Development of Recommendations for the Manufacture and Characterization of Stem Cell Products

The FDA recommendations and requirements for characterization of stem cell products derive from several sources.
These sources include the applicable regulations found in the Code of Federal Regulations (CFR), relevant guidance documents, and interactions with the scientific community.

CODE OF FEDERAL REGULATIONS
The Code of Federal Regulations forms the backbone of the FDA’s regulatory framework for innovative products such as those derived from stem cells. The applicable regulations include those outlined for the requirements for investigational new drug (IND) applications at 21 CFR 312; the General Biological Product Standards at 21 CFR 610; and current good manufacturing practices (cGMP) at 21 CFR 210, 211, among others. The intent of these various regulations is to ensure to the extent possible that patients receive safe investigational products that are likely to convey the intended therapeutic effect within the context of defined clinical studies. The ultimate goal is to collect sufficient safety and efficacy data to permit marketing approval and/or widespread use in patients who need these products.

RELEVANT GUIDANCE DOCUMENTS
The various regulations are intended to be broadly applicable to the diverse universe of products. Thus, guidance documents are intended to further define the FDA’s expectations for a specific class of products. The FDA, either alone or in partnership with other organizations such as the International Conference on Harmonization (ICH) has issued a number of useful documents that encompass most biologic products, including those applicable to stem cells. Numerous examples are mentioned throughout this chapter and are listed in the references section of this chapter. The principles underlying those documents will be useful in guiding the development of many stem cell–based therapies. Information regarding Center for Biologics Evaluation and Research (CBER) regulatory documents can be obtained by email (OCTMA@cber.fda.gov or matt@cber.fda.gov). Most documents are available for downloading from a CBER Web site (www.fda.gov/cber/guidelines.htm).

SCIENTIFIC INTERACTIONS
CBER reviewers have regular internal meetings to discuss relevant scientific issues and develop consistency in oversight of cell therapy products. Staff within the FDA’s OCTGT also participate in many outreach activities such as regulatory and scientific meetings in which interested parties can interact and communicate with the people who regulate the cell therapy arena. These interactions enrich both FDA staff and members of the regulated community by providing gains in mutual understanding and appreciation of perspectives, outside of formal regulatory interactions. Within the OCTGT, a number of review scientists also maintain active research programs in cell or developmental biology or other areas relevant to stem cells and other products regulated by the FDA. This approach, deemed the research–reviewer model, is a major factor allowing the FDA to maintain state-of-the-art expertise and scientific credibility during the complex task of regulating stem cells and developing policy that affects the entire field of stem cell therapeutic use. Figure 73–1 illustrates three crucial cellular signaling pathways that are active research areas within CBER’s Division of Cellular and Gene Therapies. The Notch-Delta pathway, the BMP pathway, and the Wnt pathway each influence cell fate specification within a variety of medically significant cellular lineages. Some direct examples of relevance to stem cell biology and related cellular therapies are listed at the bottom of the illustration. The cumulative experience of FDA reviewers is also used to develop guidance documents, several of which are relevant to the manufacture of cell therapy products.

The experience of the cell therapy community can also play a key role in shaping the FDA recommendations that pertain to regulation of cell therapy products. The experience of stem cell manufacturers is communicated in meetings with the FDA staff, at presentations at scientific meetings, and at presentations to the FDA’s BRMAC. BRMAC meetings allow the FDA to obtain advice in a public forum on scientific issues that affect stem cell experiments. This format allows all interested parties to participate. Transcripts of these meetings are available on the CBER Web site (http://www.fda.gov/cber/). The BRMAC’s advice on issues such as characterization of neuronal stem cell therapy products has been valuable as the CBER staffs develop stem cell policy.

