Adrian P. Gee *Editor*

Cell Therapy

cGMP Facilities and Manufacturing

Second Edition

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Preface

Cellular and gene therapies are now producing very promising results and even potential cures. This has resulted in a dramatic increase in the number of academic institutions and biotechnology companies involved in the field. Many of these are engaged in conducting early phase clinical trials, which necessitates the use of facilities that comply with current Good Manufacturing Practices (cGMP) to prepare the therapeutic products. For those new to these regulations, it can be intimidating knowing how to start to design, build and run a cGMP-compliant facility. The purpose of this book is to update our original publication "Cellular Therapy: cGMP Facilities and Manufacturing" published in 2009. This book grew out of the Production Assistance for Cellular Therapy (PACT) contract program supported by the National Heart Lung and Blood Institute (NHLBI) of the U.S. National Institutes of Health. The second volume is also supported by the NHLBI and marks the termination of the third version of PACT.

In this updated and expanded volume, we provide basic advice to those manufacturing products for early phase clinical trials on the approaches used by a variety of facilities and individuals to comply with the regulations. This information is primarily intended for academic facilities and smaller or start-up biotechnology firms. It covers international governmental regulations for cellular therapies, the design and qualification of new facilities, operational activities, such as cleaning, environmental monitoring, equipment qualification, validation and document generation and management. It also discusses the roles played by professional accreditation organizations, standards and governmental agencies and funding organizations.

Our aim is to provide a repository of information that can be easily accessed and a listing of individuals whom the reader can contact to discuss the topics covered. Since much of the information contained is based upon governmental regulations it is strongly suggested that the reader keep abreast of current requirements whenever implementing any of the suggestions in this volume.

The editor would like to thank all of the authors for their contributions, especially during the time of the COVID-19 pandemic! They were all a joy to work with and simplified my task enormously. I would especially like to thank Laarni Ibenana and Lisa Davis of Emmes for their help in manuscript management and

organization and Lis Welniak of NHLBI for her support of the book. My gratitude is also owed to all my colleagues at the Baylor College of Medicine Center for Cell and Gene Therapy in Houston for their help and encouragement.

As I near retirement, I should like to dedicate this volume to all those with whom I have had the pleasure of working and collaborating over the last 40 years. They have made my time in this area both enjoyable and stimulating. It has been incredible to see the evolution of these new treatments and I shall continue to monitor their progress with fascination.

Houston, TX, USA Adrian P. Gee

Contents

Part I
Regulatory

Regulation of Cellular Therapy in the United States

Nicole Fisher, Laarni Ibenana, Ashraf El Fiky, and Robert Anderson

1 Introduction

In the United States, the Food and Drug Administration (FDA), a federal agency within the Department of Health and Human Services, is responsible for regulatory oversight of cell therapy products. The FDA is organized into centers that report to the FDA Commissioner, each with jurisdiction for products by class. The FDA centers most relevant to the oversight of cell therapies are the Center for Biologics Research and Evaluation (CBER) and the Center for Devices and Radiological Health (CDRH). This chapter discusses the framework under which the FDA regulates human cells, tissues, and cellular and tissue-based products (HCT/Ps) and discusses applicable requirements. 21 CFR 1271, applicable to all HCT/Ps, is discussed in detail, including donor eligibility requirements and current good tissue practices (cGTPs).

2 Regulatory Authority for Oversight of Cellular Therapy Products

The legislative framework under which the FDA operates is shaped by federal laws, including the Food, Drug, and Cosmetic (FD&C) Act of 1938 [1] and the Public Health Service (PHS) Act of 1944 [2], which were enacted by Congress under the authority of the United States Constitution. Both the FD&C Act and the PHS Act, along with other permanent laws in the United States, are codified in the *United States Code* (U.S.C.); the laws promulgating the FD&C Act begin at 21 U.S.C. 301, and the PHS Act starts at 42 U.S.C. Based on the laws established through the

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FD&C Act and the PHS Act, the FDA issues regulations in accordance with the Administrative Procedure Act [3], which permits public input through a "notice and comment rulemaking" process [4]. FDA regulations are found in Title 21 of the Code of Federal Regulations (CFR). Proposed rules and the mechanisms by which the public may submit comments are published daily in the Federal Register. The FDA reviews and assesses each comment received by the public prior to the issuance of the regulation, or final rule, via the Federal Register. The Federal Register notice not only contains the language of the regulation, but it also contains a preamble, which includes relevant background information and the FDA's responses to the comments received from the public. The preamble of a final rule provides insight into the FDA's thinking on regulations. The FDA shares their current interpretation of the regulations through guidance documents. The FDA issues guidance documents in accordance with the Good Guidance Practice regulation, 21 CFR 10.115, which specifies that guidance documents are not legally binding. A searchable list of all available FDA guidance documents is available through the FDA's website [5], and a list of guidance documents applicable to cellular therapies is included at the end of this chapter.

3 Brief History of Cell Therapy Regulations

The FDA first defined somatic cell therapy products in 1993 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 in biological characteristics ex vivo, to be administered to humans and applicable to the prevention, treatment, cure, diagnosis, or mitigation of disease or injuries" [6]. In the same Federal Register notice in 1993, the FDA announced how it would apply the existing statutory framework, applicable at the time to other therapeutic products, to human cell therapy products, i.e., cell therapy products would be regulated as drugs, biologics, and/or medical devices and would be subject to regulations promulgated under both the PHS Act and the FD&C Act. In response to reports that communicable diseases such as HIV and hepatitis were being transmitted via transplanted human tissue, the FDA issued an interim rule [7] to address the immediate need to implement additional oversight of these products to protect public health. The interim rule, which required donor screening and testing of human cellular and tissue-based products, was promulgated under Section 361 of the PHS Act [42 U.S.C. 264], which authorizes the creation and enforcement of regulations deemed necessary to prevent the introduction, transmission, or spread of communicable diseases. In 1997, the final rule was issued as 21 CFR 1270 [8]. The same year, the FDA issued their Proposed Approach to Regulation of Cellular and Tissue-Based Products [9] to provide a unified and comprehensive regulatory approach to the regulation of HCT/Ps, with the goal of protecting public health while minimizing the regulatory burden required for innovative products to reach the market. Under the risk-based approach, lower-risk products meeting certain criteria would

be regulated under Section 361 of the PHS Act and would be required to comply with 21 CFR 1271, but would not require premarket approval. Higher-risk products would be regulated as drugs, devices, and/or biological products under Section 351 of the PHS Act [42 U.S.C. 262] and the FD&C Act. These products would be subject to the applicable regulations in 21 CFR, including Part 1271 and good manufacturing practices (cGMPs) in 21 CFR 210 and 211, and would require premarket approval.

The FDA published three final rules, which comprise 21 CFR 1271, to promulgate the tiered approach to the regulation of HCT/Ps [10]:

- "Human Cells, Tissues, and Cellular and Tissue-Based Products; Establishment Registration and Listing" [11]
- "Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products" [12]
- "Current Good Tissue Practice for Manufacturers of Human Cells, Tissues, and Cellular and Tissue-Based Products; Inspection and Enforcement" [13]

Part 1271 became fully effective on May 25, 2005, and provided the basis for the regulation of all HCT/Ps and is the sole regulation applicable to Section 361 HCT/Ps. The good tissue practice final rule [13] changed the definition of "tissue" in §1270.3(j) to render Part 1270 applicable only to tissue recovered before May 25, 2005; however, in December 2020, the FDA published a proposed rule to revoke Part 1270 because it is unlikely that any tissue recovered prior to May 25, 2005, remains available for implantation [14].

FDA oversight of HCT/Ps continues to evolve with scientific innovation. The expedited development of innovative regenerative medicine therapies, including HCT/Ps, was one area of focus in the 21st Century Cures Act (Cures Act) passed in 2016 [15]. As part of the comprehensive policy framework implemented to support the expedited development of regenerative therapies, the FDA published four guidance documents (included in the list at the end of this chapter). Two of the guidance documents provide further interpretation on the requirements of Part 1271, and one guidance discusses the expedited development of Regenerative Medicine Advanced Therapy (RMAT) products [16]. The Cures Act amended Section 506 of FD&C Act to include RMAT designation for a cell therapy, therapeutic tissue engineering product, and human cell and tissue product, intended to treat, modify, reverse, or cure a serious or life-threatening disease or condition. RMAT designation does not apply to products regulated solely under Section 361 of the PHS [17]. RMAT designation and accelerated approval pathways available for RMAT products are discussed later in this chapter.

4 Definition of HCT/P

HCT/Ps are defined in § 1271.3(d) as "articles containing or consisting of human cells or tissues that are intended for implantation, transplantation, infusion, or transfer into a human recipient." Examples of HCT/Ps listed in § 1271.3(d) include the

bone, ligament, skin, dura mater, heart valve, cornea, hematopoietic stem/progenitor cells derived from peripheral and cord blood, manipulated autologous chondrocytes, epithelial cells on a synthetic matrix, and semen or other reproductive tissue. The following items do not meet the definition of HCT/P:

- Vascularized human organs for transplantation or blood vessels recovered with an organ, as defined in 42 CFR 121.2, which are intended for use in organ transplantation and labeled "For use in organ transplantation only"
- Whole blood or blood components or blood derivative products subject to listing under Parts 607 and 207
- Secreted or extracted human products, such as milk, collagen, and cell factors (except semen)
- Minimally manipulated bone marrow for homologous use and not combined with another article (except for water, crystalloids, or a sterilizing, preserving, or storage agent, if the addition of the agent does not raise new clinical safety concerns with respect to the bone marrow)
- Ancillary products used in the manufacture of HCT/P
- Cells, tissues, and organs derived from animals other than humans
- In vitro diagnostic products

5 Determining Statutory Authority Applicable to an HCT/P

Under the tiered, risk-based approach to the regulation of HCT/Ps, the FDA implemented criteria to determine which HCT/Ps are to be regulated under the authority of Section 361 of the PHS Act. Section 361 HCT/Ps do not require premarket approval and are subject only to the regulations in 21 CFR 1271. Per § 1271.10(a), an HCT/P is regulated only under Section 361 of the PHS Act if it meets all of the following criteria:

1) The HCT/P is minimally manipulated.
2) The HCT/P is intended for homologous use only (as reflected by the labeling, advertising, or other indications of the manufacturer's objective intent).
3) The manufacture of the HCT/P does not involve the combination of the cells or tissues with another article, except for water, crystalloids, or a sterilizing, preserving, or storage agent (provided that the addition of water, crystalloids, or the sterilizing, preserving, or storage agent does not raise new clinical safety concerns with respect to the HCT/P).
4) Either:
    a) The HCT/P does not have a systemic effect and is not dependent upon the metabolic activity of living cells for its primary function.
    b) The HCT/P has a systemic effect or is dependent upon the metabolic activity of living cells for its primary function and:
        i) Is for autologous use

ii) Is for allogeneic use in a first-degree or second-degree blood relative; or
iii) Is for reproductive use

Figure 1 illustrates the application of the criteria in § 1271.10(a) to determine if an HCT/P is a 361 product.

The guidance document "Regulatory Considerations for Human Cells, Tissues, and Cellular and Tissue-Based Products: Minimal Manipulation and Homologous Use" [18] provides insight and examples regarding the FDA's interpretation of the definitions "minimal manipulation" and "homologous use." Key points from the guidance document are described below.

5.1 Minimal Manipulation

Minimal manipulation is defined in § 1271.3(f) as follows:

- For structural tissue, processing that does not alter the original relevant characteristics of the tissue relating to the tissues utility for reconstruction, repair, or replacement
- For cells or nonstructural tissues, processing that does not alter the relevant biological characteristics of cells or tissues

Therefore, in order to apply the definition, one must first determine if the HCT/P in question is structural tissue or cells/nonstructural tissue, based on the characteristics in the donor (i.e., before any recovery or processing steps). The guidance describes structural HCT/Ps as "those that physically support or serve as a barrier or conduit, or connect, cover, or cushion" and provides the following examples: the bone, skin, amniotic membrane and umbilical cord, blood vessel, adipose tissue, articular cartilage, nonarticular cartilage, and tendon or ligament. Cells or nonstructural tissue that have "metabolic or other biochemical roles in the body such as hematopoietic, immune, and endocrine functions" are considered cells/nonstructural HCT/Ps; examples provided in the guidance include reproductive cells/tissues, cord blood, lymph nodes/thymus, parathyroid glands, peripheral nerve, and pancreatic tissue.

Additional terms included in the definition of minimal manipulation, such as "original relevant characteristics" and "relevant biological characteristics," also need to be considered when applying the criteria. For structural tissue, "relevant" tissue characteristics are those traits that contribute significantly to the tissue's ability to reconstruct, repair, or replace, and "original" applies if the trait was present in the donor. The guidance cites the following examples of the relevant characteristics of structural tissues: strength, flexibility, cushioning, covering, compressibility, and response to friction and shear. The meaning of "relevant biological characteristics" for cells/nonstructural tissue is similar to "relevant" for structural tissue in that it refers to traits in the donor that play a role in the cell or tissue's function (e.g., differentiation, proliferation potential, and metabolic activity).

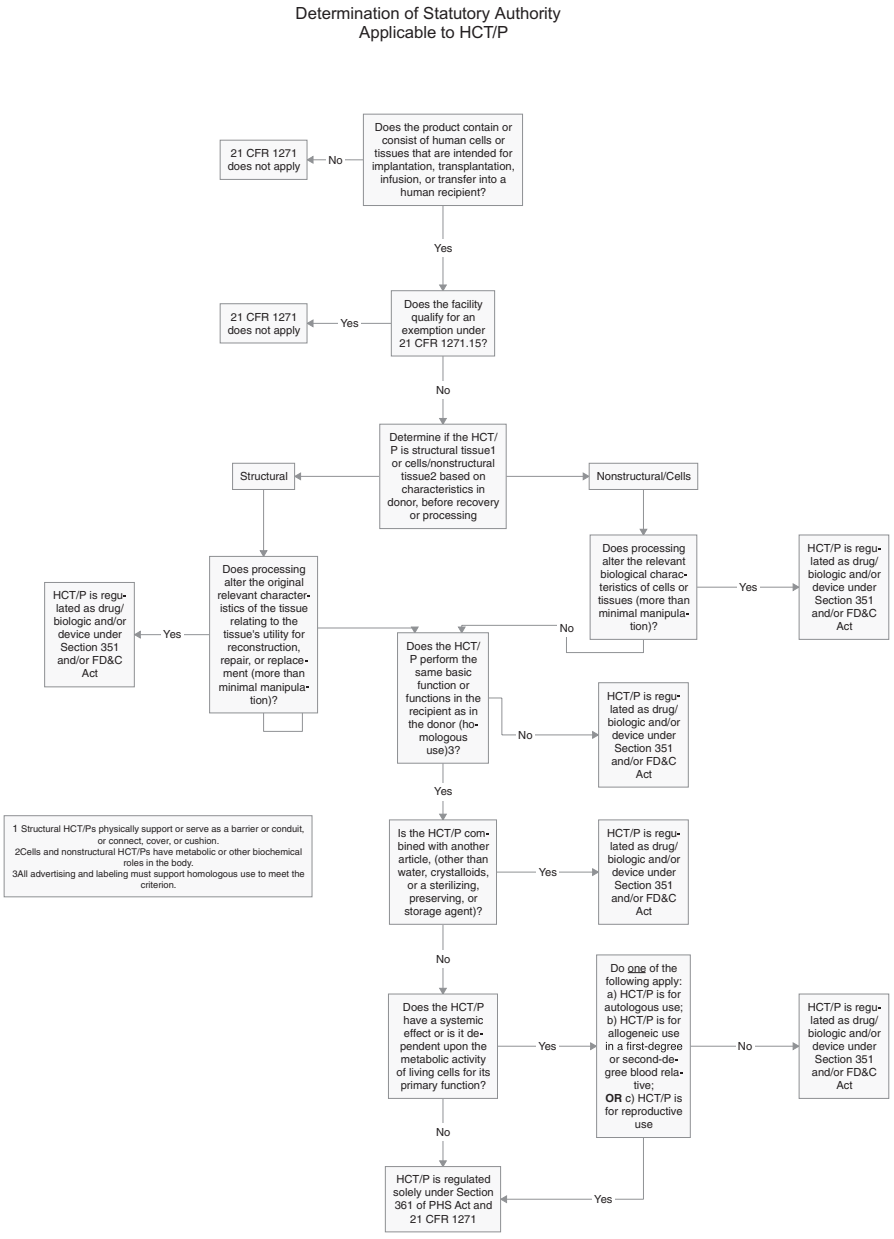

Fig. 1 Flowchart depicting application of criteria in 21 CFR 1271.10(a) to determine statutory authority applicable to an HCT/P

5.2 Homologous Use

Homologous is defined in § 1271.3(c) as "repair, reconstruction, replacement, or supplementation of a recipient's cells or tissues with an HCT/P that performs the same basic function or functions in the recipient as in the donor." The recipient cells/tissue do not need to be identical to the donor cells/tissue to meet the definition. The guidance further defines "repair," "reconstruction," "replacement," and "supplementation." To meet the "same basic function or function" part of the definition of homologous use, any of the basic functions (i.e., those that are well understood and commonly ascribed to the HCT/P) expected in the recipient must be a basic function in the donor. An HCT/P may still meet the definition of the "same basic function" even if it is used in a different location in the recipient.

Another key component of the criteria related to homologous use is the manufacturer's intent for use, as reflected by the labeling, advertising, and other circumstances surrounding the distribution of the product, including written or oral statements by the manufacturer or its representatives. If any of these refer to nonhomologous uses, the HCT/P would not meet the criterion.

If the HCT/P does not meet the criteria in § 1271.10(a), and the establishment that manufactures the HCT/P does not qualify for any of the below exceptions in § 1271.15, the HCT/P will be regulated as a drug, device, and/or biological product under the FD&C Act and/or Section 351 of the PHS Act. These products, discussed in more detail later in this chapter, are subject to 21 CFR 1271, additional regulations in 21 CFR specific to drugs, biological products, or medical devices, and are subject to premarket review [10].

Per § 1271.15, the following entities are exempt from the requirements in Part 1271:

- Establishments that use HCT/Ps solely for nonclinical scientific or educational purposes.
- Establishments that remove HCT/Ps from an individual and implant the HCT/P into the same individual during the same surgical procedure.
  - For additional information regarding this exemption, refer to the Guidance Document "Same Surgical Procedure Exception under 21 CFR 1271.15(b): Questions and Answers Regarding the Scope of the Exception" [19].
- Carriers that accept, receive, carry, or deliver HCT/Ps as part of their usual business as a carrier.
- Establishments that do not recover, screen, test, process, label, package, or distribute, but only receive or store HCT/P's solely for implantation, transplantation, infusion, or transfer within the same facility.
- Establishments that only recover reproductive cells or tissue for immediate transfer into a sexually intimate partner of the donor.
- An individual that only recovers HCT/Ps under contract with a registered establishment and sends recovered HCT/Ps directly to the registered establishment.
  - Note: the individual is exempt from registration and listing; however, regulations pertaining to the manufacturing step(s) performed are still applicable.

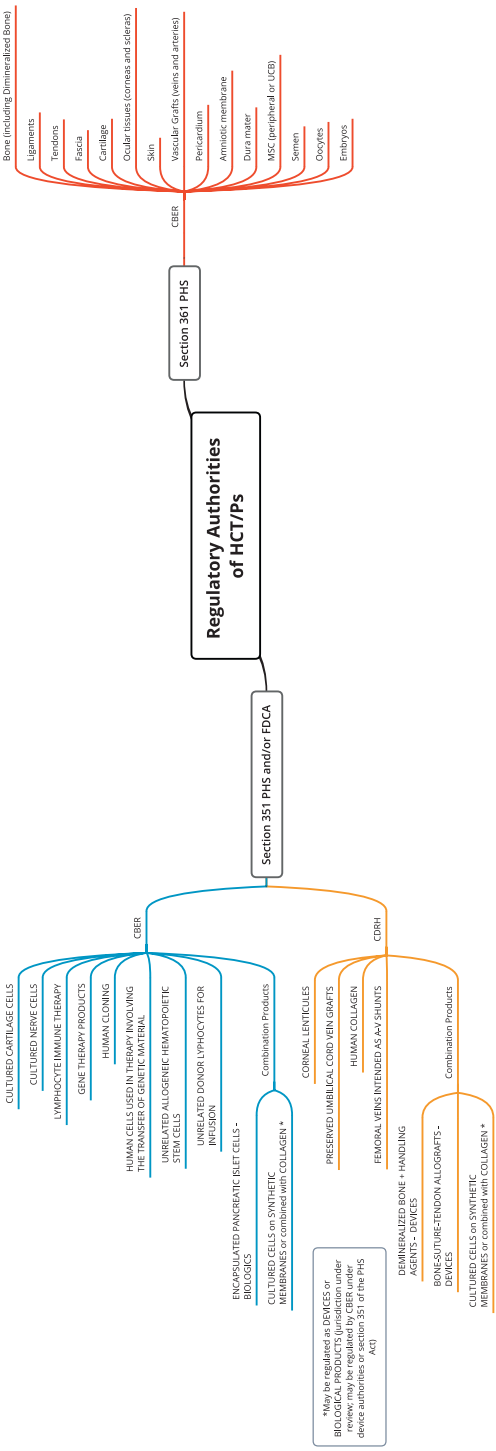

Fig. 2 List of product types, separated by FDA center responsible for review, considered Section 361 HCT/Ps or HCT/Ps regulated under Section 351 and/or the FD&C Act

Figure 2 illustrates the types of HCT/Ps that may be regulated under Section 361 (if all criteria are met) and those that the FDA considers to be regulated as drugs, biologics, and/or devices [20]. Section 361 HCT/Ps are regulated under CBER. As discussed in more detail in the section "Requirements for HCT/Ps Regulated as Drugs, Biologics, and/or Medical Devices," HCT/Ps regulated as drugs, biologics, and/or devices are regulated under CBER or CDRH. Combination products (i.e., those with multiple constituent parts that are drugs, biologics, and/or devices) are assigned to an FDA center based on their primary mode of action [21]. HCT/P combination products are typically assigned to either CBER or CDRH. The FDA has several mechanisms to assist with classification and/or center assignment, including the pre-RFD (informal) and RFD (formal) processes, discussed below.

6 Classification and Jurisdiction Assistance

The FDA offers several mechanisms by which a manufacturer of an HCT/P may obtain a recommendation or decision regarding classification and/or jurisdiction.