Another important resource that facilitates development of recommendations for regulation of stem cells involves the FDA–National Institutes of Health (NIH) interagency interactions through formalized Memoranda of Understanding Agreements (MOUs) that allow bidirectional input on translational research. The FDA benefits from the scientific expertise of NIH program managers and intramural scientists, and the NIH benefits from the FDA scientific perspectives on important funding initiatives to expedite translation of research from bench to bedside. For example, under the MOU, the NIH can involve FDA expertise in Request for Application (RFA) design for translational research. In addition to these MOUs, there is increasing participation by the FDA in expert advisory panels that develop recommendations for NIH translational research programs. These feedback mechanisms serve the FDA-regulated community, the NIH-funded research community, and the public by focusing scientific and funding resources on developing novel technologies such as stem cell use to address challenges to public health.

In summary, the FDA receives input and feedback from a variety of sources in formulating recommendations regarding stem cell manufacturing and characterization. The recommendations can evolve with advances in technology and through accumulating experience. For particular IND decisions, the FDA considers the potential risks and benefits of each cell therapy product and each proposed clinical trial when making its recommendations. This case-by-case approach, which takes into account the severity of the disease and the proposed patient population, permits flexibility in product manufacture and characterization.
Regulatory Approaches for Stem Cell Products

CORD BLOOD, PERIPHERAL BLOOD, AND BONE MARROW–DERIVED STEM CELLS

Several different regulatory approaches can be applied to hematopoietic stem cells (HSCs) such as those derived from cord blood, peripheral blood, and bone marrow. The regulatory approach taken for a specific product is determined by the clinical use and method of manufacturing of the HSC product. For both umbilical cord blood and unrelated allogeneic peripheral blood stem cells, the FDA has called for data to determine the feasibility of regulating some of the lower risk uses of HSCs through a standards-based approach. This proposed approach requires cell-processing establishments to register, ensure that they meet standards for safety, and use FDA-approved donor screening test kits. Finally, autologous or allogeneic cord blood, peripheral blood, or bone marrow stem cell products will be regulated under IND if more than minimal manipulation occurs, the cell product is combined with a drug or device, or the intended use is considered to be nonhomologous. Although the field of stem cell biology has produced evidence that stem cell activity for a variety of lineages can be demonstrated in cord blood, peripheral blood, or bone marrow cell preparations, the FDA will regard use of these products for anything beyond hematopoietic reconstitution as nonhomologous use until a clearer scientific picture emerges.

STEM CELL INVESTIGATIONAL NEW DRUG REGULATION

The remainder of this chapter discusses stem cell products that will be regulated under IND. These are products that are more than minimally manipulated, combined with a drug or device, metabolically active, or nonhomologous in their intended use. When an IND application or pre-IND information package is
reviewed in the OCTGT, reviewers from three disciplines are assigned including (1) a product expert, (2) a pharmacology/toxicology expert, and (3) a clinical expert. However, the information in this chapter focuses on product issues such as manufacturing and characterization of stem cell products.

In the review of IND applications, primary FDA objectives are to ensure the safety and rights of subjects in clinical investigations. An additional objective during phases 2 and 3 is to help ensure that the quality of the scientific evaluation of the investigational product is adequate to permit an evaluation of its safety and effectiveness (21 CFR 312.22(a)). FDA reviewers assess whether sufficient information has been provided to ensure the proper identification, quality, purity, and strength of the investigational product (21 CFR 312.23(a)(7)(i)). For investigational cell therapy products such as stem cells, alternative terms, such as safety, identity, purity, and potency are generally used.

**STEM CELLS AND XENOTRANSPLANTATION**

Any stem cell product derived from a nonhuman source would be considered a xenotransplantation product. Also, therapeutic use of stem cell products grown on feeder layers of nonhuman origin falls into the category of xenotransplantation. The FDA has a regulatory framework in place to regulate such products and has no policy or intent to prohibit use of stem cells grown under such conditions. The Guidance to Industry document “Source Animal, Product, Preclinical, and Clinical Issues Concerning the Use of Xenotransplantation Products in Humans” includes human body fluids, cells, tissues, or organs that have had ex vivo contact with nonhuman animal cells, tissues, or organs as xenotransplant products. The major issue is to ensure that the human stem cell line is free from infectious agents of nonhuman origin. The possible presence of xenozoonotic infectious agents is a concern that would not necessarily apply to stem cell products grown under conditions that exclude contact with nonhuman feeder cells.

**COMBINATION PRODUCTS**

One area of intense development is the use of stem cells in combination with drugs, recombinant proteins, or devices. Examples include combinations of stem cells with scaffolds for bone or organ replacement or for extracorporeal liver or kidney assist devices. Such applications fall under the category of combination products (defined in 21CFR 3.2(e)). The regulatory path for these applications can involve experts from several FDA centers using mechanisms of consultative or collaborative review. The center responsible for organizing the review will be assigned based on a determination of the primary mode of action of the combined product. The FDA’s newly formed Office of Combination Products will serve to facilitate the review process and ensure quality, clarity, and efficiency of application reviews that involve multiple FDA centers.

**CHEMISTRY, MANUFACTURING, AND CONTROL REVIEWER GUIDANCE FOR CELL THERAPY PRODUCTS**

CBER has issued a guidance document intended to facilitate consistency and efficiency of review of cell therapy products, including stem cells. This document is based on current best review practices and can be consulted by IND sponsors to gain a thorough perspective on the types and organization of information that CBER reviewers are looking for during review of a cell therapy IND. The document is entitled “Draft Guidance for Reviewers: Instructions and Template for Chemistry, Manufacturing, and Control (CMC) Reviewers of Human Somatic Cell Therapy Investigational New Drug Applications (INDs)” and is available for public examination and comment. Another guidance document “Guidance for Industry: Guidance for Human Somatic Cell Therapy and Gene Therapy” discusses many scientific issues relevant to stem cell therapies.

**General Expectations for Control of Manufacturing and Product Characterization**

**SAFETY, IDENTITY, PURITY, AND POTENCY**

A major goal of FDA oversight of cellular therapies is to ensure safety of patients who receive an investigational product. Careful attention to the details of manufacturing and product characterization are key factors in ensuring safety. These are also important factors in assessment of purity of the final product that will be administered to patients. Assessment of identity and purity is a challenge for cellular products, especially stem cells. Identity or purity assessments can involve biologic, biochemical, and functional characterization and should be designed to determine how well the characteristics of the cell population conform to the expected and desired properties.

Another important and challenging goal of stem cell product characterization is assessment of the potency of the final product. Potency is the “specific ability of the product, as indicated by appropriate laboratory tests...to effect a given result” (21CFR 600.3). Although the mechanism of action is not necessarily known, the outcome of a laboratory test for potency should correlate very well with the desired clinical effect. Because stem cells are intended to undergo differentiation before mediating their clinical effect, the problem of what to measure and how to correlate the measurement with a final outcome is a great challenge. Because direct measurement of stem cell potency may be challenging because of lack of appropriate in vitro or in vivo assays, use of other means to demonstrate potency could be adequate for initiation of clinical trials. However, development of a valid potency assay for stem cell product lot release will be required for FDA licensure. It is generally expected that a potency assay will be in place before phase 3. An expanded discussion of this important topic is presented in the subsection on potency in the section on testing of stem cell products.

In summary, as with all biologic therapeutic products, assessments of safety, purity, and potency of stem cell products is crucial in product development.

**CONTROL OF PROCESS AND PRODUCT**

As with all complex biologic products, regulation of the manufacturing process is as important as characterization and
testing of the final product in ensuring the safety, purity, and
potency of stem cell products. Thus, thorough characterization
of starting materials and manufacturing intermediates is
required to ensure that the final cellular product is acceptable
for administration to humans.