1) The TRG Rapid Inquiry Program (TRIP) is a temporary program operating as part of the FDA's Tissue Reference Group (TRG). The TRG was created as specified in the 1997 Proposed Approach document and is further described below. When the TRIP was originally announced, it was effective from June 12, 2019, to December 31, 2019. The FDA has announced several extensions of the program and, most recently, announced in July 2020 that the program would be extended through March 31, 2021 [18]. At the time of publication of this chapter, it was not clear if the program will be extended past that date. Through TRIP, manufacturers may obtain a nonbinding assessment regarding the regulation of their specific HCT/P. Requests should be submitted via email for each HCT/P and should contain the information specified on the TRIP website. The FDA aims to respond with their assessment within 1 week [22].
2) The TRG is another mechanism by which a manufacturer may obtain a nonbinding recommendation regarding the application of the criteria in § 1271.10(a) to a specific HCT/P, including which FDA center will have primary jurisdiction. The TRG is composed of three representatives from CBER and three from CDRH, including the product jurisdiction officer at each center. Representatives from the Office of Combination Products (OCP) and from the Office of the Chief Counsel attend the meetings. Submissions to the TRG may be sent via mail, email, or fax and should contain the information specified on the TRG website. The TRG aims to respond within 60 days; however, if the manufacturer does not agree with the agency's recommendation, they may submit a Request for Designation (RFD) or pre-RFD as described below [23, 24].
3) An RFD may be submitted to the OCP to obtain a formal decision regarding the classification of an HCT/P, including which FDA center will have primary jurisdiction. 21 CFR 3.7 contains the information required for an RFD, and addi-

Table 1 Applicability of 21 CFR 1271 to Section 361 HCT/Ps

21 CFR 1271 Subpart	Applicability to 361 HCT/Ps
Subpart A – general provisions	Applicable to all 361 HCT/Ps
Subpart B – procedures for registration and listing	Applicable to all 361 HCT/Ps
Subpart C – donor eligibility	Applicable to all 361 HCT/Ps
Subpart D – current good tissue practice	Applicable only to nonreproductive 361 HCT/Ps
Subpart E – additional requirements for establishments described in 21 CFR 1271.10 (reporting and additional labeling requirements)	Applicable only to nonreproductive 361 HCT/Ps
Subpart F – inspection and enforcement of establishments described in 21 CFR 1271.10	Applicable to all 361 HCT/Ps

tional information on the process can be found in the April 2011 guidance document "How to Write a Request for Designation (RFD)" [25]. The Agency aims to respond within 60 days.

4) A pre-RFD may also be submitted to obtain a nonbinding, informal feedback on the classification of the HCT/P, which FDA center will have primary jurisdiction, and/or for advice regarding the preparation of the RFD. Additional information can be found in the February 2018 guidance document "How to Prepare a Pre-Request for Designation (Pre-RFD)" [26]. The Agency aims to respond within 60 days.

7 HCT/Ps Regulated Solely Under Section 361 of the PHS Act

As described above, if an HCT/P meets all of the criteria included in § 1271.10(a), it is subject only to regulation under Section 361 of the PHS Act. Section 361 HCT/Ps are regulated by the CBER and must comply with the regulations in Part 1271, as shown in Table 1 subparts E and F, which are only applicable to 361 HCT/Ps and are discussed below. Subparts B through D, which also apply to HCT/Ps regulated as drugs, biologics, or devices, are discussed in greater detail later in this chapter under the section "Requirements of 21 CFR 1271 Applicable to All HCT/Ps, Including Registration and Listing, Good Tissue Practices, and Donor Eligibility Determination."

7.1 Reporting [21 CFR Subpart E]

Reporting of adverse reactions and deviations is required for nonreproductive 361 HCT/Ps.

7.1.1 Adverse Reaction Reports [§ 1271.30(a)]

An adverse reaction is defined in § 1271.3(y) as "a noxious and unintended response to any HCT/P for which there is a reasonable possibility that the HCT/P caused the response." Situations in which the HCT/P is one of several possible causes of the issue, or those in which the relationship between the issue and the HCT/P is "unlikely" (yet still possible), would meet the definition of an adverse reaction [27].

Under § 1271.350(a), any adverse reaction involving a communicable disease related to an HCT/P made available for distribution must be investigated. The adverse reaction must be reported to the FDA if it:

- Is fatal
- Is life-threatening
- Results in permanent impairment of a body function or permanent damage to body structure
- Necessitates medical or surgical intervention, including hospitalization
- Includes an action related to the treatment or prevention of communicable disease or infection that is not routinely expected after the administration of an HCT/P [27]

It is important to note that if the adverse reaction does not involve communicable disease transmission (e.g., graft failure), it does not need to be reported to the FDA [27]. Adverse reactions required to be reported to the FDA must be submitted using an FDA Form 3500A within 15 calendar days of initial receipt of the information. The 2016 guidance document "Investigating and Reporting Adverse Reactions Related to Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps) Regulated Solely under Section 361 of the Public Health Service Act and 21 CFR Part 1271" [28] contains detailed recommendations for investigating and reporting complaints of adverse reactions and includes instructions on how to complete the Form FDA 3500A for these products.

7.1.2 Reports of HCT/P Deviations [§ 1271.30(b)]

An HCT/P deviation is defined in § 1271.3(dd) as "an event (1) that represents a deviation from applicable regulations in this part or from applicable standards or established specifications that relate to the prevention of communicable disease transmission or HCT/P contamination or (2) that is an unexpected or unforeseeable event that may relate to the transmission or potential transmission of a communicable disease or may lead to HCT/P contamination."

Under § 1271.350(b), all HCT/P deviations related to a distributed HCT/P must be investigated, and deviations related to a core cGTP requirement ([§ 1271.150(b)], described below) must be reported to the FDA within 45 days of discovery using Form FDA 3486. The report should contain a description of the HCT/P deviation; information relevant to the event and the manufacture of the HCT/P involved; and information on all follow-up actions that have been or will be taken in response to

the HCT/P deviation (e.g., recalls). Refer to the 2017 guidance document "Deviation Reporting for Human Cells, Tissues, and Cellular and Tissue-Based Products Regulated Solely Under Section 361 of the Public Health Service Act and 21 CFR Part 1271" [29] for additional information.

7.2 Additional Labeling Requirements [21 CFR Subpart E]

In addition to the labeling requirements in §§ 1271.55, 1271.60, 1271.65, and 1271.90, which pertain to donor eligibility determination and quarantined HCT/Ps, the labeling requirements described in this section apply to nonreproductive 361 HCT/Ps.

Each HCT/P made available for distribution must be labeled clearly and accurately [§ 1271.370(a)] with the following information per § 1271.370(b):

- Distinct identification code affixed to the HCT/P container and assigned in accordance with § 1271.290(c) as described under "good tissue practices".
- Description of the type of HCT/P.
- Expiration date, if any.
- Warnings required under §§ 1271.60(d)(2), 1271.65(b)(2), or 1271.90(c), if applicable and physically possible.
  - If there is not sufficient space on the label, the warnings must accompany the HCT/P instead.

The following information must either appear on the HCT/P label or accompany the HCT/P per § 1271.370(c):

- Name and address of the establishment that determines that the HCT/P meets release criteria and makes the HCT/P available for distribution
- Storage temperature
- Other warnings, where appropriate
- Instructions for use when related to the prevention of the introduction, transmission, or spread of communicable diseases

7.3 FDA Inspections and Enforcement Actions [21 CFR Subpart F]

The FDA will conduct inspections under 21 CFR 1271.400(a) in order to determine compliance with the applicable requirements of Part 1271, which may include, but is not limited to, an assessment of the establishment's facilities, equipment, finished and unfinished materials, containers, processes, HCT/Ps, procedures, labeling, records, files, papers, and controls required to be maintained under Part 1271. Inspections may be conducted with or without prior notice but typically occur

during normal business hours, and the frequency of inspection is at the agency's discretion [10].

Based on the inspection or other available information, the FDA may take enforcement action(s) to prevent the introduction, transmission, or spread of communicable diseases. Possible advisory, administrative, and judicial actions for Section 361 HCT/Ps include untitled letters, warning letters, orders of retention/recall/destruction or order of cessation of manufacturing, or prosecution [20].

7.3.1 Enforcement Discretion

As described above, if an HCT/P does not meet the criteria § 1271.10(a), the product is considered a drug, device, and/or biological product under the FD&C Act and/or Section 351 of the PHS Act and is subject to premarket approval, in addition to the requirements discussed later in this chapter. To allow manufacturers time to determine if they need to submit an Investigational New Drug (IND) application or a marketing application and to prepare the application, the FDA announced its plan to exercise enforcement discretion in the 2017 guidance document "Regulatory Considerations for Human Cells, Tissues, and Cellular and Tissue-Based Products: Minimal Manipulation and Homologous Use" [18]. In the 2017 guidance, the FDA stated that over a period of the subsequent 36 months, ending in November 2020, the agency would exercise enforcement discretion provided that the use of the HCT/P was not associated with reported safety concerns or potential significant safety concerns. In July 2020, the FDA updated the guidance to extend the enforcement discretion period through May 31, 2021. It is not clear at the time of publication if the enforcement discretion period will be extended again.

The enforcement discretion focuses on products with high-risk routes of administration, such as intravenous, intraocular, or central nervous system injection/infusion and aerosol inhalation, and those that are intended for nonhomologous use, particularly those intended to be used for the prevention or treatment of serious and/or life-threatening diseases [18]. The FDA has issued over 15 untitled letters and warning letters during the enforcement discretion period [30].

8 Requirements for HCT/Ps Regulated as Drugs, Biologics, and/or Medical Devices

HCT/Ps that do not meet the criteria in § 1271.10(a) for regulation under Section 361 of the PHS Act are regulated as drugs, devices, and/or biological products under the FD&C Act and/or Section 351 of the PHS Act. Section 351 of the PHS Act defines a biological product as a "virus, therapeutic serum, toxin, antitoxin, vaccine, blood, blood component or derivative, allergenic product, or analogous product, … applicable to the prevention, treatment, or cure of a disease or condition of human beings."

HCT/Ps regulated under Section 351 of the PHS Act also meet the definition of drugs and/or devices under the FD&C Act. Section 201(g) of the FD&C Act defines a drug as:

> (A) articles recognized in the official United States Pharmacopoeia, official Homoeopathic Pharmacopoeia of the United States, or official National Formulary, or any supplement to any of them; and (B) articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals; and (C) articles (other than food) intended to affect the structure or any function of the body of man or other animals; and (D) articles intended for use as a component of any articles specified in clause (A), (B), or (C) …

Section 201(h) of the FD&C Act defines a device as "an instrument, apparatus, implement, machine, contrivance, implant, in vitro reagent, or other similar or related article, including any component, part, or accessory, which is (1) recognized in the official National Formulary, or the United States Pharmacopeia, or any supplement to them, (2) intended for use in the diagnosis of disease or other conditions, or in the cure, mitigation, treatment, or prevention of disease, in man or other animals, or (3) intended to affect the structure or any function of the body of man or other animals, and which does not achieve its primary intended purposes through chemical action within or on the body of man or other animals and which is not dependent upon being metabolized for the achievement of its primary intended purposes."

HCT/Ps that meet the definition of biological drug products are regulated at the FDA by CBER under Section 351 of the PHS Act and the FD&C Act. These HCT/Ps are subject to regulations promulgated under both Acts, including 21 CFR 210 and 211 (cGMPs), 21 CFR Parts 600-680 (the Biological Product Regulations), and 21 CFR 1271 (Registration and Listing, Donor Eligibility, and cGTPs) [31].

HCT/Ps that meet the definition of device are regulated by CDRH under the FD&C Act. As discussed above, products with more than one constituent drug, device, and/or biological part are considered combination products, which are assigned to a lead FDA center based on their primary mode of action [21]. HCT/P combination products are typically assigned to either CBER or CDRH; refer to Fig. 2. Note that this chapter focuses on the requirements for HCT/Ps regulated as biologics. Regulations applicable to devices include, but are not limited to, 21 CFR 801 (Labeling), 21 CFR 807 (Registration and Listing), 21 CFR 807 Subpart F (Premarket Notification), 21 CFR 814 (Premarket Approval), 21 CFR 812 (Investigational Device Exemption), and 21 CFR 820 (Quality System Regulation). The FDA's website [32] should be consulted for comprehensive information regarding the regulation of medical devices.

8.1 Biologic Product Licensing

Biological drug products are required to be licensed under Section 351 of the PHS Act; the licensing provisions are included in 21 CFR 601. Form FDA 356h contains the required information for a Biologics License Application (BLA), which is a

comprehensive data package containing data demonstrating that the product meets prescribed requirements of safety, purity, and potency. Briefly, the BLA includes applicant information, product/manufacturing information, data from preclinical studies, data from clinical studies, and labeling [33]. Refer to the section entitled "Special Considerations for Cord Blood" for information specific to BLAs for cord blood.

Per 21 CFR 601.2(d), the FDA will issue the license if it determines that "the establishment(s) and the product meet applicable requirements to ensure the continued safety, purity, and potency of such products." A product's effectiveness for its intended uses must be demonstrated as part of the statutory requirement for potency [21 CFR 600.3(s)] [6]. The biological drug product must comply with the conditions of licensure in the FDA-approved BLA, along with the Biologics regulations [21 CFR 600-680] to ensure the product is safe, pure, potent, effective, and appropriately labeled [31]. As this chapter does not discuss the Biologics regulations in-depth, the relevant regulations should be referenced for specific requirements. Figure 3 contains a timeline of HCT/P approvals.

8.2 Investigational New Drug Application Regulations

In order to generate the safety and effectiveness data needed for a BLA, the product is studied in human clinical trials under an Investigational New Drug (IND) application in accordance with the regulations in 21 CFR 312.

The list below contains the IND content required per 21 CFR 312.23; however, the IND application is discussed in greater detail in a subsequent chapter.

- Form FDA 1571
- Table of contents
- Introductory statement and general investigational plan
- Investigator's brochure
- Clinical protocol
- Chemistry, manufacturing, and control information
- Pharmacology and toxicology information
- Previous human experience
- Additional information, as relevant

The clinical trial cannot be initiated until the IND is in effect, and an IND must be in effect for the product to be lawfully shipped for use in the clinical trial(s). An IND goes into effect 30 days after it is received by the FDA (unless FDA notifies the sponsor otherwise) provided the sponsor also complies with the requirements in 21 CFR 50 (Protection of Human Subjects) and 21 CFR 56 (Institutional Review Boards). Per 21 CFR 312.42, the FDA may place an IND on clinical hold at any time. A clinical hold is issued to delay the start of a proposed clinical study or suspend conduct of an ongoing study. When an ongoing study is placed on clinical hold, no new subjects may be recruited to the study and placed on the

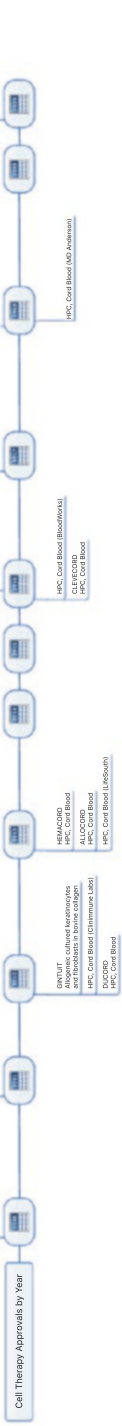

Fig. 3 Approval timeline of licensed HCT/Ps

investigational drug; patients already in the study should be taken off therapy involving the investigational drug unless specifically permitted by the FDA in the interest of patient safety. The grounds for a clinical hold are specified in 21 CFR 312.42(b) and include:

- Human subjects are or would be exposed to an unreasonable and significant risk of illness or injury.
- The clinical investigators named in the IND are not qualified by reason of their scientific training and experience to conduct the investigation described in the IND.
- The investigator brochure is misleading, erroneous, or materially incomplete.
- The IND does not contain sufficient information required under § 312.23 to assess the risks to the subjects of the proposed studies.
- The IND is for the study of an investigational drug intended to treat a life-threatening disease or condition that affects both genders and men or women with reproductive potential who have the disease or condition being studied are excluded from eligibility.
- The (Phase 2 or 3) plan or protocol for the investigation is clearly deficient in design to meet its stated objectives.

Typically, the basis for hold is related to patient safety risks or the IND does not contain sufficient information. A few examples of scenarios in which a clinical hold may be issued include a product with a potentially hazardous impurity profile, inadequate preclinical animal data to support the proposed clinical trial, or inadequate safety assessments during the clinical trial [34]. Imposition of the study hold may be initially communicated via telephone or other rapid method by the end of the 30-day review. A written explanation of the clinical hold issues will be sent to the sponsor within 30 days of the notification of clinical hold. The sponsor must submit a complete response to all deficiencies; refer to the guidance document "Submitting and Reviewing Complete Responses to Clinical Holds" [35]. The FDA will review the responses within 30 days of receipt; however, the investigation may not resume until notification is received from the FDA [34].

Additional requirements including investigational product labeling and responsibilities of sponsors and investigators are outlined in 21 CFR 312. Refer to the section entitled "Special Considerations for Cord Blood" for information specific to INDs for cord blood.

8.3 Current Good Manufacturing Practices

Because biologic products are a subset of drugs, they are also subject to cGMPs as described in 21 CFR 210 and 21 CFR 211. The cGMPs govern the methods to be used in, and the facilities or controls to be used for, the manufacture, processing, packing, or holding of a drug to ensure that it meets the requirements for safety, identity, strength, quality, and purity [21 CFR 210.1(a)]. 21 CFR 210.1(b) states that

failure to comply with the requirements in Part 211 means that the product is considered adulterated under Section 501(a)(2)(B) of the FD&C Act and subject to regulatory action.

Part 211 contains the following subparts:

- Subpart A – general provisions
- Subpart B – organization and personnel
- Subpart C – buildings and facilities
- Subpart D – equipment
- Subpart E – control of components and drug product containers and closures
- Subpart F – production and process controls
- Subpart G – packaging and labeling control
- Subpart H – holding and distribution
- Subpart I – laboratory controls
- Subpart J – records and reports
- Subpart K – returned and salvaged products

HCT/Ps must also comply with cGTPs in 21 CFR 1271, Subparts C and D. According to § 1271.150(d), if Part 1271 conflicts with a requirement in Part 210 or 211, the regulations more specifically applicable to the product in question (i.e., 21 CFR 1271) will supersede the more general [13].

There is significant overlap between the cGTPs and cGMPs, and, in many cases, both sets of requirements require the same manufacturing practice; however, several requirements of the cGTPs would not be included in routine cGMP practice (e.g., predistribution shipment, audits, prohibition on pooling, tracking, etc.). In addition, there are cases in which a corresponding cGMP requirement partially covers a cGTP requirement (e.g., the quality program requirement in § 1271.160) [27]. The more specific regulations (i.e., cGTPs) are discussed in this chapter; refer to "Requirements of 21 CFR 1271 Applicable to All HCT/Ps, Including Registration and Listing, Good Tissue Practices, and Donor Eligibility Determination." However, it is important to note that HCT/Ps regulated as biological drug products must also comply with cGMPs in Parts 210 and 211.

8.4 Donor Eligibility and Good Tissue Practices

Under 21 CFR 210.1(c), HCT/Ps regulated as biological drug products are subject to the donor-eligibility and applicable cGTPs, both of which are described below under "Requirements of 21 CFR 1271 Applicable to All HCT/Ps, Including Registration and Listing, Good Tissue Practices, and Donor Eligibility Determination."

8.5 Registration and Listing

Registration and listing requirements for HCT/Ps regulated as biological drug products are discussed below under "Requirements of 21 CFR 1271 Applicable to All HCT/Ps, Including Registration and Listing, Good Tissue Practices, and Donor Eligibility Determination."

8.6 Enforcement Actions

HCT/Ps regulated as biological drug products are subject to inspection and enforcement actions under both the PHS and FD&C Acts. CBER's Compliance Program Guidance Manual 7345.848 [31], summarized in this section, describes FDA's approach to conducting inspections of establishments involved in the manufacture of HCT/Ps regulated as biological products.

To ensure compliance with applicable regulations and conditions in the FDA-approved BLA, a cGMP-focused inspection is conducted at least biennially (or more frequently, if determined to be necessary). Prelicense inspections are performed for new biological products seeking a license, and pre-approval inspections are performed as part the approval process for significant changes to a biologics license application. As part of their risk-based approach to inspection, the FDA has identified three critical elements (standard operating procedures, training, and records) and seven key systems (quality, facilities/equipment, materials, production, packaging/labeling, laboratory control, donor eligibility). A Level I inspection covers the three critical elements and at least four of the key systems, including the quality system and production system. A Level II inspection covers the three critical elements, the quality system, and one additional key system on a rotating basis. Level I inspections are conducted for the initial inspection of an establishment; establishments under a Consent Decree of Permanent Injunction or under a Notice of Intent to Revoke; for follow-up inspections to verify an establishment's implementation of corrective action after regulatory action has been taken; establishments that have implemented significant changes since the prior inspection; or establishments whose previous two inspections were Level II. Level II inspections are conducted for establishments with a satisfactory history of compliance; establishments for which one of the two previous biennial inspections was Level I; or when inspection preparation procedures did not reveal significant safety or quality trends. Possible enforcement actions include untitled letters, warning letters, license revocation or suspension, seizure, injunction, and prosecution [31].

9 Requirements of 21 CFR 1271 Applicable to All HCT/Ps, Including Registration and Listing, Good Tissue Practices, and Donor Eligibility Determination

9.1 HCT/P Establishment Registration and Listing

All establishments that manufacture HCT/Ps regulated under Section 361 or as biological drug products must register and list with CBER, unless exempt per § 1271.15. As described in 21 CFR 1271 Subpart B, new establishments must register and list their HCT/Ps within 5 days after beginning operations, using the electronic HCT/P establishment registration system (eHCTERS). Registration must be updated annually, and listing changes must be submitted within 6 months of making the change. Establishments may request a waiver from the electronic submission requirement as described in § 1271.23. Additional information, including how to use and access the eHCTERS, can be found on FDA's Tissue Establishment Registration website [36].

Establishments that manufacture biological drug products under an IND [21 CFR Part 312] are not required to register and list their HCT/Ps with CBER until the products are approved. Once the investigational HCT/P is approved through a BLA, the establishment must register and list under 21 CFR 207, rather than 21 CFR 1271 [10].

9.2 Good Tissue Practices

The cGTPs are requirements in Subparts C and D of Part 1271 that "govern the methods used in, and the facilities and controls used for, the manufacture of HCT/Ps, including but not limited to all steps in recovery, donor screening, donor testing, processing, storage, labeling, packaging, and distribution" [§ 1271.150(a)]. These requirements apply to all HCT/Ps, whether regulated solely as Section 361 HCT/Ps or as biological drug products.

The following core cGTPs are referenced in § 1271.150(b):

- Donor requirements relating to:
  - Donor eligibility determinations [§ 1271.50]
  - Donor screening [§ 1271.75]
  - Donor testing [§§ 1271.80 and 1271.85]
- Facilities requirements [§ 1271.190(a) and (b)]
- Environmental control requirements [§ 1271.195(a)]
- Equipment requirements [§ 1271.200(a)]
- Supply and reagent requirements [§ 1271.210(a) and (b)]
- Recovery requirements [§ 1271.215]

- Processing and process control requirements [§ 1271.220]
- Labeling control requirements [§ 1271.250(a) and (b)]
- Storage requirements [§ 1271.260 (a) through (d)]
- Receipt, predistribution shipment, and distribution requirements [§ 1271.265 (a) through (d)]

In addition to the core cGTPs, each establishment must comply with the requirements applicable to the operations it performs [§ 1271.150(c)(1)(i)]. If an establishment determines that a particular regulation is not applicable, the associated justification must be documented [§ 1271.150(e)], and exemptions from or alternatives to the requirements may be requested under § 1271.155.

The following sections briefly describe each of the cGTP requirements specified in Part 1271 Subpart D and summarized in the 2011 guidance document "Current Good Tissue Practice (CGTP) and Additional Requirements for Manufacturers of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps)" [27]. Donor eligibility requirements [Part 1271 Subpart C] are discussed in the subsequent section.

9.2.1 Quality Management Program [§ 1271.160]

An establishment that performs any step in the manufacture of HCT/Ps must establish and maintain a quality program that is appropriate for the functions it performs and the HCT/Ps it manufactures. The quality program is responsible for ensuring compliance with the core cGTPs and that appropriate corrective actions are taken, as necessary, to remediate issues. Under the quality program, the establishment must implement procedures for receiving, investigating, evaluating, and documenting information related to the core CGTP requirements, including complaint investigation and appropriate follow-up activities, such as reporting under § 1271.350. Other responsibilities of the quality program include ensuring personnel are appropriately trained to perform their assigned duties, suitable monitoring systems are in place, computer software is verified/validated as appropriate, and that procedures are implemented and maintained per § 1271.180. A quality audit of activities related to the core cGTPs must be performed periodically; however, the FDA recommends performing a quality audit at least annually.