The first step of stem cell product manufacture is acquisi-
tion of stem cells from a suitable donor source. Whether human or animal donors are used, the principles of donor
suitability are similar and rely on detailed knowledge of
medical status and history of the donor. Infectious adventi-
tious agents are of primary concern in terms of safety. For nonhuman stem cells, Public Health Service (PHS) and
FDA guidance documents put forth extensive recommendations for animal husbandry, health surveillance, disease
screening, and veterinary care of donor animals. For human
sources the FDA advises medical history screening and
infectious agent testing and may recommend genetic screen-
ing or testing depending on patient populations, donor
pool, and evolution of genetic testing technology. Details
of donor suitability and screening are described in more
detail in the subsection on donor screening in the section
on manufacturing and characterization issues for stem cell
products.

Other important factors include genetic status of donor
cells, especially those that may cause adverse events in recipients. This consideration is often of concern for human donors
but could be important for both sources. Elimination of unde-
sirable genetic traits in donor stem cells will help ensure
that the recipient does not develop a donor-derived medical
condition.

CURRENT GOOD MANUFACTURING PRACTICE
The principles of cGMP, set forth in 21 CFR 210 and 211,
apply to stem cell products and encompass both the manu-
facturing facility and the specific product prepared within the
facility. Although full implementation of cGMP is expected by
licensure, incorporation of cGMPs at earlier stages of
product development is expected but can be staged in a
manner consistent with the phase of product development.
For example, cGMP includes appropriate written protocols
for each stage of product manufacturing and characterization.
At later stages of product development, standard operating procedures (SOPs) that document all significant information
relating to stem cell production should be used. Quality over-
sight by the sponsor is important for each stage of develop-
ment and involves both quality control (QC) and quality
assurance (QA) mechanisms. This means that the person(s)
responsible for assurance that the production and characteri-
zation testing have all been performed properly and have met
specified criteria (QA) are separate from and not direct subor-
dinates of the person(s) responsible for conducting these tests
and filing these reports (QC).

The cGMPs also stipulate development of validated assays
that must be in place by product licensure. Data regarding
assay performance (specificity, sensitivity, and reliability)
should be submitted to the FDA as part of the validation
process.

Manufacturing and Characterization
Issues for Stem Cell Products
This section describes in detail, the various recommendations
currently used by FDA reviewers to ensure that safe, pure, and
potent cell products are administered to patients in clinical
investigations. Figure 73–2 illustrates some of the complexities
of stem cell biology and summarizes some of the challenges
associated with development of stem cell therapies.

COMPONENTS AND CHARACTERIZATION
Although the goal of stem cell manufacturing is to produce a
safe, pure, and efficacious product, the complexity of the
process necessitates carefully controlling all the manufacturing
procedure and of the components used. Thus, all components
used to manufacture the cellular product should be carefully
described. This includes the source of each component and
a summary of testing performed on each component.

Donor Screening
Donor screening is an important consideration in ensuring
safety of stem cell products. The description of stem cell
sources should include tissue of origin and type of cell, such
as hematopoietic, neuronal, or embryonic stem cells and such
details as whether or not donor cells are mobilized or activated
in vivo in the donor. The collection method and use of any
devices during collection are also important details.

Appropriate infectious disease screening procedures must
be performed. The FDA has issued several draft guidances or
proposed rules for industry on donor screening that should be
consulted. If autologous cells are used, screening is relevant
to the tissue culture methods used during the manufacture of

Figure 73–2. A hypothetical cellular differentiation pathway is used to
illustrate the complexity of regulatory oversight of stem cell products.
Mechanisms that control biologic processes such as self-renewal or lineage
commitment include changes in gene and protein expression that lead to
differentiation (steps in development, blue text) and interactions with the host
through microenvironmental cell–cell interactions or growth factors (influences
on development, green text). Regulatory concerns that are associated with
these processes during in vitro stem cell manufacture are shown (black text).
(See CD-ROM for color version of this figure.)
the product could propagate or spread viruses or other adventitious agents (e.g., human immunodeficiency virus-1 [HIV-1], cytomegalovirus [CMV]). For allogeneic stem cell products, there are many specific agents of concern and tests are designed for specific and nonspecific pathogen detection. Donor screening and testing should be performed for adventitious agents, such as HIV-1, HIV-2, hepatitis B virus (HBV) (surface and core antigen), hepatitis C virus (HCV), human T-lymphotropic virus types 1 and 2 (HTLV-1, HTLV-2), CMV, Epstein-Barr virus (EBV), and others, as appropriate. FDA-licensed or FDA-approved test kits should be used in these detection assays, when available.

In addition to infectious agent screening, an IND application should include a description of other serologic, diagnostic, and clinical history data obtained from the donor. It may be important to conduct other characterizations such as typing for genetic polymorphisms and major histocompatibility complex (MHC) loci. If cord blood or other maternally derived tissue is used but not banked, one should document testing performed on donor mothers.

For embryonic stem cell sources, the donor screening could include the following: (1) screening of egg/sperm donors for infectious diseases (HIV-1 and HIV-2, HTLV-1 and HTLV-2, HBV, HCV, CMV, and other agents of concern); (2) evaluation of egg/sperm donors’ medical histories; and (3) genetic testing for selected, relevant disorders. In addition, archiving of donor blood or tissue samples could be important for later testing if adverse events are later associated with a stem cell therapy.

**Derivation of Stem Cell Lines**

The origin of the stem cells including the source tissue, collection methods, and methods of propagation are all important details that should be reported to the FDA. If stem cell products are derived from nonhuman sources, they fall under the definition of xenotransplantation products. The draft guidelines on “Source Animal, Product, Preclinical, and Clinical Issues Concerning the Use of Xenotransplantation Products in Humans” and the “PHS Guideline on Infectious Disease Issues in Xenotransplantation” describe additional steps that may be necessary to address the infectious disease risks that may be posed by the use of xenotransplantation products. Animal husbandry, herd health surveillance, adventitious agent testing of stem cell products, and archiving of animal and patient materials are all important issues associated with use of xenotransplantation products.

For embryonic stem cell lines, procedures used to acquire donor eggs and sperm, to perform in vitro fertilization, and to isolate cleavage-stage embryos should be described in detail. Details regarding origin and characterization of feeder layers should be included and cover species of origin, whether feeders are primary cultures or established cell lines, maintenance of the feeder layers, stability of feeder lines (if established lines), details regarding any cell banks, and passage numbers of cells in the banks and in production. If murine feeders are used, details should include the mouse strain, a description of animal husbandry and housing, mouse colony health status, tissue of origin, and the method of feeder harvest and propagation should be provided.

A description of the assessments used to characterize stem cell lines should be provided and could include but is not restricted to the following: (1) a demonstration of pluripotency, (2) karyotype and chromosomal analysis, (3) growth and proliferation characteristics, (4) expression of molecular markers indicative of undifferentiated stem cells, and (5) stability of cell lines over time in tissue culture and following extended periods of cryopreservation. Chapter 77 of Volume 1 gives greater details regarding important information for FDA evaluation of embryonic stem cell products.

If human feeder cells are used during propagation of any stem cell product, donor screening and adventitious agent testing of the feeder layers are also important to ensure the safety of the cell lines grown in contact with the feeders.

If cell lines are used as feeders for propagation of stem cells, the sections on cell banks (discussed later) are applicable to both the stem cells and the feeder cell lines.

**Cell Bank System**

For some types of stem cells and feeder cell lines, cell banks will be established to ensure that a well-characterized, safe source for manufacturing is available for as long as the product is intended to be available. In these cases, there should be detailed information relating to the cell bank system used in product manufacture, such as history, source, derivation, characterization, and frequency of testing for each master cell bank (MCB) and working cell bank (WCB), if used. Several guidance documents regarding establishment and characterization of cell banks are available and should be consulted.