9.2.2 Personnel [§ 1271.170]

The establishment must have sufficient personnel with the necessary education, experience, and training to perform their assigned responsibilities. Personnel must perform only those activities they are trained and/or qualified to perform, and retraining should be performed as needed.

9.2.3 Procedures [§ 1271.180]

Procedures for all core cGTP requirements must be established and maintained. The procedures should be designed to prevent circumstances that increase the risk of the introduction, transmission, or spread of communicable diseases and must be readily available to personnel performing the associated operations. The procedures must be reviewed and approved before implementation and should be re-reviewed periodically to ensure continued compliance.

9.2.4 Facilities [§ 1271.190]

Facilities must be suitably designed and maintained to prevent contamination and/or mix-ups. A cleaning program supported by environmental monitoring should be implemented as appropriate, and the facility should use physical segregation or implement other control systems for each operation to prevent improper labeling, mix-ups, and/or contamination. Procedures for cleaning and sanitation, including detailed cleaning methods and schedules, must be implemented, and associated records must be retained for 3 years after their creation [§ 1271.190(d)(2)].

9.2.5 Environmental Control and Monitoring [§ 1271.195]

Environmental conditions that could possibly contribute to contamination of HCT/Ps or equipment must be adequately controlled and monitored. The establishment needs to determine the appropriate level of control for each manufacturing step; however, the following controls or systems should be implemented, as appropriate:

- Temperature and humidity controls
- Ventilation and air filtration
- Cleaning and disinfecting of rooms and equipment to ensure aseptic processing operations
- Maintaining equipment used to control conditions necessary for aseptic processing operations

Each environmental control system, including equipment, must be inspected periodically to ensure proper functioning of the system.

Each facility should also determine what environmental monitoring system is appropriate for each area of operation, including the type and frequency of environmental monitoring, necessary cleaning verification, and appropriate alert/action limits. The 2004 guidance document "Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice" [37] and the 2011 guidance document "Current Good Tissue Practice (CGTP) and Additional Requirements for Manufacturers of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps)" [38] should be consulted during the development of the environmental control and monitoring program.

9.2.6 Equipment [§ 1271.200]

Equipment used in the manufacture of HCT/Ps must be appropriately designed, installed, and maintained to prevent contamination. While not specifically stated in § 1271.200, equipment installation qualification, operation qualification, and performance qualification are recommended. Procedures must be implemented for cleaning and maintaining equipment, and each facility should determine the appropriate cleaning procedures and schedules, taking into consideration any recommendations from the equipment manufacturer.

Routine equipment calibration is required; the facility should determine the appropriate calibration schedule for each piece of equipment, taking into consideration any recommendations from the equipment manufacturer. Records related to cleaning, maintenance, and calibration must be maintained and displayed on or near each piece of equipment or made readily available to associated personnel.

9.2.7 Supplies and Reagents [§ 1271.210]

Any materials used during the manufacture of an HCT/P, including those that do not come into direct contact with the product, are considered supplies and reagents. Supplies and reagents must be verified to meet specifications before being used in manufacturing. Verification that reagents meet specifications may be performed by testing samples or reviewing the Certificate of Analysis (COA) or other documentation such as a specification sheet or manufacturer's package insert. Verification can be performed by the establishment that uses the supply or reagent or by the material vendor. Reagents used in processing and preserving HCT/Ps must be sterile, where appropriate, and any processes used to produce in-house reagents must be validated and/or verified. Records related to supply/reagent receipt, verification, and use should be maintained.

9.2.8 Recovery [§ 1271.215]

Recovery is defined in § 1271.3(ii) as "obtaining from a human donor cells or tissues that are intended for use in human implantation, transplantation, infusion, or transfer." Establishments that recover HCT/Ps must implement controls over procedures, personnel, equipment, and supplies/reagents to ensure their processes do not cause or contribute to contamination. Establishments that are contracted to recover HCT/Ps for a processing facility do not have to register and list with the FDA but are required to meet other requirements in Part 1271 per § 1271.15(f).

9.2.9 Processing and Processing Controls [§ 1271.220]

Processing is defined in § 1271.3(ff) as "any activity performed on an HCT/P, other than recovery, donor screening, donor testing, storage, labeling, packaging, or distribution, such as testing for microorganisms, preparation, sterilization, steps to inactivate or remove adventitious agents, preservation for storage, and removal from storage." Establishments must use aseptic technique and implement appropriate in-process controls and monitoring, such as statistical process control or review of in-process testing results, to ensure that processing steps do not cause or contribute to contamination of the HCT/P. The cGTP guidance [27] contains additional considerations specific to musculoskeletal HCT/Ps, including preprocessing (predisinfection) cultures.

Pooling of HCT/Ps from two or more donors is not acceptable; however, an exemption or alternative can be requested under § 1271.155 if pooling is necessary to obtain the required dose for administration. Although discouraged due to the risk of contamination, cellular products obtained from a single donor may be pooled together provided all other applicable requirements, including tracking and labeling, are met.

9.2.10 Process Changes [§ 1271.225]

Changes to processing steps [§ 1271.220] must be verified or validated in accordance with § 1271.230 and approved by a responsible person prior to implementation.

9.2.11 Process Validation [§ 1271.230]

When the results of processing [§ 1271.220] cannot be fully verified by inspection or testing, the process must be validated according to established procedures. Validation is "confirmation by examination and provision of objective evidence that particular requirements can consistently be fulfilled; process validation is establishing by objective evidence that a process consistently produces a result or HCT/P meeting its predetermined specifications" [§ 1271.3(kk)]. Verification is "confirmation by examination and provision of objective evidence that specified requirements have been fulfilled" [§ 1271.3(ll)]. Validation is performed before a process is used to manufacture HCT/Ps, while verification of a process is performed on all products after a process has been completed. If any written representation is made that processing methods reduce the risk of transmission of communicable disease (e.g., representation of sterility or pathogen inactivation of an HCT/P), the process referenced must be fully verified or validated.

Part 1271 does not include specific requirements for how to conduct a validation; however, guidance documents "Validation of Procedures for Processing of Human Tissues Intended for Transplantation" [38] and "Process Validation: General Principles and Practices" [39] may be referenced for additional information.

9.2.12 Labeling Controls [§ 1271.250]

Label control procedures, including label verification, must be implemented to ensure proper HCT/P identification and to prevent mix-ups. Specific labeling requirements are included in the following regulations:

- Records accompanying an HCT/P [§ 1271.55]
- HCT/Ps in quarantine [§ 1271.60]
- Storage and use of HCT/Ps from a donor determined to be ineligible [§ 1271.65]
- HCT/Ps from donors excepted from the donor eligibility requirements [§ 1271.90]
- Tracking of HCT/Ps [§ 1271.290]
- Labeling [§ 1271.370]

9.2.13 Storage [§ 1271.260]

Storage areas must be controlled to prevent mix-ups and contamination of HCT/Ps, supplies, and reagents. Systems must also be in place to identify in-process HCT/Ps, quarantined HCT/Ps, and HCT/Ps that have met all release criteria and are available for distribution. HCT/Ps must be clearly identified as in quarantine, either in a physically labeled separate location or using a validated computer system, until donor eligibility has been completed.

HCT/Ps must be maintained at an appropriate temperature during storage and during each step of the manufacturing process. Environmental control and monitoring records, discussed above, should be reviewed periodically to ensure that temperatures have remained within the established acceptable limits. Corrective actions taken in response to temperature excursions should be documented.

In accordance with § 1271.260(c), an expiration must be assigned to each HCT/P, if appropriate (e.g., fresh HCT/Ps and those that are thawed before administration). Factors to consider when assigning an expiration include the HCT/P type, processing steps (including preservation method), storage conditions, and packaging.

9.2.14 Receipt, Predistribution Shipment, and Distribution of an HCT/P [§ 1271.265]

Each incoming HCT/P must be evaluated for damage or signs of contamination and dispositioned based on pre-established criteria. The shipping container and HCT/P container and packaging should be inspected for signs of damage. The HCT/P should also be inspected and, if possible, cultured before processing. Based on the results of the preprocessing culture, which may be performed by the recovery establishment or by the processing facility, additional steps may be performed to reduce bioburden. If culture of the HCT/P is not possible, other methods of evaluation should be used, such as evaluation of storage solutions with pH indicators or visual confirmation of container closure integrity.

If an HCT/P is shipped within the establishment or between establishments before all release criteria have been met, the sender must document that the HCT/P has met pre-established criteria designed to prevent communicable disease transmission before the "predistribution shipment." In addition, the HCT/P must be kept in quarantine during shipment and upon receipt.

An HCT/P may be made available for distribution if it meets all release criteria [§ 1271.3(z)]. To make this determination, a responsible person must review all documentation associated with the HCT/P including, but not limited to, donor eligibility, recovery, processing, storage, and tracking records. Packaging and shipping containers must be designed to protect the HCT/P from contamination and appropriate shipping conditions must be established. Documentation pertaining to receipt, predistribution, and distribution should contain the following:

- Identification of the HCT/P
- Identification of the sender or establishment
- Activities performed on the HCT/P and associated results
- Date(s) of activity
- Quantity of HCT/P received or distributed
- Disposition of the HCT/P, including shipment consignee

9.2.15 Records [§ 1271.270]

Documentation required throughout Part 1271 must be accurate, legible, traceable, sufficiently detailed, and maintained contemporaneously. A records management system relating to the core cGTP requirements must be established and maintained. Pertinent manufacturing records, including those required to release the HCT/P, must also be maintained under the system. Original or true copies of the records must be maintained for 10 years after their creation, unless otherwise stated in Part 1271 (e.g., retention of cleaning and sanitation activities are retained for 3 years per § 1271.190(d)(2)). Records pertaining to a particular HCT/P must be retained for at least 10 years after the date of administration, or if the administration date is not known, then at least 10 years after the latest date of distribution, disposition, or expiration.

9.2.16 Tracking [§ 1271.290]

Facilities that perform any step in the manufacture of an HCT/P must use a tracking system that enables forward and backward tracing of the HCT/P from the donor to the final disposition/consignee. Establishments partially involved in the manufacturing of an HCT/P may use the system implemented by another establishment, provided it meets all requirements. Facilities that do not handle the HCT/P (i.e., a testing facility that receives a blood specimen) are not subject to the tracking requirements.

Table 2 HCT/P donor screening and testing requirements

Communicable disease or agent	Applicable to HCT/P type	Screening	Testing
HIV-1	All HCT/P	Required	Antigen and nucleic acid testing
HIV-2	All HCT/P	Required	Antigen testing
Hepatitis B virus	All HCT/P	Required	Nucleic acid, surface antigen, and core antigen testing
Hepatitis C virus	All HCT/P	Required	Antigen and nucleic acid testing
Human TSE, including Creutzfeldt-Jakob disease	All HCT/P	Required	None
Treponema pallidum	All HCT/P	Required	Required
West Nile virus	Living donors only	Required	Nucleic acid testing
Zika virus	All HCT/P	Required	None
Sepsis	All HCT/P	Required	None
Vaccinia	All HCT/P	Required	None
HTLV-1/2	Viable, leukocyte-rich HCT/Ps only	Required	Antigen testing
CMV[1]	Viable, leukocyte-rich HCT/Ps only	**Not** Required	Antigen testing (total IgG and IgM)
Chlamydia trachomatis	Reproductive HCT/Ps only	Required	Test must be labeled for the detection in an asymptomatic, low-prevalence population
Neisseria gonorrhea	Reproductive HCT/Ps only	Required	Test must be labeled for the detection in an asymptomatic, low-prevalence population

Note: Screening and testing requirements sourced from [40, 41]
[1]Though CMV is not a relevant communicable disease agent or disease, donors of viable, leukocyte-rich HCT/Ps must be tested per § 1271.85(b)(2) ([40]).

As part of the tracking system, the HCT/P must be assigned and labeled with a distinct identification code that relates the HCT/P to the donor and all records pertaining to the HCT/P. Except in the cases of autologous, directed reproductive donations, or first-degree or second-degree blood relative donations, the code must be created for tracking purposes and may not include the name, social security number, or medical record number. The identification code is not required to be on all records concerning the donor, but it must be affixed to the HCT/P container per § 1271.370(b)(1).

Complaint File [§ 1271.320]

A complaint is "any written, oral, or electronic communication about a distributed HCT/P that alleges (1) that an HCT/P has transmitted or may have transmitted a communicable disease to the recipient of the HCT/P or (2) any other problem with an HCT/P relating to the potential for transmission of communicable disease, such as the failure to comply with current good tissue practice" [§ 1271.3(aa)]. Procedures

for handling complaints related to the core cGTP requirements, including review, evaluation, documentation, and investigation (as appropriate), must be implemented.

Any complaints related the core cGTP requirements must be reviewed and evaluated to determine if the complaint is related to an HCT/P deviation or to an adverse reaction and to determine if a report is required under § 1271.350. An investigation must be conducted for a reportable event. Non-reportable events must be evaluated to determine if an investigation is needed; the associated justification must be documented. A designated complaint file containing all related information to the complaint must be maintained.

9.3 Donor Eligibility, Screening, and Testing

Also part of cGTP, Part 1271 Subpart C contains the requirements for donor eligibility determination, including donor screening and testing, which are further discussed in the 2007 guidance document "Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps)" [40] and summarized in this section. Per § 1271.45(c), "an HCT/P must not be implanted, transplanted, infused, or transferred until the donor has been determined to be eligible" through donor screening and testing, except in the limited circumstances specified under §§ 1271.60(d), 1271.65(b), and 1271.90 and described at the end of this section. Until donor eligibility has been determined, the HCT/P must be segregated and clearly identified as in quarantine. During donor screening, relevant medical records such as a current donor medical history interview, a current report of the physical assessment of a cadaveric donor, the physical examination of a living donor, coroner/autopsy reports, and laboratory test results (other than donor testing) are reviewed for risk factors for, and clinical evidence of, the relevant communicable diseases included in Table 2.

HCT/P donor testing must be conducted at an FDA-registered facility by a CLIA-certified laboratory (or laboratory meeting equivalent requirements, as determined by the Centers for Medicare and Medicaid Services (CMS)). Donor testing for the diseases/agents in Table 2 must be performed using available FDA licensed, approved or cleared donor screening tests in accordance with the manufacturer's instructions. If cadaveric samples are being tested, the donor screening test must be specifically labeled for cadaveric specimens.

Once donor eligibility has been determined, a statement indicating if the donor has been determined to be eligible or ineligible and a summary of the records used to make the determination must accompany the HCT/P during distribution, even during transfer within the same facility. Refer to § 1271.55(b) for the requirements of the summary of records. Additionally, the distinct identification code described above [§ 1271.290] must be affixed to the HCT/P container. Records pertaining to a particular HCT/P must be maintained for at least 10 years after the date of its administration, or, if the date of administration is not known, for at least 10 years after the date of distribution, disposition, or expiration, whichever is latest [§ 1271.55(d)(4)].

9.3.1 Exemptions from Donor Eligibility Requirements

Donor eligibility determination is not required for the following HCT/Ps described in § 1271.90(a):

- Cells and tissues for autologous use [§ 1271.90(a)(1)].
- Reproductive cells or tissue donated by a sexually intimate partner of the recipient for reproductive use [§ 1271.90(a)(2)].
- Cryopreserved cells or tissue for reproductive use, other than embryos, exempt at the time of donation as described in the two bullets above, that are subsequently intended for directed donation, provided that:
  - Additional donations of suitable cells and tissues are unavailable due to the infertility or health condition of a donor of the cryopreserved reproductive cells or tissue
  - Appropriate measures are taken to screen and test the donor(s) before transfer to the recipient [1271.90(a)(3)]
- A cryopreserved embryo, originally excepted under § 1271.90(a)(2) at the time of cryopreservation, that is subsequently intended for directed or anonymous donation. When possible, appropriate measures should be taken to screen and test the semen and oocyte donors before transfer of the embryo to the recipient [1271.90(a)(4)].

HCT/Ps exempt from donor eligibility determination under § 1271.90(a) require at least one of the following additional labeling statements ([41]):

- "For autologous use only," if the HCT/P is for autologous use (exempt under 1271.90(a)(1)).
- "Not evaluated for infectious substances," if all otherwise applicable screening and testing have not been performed as required (exempt under 1271.90(a)(1 through 4)).
  - For example, the label for an HCT/P exempt from donor screening per § 1271.90(a)(1) would require both statements "For autologous use only" and "Not evaluated for infectious substances."
- "WARNING: Advise recipient of communicable disease risks", if the donor eligibility determination has not been completed or if screening or testing indicates the presence or risk of relevant communicable disease agents (exempt under 1271.90(a)(2 through 4)).
- Biohazard legend shown in § 1271.3(h), if donor screening or testing indicates the presence of relevant communicable disease agents or diseases and/or risk factors for, or clinical evidence of, relevant communicable disease agents or diseases.
- "WARNING: Reactive test results for (name of disease agent or disease)" if HCT/Ps are recovered under § 1271.90(a) from donors who have positive or reactive test results for any relevant communicable disease agent or disease.

- "Advise recipient that screening and testing of the donors were not performed at the time of cryopreservation of the reproductive cells or tissue, but have been performed subsequently," if the reproductive tissue will be donated to a directed recipient under § 1271.90(a)(3) or a directed or anonymous recipient under § 1271.90(a)(4) and the screening and testing is performed before transfer to the recipient rather than at the time of recovery.

Under § 1271.60(d), HCT/Ps may be used prior to completion of donor eligibility determination if there is a documented urgent medical need, which is defined in § 1271.3(u) as "no comparable HCT/P is available and the recipient is likely to suffer death or serious morbidity without the HCT/P." The HCT/P must be labeled "Not evaluated for infectious substances," and "WARNING: Advise patient of communicable disease risk," and the manufacturer must document that the physician was informed that donor testing and screening were not completed. The results of any completed screening or testing should accompany the product, and the donor eligibility determination must be completed as soon as possible.

An HCT/P from a donor that has been determined to be ineligible through testing and/or screening may only be used for implantation, transplantation, or transfer in the following three circumstances outlined in § 1271.65(b):

- The HCT/P is for allogeneic use in a first-degree or second-degree blood relative.
- The HCT/P consists of reproductive cells or tissue from a directed reproductive donor.
- There is an urgent medical need for the HCT/P based upon a physician's request documented by the establishment.

The biohazard legend [§ 1271.3(h)] and the statements "WARNING: Advise patient of communicable disease risk," and, in the case of reactive or positive test results, "WARNING: Reactive test results for (name of disease agent or disease)" must be included on the label of an HCT/P from an ineligible donor.

10 Special Considerations for Cord Blood

Peripheral or umbilical cord blood stem cells for autologous use or allogeneic use in a first- or second-degree blood relative are regulated under Section 361 of the PHS Act and do not require premarket notification, provided they meet the criteria in § 1271.10(a). However, hematopoietic stem/progenitor cells from placental/umbilical cord blood, sourced from an unrelated allogeneic cord blood donor (HPC, cord blood), are regulated as biologics under the PHS Act and drugs under the FD&C Act. Accordingly, they are subject to the regulations discussed above including, but not limited to, cGMPs, registration and listing, donor eligibility, and cGTPs. Manufacturers of minimally manipulated hematopoietic stem/progenitor cells from placental/umbilical cord blood (typically cord blood banks), sourced from an unrelated allogeneic cord blood donor and intended for hematopoietic and immunologic

reconstitution, are encouraged to refer to the 2014 guidance document "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" [42]. Hematopoietic stem/progenitor cells that are more than minimally manipulated or for a different indication require either an IND or other appropriate premarketing application. To develop the regulatory framework for HPC, cord blood, the FDA worked with industry to develop product standards and establishment and processing controls based on clinical trial data that demonstrated the safety and effectiveness of the cells. If HPC, cord blood manufacturers follow the recommendations in the 2014 guidance, they may reference clinical data submitted to FDA-1997-N-0010 (Legacy Docket number 97N-0497) as part of their BLA.

During the public comment period for the initial HPC, cord blood licensure guidance, the FDA received comments emphasizing the importance of continued availability of HPC, cord blood units that may not meet standards for licensure. In response to the comments, the FDA issued IND guidance specific to HPC, cord blood, "Investigational New Drug Applications for Minimally Manipulated, Unrelated Allogeneic Placental/Umbilical Cord Blood Intended for Hematopoietic and Immunologic Reconstitution in Patients with Disorders Affecting the Hematopoietic System" [43]. The individual or entity that submits an IND is considered a sponsor [21 CFR 312.3(b)], which may be a cord blood bank, a transplant center, or a cord blood registry involved in distribution coordination. The physician that is responsible for transplant is an investigator under the IND, while a sponsor that submits the IND and is directly responsible for the transplant is a sponsor-investigator [21 CFR 312.3(b)]. Specific requirements for sponsors and investigators may be found in 21 CFR 312. The guidance document includes a summary of the minimum information that should be included in the IND, in addition to additional applicable requirements in 21 CFR 312. HPC, cord blood units may be made available for clinical use once the IND goes into effect under 21 CFR 312.40(b).

11 Gene Therapy Products

Some cellular therapy products are combined with gene therapy techniques, such as replacing or inactivating a disease-causing gene or introducing a new or modified gene to help treat a disease. Gene therapies can use a variety of in vivo or ex vivo techniques such as plasmid DNA, viral vectors, bacterial vectors, or gene editing technology (e.g., CRISPR) [44]. Gene therapy products are subject to the same regulatory requirements as for biological drug products described earlier including, but not limited to, premarket approval, IND regulations, and cGMP requirements. Gene therapy products that also incorporate an HCT/P, such as genetically modified cells from an allogeneic donor or autologous cells that are genetically modified before reinfusion, must also meet the applicable requirements in 21 CFR 1271 including donor eligibility and other cGTPs. Although regulated under the same

Table 3 Cell and gene therapy guidance documents

Guidance document title	Year
Guidance for Human Somatic Cell Therapy and Gene Therapy	1998
Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products	2007
Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Somatic Cell Therapy Investigational New Drug Applications (INDs)	2008
Considerations for Allogeneic Pancreatic Islet Cell Products	2009
Cellular Therapy for Cardiac Disease	2010
Potency Tests for Cellular and Gene Therapy Products	2008
Clinical Considerations for Therapeutic Cancer Vaccines	2009
Preparation of IDEs and INDs for Products Intended to Repair or Replace Knee Cartilage	2011
Preclinical Assessment of Investigational Cellular and Gene Therapy Products	2013
IND Applications for Minimally Manipulated, Unrelated Allogeneic Placental/Umbilical Cord Blood Intended for Hematopoietic and Immunologic Reconstitution in Patients with Disorders Affecting the Hematopoietic System	2013
BLA for Minimally Manipulated, Unrelated Allogeneic Placental/Umbilical Cord Blood Intended for Hematopoietic and Immunologic Reconstitution in Patients with Disorders Affecting the Hematopoietic System	2014
Determining the Need for and Content of Environmental Assessments for Gene Therapies, Vectored Vaccines, and Related Recombinant Viral or Microbial Products	2015
Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products	2015
Design and Analysis of Shedding Studies for Virus or Bacteria-Based Gene Therapy and Oncolytic Products	2015
Use of Donor Screening Tests to Test Donors of Human Cells, Tissues and Cellular and Tissue-Based Products for Infection with *Treponema pallidum* (Syphilis)	2015
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	2016
Recommendations for Microbial Vectors Used for Gene Therapy	2016
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)	2016
Investigating and Reporting Adverse Reactions Related to Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps) Regulated Solely under Section 361 of the Public Health Service Act and 21 CFR Part 1271	2016
Deviation Reporting for Human Cells, Tissues, and Cellular and Tissue-Based Products Regulated Solely Under Section 361 of the Public Health Service Act and 21 CFR Part 1271	2017
Same Surgical Procedure Exception under 21 CFR 1271.15(b): Questions and Answers Regarding the Scope of the Exception[1]	2017
Donor Screening Recommendations to Reduce the Risk of Transmission of Zika Virus by Human Cells, Tissues, and Cellular and Tissue-Based Products	2018
Expedited Programs for Regenerative Medicine Therapies for Serious Conditions[1]	2019
Evaluation of Devices Used with Regenerative Medicine Advanced Therapies[1]	2019
Regulatory Considerations for Human Cells, Tissues, and Cellular and Tissue-Based Products: Minimal Manipulation and Homologous Use[1]	2020

(continued)

Table 3 (continued)

Guidance document title	Year
Human Gene Therapy for Retinal Disorders	2020
Human Gene Therapy for Rare Diseases	2020
Human Gene Therapy for Hemophilia	2020
Testing of Retroviral Vector-Based Human Gene Therapy Products for Replication Competent Retrovirus During Product Manufacture and Patient Follow-up	2020
Long Term Follow-up After Administration of Human Gene Therapy Products	2020
Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs)	2020

Note: All FDA guidance documents are available from FDA's website [5]
[1]Guidance document issued under regenerative medicine framework

statutory requirements as other biological drug products, gene therapy products pose unique challenges for which the FDA has issued specific guidance documents to address. For example, because gene therapy products are intended to result in permanent or long-acting changes in the body, patients may be at risk for delayed adverse events. The FDA published the 2020 guidance document "Long-Term Follow-Up After Administration of Human Gene Therapy Products" [45] which provides recommendations for the study design of long-term follow-up (LTFU) observations and discusses factors that should be considered when determining the need for LTFU (e.g., product characteristics, patient-related factors, and existing preclinical/clinical data). Another guidance document published in 2020, "Testing of Retroviral Vector-Based Human Gene Therapy Products for Replication Competent Retrovirus During Product Manufacture and Patient Follow-up" [46], discusses FDA recommendations to ensure there is no replication-competent retrovirus present in a retroviral vector-based product and no signs of retroviral infection in patients who have received the product. Guidance documents applicable to gene therapy products are included in Table 3.