For some stem cell products cell banks may not be established and not all of the testing described in the following sections may be possible.

**Master Cell Bank.** MCB characterization should include sufficient testing to establish the safety, identity, purity, and stability of the cells. MCB testing should establish microbiologic safety including sterility, freedom from mycoplasma, freedom from the presence of specific pathogens, and in vivo and in vitro testing for adventitious viral agents as appropriate. Cells of human origin, unless autologous, should be tested for human viruses such as CMV, HIV-1 and HIV-2, HTLV-1 and HTLV-2, EBV, HBV, and HCV, as appropriate. Cell lines that are exposed to bovine or porcine components (e.g., serum, serum components, trypsin) should be tested (9CFR113.47). In vitro and in vivo adventitious agent tests are nonspecific screens designed to detect the presence of a wide spectrum of viruses that could be introduced during manufacturing or were present in the starting stem cell population. The in vitro adventitious virus assay can sometimes be used to identify certain viruses. The in vivo and in vitro virus tests detect complimentary virus types (Table 73–1).

Identity is an important characteristic of the MCB and should include tests to unambiguously distinguish the specified cells through physical or chemical characteristics of the cell line
such as cell surface marker phenotype, genotype, or other markers. Purity of bank cells should also be established and include identification and quantification of any contaminating cells.

Finally, activity of cells should be characterized by some criteria related to the proposed use of the stem cells. For example, committed stem cells in a given lineage should be demonstrated to express lineage and developmental stage specific genes or generate appropriate progeny in in vitro assays.

For a licensed product or one in phase 3 trials, lot release testing of final product requires a demonstration of potency. Such testing should be performed throughout manufacturing, including manufacture of cell banks, to evaluate the manufacturing process itself and to ensure the quality and consistency of the product lots. Control of the manufacturing process and the final products, including testing for adventitious viral agents and assessments of other product characteristics such as identity, purity (including endotoxin), viability, and potency. Such testing should be performed throughout manufacturing, including manufacture of cell banks, to evaluate the manufacturing process itself and to ensure the quality and consistency of the product lots. Control of the manufacturing process is crucial to consistently produce an equivalent product from lot to lot. Consistency is needed to identify the critical parameters necessary to ensure the desired clinical effect.

Product testing is designed to determine how well the product meets the specifications used for intermediate and final product release criteria. Specifications are the quality standards such as tests, analytical procedures, and acceptance.
criteria, which confirm the quality of products and other materials used in the production of a product. Acceptance criteria mean numerical limits, ranges, or other criteria for the tests described. The proposed specifications should be appropriate to the stage of product development keeping in mind that release criteria should be refined and tightened as product development progresses toward licensure.

Release tests and specifications for stem cell products should include, but are not limited to, microbiologic testing, identity, purity, potency, viability, and cell number. Criteria for each is discussed in the following sections.

Microbiologic Testing
Microbiologic testing should be performed on stem cell banks, feeder cells, in-process cultures, and the final product, as appropriate. Sterility testing on the final product should be performed as described in 21 CFR 610.12 or as described in United States Pharmacopoeia (USP) <71> Sterility Testing (under 21 CFR 610.9). Alternative test methods can be proposed and, if found to be adequate, may be used but must be validated to be equivalent to the prescribed testing before product licensing.

If antibiotics are used in product manufacturing, they should be removed before sterility testing. If the antibiotics cannot be removed, the bacteriostasis and fungistasis testing as described in USP <71> Sterility Tests may be necessary to ensure that any residual antibiotic present in the product does not interfere with the results of sterility testing.

Results of sterility testing are part of required final product specifications. If the final product is frozen before use, sterility testing should be performed immediately before cryopreservation and acceptable results should be confirmed before administration. If a stem cell product undergoes further manufacturing after thawing (e.g., as washing, culturing, combination with a device), further sterility testing may be necessary. In certain circumstances, stem cell products can be administered to patients before completion of 14-day sterility tests. If cells must be administered before obtaining the results from 14-day sterility testing, sterility testing should be initiated on a sample taken 48 to 72 hours before final harvest or after the last refeeding of the cultures and the sterility test cultures should be negative before release of the product. This test should be continued for the full 14 days even after the product has been given to the patients. Also, a Gram stain should be performed and be negative before administration and a sterility test on the final formulated product should be initiated and continued for the entire 14 days. In these cases, sterility lot release criteria should be a no-growth result from the 48- to 72-hour sterility test and a negative Gram stain. In addition, contingency procedures should be developed in case the more extended sterility tests show that the product the patient received was contaminated. Contingency procedures should include contacting the treating physician, the patient, the FDA, and the institutional review board (IRB). In addition, plans to determine the type of contamination should be in place.

In-process sterility testing at critical points during manufacturing is encouraged to ensure safety of stem cell products. Routine testing during extended culture periods and after critical points in manufacturing, such as when cells have undergone activation or other modification, adds an additional margin of safety and tests the robustness of the manufacturing process. Appropriate in-process testing is based on the manufacturing scheme and the test method used for in-process sterility testing is at the discretion of the sponsor.

Mycoplasma testing should be performed on the product when there is the best chance of detecting contamination, such as after pooling of cultures but before cell washing. Mycoplasma testing should include both cells and supernatant. Results from culture-based mycoplasma tests are sometimes unavailable before administration of cellular products. In this case the use of polymerase chain reaction (PCR)-based mycoplasma assays is acceptable during the investigational phases of product development. However, before product licensing, the PCR test must be shown equivalent to recommended assays as described in §610.9.

Adventitious virus testing is necessary for stem cell lines and feeder cells, when these are used. In vitro viral testing should be conducted on the stem cell MCB and end of production cells (one-time test), when appropriate. In vivo viral assays should also be conducted on the MCB.

For human stem cell products, there should also be selected species-specific testing for adventitious viruses. Selection of the virus-specific tests is dependent on the origin of the stem cells and any feeder layer cells used. Whenever FDA-approved tests are available, these tests should be used. When human cell lines are used as the therapeutic product, there should be testing for human pathogens. PCR-based test tests for CMV, HIV-1 and HIV-2, HTLV-1 and HTLV-2, EBV, HBV, HCV, and other human viral agents should be included, as appropriate.

For more information on adventitious agent testing, one should refer to reference 10 and ICH guidance Q5A: “Guidance on Viral Safety Evaluation of Biotechnology Products Derived From Cell Lines of Human or Animal Origin.”

Identity
The identity of the stem cell MCB and the final product should be determined by established assays. These assays should be able to distinguish the MCB or final product from cell products being manufactured in the same facility (21 CFR 610.14). If the final product consists of one or more differentiated or undifferentiated cell types, tests should be in place to distinguish between the cell types that might be present. If feeder layers were used to propagate a stem cell product, tests that distinguish the stem cell and feeder cells should be used. Identity testing for the MCB should also include testing to distinguish between multiple cell lines used to produce a single final product. Appropriate tests might include assays for cell surface markers or genetic polymorphisms. Identity acceptance criteria for MCBs and final products should be established and based on measurements of different cell types in a stem cell product. These measures reflect on the reliability, reproducibility, and
robustness of the manufacturing process and may be important measures in terms of safety and efficacy of stem cell products.

Purity

The purity of a stem cell product could be defined as freedom from extraneous material and cells, except that which is unavoidable in the manufacturing process (21 CFR 610.13). In addition to unintended cell types such as feeder cells or undesirable differentiated cells, testing for purity should include assays for endotoxin and for reagents or components used during manufacture, such as cytokines, growth factors, antibodies, and serum. Further information is available in ICH Q3 on “Impurities.”