12 RMAT Designation

Section 506(g)(8) of the FD&C Act defines regenerative medicine therapy as "cell therapy, therapeutic tissue engineering products, human cell and tissue products, and combination products using any such therapies or products, except for those regulated solely under section 361 of the Public Health Service Act [42 U.S.C. 264] and Part 1271 of Title 21, Code of Federal Regulations." Combination products with a biological primary mode of action and gene therapy products may also be considered regenerative medicine therapies [47].

Regenerative therapies intended to treat, modify, reverse, or cure serious conditions are eligible for several of FDA's expedited development programs including fast-track designation, breakthrough therapy designation, accelerated approval, priority review, and/or RMAT designation; more than one designation may be granted

to one product [47]. Fast-track designation, breakthrough designation, accelerated approval, and priority review are discussed in depth in the 2014 guidance document "Expedited Programs for Serious Conditions – Drugs and Biologics" [48]. RMAT designation is discussed in the 2019 guidance "Expedited Programs for Regenerative Medicine Therapies for Serious Condition*s*" 47).

As described in the 2019 guidance [47], an investigational drug is eligible for RMAT designation if it meets the definition of regenerative medicine therapy described above; it is intended to treat, modify, reverse, or cure a serious condition; and preliminary clinical evidence indicates that the regenerative medicine therapy has the potential to address unmet medical needs for such condition. The determination of whether the preliminary clinical evidence is sufficient to support RMAT designation is made by the agency on a case-by-case basis. The evidentiary standard for RMAT designation is more than that for fast-track designation, which only requires the product to have the potential to address an unmet medical need, and the said potential may be demonstrated without clinical data (i.e., with in vitro or animal model data). However, unlike breakthrough designation, RMAT designation does not require evidence that the treatment offers substantial improvement over available therapies. The request for RMAT designation should be submitted to the IND, either with the original application or as an IND amendment, and the FDA will respond to the request within 60 days.

The advantages of RMAT designation include the same benefits as fast-track and breakthrough therapy designation programs, including early interactions with FDA and rolling review of the BLA (i.e., sections of the BLA can be submitted to FDA as they are completed, rather than waiting until the entire BLA is complete). Products granted RMAT designation may also be eligible for Priority Review or Accelerated Approval. A product may be eligible for Priority Review at the time of BLA submission if approval of the product would lead to a significant improvement in the safety or effectiveness of the condition. CBER has a 6-month review target for BLAs submitted for products granted Priority Review. Accelerated Approval may be granted for "products for a serious or life-threatening disease or condition… upon a determination that the product has an effect on a surrogate endpoint that is reasonably likely to predict clinical benefit, or on a clinical endpoint that can be measured earlier than irreversible morbidity or mortality that is reasonably likely to predict an effect on irreversible morbidity or mortality or other clinical benefit, taking into account the severity, rarity, or prevalence of the condition and the availability or lack of alternative treatments." Additional details regarding the Accelerated Approval pathway, including the use of surrogate endpoints, may be found in the 2014 guidance "Expedited Programs for Serious Conditions – Drugs and Biologics" [47].

13 Conclusions

All HCT/Ps, unless exempt, are subject to 21 CFR 1271, which focuses on the prevention of communicable disease transmission, including good tissue practices and donor screening/testing. Under the tiered approach to the regulation of HCT/Ps first introduced by the FDA in 1997, lower-risk products are required to comply with 21 CFR 1271 only and are not subject to premarket approval, while higher-risk products are regulated as biological drug products, which are subject to additional parts of 21 CFR as well as 21 CFR 1271 and are required to be licensed under a BLA. Some HCT/Ps may be studied under an IND to generate data necessary to support BLA approval. The FDA issues guidance documents to share their current interpretation of the regulations and has released guidance documents on many topics related to HCT/Ps including gene therapies and RMAT designation. Regulatory requirements are likely to continue evolving as scientific innovation surrounding HCT/Ps advances.

References

1. Food, Drug, and Cosmetic Act. (1938). 21 U.S.C. § 301 et. seq.
2. Public Health Services Act. (1944). 42 U.S.C.
3. Administrative Procedures Act. (1946). 5 U.S.C. § 551 et. seq.
4. U.S. Food and Drug Administration website. (2018). *What is the difference between the Federal Food, Drug, and Cosmetic Act (FD&C Act), FDA regulations, and FDA guidance?* Updated March 28, 2018. https://www.fda.gov/about-fda/fda-basics/what-difference-between-federal-food-drug-and-cosmetic-act-fdc-act-fda-regulations-and-fda-guidance. Last accessed 3 Jan 2021.
5. U.S. Food and Drug Administration website. (2020). *Search for FDA guidance documents*. Updated December 10, 2020 https://www.fda.gov/regulatory-information/search-fda-guidance-documents. Last accessed 3 Jan 2021.
6. U.S. Food and Drug Administration. (1993). *Application of current statutory authorities to human somatic cell therapy products and gene therapy products; Notice. 58 FR 53248*. Published October 14, 1993. https://tile.loc.gov/storage-services/service/ll/fedreg/fr058/fr058197/fr058197.pdf. Last accessed 3 Jan 2021.
7. U.S. Food and Drug Administration. (1993). *Human tissue intended for transplantation; Interim Rule. 58 FR 65514*. Published December 14, 1993. https://tile.loc.gov/storage-services/service/ll/fedreg/fr058/fr058238/fr058238.pdf. Last accessed 3 Jan 2021.
8. U.S. Food and Drug Administration. (1997). *Human tissue intended for transplantation; Final Rule. 62 FR 40444. Published July 29, 1997*. https://www.govinfo.gov/content/pkg/FR-1997-07-29/pdf/97-19819.pdf. Last accessed 3 Jan 2021.
9. U.S. Food and Drug Administration. (1997). *Proposed approach to regulation of cellular and tissue-based products; Availability and public meeting. 62 FR 9721*. Published March 4, 1997. https://www.federalregister.gov/documents/1997/03/04/97-5240/proposed-approach-to-regulation-of-cellular-and-tissue-based-products-availability-and-public. Last accessed 3 Jan 2021.
10. U.S. Food and Drug Administration. (2007). *Regulation of human cells, tissues, and cellular and tissue-based products (HCT/Ps) small entity compliance guide*. Guidance for Industry. August 2007. https://www.fda.gov/media/70689/download. Last accessed 3 Jan 2021.

11. U.S. Food and Drug Administration. (2001). *Human cells, tissues, and cellular and tissue-based products; Establishment registration and listing; Final Rule. 66 FR 5447*. Published January 19, 2001. https://www.govinfo.gov/content/pkg/FR-2001-01-19/pdf/01-1126.pdf. Last accessed 3 Jan 2021.
12. U.S. Food and Drug Administration. (2004). *Eligibility determination for donors of human cells, tissues, and cellular and tissue-based products; Final rule and notice. 69 FR 29786*. Published May 25, 2004. https://www.govinfo.gov/content/pkg/FR-2004-05-25/pdf/04-11245.pdf. Last accessed 3 Jan 2021.
13. U.S. Food and Drug Administration. (2004). *Current good tissue practice for human cell, tissue, and cellular and tissue based product establishments; Inspection and enforcement; Final Rule. 69 FR 68612*. Issued November 24, 2004. https://www.govinfo.gov/content/pkg/FR-2004-11-24/pdf/04-25798.pdf. Last accessed 3 Jan 2021.
14. U.S. Food and Drug Administration. (2020). *Revocation of the regulations for human tissue intended for transplantation and human dura mater*; Proposed Rule. 85 FR 82990. Issued December 21, 2020. https://www.govinfo.gov/content/pkg/FR-2020-12-21/pdf/2020-27828.pdf. Last accessed 3 Jan 2021.
15. 21st Century Cures Act. (2016). *H.R. 34, 114th congress*. https://www.fda.gov/about-fda/jobs-and-training-fda/title-21-21st-century-cures-act-positions. Last accessed 3 Jan 2021.
16. U.S. Food and Drug Administration website. (2019). *Framework for the regulation of regenerative medicine products*. Updated May 21, 2019. https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/framework-regulation-regenerative-medicine-products. Last accessed 3 Jan 2021.
17. U.S. Food and Drug Administration website. (2020). *Regenerative medicine advanced therapy designation*. Updated October 5, 2020. https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/regenerative-medicine-advanced-therapy-designation. Last accessed 3 Jan 2021.
18. U.S. Food and Drug Administration. (2020). *Regulatory considerations for human cells, tissues, and cellular and tissue-based products: Minimal manipulation and homologous use*. Guidance for Industry. July 2020 https://www.fda.gov/media/109176/download. Last accessed 3 Jan 2021.
19. U.S. Food and Drug Administration. (2017). *Same surgical procedure exception under 21 CFR 1271.15(b): Questions and answers regarding the scope of the exception*. Guidance for Industry. November 2017. https://www.fda.gov/media/89920/download. Last accessed 3 Jan 2021.
20. U.S. Food and Drug Administration. (2020). *Compliance program guidance manual 7341.002, Inspection of human cells, tissues, and cellular and tissue-based products (HCT/Ps)*. https://www.fda.gov/media/73949/download. Last accessed 3 Jan 2021.
21. U.S. Food and Drug Administration website. (2020). *Frequently asked questions about combination products*. Updated April 9, 2020. https://www.fda.gov/combination-products/about-combination-products/frequently-asked-questions-about-combination-products#review. Last accessed 3 Jan 2021.
22. U.S. Food and Drug Administration website. (2020). *TRG Rapid Inquiry Program (TRIP)*. Updated August 5, 2020. https://www.fda.gov/vaccines-blood-biologics/trg-rapid-inquiry-program-trip. Last accessed 3 Jan 2021.
23. Center for Biologics Evaluation and Research. (2020). *SOPP 8004: Tissue reference GroupVersion 5*. Effective January 16, 2020. https://www.fda.gov/media/109176/download. Last accessed 3 Jan 2021.
24. U.S. Food and Drug Administration website. (2018). *Tissue reference group*. Updated March 26, 2018. https://www.fda.gov/about-fda/fda-basics/what-difference-between-federal-food-drug-and-cosmetic-act-fdc-act-fda-regulations-and-fda-guidance. Last accessed 3 Jan 2021.
25. U.S. Food and Drug Administration. (2011). *How to write a request for designation (RFD)*. Guidance for Industry. April 2011. https://www.fda.gov/media/80495/download. Last accessed 3 Jan 2021.

26. U.S. Food and Drug Administration. (2018). *How to prepare a pre-request for designation (Pre-RFD)*. Guidance for Industry. February 2018 https://www.fda.gov/media/102706/download. Last accessed 3 Jan 2021.
27. U.S. Food and Drug Administration. (2011). *Current Good Tissue Practice (CGTP) and additional requirements for manufacturers of human cells, tissues, and cellular and tissue-based products (HCT/Ps)*. Guidance for Industry. December 2011 https://www.fda.gov/media/82724/download. Last accessed 3 Jan 2021.
28. U.S. Food and Drug Administration. (2016). *Investigating and reporting adverse reactions related to human cells, tissues, and cellular and tissue-based products (HCT/Ps) regulated solely under Section 361 of the Public Health Service Act and 21 CFR Part 1271*. Guidance for Industry. March 2016 https://www.fda.gov/media/91082/download. Last accessed 3 Jan 2021.
29. U.S. Food and Drug Administration. (2017). *Deviation reporting for human cells, tissues, and cellular and tissue-based products regulated solely under Section 361 of the Public Health Service Act and 21 CFR Part 1271*. Guidance for Industry. September 2017. https://www.fda.gov/media/107703/download. Last accessed 3 Jan 2021.
30. Wilson Sonsini website. (2020). *FDA delays regulatory enforcement of stem cells, other cellular and tissue products*. August 5, 2020. https://www.wsgr.com/en/insights/fda-delays-regulatory-enforcement-of-stem-cells-other-cellular-and-tissue-products.html. Last accessed 3 Jan 2021.
31. U.S. Food and Drug Administration. (2010). *Compliance program guidance manual 7345.848, Chapter 45 Biological drug products inspection of biological drug products (CBER)*. October 1, 2010. https://www.fda.gov/media/73834/download. Last accessed 3 Jan 2021.
32. U.S. Food and Drug Administration website. (2020). *Device advice: Comprehensive regulatory assistance*. Updated August 2020. https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance. Last accessed 3 Jan 2021.
33. U.S. Food and Drug Administration website. (2020). *FDA website Biologics License Applications (BLA) process (CBER)*. Updated November 20, 2020. https://www.fda.gov/vaccines-blood-biologics/development-approval-process-cber/biologics-license-applications-bla-process-cber. Last accessed 3 Jan 2021.
34. U.S. Food and Drug Administration website. (2015). *IND application procedures: Clinical hold*. Updated October 9, 2015. https://www.fda.gov/drugs/investigational-new-drug-ind-application/ind-application-procedures-clinical-hold. Last accessed 3 Jan 2021.
35. U.S. Food and Drug Administration. (2000). *Submitting and reviewing complete responses to clinical holds*. Guidance for Industry. October 2000. https://www.fda.gov/media/72548/download. Last accessed 3 Jan 2021.
36. U.S. Food and Drug Administration website. (2019). *Tissue establishment registration*. Updated November 5, 2019. https://www.fda.gov/vaccines-blood-biologics/biologics-establishment-registration/tissue-establishment-registration. Last accessed 3 Jan 2021.
37. U.S. Food and Drug Administration. (2004). *Sterile drug products produced by Aseptic Processing – Current good manufacturing practice*. Guidance for Industry. September 2004. https://www.fda.gov/media/71026/download. Last accessed 3 Jan 2021.
38. U.S. Food and Drug Administration. (2002). *Validation of procedures for processing of human tissues intended for transplantation*. Guidance for Industry. March 2002. https://www.fda.gov/media/73242/download. Last accessed 3 Jan 2021.
39. U.S. Food and Drug Administration. (2011). *Process validation: General principles and practices*. Guidance for Industry. January 2011. https://www.fda.gov/files/drugs/published/Process-Validation%2D%2DGeneral-Principles-and-Practices.pdf. Last accessed 3 Jan 2021.
40. U.S. Food and Drug Administration. (2007). *Eligibility determination for donors of human cells, tissues, and cellular and tissue-based products (HCT/Ps)*. Guidance for Industry. August 2007. https://www.fda.gov/media/73072/download. Last accessed 3 Jan 2021.
41. U.S. Food and Drug Administration website. (2019). *Testing donors of human cells, tissues, and cellular and tissue-based products (HCT/P): Specific requirements*. Updated May 3, 2019. https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/testing-donors-

human-cells-tissues-and-cellular-and-tissue-based-products-hctp-specific-requirements. Last accessed 3 Jan 2021.
42. U.S. Food and Drug Administration. (2014). *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*. Guidance for Industry. March 2014. https://www.fda.gov/media/86387/download. Last accessed 3 Jan 2021.
43. U.S. Food and Drug Administration. (2014). *Investigational new drug applications for minimally manipulated, unrelated allogeneic placental/umbilical cord blood intended for hematopoietic and immunologic reconstitution in patients with disorders affecting the hematopoietic system*. Guidance for Industry. March 2014. https://www.fda.gov/media/89441/download. Last accessed 3 Jan 2021.
44. U.S. Food and Drug Administration website. (2018). *What is gene therapy?* Updated July 25, 2018. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/expedited-programs-regenerative-medicine-therapies-serious-conditions. Last accessed 3 Jan 2021.
45. U.S. Food and Drug Administration. (2020). *Long term follow-up after administration of human gene therapy products*. Guidance for Industry. January 2020. https://www.fda.gov/media/113768/download. Last accessed 3 Jan 2021.
46. U.S. Food and Drug Administration. (2020). *Testing of retroviral vector-based human gene therapy products for replication competent retrovirus during product manufacture and patient follow-up*. Guidance for Industry. January 2020. https://www.fda.gov/media/113790/download. Last accessed 3 Jan 2021.
47. U.S. Food and Drug Administration. (2019). *Expedited programs for regenerative medicine therapies for serious conditions*. Guidance for Industry. February 2019. https://www.fda.gov/media/120267/download. Last accessed 3 Jan 2021.
48. U.S. Food and Drug Administration. (2014). *Expedited programs for serious conditions–Drugs and biologics*. Guidance for Industry. May 2014. https://www.fda.gov/media/86377/download. Last accessed 3 Jan 2021.

Regulatory Landscape and Emerging Trends in Advanced Therapy Manufacturing: An EU Perspective

Mohamed Abou-el-Enein

1 The Legal Framework for ATMP Development and Manufacturing in the European Union (EU)

Advanced therapy medicinal products (ATMPs) are a heterogeneous group of biologicals comprising somatic cell therapy medicinal products (SCTMP), tissue-engineered products (TEP), gene therapy medicinal products (GTMP), and combined ATMPs where a medical device becomes an additional integral part of the product. This class of products offers a multitude of novel therapeutic approaches, including gene correction, identifying and killing unwanted populations of cells (e.g., malignant cells, autoreactive immune cells), or differentiation of cells into specialized functional tissues. For a cell product to be classified as an Advanced Therapy Medicinal Product (ATMP), it should fulfill at least one of these two conditions: (1) the cells have been subject to substantial manipulation where the biological characteristics, functions, or properties relevant for the therapeutic effect have been altered, and/or (2) these cells are intended for the same essential function(s). ATMPs are defined in Annex I, Part IV of Directive 2001/83/EC, which was amended by the ATMP Regulation 1394/2007, introducing the definitions for TEP and combined ATMPs. To update definitions and technical requirements for GTMP and SCTMP as well as setting up ones for TEP, the Regulation 1394/2007 mandated the establishment of guidelines and Directive 2009/120/EC, amending Directive 2001/83/EC.

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The ATMP Regulation also outlined specific incentives for small- and medium-sized enterprises (SME) developing ATMPs. Of note, for combined ATMPs, the device component should also comply with the new medical device regulation (EU 2017/745). Overall, the ATMP Regulation helped consolidating these products as medicines and harmonized their marketing authorization process.

For ATMPs to be placed on the EU market, developers must apply for marketing authorization through a mandatory centralized procedure coordinated by the European Medicines Agency (EMA). Products granted marketing authorizations under the centralized procedure are subsequently available to healthcare systems and patients throughout the 27 Member States and in Iceland, Lichtenstein, and Norway (EEA states). The European Commission grants this type of authorization following the scientific assessment of the marketing authorization application by the Committee for Medicinal Products for Human Use (CHMP) of the EMA. With the introduction of Regulation (EC) No 1394/2007, within EMA, the Committee for Advanced Therapies (CAT) was created and became responsible for preparing a draft opinion on each ATMP application, as well as to monitor scientific developments in the field closely. Both the CAT and CHMP independently vote on whether, in their opinion, the risk/benefit of a new therapeutic modality is positive or negative and convey their views to the EC, including where necessary details of any divergent views.

The ATMP regulation also amended Directive 2001/83/EC, adding a provision (Article 3.7) to exempt ATMPs from the Directive under specific situations, known as the hospital exemption. Such ATMP must be prepared on a nonroutine basis according to specific quality standards and used within the same Member State in a hospital under the exclusive professional responsibility of a medical practitioner, in order to comply with an individual medical prescription for a custom-made product for an individual patient. The appropriate national competent authority must authorize their manufacture and ensure traceability and pharmacovigilance are equivalent to an authorized ATMP. Importantly ATMP supplied under hospital exemption cannot be imported or exported, and the details of how this should be implemented were left to Member States to decide.

Like other medicinal products, ATMPs must be manufactured in compliance with good manufacturing practice (GMP) as defined in the European Commission Directives 91/356/EEC and amended by Directive 2003/94/EC and 91/412/EEC, respectively [1, 2]. EudraLex Volume 4 of "The rules governing medicinal products in the European Union" contains guidance for interpreting the principles and guidelines of these GMP standards. Regulation (EU) No 1252/2014 came into force to supplement Directive 2001/83/EC and provide a legal framework of GMP principles for active pharmaceutical ingredients, which was only available in EudraLex guidance Part II. In 2018, Part IV guideline of the EudraLex came into operation to define the GMP requirements that should apply to ATMP manufacturing in case of either marketing authorization (authorized ATMPs) or clinical trial settings (investigational ATMPs, iATMP) [3]. The guideline states the need for manufacturers to establish a pharmaceutical quality system to guarantee appropriate measures are being taken during the manufacturing process of ATMPs (a standard requirement for all medicines). It is also noted that the size of the company or institution and the

complexity of activities shall be taken into consideration when implementing the quality system. Adherence to GMP standards should be established by:

- Ensuring the availability of adequately trained personnel with precise allocations of responsibilities.
- Providing premises and equipment with adequate maintenance, suitable for the intended use.
- Implementing an acceptable documentation system, with specifications for materials, intermediates, bulk products, active substance, and finished product and a clear understanding of the manufacturing process.
- Ensuring the manufacturing process is sufficient to guarantee consistent production, product quality, and the compliance thereof with present meaningful specifications.
- Implementing a quality control (QC) system that is operationally independent of the manufacturing.
- Putting in place arrangements for the prospective evaluation of planned changes and their approval before implementation, taking into account regulatory requirements, and after implementation assessing and evaluating implemented process changes.
- Identifying quality defects and process deviations as soon as possible, investigating potential causes, and taking appropriate corrective and/or preventive measures.
- Implementing acceptable systems to ensure traceability of manufactured ATMPs as well as corresponding starting and critical raw materials.

2 EU Versus US Legislative Frameworks: Identifying Commonalities and Differences

When developing an ATMP, a manufacturer will need to adhere to the regulatory framework applicable in the country where the activities will take place. In the EU, there is a multilayered legal structure composed of regulations, directives, and guidelines. Regulations are legally binding and come into force, as written, on a set date in all Member States. Directives must be translated into national legislation by the required date but leave flexibility as to how this is achieved and whether more stringent provisions are included. In contrast to regulations and directives, guidelines are nonlegally binding but are essential to elaborate on the practical operation of legislation. Finally, European Pharmacopoeia (Eur. Ph.) lays down common, compulsory quality standards for all medicinal products in Europe.

Similarly, in the USA, the framework set up by the US Food and Drug Administration (FDA) to regulate pharmaceuticals relies on several levels of legislation. There exist the legally binding Code of Federal Regulations (CFR): Title 21 Parts 1-1499 [4], Level 1 and 2, in conjunction with nonbinding guidance documents and the United States Pharmacopeia (USP) to guide developers. A schematic overview of the EU and US legislative frameworks throughout the entire development cycle of cell-based therapies is provided in Fig. 1.

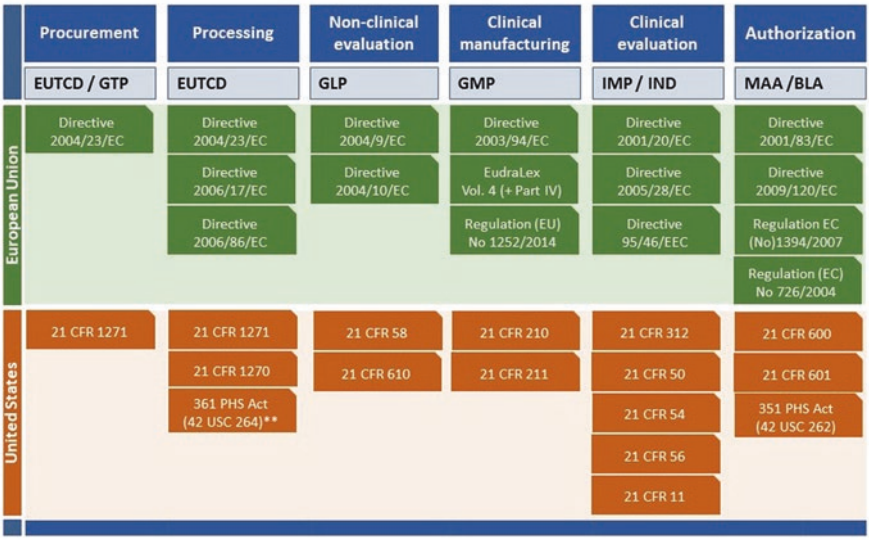

Fig. 1 Comparative overview of regulatory requirements at each stage of cell-based therapy development in EU vs US. (Adapted from Ref. (30))
***Centralized procedure, ** Nondrug. Non-device only. Abbreviations:** *BLA* **Biologics License Application,** *CFR* **Code of Federal Regulations,** *EC* **European Commission,** *EUTCD* **European Tissues and Cells Directive,** *GCP* **good clinical practice,** *GMP* **good manufacturing practice,** *GLP* **good laboratory practice,** *GTP* **good tissue practice,** *IMP* **investigational medicinal product,** *IND* **investigational new drug,** *MAA* **marketing authorization application,** *PHS* **Public Health Service,** *USC* **US Code**

To ensure GMP compliance, similar to EU, the US FDA sets minimum GMP standards that should be implemented, independent of the clinical trial stage, including assurance of product sterility, quality oversight, facility control, adequate documentation, and traceability. Both regulatory jurisdictions employ GMP inspections to assess manufacturing sites for compliance to GMP standards. In the US, this process is performed as part of an Investigational New Drug (IND) application for phase 1 studies, i.e., under Section 501(a) [2](B) of the Food, Drug, and Cosmetic Act (FD&C Act). These studies are explicitly exempt from GMP regulations described in CFR Title 21, Part 211 unless and until they are used (or have previously been used) in phase 2 trials or later, at which they are required to register the site with the US FDA [5]. It is, therefore, left to the developer to decide whether to implement full GMP from the beginning of their clinical development program. In contrast, full GMP compliance is required for all clinical trials, including first-in-human (FIH) within the EU. Manufacturing authorizations are a national responsibility and issued by the relevant authority in the country where they are situated, but are mutually recognized across the EU/EEA. Batch release is the responsibility of a qualified person (QP), who is independent of the manufacturer, even where employed by them. Detailed differences between both legislative frameworks are listed in Table 1.

Table 1 Summary of key differences between US and EU Regulatory frameworks for cell-based therapy manufacturing [6–15]

Category	EU	US
Starting material testing	Number of containers to be sampled: Each container (EU GMP guideline EudraLex volume 4, (part 1, chapter 5: Production) and annex 8 (20) (21))	Number of containers to be sampled: No strict requirements (22, 23)
	Annex 8 permits to deviate from this requirement	Variability of the material and the quality history of the supplier should be considered (24)
Product quality (potency assay)	Potency assay is required with acceptance criteria limits for phase 1/ first-in-human trials	Limited quantitative information on biological activity/attributes is sufficient in early-phase trial
	The assay should be fully validated at the latest before the start of phase 3/pivotal trial (3, 25)	Potency assay is required with acceptance criteria limits at the start of phase 3/pivotal trial. should be fully validated before licensure (26)
GMP compliance and final product release by QP	Each manufacturing site must have at least one QP	QA department/company serving as a legal entity and no QP is required
	QP is personally responsible for releasing both investigational and authorized products	QA department/company takes personal responsibility for potential arising issues with product safety
	QP should serve as an independent legal entity responsible for potential arising issues with product safety	
Process validation	Minimum of three validation batches are required	No lower limit for the number of validation batches (27, 28)
	Continuous lifecycle validation is recommended	Continuous lifecycle validation is recommended
Methods for process validation	Three different approaches (traditional, continuous process verification, and hybrid) are proposed	No specific approaches proposed
Cleanroom air classification standards	EU follows the pharmaceutical cleanroom classification (PCQ) for sterile medicinal products (grades A, B, C, and D as described in annex 1 to the European GMP guideline)	The USA follows the ISO Standards for cleanroom air quality classification (ISO 5, 6, 7, and 8 as described in EN ISO 14644)
	EU GMP requires first to classify and then monitor cleanrooms for airborne particles both ≥0.5 microns and ≥ 5 microns in size	US GMP only requires the classification and monitoring of cleanrooms for airborne particles ≥0.5 microns
		Open-process manufacturing steps: US-based cleanrooms are built to house grade A/ISO 5 biosafety cabinets in a grade C/ISO 7 background and, therefore, are not compatible with EU grade A biological safety cabinet (BSC) in a grade B background

(continued)

Table 1 continued

Category	EU	US
Manufacturing equipment and other relevant devices	CE label is required to ensure conformity with safety, health, and environmental protection requirements	A multitude of regulatory pathways to marketing exist by assessing the device effectiveness and risk of causing harm
Combination products	Definition applies in the case of a medical device is an integral part of the final product	Definition applies if a biological product and a device or a drug and a biological product includes: (a) are cross-labeled in case product components are packaged separately, (b) co-packed, and (c) are physically, chemically or otherwise combined (e.g., cells on a matrix component)
	No specific guidance on GMP manufacturing for combination products	Provides guidance on GMP manufacturing for combination products
	Notified body (NB) based system is somewhat inefficient (sponsors identifies the NB and may end up choosing the ones with the most lax operating standards)	CHDR and CBER/CDER falls under one umbrella within the FDA to facilitate processes and communications
Traceability	Product should be traceable from delivery to the clinical site until patient administration	Requirement for retention of records for human cells, tissues, and cellular and tissue-based products is at least 10 years after the date of product administration
	If ATMP contains cells or tissues of human origin or traceability from the donor of cells, or tissues to the recipient of a product should be ensured	
	Data should be kept for 30 years after the expiry date of the product (unless the authorities mandate a longer period) (29)	

Abbreviations: *CBER/CDER* Center for Biologics/Drug Evaluation and Research, *CHDR* Center for Human Drug Research, *FDA* Food and Drug Administration, *GMP* good manufacturing practice, *ISO* International Standards Organization, *QA* quality assurance, *QP* qualified person. *Life cycle approach: Once sufficient knowledge has been established about product and process (i.e., the process qualification phase), the validation batches can be manufactured. Moreover, (commercial) batches are considered verification batches; thus, validation is an ongoing process until product discontinuation

3 Process and Product Development: The Case of Chimeric Antigen Receptor (CAR) T Cells

CAR T cell therapy is a therapeutic approach, for which T cells are genetically modified ex vivo to express a synthetic receptor on their surface that redirect T-cell

specificity toward tumor-associated antigens. CAR T cells directed against the B-cell epitope CD19 have demonstrated high response rates in patients with chemo-refractory or relapsed hematologic malignancies, for which only limited treatment options were available. As of October 05, 2020, two CD19-directed CAR T cell products tisagenlecleucel (Kymriah®, Novartis) and axicabtagene ciloleucel (Yescarta®, Kite Pharma/Gilead) are licensed for several markets, including the US and EU [16]. A third CD19 CAR-T cell product, brexucabtagene autoleucel (Tecartus™, Kite Pharma/Gilead), has been approved only in the USA.

A typical manufacturing workflow for a CAR-T cell product is described in Fig. 2.

Manufacturing of CAR T cell products poses a number of challenges, including:

- Variability of the cellular starting material and difficulty to obtain sufficient numbers of T lymphocytes.
- Complex, and in some instances, multiple mechanisms of action.
- Difficulty in developing sensitive and reproducible analytical tools for characterization and control.
- High cost and limited availability of vector manufacturing capacity.
- Significant use of complex biological raw materials and their inherent variability.
- Overreliance on labor-intense manual process steps that introduce the risk of contamination and batch-to-batch variation.
- Living cells are not well-suited as reference materials, and for autologous products this becomes a barrier. This impacts the ability to measure potency and assess process and product consistency.

The following sections describe the challenges faced by developers and ongoing efforts to optimize CAR T cell manufacturing.

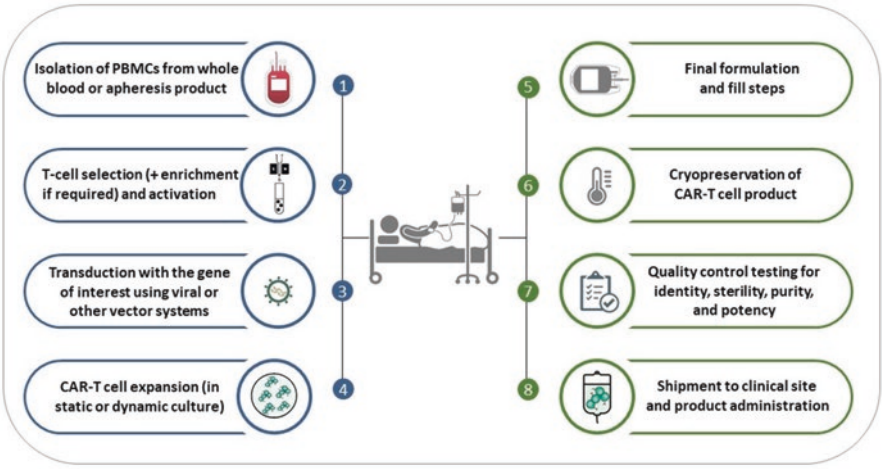

Fig. 2 CAR-T cell manufacturing and delivery workflow

3.1 Cellular Starting Material

The availability of raw materials of a suitable quality and quantity, whether as starting material or as reagent used during production, has been an issue with cell therapy products. For instance, isolation of sufficient T cells can be challenging with autologous CAR T cell products owing to defects in lymphocytes due to the underlying disease, which can be exacerbated by prior chemotherapy. In addition, cellular impurities (unwanted cell populations) such as erythrocytes, platelets, granulocytes and neutrophils can be present in the apheresis product. While these are unlikely to pose a safety concern in the autologous setting, they can impact the expansion capacity of T cells and the overall manufacturing process. Despite the existance of certain manufacturing steps to optimize the quality and quantity of the cell populations, these limitations has encouraged some developers to explore allogeneic approaches for CAR T cell manufacturing, which are not free from challenges themselves.

3.2 Process Control Strategies

One of the most critical aspects of CAR T cell process development is implementing process control analytics to ensure the reliability of the manufacturing process and verify product quality at release. Many of the aspects discussed above, such as variability of the starting material and other raw materials, pose a challenge when developing a control strategy for the process. The objective is to develop a robust process to compensate for these sources of variability, especially the donor source, which cannot otherwise be controlled. Ultimately the process itself dictates the resulting quality of the product. The complexity of these products means establishing control is also reliant on suitable analytical methods to characterize the process and process intermediates. Process characterization relies on identifying the critical quality attributes, which requires an understanding of the mechanism/s of action. Examples of these attributes are purity/impurities and phenotype/memory subsets after selection, gene delivery efficacies, cell count, and viability assessment at specific checkpoints, and T-cell exhaustion markers during expansion. These limitations are still being faced by developers, even after achieving marketing authorization.

3.3 Off-the-Shelf Production

Efforts are underway to establish manufacturing processes with allogeneic donor material for larger-scale batch production and cryopreserved off-the-shelf storage solutions. This may offer the immediate availability of manufactured and banked

products to provide prompt supply for patients with a risk of rapid disease progression. Other benefits relate to the potential to enable re-dosing or repeated dosing, or administering several CAR T cell products directed against various target antigens. Off-the-shelf production also has the potential to diminish other risk factors of autologous manufacturing, such as insufficient starting material or the need to remove critical cellular impurities, e.g., circulating tumor cells. Nevertheless, there are key risks associated with the use of allogeneic cells, such as graft-versus-host disease (GvHD) and immunogenicity-mediated rejection [17], that need to be taken into consideration and addressed adequately during development. Currently, most efforts aiming to reduce these risks are attributed to implementing genome-editing technologies by knocking out the endogenous TCRα gene or creating targeted gene disrupting insertions in the TCRα-chain locus to mitigate GvHD. Other strategies aim to knockout both the TCR to block GvHD development and ß2-microglobulin to block the human leukocyte antigen (HLA) I molecule expression for preventing immunogenic events [18].

4 Automation in Cell and Gene Therapy Manufacturing

Autologous products by their nature cannot be scaled up, leaving only scale-out as an option. Therefore, enough replicates of the process are required to meet expected demand along with a system to schedule orders, donations, and manufacturing to ensure there is sufficient capacity. Each manufacturing workstation must be adequately segregated from other workstations to avoid cross-contamination and be staffed and supplied with materials in a timely manner. As such, autologous manufacturing requires dedicated space to be available 24/7 and efficient coordination of operational activities. In addition, many autologous products are still manufactured using mostly manual processes with multiple open-handling steps, such as tissue culture flasks or small bioreactors. These approaches are highly dependent on a well-trained operative, and all manufacturing steps should be performed in a higher-grade clean room (e.g., EU A in B background). Scale-out manufacturing also impacts quality control activites, which needs to have sufficient capacity not just for the volume of samples but also because those samples are both unstable, and the results can be time-sensitive, especially when the product is not cryopreserved. From a commercial perspective, this means facilities and staff costs are likely to be higher than traditional biotech industrial processes, with less scope for cost reduction.

Automated and functionally closed systems for the manufacturing of autologous products would, therefore, be advantageous. Automation can also extend to cover process analytics. While not discussed here, automating the manufacturing process of allogeneic products at scale is similarly favorable.

4.1 Automation Platforms

The current trend is to move toward (semi)automatic systems that allow almost closed processing of cell-based and genetically engineered products. For instance, "functionally closed" cell isolation, expansion, and transduction systems, such as the CliniMACS Prodigy® (Miltenyi Biotech) or the Cocoon® (Lonza), are currently being employed by a number of developers. Others are using only bioreactors, such as the Quantum Cell Expansion System (Terumo BCT, Inc.), representing a platform for expansion of both adherent or suspension cells. Example of other cell expansion tools are the Xuri™ cell platform W25 (GE Healthcare) and the G-Rex® Technology (Wilson Wolf Corp.). The Xuri™ system allows to expand T and NK cells under controlled conditions by applying culture bags connected to an environmental control unit (CO2/O2/air mix controller, gas flow controller, automated pH controller, dissolve oxygen measurement device) lying on a rocking temperature-maintaining platform and connected to an intuitive system control (32). The G-Rex® bioreactor employs a gas-permeable membrane to culture cells at high cell density in a flask-like device. This device can be easily placed into an incubator and attached to a closed system tubing and pump system fluid exchange, to avoid reopening the bioreactor for these processing steps [19].

There are also novel platforms to enable fully automated final formulation and fill/finish of ATMPs, such as the Finia® Fill and Finish system (Terumo BCT, Inc.) [20] or the Invetech automated class C/D formulation and filling platform [21]. The availability of platforms, such as the VIA Freeze™ System (GE Healthcare Life Sciences) [22] or the CoolCell® cryopreservation systems and ThawSTAR™ automated cell thawing systems (BioCision) [23], has enabled the automated controlled rate freezing, storage, and thawing of cell products. Also, automated liquid-nitrogen freezers such as the BioArchive® (Thermogenesis) allow for fast retrieval of products, ensuring traceability and avoiding the exposure to transient warming events.

4.2 Automation of Process Monitoring

In general, automation of unit operations combined with automated process monitoring can improve the overall control of a particular manufacturing process. Moreover, product quality might be enhanced due to improved repeatability compared to manual handling steps. This assumes the system used is capable of controlling the critical process parameters associated with each unit operation. Automation does not replace the need for process characterization and setting process specifications, meaning the system should be sufficiently adjustable to perform these activities. Implementing automated manufacturing processes in combination with process analytical technology (PAT) that provides real-time analytics could, in some cases, provide superior control. For example, existing bioreactors already allow feeding to be controlled by monitoring glucose, lactate, oxygen, or other metabolites, meaning

the system can compensate for batch-to-batch variation. Given the nature of autologous products, real-time adjustment of parameters has the potential to adjust for donor variation, as opposed to the fixed parameters typically employed where PAT is not used.

4.3 Opportunities Associated with Automation

There are various opportunities where automation can enhance the process development and manufacturing of cell therapies. Fully automated systems that enable the complete elimination of manual handling steps, including cell processing and material transfer from one operation unit to the other, would significantly lower the risk for human processing errors. However, these advantages are associated with high prices of equipment and reagents and reduced flexibility, particularly in case of autologous-based manufacturing, where scaling out is the only feasible approach, as mentioned earlier. An ideal system would also be available in different scales such that small-scale model processes can be used for process characterization and process development activities. The availability of different scale systems is also likely required for allogeneic products to allow for changes in the volume of products manufactured from first-in-human (FIH) to commercial scale.

5 Models of Distribution for Advanced Therapies Manufacturing

When devising a production strategy for clinical and later commercial manufacturing of cell therapy products, developers must weigh challenges and opportunities of traditional centralized strategies against decentralized manufacturing with multisite scenarios. In centralized manufacturing, all relevant steps of the process are covered and carried out in one facility, while in a decentralized approach, these steps are distributed throughout a geographical network of manufacturing labs. The decision which strategy would be the most efficient for manufacturing a specific product should be made in the early phase of product development to avoid delays and additional costs and challenges due to comparability issues (ICH Q5E, [24]).

5.1 Advantages of Decentralized Manufacturing

While centralized manufacturing enables the most comforting oversight of the manufacturing process and the resulting product, in decentralized manufacturing, the developer surrenders part of the control to other sites for closer patient access [25].

For most nonindustrial clinical/academic sites, a decentralized model would require considerable efforts to operate each site reliably and efficiently. As stated in EudraLex Volume 4 GMP guideline, the EMA acknowledges that the unique scenario of ATMP production compared to conventional medicinal products entails the adoption of decentralized manufacturing under certain circumstances. The document states that "There may be cases where manufacturing of the ATMP needs to take place in sites close to the patient (e.g., ATMPs with short shelf-life, clinical advantage of using fresh cells as opposed to freezing the starting materials/finished product, etc.). In such cases, manufacturing of the ATMPs may need to be decentralized to multiple sites so as to reach to patients across the EU. This scenario may occur both in the context of authorized ATMPs as well as in the context of investigational ATMPs."

Decentralized manufacturing strategies enhance proximity to treatment centers, reducing logistical challenges (cross-border transport, especially to other third countries) and transportation costs [25]. Nevertheless, complying with the necessary standards and regulatory requirements must be ensured across different regional sites and accepted by the relevant regulatory authorities. Overall, for decentralized manufacturing of ATMPs to succeed, essential parameters such as the starting material, production workflow, QC methods, and batch release specifications have to be consistent among all sites.

5.2 Regulatory Expectations for Decentralized Manufacturing

Regulatory expectations for decentralized manufacturing and quality assurance under EU legislation are formulated in paragraphs 11.46(a), 11.48, 11.50, and 11.51 of the EudraLex Volume 4 Part IV GMP guideline [3]. These guidelines highlight the need for (a) establishing a leading "central" site responsible for oversight of decentralized sites by ensuring adequately qualified and trained personnel for decentralized batch certification and release process duties along with regular audits to evaluate compliance, (b) a "written contract/technical agreement between the central site and the decentralized sites establishing the responsibilities of each party, including the responsibility of the QP," (c) allowing the "central" site QP to access and rely on data or information transmitted to them by decentralized sites, and (d) handling deviations at decentralized sites. Any deviation at a decentralized site needs to be approved in writing by a qualified and responsible person by involving the QP; appropriate actions should be implemented to identify the root cause of the deviation and take corrective and preventive measures. Moreover, any signs of quality defects, deviations, or nonconformities at decentralized sites need to be immediately reported to the leading site. Nevertheless, remote QP surveillance can be challenging, which raises the need for a QP to be present for a certain period of time on each site, adding to the operations' overall cost.

6 Conclusions

While the regulatory framework for medicinal products is well developed in the European Union, novel classes of products, such as ATMPs, pose a challenge to developers and competent authorities trying to keep pace with the rapidly emerging technological advances in this sector [26]. Product manufacturing remains one of the major issues raised during the regulatory evaluation of ATMPs [27]. As such, a key focus for regulators is to provide a reasonable degree of flexibility to stimulate further innovation while protecting patient safety and ensuring the safety, quality, and efficiency of product manufacturing. Moreover, the regulatory framework needs to account for the inevitable variances between different advanced therapies while simultaneously enabling higher standardization and harmonization among different Member States. Similarly, developers need to exert more effort to establish compliant processes and design well-thought-out clinical testing programs [27]. These rapid developments will also bring up new concepts in manufacturing strategies, such as automation and off-the-shelf solutions.

With an automated functionally closed system and standardized manufacturing approach, it may become feasible to bring cell manufacturing to regions that are not in the vicinity of larger manufacturing centers. This could significantly reduce waiting times for product manufacturing, shorten transport times, and may eventually lower product manufacturing costs. This model closely follows the model of regional blood banks, which allow for the availability and distribution of blood products also to remote healthcare centers. For us to achieve the goal of having cost-effective and accessible cell therapies, efforts should continue to explore the feasibility of recent technologies while capturing and addressing the many variables influencing cell therapy manufacturing processes and associated costs [28, 29].