The ability of stem cells to adopt a variety of cell fates makes determination of purity a challenge. However, unwanted, unintended differentiated cell types could affect the function, efficacy, and safety of a stem cell product. In addition, although a determination of the purity of a stem cell product may not currently be correlated with safety or efficacy outcomes, reproducibility of manufacturing conditions can be assessed by this measurement. Accumulation of this data will allow correlation of clinical outcomes with purity assessments.

Viability

Viability of the final product is an important measurement that impacts safety and efficacy of stem cell products and indicates the robustness of the manufacturing process. Drastically reduced viability of individual lots can be an important warning regarding manufacturing conditions or presence of toxic impurities. Great variability in viability can potentially indicate problems with the manufacturing process. For somatic cellular therapies, the minimum acceptable viability specification of the final product is generally set at 70%. If this level cannot be achieved, data should be generated to justify the lower viability specification by demonstrating that dead cells and cell debris do not affect the safe administration or therapeutic effect of the stem cell product.

Cell Number and Dose

For stem cell products, there should be specifications for the minimum number of viable and functional cells as part of the product testing and lot release. The number of viable cells administered to a patient or in preclinical studies is important in following the safety and efficacy of stem cell products. Determination of a suitable dose in terms of both safety and efficacy is a challenge due to the ability of stem cells to self-renew and to differentiate. However, every attempt should be made to design appropriate preclinical studies to address this challenge. Such studies, as well as any applicable clinical experience, should be used in setting the specifications for cell number in stem cell product doses.

Potency

A major challenge with regard to stem cells is assessing biologic activity or potency. A suitable potency assay should measure relative biologic function of the product and should be useful in establishing a safe and efficacious dose for administration to patients. However, determination of the specific biologic functions of a stem cell that predict its useful and safe cell fate is a challenging necessity. Depending on the specific stem cell product, a variety of possible approaches can be envisioned. The FDA does not have specific requirements for particular potency assessments and will consider the merits of individual proposals in the context of overall risk–benefit considerations depending on the disease indication and the patient population. Potency can be established using several assays, which should include a quantitative assay but may also include qualitative biologic assays. In terms of product development, potency assays should be in place by the end of IND phase 2 and should consists of in vivo or in vitro tests that measure an appropriate biologic activity. This assay should be validated by licensure.

For embryonic stem cells, assays that display desirable activities could include demonstration of in vitro differentiation such as demonstration of pluripotency by ability to generate the three different embryonic tissues. For stem cells with a more restricted lineage potential, in vitro differentiation into known progeny lineages such as lymphoid and myeloid differentiation from HSCs or adipogenesis, chondrogenesis, and osteogenesis from mesenchymal stem cells are significant ways to demonstrate maintenance of desirable biologic activity. Other approaches to indicate maintenance of biologic activity could include expression of cell surface markers, proteins, or gene expression profiles using reverse transcriptase (RT)-PCR, microarrays, or proteomics techniques.

Summary

Stem cells are among the most complex biologics to date. FDA recommendations for stem cell–based product manufacture and characterization incorporate the tremendous experience gained in many cell therapy clinical trials and from experience with the entire field of stem cell research. In advising sponsors about their stem cell development program, the FDA recognizes the need for flexibility in its recommendations and will consider many factors, including the intended target population, the seriousness of the disease under study, and the potential benefits and risks from the investigational product. The agency continually updates and reassesses recommendations for stem cell production and testing based on the growing experience and on feedback from a variety of sources.

It is important to note that the information in this chapter represents guiding principles and general information and should be used in conjunction with consultation from FDA staff. The FDA encourages new investigators to consult with FDA staff before submission of an IND. The formal process for FDA consultation is a pre-IND meeting. Sponsors may request information about the pre-IND and IND process through CBER’s Office of Communication, Training, and Manufacturers Assistance (OCTMA) at 301-827-2000.
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