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References

1. European Commission. (1989). *EudraLex Volume 4 Good Manufacturing Practice (GMP) guidelines*.
2. European Commission. (2003). EC Directive 2003/94/EC.
3. European Commission. (2017). EudraLex “The rules governing medicinal products in the European Union” volume 4: Good manufacturing practice, Part IV: Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products.
4. U.S. Food & Drug Administration. (2019). *TITLE 21: Food and drugs, Chapter I: Food and drug administration.* Department of Health and Human Services, Subchapter C. Drugs: General, PART 211: Current good manufacturing practice for finished pharmaceuticals.
5. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research

(CBER), Office of Regulatory Affairs (ORA). (2008). *Guidance for industry: CGMP for phase 1 investigational drugs*.
6. European Commission. EudraLex "The Rules Governing Medicinal Products in the European Union" Volume 4: EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use, Part 1, Chapter 5: Production.
7. European Commission. EudraLex "The Rules Governing Medicinal Products in the European Union" Volume 4: EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use. Annex 8 "Sampling of starting and packaging materials".
8. Federal Drug Agency (FDA). (2019). *21CFR211.84 testing and approval or rejection of components, drug product containers, and closures*.
9. Federal Drug Agency (FDA). (2019). 21CFR211.160(b): Subpart I--Laboratory Controls, Sec. 211.160 General requirements.
10. Federal Drug Agency (FDA). (2018). *How does FDA interpret the regulations (21 CFR part 211) regarding the establishment of expiry dating for chemicals, reagents, solutions, and solvents?* Question 4 in Questions and Answers on Current Good Manufacturing Practices—Records and Reports.
11. European Medicines Agency (EMA), Committee for Advanced Therapies (CAT). (2018). *Guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells*. EMA/CAT/GTWP/671639/2008 Rev. 1.
12. Federal Drug Agency (FDA), Center for Biologics Evaluation and Research (CBER). (2011). *Guidance for industry: Potency tests for cellular and gene therapy products*.
13. Federal Drug Agency (FDA). (2019). *Questions and answers on current good manufacturing practices – Production and process controls*.
14. European Commission. (2015). *EudraLex Volume 4: EU guidelines for good manufacturing practice for medicinal products for human and veterinary use* – Annex 15: Qualification and Validation.
15. European Commission (2019). *Guidelines on good clinical practice specific to advanced therapy medicinal products*.
16. Elsallab M, Levine BL, Wayne AS, Abou-El-Enein M (2020) CAR T-cell product performance in haematological malignancies before and after marketing authorisation [Internet]. 21, The Lancet Oncology. Elsevier Ltd; e104–16. https://doi.org/10.1016/S1470-2045(19)30729-6. Last accessed 19 Oct 2020.
17. Depil, S., Duchateau, P., Grupp, S. A., Mufti, G., & Poirot, L. (2020). 'Off-the-shelf' allogeneic CAR T cells: Development and challenges. *Nature Reviews Drug Discovery, 19*(3), 185–199.
18. Ren, J., Liu, X., Fang, C., Jiang, S., June, C. H., & Zhao, Y. (2016). Multiplex genome editing to generate universal CAR T cells resistant to PD1 inhibition. *Clinical Cancer Research*.
19. Khalil, M., Foster, A., & Gagliardi, C. (2018). A simplified method for transduction and expansion of T cells for clinical application. *Blood, 132*(Supplement 1), 4555.
20. Terumo, B.C.T. *FINIA® automated fill and finish system*. https://www.terumobct.com/finia. Last accessed 19 Oct 2020.
21. Invetech. *Automated GMP formulation and fill systems*. https://www.invetechgroup.com/cell-therapy-formulation-fill/. Last accessed 19 Oct 2020.
22. GE Healthcare Life Sciences. Freezing of T cells using the VIA Freeze™ system. https://www.gehealthcare.com/article/chill-out-harnessing-new-freezing-technology-to-help-deliver-the-future-of-medicine/. Last accessed 19 Oct 2020.
23. BioCision. *Controlled cell freeze and thaw platforms*.
24. European Medicines Agency (EMA). (2005). *ICH Q5E: Comparability of biotechnological/biological products*. Note for guidance on biotechnological/biological products subject to changes in their manufacturing process (CPMP/ICH/5721/03).
25. Harrison, R.P., Ruck, S., Medcalf, N., Rafiq, Q.A. (2017). Decentralized manufacturing of cell and gene therapies: Overcoming challenges and identifying opportunities. *Cytotherapy*

[Internet]. 2017; Oct; 19(10): 1140–1151. https://linkinghub.elsevier.com/retrieve/pii/S1465324917306321. Last accessed 19 Oct 2020.

26. Abou-El-Enein, M., Elsanhoury, A., & Reinke, P. (2016). Overcoming challenges facing advanced therapies in the EU market. *Cell Stem Cell [Internet], 19*(3), 293–297. https://doi.org/10.1016/j.stem.2016.08.012. Last accessed 19 Oct 2020.
27. Elsallab, M., Bravery, C. A., Kurtz, A., & Abou-el-enein, M. (2020). Mitigating deficiencies in evidence during regulatory assessments of advanced therapies: A comparative study with other biologicals. *Molecular Therapy – Methods & Clinical Development [Internet], 2020*, 269–279. https://doi.org/10.1016/j.omtm.2020.05.035. Last accessed 19 Oct 2020.
28. Abou-El-Enein, M., Römhild, A., Kaiser, D., Beier, C., Bauer, G., Volk, H.-D., et al. (2013). Good Manufacturing Practices (GMP) manufacturing of advanced therapy medicinal products: A novel tailored model for optimizing performance and estimating costs. *Cytotherapy [Internet]*. 2013; Mar [cited 2013 Mar 19]; 15(3): 362–383. http://www.ncbi.nlm.nih.gov/pubmed/23579061. Last accessed 19 Oct 2020.
29. Abou-El-Enein, M., Bauer, G., Medcalf, N., Volk, H.-D. & Reinke, P. (2016). Putting a price tag on novel autologous cellular therapies. *Cytotherapy [Internet]*. 2016 Aug; 18(8): 1056–1061. http://linkinghub.elsevier.com/retrieve/pii/S1465324916303796. Last accessed 19 Oct 2020.
30. The British Standards Institution. (2006). *PAS 83:2012 developing human cells for clinical applications in the European Union and the United States of America – Guide*. pp. 1–74.

Australian Cellular Therapy Regulations

Rosemarie Bell

1 Determination of a Biological

The Therapeutic Goods Administration (TGA) is responsible for the safety, consistency and ongoing surveillance of therapeutic goods. With the Regulatory Framework for Biologicals established in 2011, a biological is determined to be a commodity that originates or contains either human cells or human tissues and is used to treat or prevent disease, health disorders, defects or injuries, diagnose a medical condition, alter a physiological process, assess disease susceptibility of a person, or body part replacement or modification. Faecal microbiota transplant products and materials that are composed of or contain live animal cells, tissues or organs also fall under the biological definition [3].

2 Types of Biologicals

Products that fall under the category of a biological may not be required to be regulated as one. Biologicals are separated into three categories:

- Excluded as biological.
- Regulated as therapeutic good (ARTG registered), but not as a biological.
- Regulated as a biological.

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3 Excluded Biologicals

Excluded biologicals [4] are not subjected to the requirements of the Therapeutic Goods Act 1989 and are not required to adhere to the following:

- Good manufacturing practice.
- Inclusion into the ARTG.
- Reporting of adverse events.
- Comply to relevant TGA Standards for biologicals and therapeutic goods.

Made under Section 7AA of the *Therapeutic Goods Act 1989*, *Therapeutic Goods* (*excluded goods*) *Determination, 2018*, Sections *4A to 4D* outlines the criteria to exclude biologicals from TGA regulations [4]. Biological categories, mandatory criteria and alternative regulatory governance external to the TGA are summarised in Table 1 Excluded Biologicals [4].

Equipment and materials used to manufacture the excluded biological may still be required to be regulated under a medical device or an IVD [4].

4 Biologicals Regulated as a Therapeutic Good

Governed by the Therapeutic Goods Act 1989, Therapeutic Goods (things that are not biologicals) Determination [5], certain commodities that fall under the definition of a biological are declared not to be a biological. These products are regulated by the TGA as a therapeutic good. Examples include, but are not limited to:

- Vaccines that do not contain viable human cells.
- Plasma-derived products.
- Products that contain plasma-derived products.
- Blood and blood components.
- Biological medicines.
- Recombinant products.
- HPCs derived from cord blood.
- In vitro diagnostic medical devices (IVDs).

5 Regulated Biologicals

Products that meet the definition of a biological and a therapeutic good in accordance with the *Therapeutic Goods Act 1989* but do not fall into the groups listed within Sections 3.0 and 4.0 are regulated as a biological [7].

Table 1 Excluded biologicals

Excluded biological	Intended use	Examples	Alternative regulatory governance (external to the TGA)
Eligible autologous human cells and tissue products	All of the following criteria must be met	Autologous haematopoietic progenitor cells to reconstitute blood after cancer treatment*	*require National Pathology Accreditation Advisory Council (NPAAC) or National Association of testing authorities (NATA) accreditation
	Collected from a patient who is under the clinical care of a registered under law, state or internal territory medical or dental practitioner	The use of autologous blood to seal cerebrospinal fluid leaks	Australian Health Practitioner Regulation Agency (AHPRA)
			State, territory and National Medical Boards
		Autologous blood donation	State, territory and National Dental Boards
		Bone grafts	Local councils
	Manufactured by that medical or dental practitioner or by persons under the professional supervision of the same practitioner (with the exception of storage and testing) in a hospital for that specific patient who is a patient of that hospital	Pancreatic islet cells	Public hospital management within the state or territory
		Skin grafts	Private hospital licensing within the state or territory
		Vascular conduits	Australian competition and consumer commission (ACCC)
	Not consumer advertised		
Fresh viable haematopoietic progenitor cells	Direct donor to host transplantation for the purpose of haematopoietic reconstitution	Bone marrow cells	
		Cord blood	
Fresh viable human organs or parts of human organs	Direct donor to host transplantation	Lungs	
		Hearts	
		Corneas	

(continued)

Table 1 (continued)

Excluded biological	Intended use	Examples	Alternative regulatory governance (external to the TGA)
Human reproductive tissue	Assisted reproductive technologies	Sperm	Australian Health Practitioner Regulation Agency (AHPRA, 2020)
		Eggs	State, territory and National Medical Boards
		Embryos for in vitro fertilisation	Public hospital management within the state or territory
		Other assisted reproductive technologies	Private hospital licensing within the state or territory
			Australian competition and consumer commission (ACCC)
			Reproductive Technology Accreditation Committee and Codes of Practice (FSA, 2020)

- Skin, bone, ocular, cardiovascular and amnion tissue-based products.
- Genetically modified cell-based products that undergo in vitro cell expansion.
- Genetically modified cell-based products that undergo in vitro cell depletion.
- Immunotherapy products consisting of human cells.
- Medical devices and cell therapy products.
- Goods that contain live animal cells, tissues and organs.
- Human cells and tissue products (stem cells) for autologous use.

6 Biological Classifications

6.1 Classification

The development of a biological product and its subsequent application for inclusion on the ARTG is fundamentally based on the classification of the biological. Based on the ascending order of risk to patients, the type of processing involved and the final directive of the product, biologicals are classified into four classes [6].

6.1.1 Class 1

Class 1 biologicals are considered low risk to public health with external governance (accreditation) and clinical oversight deemed appropriate by the TGA. Class 1 biologicals can be supplied if they comply to all standards applicable to the product, are mentioned within Schedule 16 of the *Therapeutic Goods Regulations* (*1990*) and included on the ARTG following a declaration of compliance [2]. It is important to note that at the time of this publication faecal microbiota transplant products are the only Class 1 biological supplied within Australia.

6.1.2 Class 4

Class 4 biologicals are high-risk products that contain either live animal cells, tissues or organs and are either composed of, contain or derived from either human cells or tissues that have been processed to genetically modify the intrinsic function/s of the donor cells or artificially change the function/s of the cell or tissue whose original function/s were not intrinsic to the cells or tissues upon donor collection. Pluripotent stem cells or biologicals derived from pluripotent stem cells are also considered Class 4 biologicals [6]. Examples include, but not be limited to, genetically modified *CAR*-T cells and induction of pluripotent stem cells (iPSCs) [6].

6.1.3 Classes 2 and 3

Class 2 and 3 biologicals are determined by the level of manipulation required to generate the product and its intended use. Class 2 biologicals undergo minimal manipulation during processing and are for homologous use only. Class 3 biologicals will have more than minimal manipulation and be either intended for homologous or nonhomologous use. Products intended for homologous use involving minimal manipulation are Class 2 biologicals [6].

7 Regulation of Autologous Human Cells and Tissue Products

7.1 Autologous Products

Autologous human cell and tissue (HCT) products are received from and given to the same person. Stem cell treatments are a common form of HCT products. Examples include:

- Skin grafts.
- Adipose-derived stem cells.

- Blood and blood components.
- Haematopoietic stem cells.
- Bone marrow-derived mesenchymal stem cells.
- Genetically altered lymphocytes.

7.2 Regulation

Autologous HCT products are regulated primarily by the TGA; however other regulatory requirements can apply depending on the source and directive of the final product. Additional regulatory authorities include Australian Health Practitioner Regulation Agency (AHRPA, 2020); state, territory and national medical boards; public hospital management within the state or territory or private hospital licensing within the state or territory or the Australian Competition and Consumer Commission (ACCC).

The Australian Regulatory Guidelines for Biologicals (ARGB) outlines the regulatory pathway for sponsors of autologous HCTs [8].

7.3 Risk-Based Regulation

A risk-based perspective categorises three levels of TGA regulation for autologous HCTs. The risk is dependent upon the type of product, risk to the patient and external parties that may oversee the governance and safety of the autologous HCT product. These categories consist of:

7.3.1 Excluded from TGA Regulation

The collection, manufacturing and use by persons under the professional supervision or by the medical or dental practitioner who is providing clinical care of a patient within a hospital and provided that the product is not advertised to consumers.

7.3.2 Regulated by the TGA with Exemptions from Certain Requirements

The collection, manufacturing using minimal manipulation for an homologous, singular indication in one clinical procedure and is used by persons under the professional supervision and/or by the medical or dental practitioner who is providing clinical care of a patient outside of a hospital.

7.3.3 Fully Regulated by the TGA

Autologous HCT products that are required to be fully regulated by the TGA are classified as a medicine if the product is inclusive within the definition of a blood, a blood component or a haematopoietic progenitor cells or a biological.

If the autologous HCT product does not meet the criteria for exclusion or exemption, it is determined to be a biological and will be regulated under the ARGT.

8 Manufacture of Biologicals and Human Cellular Therapy Products

For products to be listed on the Australian Register of Therapeutic Goods ARTG, the regulatory requirements for either a blood, blood component, haematopoietic progenitor cells or a biological, a manufacturing licence must be held by the manufacturer. Overseas manufacturers must be granted a GMP clearance by the TGA. Upon licensing, the TGA will monitor the product through periodic inspections of the manufacturer to ensure ongoing regulatory compliance. In addition, the manufacturers are required to perform post-marketing evaluation on the licensed products to identify potential risks and adverse events.

To be included within the ARTG, the TGA will require a review of preclinical and clinical data in addition to data acquired during manufacturing (i.e. from process qualification runs). Manufacturers of biologicals and human cellular therapy products are required to demonstrate compliance with the Australian Code of GMP for human blood and blood components, human tissues and human cellular therapy products (cGMP) [9]. The cGMP is applicable for all manufacturers who wish to undertake the quality assurance, donor selection, collection, processing, in process and release testing, storage and release of supply of human blood and blood components, human cellular therapies and human tissues. Licensing requirements for the manufacture of these products are documented within Part 3–3 of the *Therapeutic Goods Act of 1989* [1].

Compliance to the PIC/s Guide to Good Medicinal Products, with the exception of Annexes 4, 5, 14 and 16, is required for manufacturers of biologicals that contain live animal cells, tissues or organs [10].

9 Therapeutic Goods Orders

To ensure that the product/s manufactured are safe and effective, additional Therapeutic Goods Orders (TGOs) are legislative documents that manufacturers must demonstrate compliance to. Relevant TGOs include, but are not limited to:

- Therapeutic Goods Order No. 88 standards for donor selection, testing and minimising infectious disease transmission via therapeutic goods that are human blood and blood components, human tissues and human cellular therapy products [11].
- Therapeutic Goods Order No. 83 human musculoskeletal tissue [12].
- Therapeutic Goods Order No. 84 cardiovascular tissue [13].
- Therapeutic Goods Order No. 86 human skin [14].
- Therapeutic Goods Order No. 87 general requirements for labelling of biologicals [15].
- Therapeutic Goods Order No. 94 (standard for haematopoietic progenitor cells derived from cord blood) [16].
- Therapeutic Goods Order No. 102 (standard for blood and blood components) [17].
- Therapeutic Goods Order No. 94 (standard for haematopoietic progenitor cells derived from cord blood) [18] stipulates that haematopoietic progenitor cells derived from cord blood must meet the requirements of the International Standards for Cord Blood Collection, Banking, and Release for Administration, Sixth edition, July 2016 [19].

In the absence of TGOs for a specific product, default standards can apply including British Pharmacopoeia European Pharmacopoeia and United States Pharmacopeia-National Formulary [20].

The incorporation of a risk-based approach to identify products of escalating risk, with the submission of a Technical Master File (for blood, blood components and haematopoietic progenitor cells), demonstrates compliance of safety, quality and efficacy of the product [21]. The documentation of every step within the manufacturing process from donor selection to release of supply ensures compliance to the cGMP and relevant TGOs and is submitted to the evaluations and inspections division of the TGA for approval prior to licensing.

10 Special Access Schemes

The Special Access Scheme (SAS) allows an unapproved biological (product that is not listed on the ARTG) to be applied under emergency or compassionate use [22]. The Special Access Scheme permits the following:

- Importation or supply of an unapproved biological for a single medical procedure.
- Importation or supply of an unapproved biological for personal importation.

There are three types of special access schemes for biologicals [22].

10.1 SAS Category A

Notified to the TGA within 28 days after administration, it is for patients who are defined as seriously ill whereby death is likely within a few months or, alternatively, if an early death may occur in the absence of early treatment.

10.2 SAS Category B

This is utilised when the patient does not fit into the Category A and the therapeutic good to be supplied is unapproved. Significant clinical justification must be presented to the TGA for consideration, with an explanation given to exclude therapeutic goods currently listed on the ARTG.

10.3 SAS Category C

This notifies the TGA of the use of the biological that has a demonstrated history of use. A list of indications is supplied, and generally the health practitioner is authorised to prescribe the product or the registered indications.

Therapeutic Goods (authorised supply of specified biologicals) Rules, 2018 [23], govern the use of Biologicals and other cellular therapy products within a special access scheme setting [23].

11 Clinical Trials

Clinical trials involving cellular therapies are classified as ‘unapproved’ therapeutic goods [24]. Sponsors and manufacturers wishing to import and/or supply unapproved therapeutic goods under the provision of a clinical trial within Australia are required to notify the TGA through the Clinical Trial Notification (CTN) Scheme or apply through the Clinical Trial Approval (CTA) Scheme.

For CTN Schemes, the sponsor will submit a CTN notification to the TGA. In addition, Human Research Ethics Committee (HREC) approval is required prior to the commencement of the trial. The HREC will review the scientific data presented inclusive of trial design, safety, risk and ethical considerations of the proposed clinical trial and approves the trial protocol. The clinical trial must adhere to the HREC approved trial protocol. The sponsor is responsible for ensuring that all notifications and approvals have been acquired prior to the commencement of the ‘unapproved’ therapeutic good.

The Australian Regulatory Framework for Biologicals does not allow for certain types of Class 4 biologicals to be supplied under a CTN scheme and must be supplied under the CTA scheme. The sponsor of the trial is required to submit a CTA application to the TGA, along with the scientific data acquired during preclinical or early phase clinical data. HREC approval of the trial design, safety and ethical consideration is required prior to the commencement of the clinical trial [24].

Clinical trials supplying 'unapproved' therapeutic goods are required to comply to the ICH Guidelines for Good Clinical Practice (GCP), with an Integrated Addendum to ICH E6(R1) by the TGA [25].

The National Health and Medical Research Council Act 1992 established the National Health and Medical Research Council (NHMRC) to provide guidance on medical research and ethical conduct (referenced within the Therapeutic Goods Regulations 1990 [2], the NHMRC's National Statement on Ethical Conduct in Human Research (the National Statement)). The conduct of a clinical trial must be in compliance with the National Statement [26].

Phase 2 and beyond clinical trials are required to be included on the ARTG. Facilities manufacturing a biological or medicine are required to hold a TGA manufacturing licence. The Therapeutic Goods (manufacturing principles) Determinations 2018 outline the GMP requirements for manufacturers [27]. Compliance to applicable GMP Standards pertaining to each clinical trial phase of product is outlined in Table 2: Manufacturing Principles of Clinical Trials [24].

12 Genetically Modified Organisms

The Office of the Gene Technology Regulator (OGTR) and Institutional Biosafety Committees in Australia and by the Health Research Council Gene Technology Advisory Committee in New Zealand are responsible for ensuring the safety of products that fall under the definition of a genetically modified organism (GMO). *The Gene Technology Act 2000* defined as a GMO to an organism that has been technically genetically modified or is an organism that has inherited traits from an organism that has been modified by gene technology [28]. Australian Gene Technology Regulations 2001 requires any organisation manufacturing and supplying a GMO to be licensed by the OGTR who ensures there is a national regulatory system for gene technology activities [29]. This system incorporates national, state and territory laws that provide the OGTR with avenues to identify and manage risks relating to the health and safety of humans in a GMO environment, restrict unauthorised use of GMOs and monitor for non-compliance.

Table 2 Manufacturing principles of clinical trials

Relevant GMP code	Clinical trial phase	Product type
PIC/s guide to good manufacturing practice for medicinal products part 1	Phase II–IV	Medicines, biologicals that are from or contain live animal cells, tissues or organs, finished products
		Placebos
Australian code of good manufacturing practice for blood and blood components, human tissues and human cellular therapy products	Phase II–IV	Human blood, blood components, HPCs, biologicals that are from or contain human cells and tissues
Annex 13 PIC/s guide to good manufacturing medicinal products	Phase 0–IV	All
ICH guideline for good clinical practice with TGA annotations	Phase 0–IV	All
Relevant TGOs	Phase II–IV	All
Default standards	Phase II–IV	All

13 Quality Management System

A Quality Management System (QMS) forms the foundational requirements of all relevant Standards and Codes [30]. Table 3, Quality Management System Fundamentals, summarises the most common inclusions of a QMS designed to comply with the regulatory requirements for cellular therapies.

14 Conclusions

The regulation of cell therapy products within Australia promotes a risk-based approach to ensure the consistency, safety and efficacy of the products supplied within Australia. Cellular therapy products manufactured within Australia for commercial and beyond phase 1 clinical trials are subject to TGA licensing requirements. Overseas manufacturers may apply for an overseas GMP certification. The scope of the licensing can be broadened to cover multiple manufacturing sites. Careful planning combined with a risk-based analysis and early notification to the TGA will ensure products are licensed in a timely fashion.

Table 3 Quality management system fundamentals

Main parameter	Documentation required
Quality system	Quality manual
	Organisational chart
	Descriptions of quality and production nominees
	Change control/management
	Management review
	Traceability
Standard operating procedures and policies	
Documentation	Document control
	Document revision
	Document archive
Suppliers	Contract management
	Approved supplier management
Facility	Cleaning of facility
	Maintenance of the facility
	Monitoring
Control of material	Material specifications
	Goods receipt and storage
	Monitoring of storage areas
	Qualification of storage areas
Validation	Process qualification
	QC assay validation
	Validation master plan
Equipment	Equipment qualification
	Equipment maintenance
	Equipment calibration
	Equipment cleaning
Continuous monitoring	Internal audits
	Trend analysis
	Continuous improvements
	Product review
Quality control	Internal and external quality control
Training	Training procedures
	Competency based assessments
	Training records
	Staff induction
	Performance review
Manufacturing	Manufacturing procedures, sampling plans
Deviations	Management of nonconformances, corrective and preventative actions, method deviations, customer complaints, adverse events
Containment	Infection control
Donor selection	Consents, acceptance criteria, unique identifiers
Donor collection	Collection procedures, identification and health checks

(continued)

Table 3 (continued)

Labelling	Labelling procedure
Storage and release	Cryopreservation procedures
	Storage procedures
	Release procedures
	Disposal procedures

References

1. Therapeutic Goods Administration. Australian Therapeutic Goods Act 1989, Federal Register of Legislation, used with permission of the Australian Government. https://www.legislation.gov.au/Details/C2020C00028. Last accessed 20 Aug 2020.
2. Therapeutic Goods Administration. Australian Therapeutic Goods Regulations 1990, Federal Register of Legislation, used with permission of the Australian Government. https://www.legislation.gov.au/Details/F2020C00410. Last accessed 20 Aug 2020.
3. Australian Government, Therapeutic Goods Administration. What is Regulated as a Biological, used with permission of the Australian Government. https://www.tga.gov.au/what-regulated-biological. Last accessed 20 Aug 2020.
4. Therapeutic Goods Administration. (2018). *Therapeutic goods (Excluded goods) determination 2018. Federal register of legislation*, used with permission of the Australian Government. https://www.legislation.gov.au/Details/F2020C00367. Last accessed 20 Aug 2020.
5. Therapeutic Goods Administration. (2019). *Therapeutic goods (Things that are not biologicals) specification, 2019. Federal register of legislation*, used with permission of the Australian Government. https://www.legislation.gov.au/Details/F2019L01669. Last accessed 20 Aug 2020.
6. Therapeutic Goods Administration. Classification of Biologicals, used with permission of the Australian Government. https://www.tga.gov.au/sites/default/files/classification-biologicals.pdf. Last accessed 20 Aug 2020.
7. Federal Register of Legislation, Therapeutic Goods Administration. (2019). *Therapeutic goods (Things that are biologicals) determination No. 1 of 2011, 2019*, used with permission of the Australian Government. https://www.legislation.gov.au/Details/F2011L00894. Last accessed 20 Aug 2020.
8. Therapeutic Goods Administration. (2019). *Australian Regulatory Guidelines for Biologicals (ARGB)* used with permission of the Australian Government. https://www.tga.gov.au/autologous-human-cells-and-tissues-products-regulation. Last accessed 20 Aug 2020.
9. Therapeutic Goods Administration. (2013). *Australian code of good manufacturing practice human blood and blood components, human tissues and human cellular therapy products,* used with permission of the Australian Government. https://www.tga.gov.au/publication/australian-code-good-manufacturing-practice-human-blood-and-blood-components-human-tissues-and-human-cellular-therapy-products. Last accessed 20 Aug 2020.
10. PIC/s Guide to Good Manufacturing Practice for Medicinal Products, Pharmaceutical Inspection Convention Pharmaceutical Inspection Co-Operation Scheme, July 2018. https://www.tga.gov.au/sites/default/files/pe009-14-gmp-guide-part1-basic-requirements-medicinal.pdf. Last accessed 20 Aug 2020.
11. Therapeutic Goods Order No. 88 – Standards for donor selection, testing, and minimising infectious disease transmission via therapeutic goods that are human blood and blood components, human tissues and human cellular therapy products. 2013, Federal Register of Legislation, Therapeutic Goods Administration, used with permission of the Australian Government https://www.legislation.gov.au/Details/F2013L00854. Last accessed 20 Aug 2020.
12. Therapeutic Goods Order No. 83 human musculoskeletal tissue, 2011, Federal Register of Legislation, Therapeutic Goods Administration, used with permission of the Australian

Government https://www.legislation.gov.au/Details/F2011L01489. Last accessed 20 Aug 2020.
13. Therapeutic Goods Order No. 84 cardiovascular tissue, 2011, Federal Register of Legislation, Therapeutic Goods Administration, used with permission of the Australian Government https://www.legislation.gov.au/Details/F2011L01490/Explanatory%20Statement/Text. Last accessed 20 Aug 2020.
14. Therapeutic Goods Order No. 86 human skin, 2011, Federal Register of Legislation, Therapeutic Goods Administration, used with permission of the Australian Government https://www.legislation.gov.au/Details/F2011L01492. Last accessed 20 Aug 2020.
15. Therapeutic Goods Order No. 87 – General requirements for labelling of biologicals, 2011, Federal Register of Legislation, Therapeutic Goods Administration, used with permission of the Australian Government, https://www.legislation.gov.au/Details/F2011L01492/Explanatory%20Statement/Tex. Last accessed 20 Aug 2020.
16. Therapeutic Goods Order No. 94 (Standard for Haematopoietic Progenitor Cells derived from Cord Blood). Federal Register of Legislation, Therapeutic Goods Administration, used with permission of the Australian Government, https://www.legislation.gov.au/Details/F2017L01288/Explanatory%20Statement/Text. Last accessed 20 Aug 2020.
17. Therapeutic Goods Order No. 102 – Standard for Blood and Blood Components, 2019, Federal Register of Legislation, Therapeutic Goods Administration, used with permission of the Australian Government. https://www.legislation.gov.au/Details/F2019L00389. Last accessed 20 Aug 2020.
18. Therapeutic Goods Order No. 94 – Standard for Haematopoietic Progenitor Cells derived from Cord Blood, 2017, Federal Register of Legislation, Therapeutic Goods Administration, used with permission of the Australian Government. https://www.legislation.gov.au/Details/F2017L01288/Explanatory%20Statement/Text. Last accessed 20 Aug 2020.
19. Foundation for the Accreditation of Cellular Therapy. (2016). *International standards for cord blood collection, banking, and release for administration*, sixth edition, the Foundation for the Accreditation of Cellular Therapy and NetCord. July 2016, Omaha, Nebraska.
20. Therapeutic Goods Administration. (2018). *From biological standards*, used with permission of the Australian Government. https://www.tga.gov.au/biological-standards. Last accessed 20 Aug 2020.
21. Strum, M. (2015). Regulation policy for cell and tissue therapies in Australia. *Tissue Engineering and Regenerative Medicine International Society, 21*(23 and 25), 2797–2800. https://doi.org/10.1089/ten.tea.2014.0576
22. Therapeutic Goods Administration. (2020). *From special access scheme*, used with permission of the Australian Government. https://www.tga.gov.au/form/special-access-scheme. Last accessed 20 Aug 2020.
23. Therapeutic Goods Administration. (2018). *Therapeutic goods (Authorised supply of specified biologicals) rules*, used with permission of the Australian Government. https://www.legislation.gov.au/Details/F2018L0043. Last accessed 20 Aug 2020.
24. Clinical Trials. (2020). *Therapeutic goods administration*, used with the permission of the Australian Government. https://www.tga.gov.au/clinical-trials. Last accessed 20 Aug 2020.
25. International Council for Harmonisation of Technical Requirements for Pharmaceuticals in Human Use (ICH). (2016). *Integrated Addendum to ICH E6 (R1): Guideline for good clinical practice, ICH harmonised guideline*. https://www.tga.gov.au/publication/note-guidance-good-clinical-practice. Last accessed 20 Aug 2020.
26. NHMRC's National Statement on Ethical Conduct in Human Research (the National Statement). (2018). https://www.nhmrc.gov.au/about-us/publications/national-statement-ethical-conduct-human-research-2007-updated-2018. Last accessed 20 Aug 2020.
27. Federal Register of Legislation. (2018). *Therapeutic goods (Manufacturing Principles) determination, therapeutic goods administration*. https://www.legislation.gov.au/Series/F2017L01574. Last accessed 20 Aug 2020.

28. Federal Register of Legislation. (2000). *Gene technology act, therapeutic goods administration*, used with the permission of the Australian Government. https://www.legislation.gov.au/Details/C2016C00792. Last accessed 20 Aug 2020.
29. Federal Register of Legislation. (2001). *Australian gene technology regulations, therapeutic goods administration*, used with the permission of the Australian Government. https://www.legislation.gov.au/Details/F2019C00781. Last accessed 20 Aug 2020.
30. Trickett, A., & Wall, D. (2011). Regulation of cellular therapy in Australia. *Pathology, 43*(6), 627–634. https://doi.org/10.1097/PAT.0b013e32834b3cfa

Landscape for Regenerative Medicine Manufacturing in Japan

Ryu Yanagisawa and Yozo Nakazawa

1 Introduction

In the current Japanese regulatory environment, there are two main statutory laws for the development and clinical application of regenerative medicines including cell therapies and cell products: the Act on the Safety of Regenerative Medicine (RM Act) and the Pharmaceuticals, Medical Devices, and Other Therapeutic Products Act (PMD Act, Revised Pharmaceutical Affairs Act) (Fig. 1) [1, 2]. These laws serve different functions in their pathways toward the clinical provision of regenerative medicines. The RM Act applies to institutions that use regenerative medicines as therapies in clinical studies or private medical practice, whereas the PMD Act applies to companies developing regenerative medical products for marketing and commercial purposes (Fig. 2) [1, 2].

2 RM Act: Regulations for Medical Therapies and Studies

Before the RM Act came into force in 2014, there were no statutory regulations designed specifically for regenerative medicine therapies in Japan, and therapies were implemented under guidelines that were not enforceable by law. The RM Act was created to standardize the process by which therapies are provided to patients in a safe manner. The RM Act sets standards and safety criteria for the manufacturing and cell processing of regenerative medicines. This Act regulates practices with unapproved regenerative medicines, and it regulates clinical and physician-led

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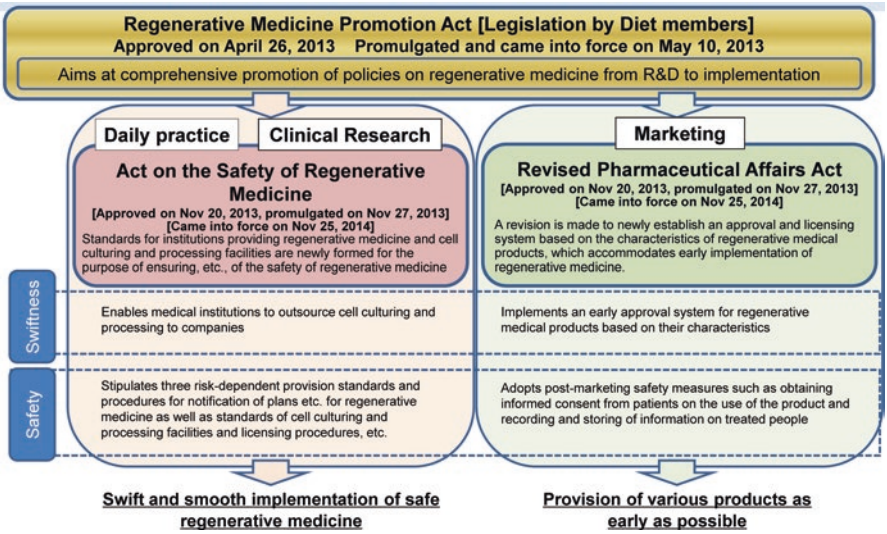

Fig. 1 Institutional framework for promoting the future implementation of regenerative medicine. Source: Ministry of Health, Labour and Welfare home page (https://www.mhlw.go.jp/english/policy/health-medical/medical-care/dl/150407-01.pdf) (accessed on 2021/05/31)

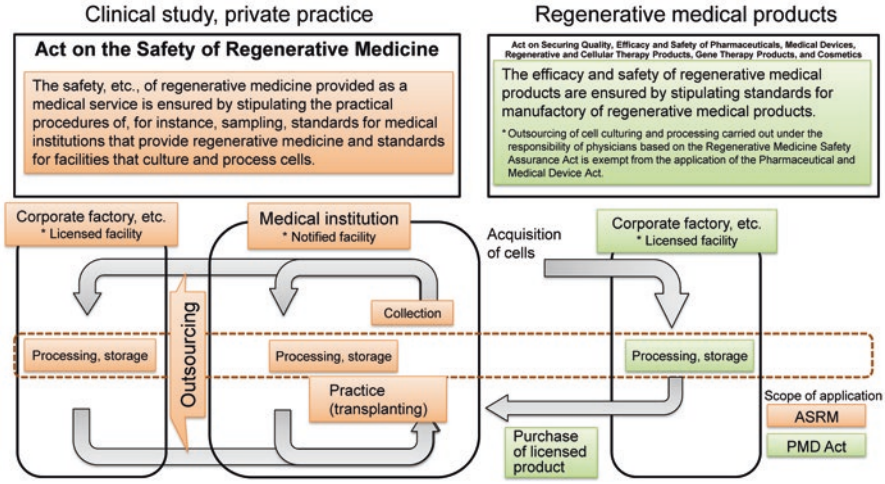

Fig. 2 Outsourcing cell culturing and processing under the RM Act (*PMD Act and RM Act*). Source: Ministry of Health, Labour and Welfare home page (https://www.mhlw.go.jp/english/policy/health-medical/medical-care/dl/150407-01.pdf) (accessed on 2021/05/31)

Table 1 Classification of regenerative medical technologies according to risk [3]

Category	Example of cells used for regenerative medicine	Example of treatments
Class I (high risk)	iPSCs, ESCs, cells into which a gene is introduced, xenogeneic cells, allogeneic cells	Transplantation of retinal pigment epithelium cells derived from autologous iPSCs, ex vivo gene therapy
Class II (medium risk)	Autologous somatic stem cells	Autologous mesenchymal stem cell infusion therapy for liver cirrhosis
Class III (low risk)	Autologous somatic cells	Cancer immunotherapy

Adapted from Konomi et al. [3]
iPSCs Induced pluripotent stem cells, *ESCs* Embryonic stem cells

studies that use processed cells within medical institutions. The recent amendment to the Act created a new system enabling outsourcing of cell processing, provided that the outsourced companies are inspected by the Pharmaceuticals and Medical Devices Agency (PMDA) and that these companies obtain a license from the Ministry of Health, Labour and Welfare (MHLW). Processed cells defined under this Act are referred to as specified cell products.

The RM Act permits institutions or certified outsourced companies to register a cell therapy under one of the three risk categories. The Act specifies Risk Class I, II, and III (high, medium, and low risk) for regenerative medical technologies based on the types of cells and risk to humans (Table 1, Fig. 3). Therapies using embryonic stem cells, induced pluripotent stem cells, and gene-modified cells such as chimeric antigen receptor T cells are categorized as Class I (high risk); therapies that use autologous somatic stem cells are categorized as Class II (medium risk); and therapies that use autologous somatic cells are categorized as Class III (low risk). There is a specific approval procedure for registration in each risk class. Under the RM Act, Classes I and II are required to receive a higher level of review from a Specially Certified Regenerative Medicine Committee and Class III is required to receive a review from a Certified Regenerative Medicine Committee. These certified committees, which can be within or outside medical institutions, operate as an Institutional Review Board and are accredited by the MHLW to examine provision plans.

3 PMD Act: Regulations for Products

The PMD Act, the official name for the revised Pharmaceutical Affairs Law, created a stand-alone category, regenerative medical products. Regenerative medical products are defined as processed human cells that are intended to be used (1) for either (a) the reconstruction, repair, or formation of structures or functions of the human body or (b) the treatment or prevention of human diseases, or (2) for gene

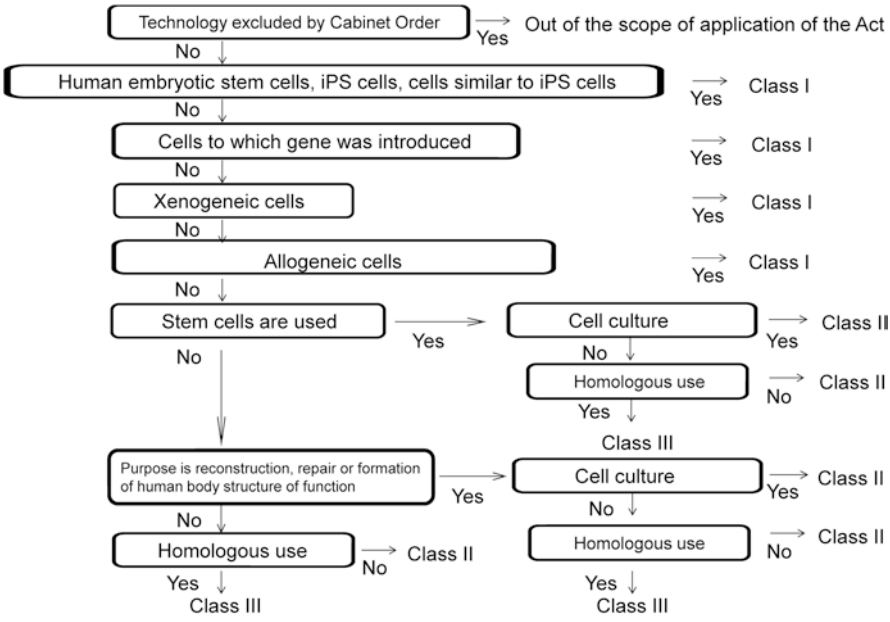

Fig. 3 Risk classification of Class I, Class II, and Class III regenerative medical technology. Source: Ministry of Health, Labour and Welfare home page (https://www.mhlw.go.jp/english/policy/health-medical/medical-care/dl/150407-01.pdf) (accessed on 2021/05/31)

therapy [4]. Blood transfusion (blood products), hematopoietic stem cell transplantation, assisted reproductive technology, except those derived from genetic engineering, and iPS cells are not included in the scope of this category [4].

The PMD Act also institutes a conditional and term-limited marketing authorization system for regenerative medical products followed by a reapproval procedure within a specified period (7 years maximum) for full approval (Fig. 4). This is due to the non-uniform nature of regenerative medical products, which require a long period of time for data collection and efficacy evaluation. Although, as a rule, regenerative medical products must undergo the traditional regulatory approval process as with pharmaceuticals and medical devices, conditional and term-limited approval is granted if the presumed efficacy and safety are demonstrated. Both traditional and conditional, term-limited approvals are determined by PMDA and MHLW.

4 Additional Regulations and Guidance Documents

Under the PMD Act, there are numerous regulations and guidance documents. Specifically, there is the Ministerial Ordinance for Good Manufacturing Practice (GMP) for Drugs and Quasi-drugs. However, given that cells and other related materials from which regenerative medical products are derived have difficulties

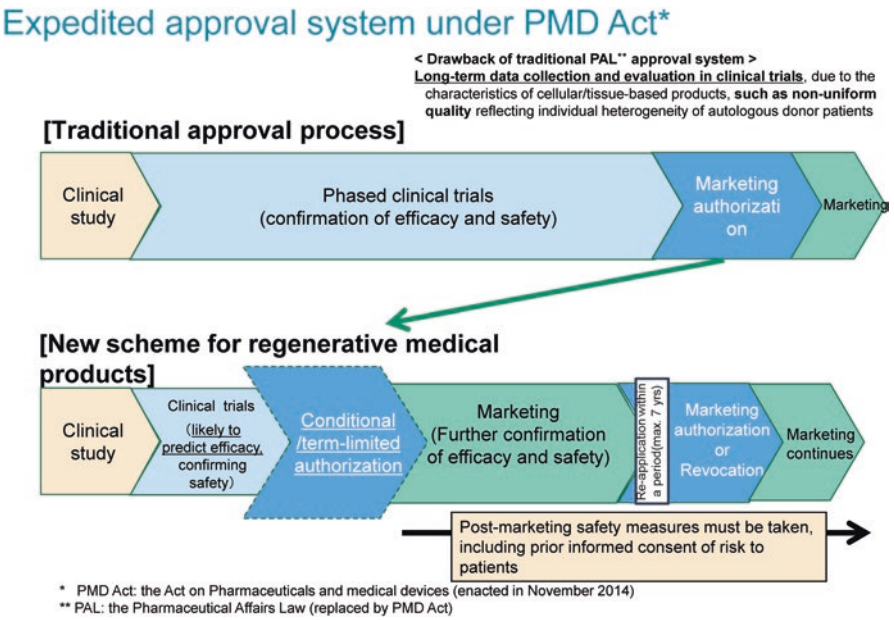

Fig. 4 Expedited approval system under the PMD Act. Source: Ministry of Health, Labour and Welfare home page (https://www.mhlw.go.jp/content/11123000/000335149.pdf) (accessed on 2021/05/31)

such as in sterile control, good gene cell and tissue (manufacturing) practices (GCTP) were introduced to indicate essential elements of a quality management system to be applied to regenerative medical products (Table 2). Additional regulations and guidance documents are summarized by Azuma K at https://link.springer.com/content/pdf/10.1007/s40778-015-0012-6.pdf (Note: regenerative medicines other than cell therapy are included) [5]. Institutions and companies are encouraged to engage in regulatory consultation provided by the PMDA.

Note that recent amendments are not included in this summary. Close monitoring of the content of future enforcement ordinances will be necessary.

Table 2 Summary of regenerative medicine scheme under the RM Act and PMD Act

	RM Act	RM Act	PMD Act	PMD Act
Scheme	Clinical research	Medical treatment	Clinical trial	Medical treatment
Purpose	Research (not for marketing approval)	Medical treatment	Application for marketing approval	Medical treatment
Review requirements before clinical use	Certified IRB approval, MHLW submission (90-day review for class I)	Certified IRB approval, MHLW submission (90-day review for class I)	30-day review by MHLW/PMDA, IRB approval	MHLW marketing approval
Responsibility for safety and quality of regenerative medicine	Physician and medical institutions	Physician and medical institutions	Physician and medical institutions (investigator-initiated trial) or company (company-sponsored trial)	Company
Manufacturing facility registration	License (outside medical institution in Japan)/accreditation (foreign)/notification (within medical institution)	License (outside medical institution in Japan)/accreditation (foreign)/notification (within medical institution)	Not required	License (domestic)/accreditation (foreign)
Manufacturing facility requirements	RM Act Art. 42,44	RM Act Art. 42,44	GMP for investigational products	GCTP
Standards for clinical practice	Provider rule (RM Act Art. 3 to 25)	Provider rule (RM Act Art. 3 to 25)	GCP	Post-market safety requirements (PMD Act Art. 68–2 to 68–15)
National Health Insurance	Not covered (in principle)	Not covered (in principle)	Partially covered	Fully covered (in principle)

Adapted from Azuma et al. [5]

IRB Institutional Review Board (Certified Regenerative Medicine Committee under the RM Act), *GCP* Good clinical practice, *GMP* Good manufacturing practice, *GCTP* Good gene, cellular, and tissue-based products manufacturing practice, *MHLW* Ministry of Health, Labour and Welfare, *PMDA* Pharmaceuticals and Medical Devices Agency, *PMD Act* Pharmaceuticals and Medical Devices Act, *RM Act* The Act on the Safety of Regenerative Medicine

References

1. Ministry of Health, Labour and Welfare website. https://www.mhlw.go.jp. Last accessed 31 May 2021.
2. Pharmaceuticals and Medical Devices Agency website. https://www.pmda.go.jp. Last accessed 31 May 2021.
3. Konomi, K., Tobita, M., Kimura, K., & Sato, D. (2015). New Japanese initiatives on stem cell therapies. Cell Stem Cell, 16, 350–352.
4. Pharmaceuticals and Medical Devices Agency website. https://www.pmda.go.jp/files/000199505.pdf. Last accessed 31 May 2021.
5. Azuma, K. (2015). Regulatory landscape of regenerative medicine in Japan. Current Stem Cell Reports, 1, 118–128.

GLP Regulations for Nonclinical Studies

Aisha Khan, Yee-Shuan Lee, and Joshua M. Hare

1 Introduction

GLP (21 CFR PART 58) [1] is a set of regulations under which laboratory studies are planned, performed, monitored, recorded, and reported. GLP standards promote quality and validity of test data so that experiments can be reproduced [1] at any time in the future. GLP principles provide a secure research environment that helps to protect data from manipulation during and after testing procedures. It also incorporates all of the organizational structures of research procedures. GLP not only regulates the personnel who work in a laboratory and research facility but also applies to computerized systems used for research purposes [2–5].

GLPs comprise a set of internationally harmonized regulations, mandated by the Environmental Directorate of the Organization for Economic Cooperation and Development (OECD) [6]. The purpose of GLPs is to foster advancement of the quality and validity of test data. These evaluations are based on safety testing for quality, rigor, and reproducibility. It is important to understand that creating and managing conditions under which laboratory studies are planned, performed, monitored, recorded, and reported is a management responsibility. GLP only applies to nonclinical studies and testing. This distinction is important because clinical studies are governed by Good Clinical Practices (GCP), the Declaration of Helsinki, and other regulations intended to protect human participant safety [3]. The role of the Study Doctor is particularly important [7] as this individual is responsible for the roles and responsibilities, oversight, and execution of all aspects of the nonclinical study [8].

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2 Elements of GLP

GLP is not a scientific management system – it is a quality management system (Fig. 1). It defines a set of quality standards for study conduct, data collection, and reporting. It does not specifically define scientific standards, but if a study follows GLPs, we can be reasonably sure that the reported results were collected as outlined in the study protocol. However, we cannot be sure that the study actually addressed a scientific hypothesis [9].

Bringing a novel cellular product from research and development (R&D) to clinical application ("bench to beside") is costly, time consuming, and demanding for most scientists, laboratories, and principal investigators [10]. The clinical protocol application (Investigational New Drug [IND] application) requires supporting data on product-manufacturing in the Chemistry, Manufacturing, and Control (CMC) section of the IND application. The nonclinical study data used to support the clinical studies is included in the application and is also subject to FDA audit and investigation. A well-structured and well-designed product development process facilitates smoother transition, optimizes resources, reduces cost, ensures integrity, and gives credibility to the data in fulfilling regulatory requirements [4, 9, 10]. The laboratory studies are designed to support the concept of therapeutic use of the cellular therapy product and should be integrated with studies on the proposed methods for manufacturing and testing the final drug product. The content of these sections should be guided by applicable GLP and GMP regulations [9, 11, 12]. GLP regulates all nonclinical safety studies that support or are intended to support applications for research regulated by the FDA. This includes, in addition to biological products, medicinal and veterinary drugs, aroma and color additives in food, and nutrition and supplements for livestock [13, 14].

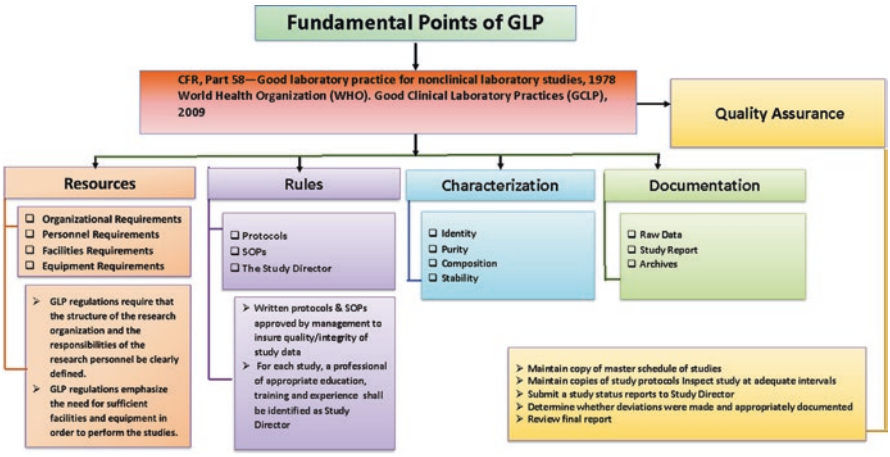

Fig. 1 Fundamental points of GLP

The objective of GLP is to provide a framework for quality system which includes [1, 9]:

- Adoption of good and safe operating procedures and use of a recording system that promotes the development of quality test data
- Comparison quality of test data to avoid duplicative testing
- Avoiding the creation of technical barriers to trade
- Prevention of human error in the performance of the job and prevention of equipment errors in measurements
- Prevention of unsafe and hazardous acts which could affect humans
- Improvement of the protection of human health and the environment

3 Nonclinical Studies Are Conducted to Support Clinical Trials

The preclinical stage of research, during which important feasibility, iterative testing, and safety data are collected [10], is conducted before the clinical trials. Pivotal nonclinical safety studies in animals must be conducted under carefully controlled conditions to achieve acceptance by regulatory agencies. Animal studies are often referred to as "nonclinical" or "preclinical," but the preferred term in GLP guidelines is "nonclinical." The main goal of nonclinical studies is to determine the product's ultimate safety profile. There is a sequence to the GLP studies which need to be performed [3–16], as shown in Fig. 2. The GLP regulations require the following studies:

3.1 Toxicology Studies to Demonstrate Safety

Successful nonclinical toxicology studies require contributions from study directors, toxicologists, pathologists, veterinarians, surgeons, regulatory specialists, and support personnel [9, 10, 13–16]. Toxicology studies are used to characterize the toxicity profile of a drug by identifying its impact on organ structure and functionality. This includes assessment of the severity and reversibility of toxicity, as well as dose ranges and their relationship to drug exposure. These toxicology studies aid in determining if, and to what degree, the biologic's toxicity is dose-dependent, species-specific, mechanism-related, and related to the method of administration [9, 10, 13–16]. By understanding the injuries that could occur to any vital organ, toxicology studies help determine the safety of a test article at its expected clinical dose. Not only do toxicology studies frame trial guidance related to duration, administration routes, and dose escalation, they also help to set the parameters for clinical monitoring [15, 17–19]. The following issue must be considered when performing toxicology studies:

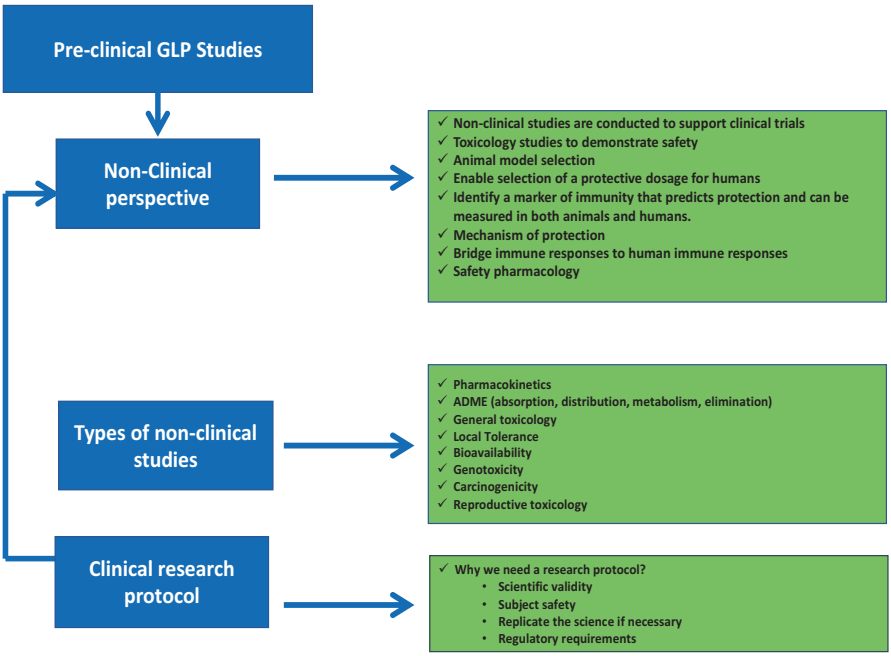

Fig. 2 Preclinical GLP studies

- *Model selection* – Animal studies must demonstrate that the therapy is likely to clinically benefit humans. Toxicology testing is required in a minimum of two species (one rodent and one non-rodent) by the US FDA and other regulatory agencies [15, 16].
- *Enabling the selection of a protective dose for humans* – Preclinical toxicity Toxicology studies help determine if the test article is safe for initial tests in humans and define the margins between therapy and overdose. Adverse events noted during preclinical evaluations help clinicians monitor test subjects for potentially unsafe effects. The knowledge of target organ effects related to toxicity help clinicians closely monitor patients and limit or withdraw therapy to prevent risk to human life or well-being. Thus, toxicology studies aid in the understanding of both the severity and reversibility of toxic effects produced by a test article on a human body Toxicology in general and on target organs specifically. Toxicology studies evaluate wide dose ranges and how they relate to systemic exposure in the body. Studies help researchers determine exposure linearity with respect to dose, metabolic saturation, accumulation of compound and steady-state exposures, and related potential adverse events and the systemic exposures where they occur [15, 16].
- *Identification of a marker of immunity* – That predicts protection and can be measured in both animals and humans [11, 17, 20].

Mechanism Toxicology

- *Off-target studies* – Specifically, these studies allow for the identification of off-target pathways that may be activated by the test article. These pathways and subsequent reactions can be effectively monitored to improve dosing [11, 17, 20].
- *Bridging of immune responses to human immune responses* – If the test article has the potential to affect immune function, additional immune toxicity testing should be considered. Information obtained from the nonclinical pharmacology studies on the ability of the test article to affect the immune system could be used in a weight-of-evidence approach to decide if additional immune toxicity studies are needed [11, 17, 20].
- *Safety pharmacology* – Studies conducted to establish the pharmacodynamic effects, the mode of action, and potential side effects should be evaluated. Consideration should be given to the significance of any issues that arise [11, 17, 20].

3.2 Pharmacokinetics

The assessment of the pharmacokinetic (PK), toxico-kinetic, and metabolism data should address the relevance of analytical methods used, the pharmacokinetic models, and the derived parameters [11, 17, 20]. The purpose of PK studies is to evaluate the absorption, distribution, metabolism, and excretion of the product in the target species. Therefore, the final product, or a formulation which has comparable characteristics in terms of bioavailability as the final product, must be administered to the target animal species at the maximum recommended dose [11, 17, 20].

- *Absorption* – Absorption is the process by which a test article enters the bloodstream [11, 17, 20]. There are many possible routes of administration, but the two most common are intravenous and oral. If a test article is administered intravenously, the absorption phase is skipped as the test article immediately enters circulation. However, many test articles are dosed orally because it makes it possible for patients to self-administer.
- *Distribution* – Distribution describes the reversible transfer of a test article from one location in the body to another [9, 11, 16, 17, 20].
- *Metabolism* – Metabolism of a test article involves enzymes, and several investigative studies may be needed to identify major metabolites and relevant metabolic pathways [9, 11, 16, 17, 20].
- *Excretion* – Excretion is the irreversible loss of a substance from the system. In most cases, all test article-related material, including parent drug and metabolites, are eventually cleared from the body. It is important to characterize which routes of excretion are most important [9, 11, 16, 17, 20].

3.3 Local Tolerance

Local tolerance studies are used to evaluate any potential adverse events at the test article administration site and are most often performed with parenteral administrations to determine whether there is any irritation or other undesired effects at the injection site [9, 11, 16, 17, 20]. To perform local tolerance studies in animal species under preclinical evaluation, the route of administration should be maintained the same as that to be employed in clinical administration.

3.4 Bioavailability

Bioavailability results provide information on the percentage of test article that is absorbed by the body as defined by quantity in plasma. The term bioavailability refers to that fraction of the pharmaceutical product administered as an unchanged test article that reaches the systemic circulation following an extravascular dose [9, 11, 16, 17, 20].

3.5 Genotoxicity

Genetic toxicology studies are conducted to assess the potential for induction of genetic mutations or chromosomal damage. Determination of a compound's potential genotoxicity is an important component of a complete safety assessment of all new products. By identifying genotoxicity at an early stage in drug discovery rather than during regulatory assessment, the likelihood of late-stage failure is reduced [9, 11, 16, 17, 20].

3.6 Carcinogenicity

The objectives of carcinogenicity studies are to identify a tumorigenic potential in animals and to assess the relevant risk in humans. Any cause for concern derived from laboratory investigations, animal toxicology studies, and data in humans may lead to a need for carcinogenicity studies [9, 11, 16, 17, 20].

3.7 Reproductive Toxicology

Reproductive toxicity studies are designed to investigate the effect of a test article on male and female fertility and reproductive performance, estrous, spermatogenesis, implantation, embryo-fetal survival and development, gestation length, parturition, lactation, pup survival and development, and the ability of offspring from the exposed parental generation to reproduce normally [9, 11, 16, 17, 20].

4 Stages of Development: Discovery to Clinical Trials

The development of clinical trials can be separated into four major stages as shown in Fig. 3. The first stage is discovery where potential new drug or therapy are being developed in the research laboratory that are non-GLP. This discovery research phase is more exploratory and are only documented in a general research capacity. This phase involves heavily in proof of concept and many trial and error to show success and efficacy.

Once the product is developed in the discovery phase, it will move into Stage 2 which is the nonclinical development phase. In this phase, all the studies should be

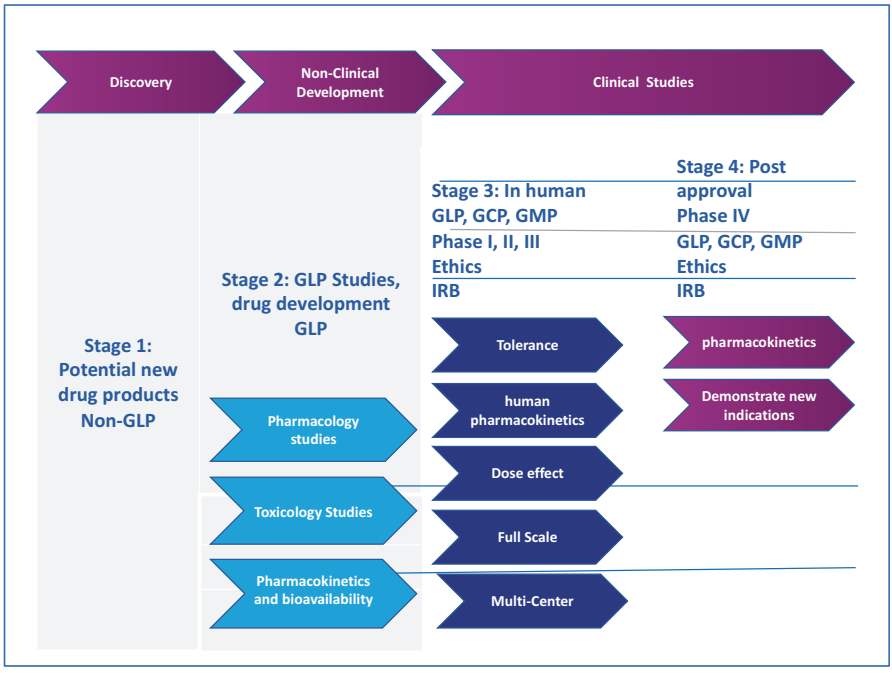

Fig. 3 Stages of development: Discovery to clinical trials

conducted under GLP because this is the most critical stage for translating from bench side into clinical application. At this stage, in vitro and in vivo studies will be performed to confirm the safety, toxicology, potency, purity, and identity of the developed drug. Performing this stage under GLP regulations is essential in order to ensure the integrity and accuracy of the data that will be used as the foundation for applying for an IND and approval of a clinical trial. In addition, process validation must be performed to validate the manufacture method that will be used to produce the clinical product. Process optimization will also occur in order to satisfy GMP regulations or to develop scale-up manufacture process. Any analytical testing performed in this stage should satisfy and adhere to GLP regulations.

The last two stages are clinical trials. Stage 3 involves Phases I, II, and III where the safety, tolerance, dose effect, and efficacy are tested first with small-scale then move toward large-scale testing on patients. Stage 4 involves Phase IV clinical trial where it is the final stepping-stone toward having the drug approved for the intended indication. In these two stages, all products are manufactured following GMP regulations where all the other testing must adhere to GLP regulations. The administration of the drug in the clinics must adhere to GCP regulations.

5 Research Protocol

The research protocol should be produced and approved by the Quality Assurance Unit (QAU) prior to initiate any studies. The goal of the research protocol is to satisfy the regulatory requirements to produce results that demonstrate scientific validity and replicability of the product and that it is safe to transition into the next stage for clinical trials.

The research protocol, similar to an IND as shown in Fig. 4, should state clearly the (1) title and purpose of the study, (2) the facility and personnel, (3) quality

Fig. 4 Sample of a study protocol

assurance, (4) study design, (5) detail description of the test articles and controls, (6) animal care and welfare, (7) method of evaluation in detail description, (8) method of euthanasia in detail, (9) statistic methods, (10) study report, and (11) data and specimen retention.

Detailed information must be obtained for any nonclinical studies in order to assure the quality of the experiments and their results. All test samples and specimen must be retained when possible, and all raw data should be recorded. These results are critical in providing the evidence to translating the product into clinical trials.

6 Background/History

6.1 Biologics Control Act of 1902 and Its Revolution

Regulation of biological products in the United States was first introduced as the "Biologics Control Act of 1902," which was the first law that implemented federal regulations of biological products in the United States [6]. This law was established in response to two tragic incidences both related to vaccines. In 1901, diphtheria patients were routinely treated with antitoxin derived from the blood serum of horses. At the time, there were no central or uniform regulations for the production of antitoxin, and it was often manufactured in local plants. In St. Louis, Missouri, this absence of regulations directly resulted in the death of 13 children after being treated with diphtheria antitoxin made from the blood of a tetanus-infected retired, milk wagon horse named Jim. Soon after this, a similar tragedy happened in Camden, New Jersey, involving deaths and injuries related to a tainted smallpox vaccine. Therefore, the Congress enacted the "Biologics Control Act of 1902."

The Laboratory of Hygiene of the Marine Hospital Services was in charge of testing biologics prior to the Biological Control Act. Following the passing of the Biological Control Act, the lab was renamed as the Hygienic Laboratory of the Public Health and Marine Hospital Service and was responsible for renewing licenses, testing products, and performing inspection to the facilities that produces the biological products. In 1948, the center was renamed as the National Institute of Health, and now it has a large role in public health research and dedicated centers to biomedical research. In 1972, the regulations have moved to the FDA [6] and later known as the "Center for Biological Evaluation and Research" (CBER).

6.2 Establishment of GLP

In the early 1970s, the FDA was alerted of poor laboratory practices and fraudulent reporting of test results in the Industrial BioTest Labs scandal. The FDA performed an in-depth facility investigation of more than 40 facilities [3]. The investigation

resulted in the FDA finalizing the GLP [7]. In response to the finding, the FDA finalized the Food Laboratory Practice (GLP) Regulations, 21 CRF part 58 on December 22, 1978, which became effective in June 1979 [4, 8]. The main purpose of GLP is to monitor the integrity and quality of product-related toxicity and safety of the intended clinical product. In 1981, the Organization for Economic Co-operation and Development (OECD) established GLP principles that are now the international standard.

7 Why GLP Regulations Are Needed for Preclinical Studies

The goal of GLP regulations is to ensure the methodologies and tools used are standardized to promote and ensure consistency, safety, reliability, integrity, and quality of test results during nonclinical and laboratory testing [1]. Nonclinical studies provide detailed information from in vitro and/or in vivo to evaluate potential harm or toxicity of the intended clinical products. A list of preclinical studies is shown in Fig. 3. For pharmacological agents, the efficacy and pharmacodynamics need to be well understood and defined. For cellular products, the characterization and potency of the cellular products need to be well defined. Other properties such as dosing, safety including evaluation of tumorigenicity, genetic stability, and viral and other contaminant due to processing are also evaluated. Analytical methods and assays developed for raw material or cell characterization, in-process testing, and quality control release of final products needed to be regulated and documented during the development phase in order ensure the consistency and comparability of data throughout the life cycle of the product [9, 11, 16, 17, 20].

Process optimization including scale-up production to support clinical application, and other components is critical for cellular products where all processes need to be validated prior to manufacturing for clinical products [9, 11, 16, 17, 20]. It is critical to produce reliable and consistent results from nonclinical studies where they are crucial and are the major determinant for translating into clinical trials for patients in an IND application. Therefore, it is important to conduct these in vitro and in vivo studies by following GLP regulations that set the minimum requirements to ensure the integrity and quality of the studies.

8 FDA's Proposed Rules

Guidelines that define GLP expectations for nonclinical studies undertaken to register new medical products have been produced by multiple regulatory agencies, starting with the FDA in 1978.

8.1 History of Relevant FDA Documents: 1970–2017

- The Congress proposed and enacted Good Laboratory Practice regulations for the FDA as part of the Federal Food, Drug, and Cosmetic Act (FD&C).
- 21 CFR Part 58 Good Laboratory Practices for Nonclinical Studies.
- The proposed regulations for Good Laboratory Practice were published in the Federal Register on November 19, 1976.
- The Good Laboratory Practice regulations, Final Rule was published in the Federal Register on December 22, 1978.
- Federal Register of October 29, 1984 (49 FR 43530), the FDA published a proposal to amend the agency's regulations in 21 CFR Part 58.
- FDA (US Food and Drug Administration). 1987. 1987 Final Rule – Good Laboratory Practice Regulations. https://www.fda.gov/ICECI/Inspections/NonclinicalLaboratoriesInspectedunderGoodLaboratoryPractices/ucm072706.htm.
- FDA (US Food and Drug Administration). 2001. Food and Drug Administration Compliance Program Guidance Manual. Chapter 48: Bioresearch Monitoring – Good Laboratory Practice (Nonclinical Laboratories). https://www.fda.gov/downloads/iceci/enforcementactions/bioresearchmonitoring/ucm133765.pdf.
- US Food and Drug Administration. "Guidance for Industry. Good Laboratory Practices. Questions and Answers," July 2007. http://www.fda.gov/downloads/ICECI/EnforcementActions/BioresearchMonitoring/UC M133748.pdf.
- CFR (Code of Federal Regulations). 2011. Title 21 – Food and Drugs. Chapter I – Food and Drug Administration. Subchapter A – General. Part 58: Good Laboratory Practice for Nonclinical Laboratory Studies. Washington, D.C.: US Government Publishing Office. https://www.gpo.gov/fdsys/granule/CFR-2011-title21-vol1/CFR-2011-title21-vol1-part58.
- FDA (US Food and Drug Administration). 2016. FDA regulations relating to Good Clinical Practice and clinical trials. https://www.fda.gov/ScienceResearch/SpecialTopics/RunningClinicalTrials/ucm155713.htm#FDARegulations.
- FDA (US Food and Drug Administration). 2017. Inspections, Compliance, Enforcement, and Criminal Investigations: Nonclinical Labs Inspections List from 10/1/2000 through 3/31/2017. https://www.fda.gov/ICECI/Inspections/NonclinicalLaboratoriesInspectedunderGoodLaboratoryPractices/UCM413586.

9 The Fundamental Points of GLP

The goal of GLP is to provide guidelines to monitor the organizational process and the conditions under which nonclinical studies are planned, performed, monitored, recorded, archived, and reported to ensure the integrity and quality of the nonclinical laboratory studies. These regulations focus on the resources, rules,

characterization, documentation, and quality assurance of the institute that will perform these nonclinical studies [1] as shown in Fig. 1.

Resources are not only the equipment, materials, and supplies needed for performing the nonclinical studies but also include the individuals who are involved in different aspects of the studies and the organizational and facility support. Providing and defining clear functions and needs ensures the quality of the results. A Study Director should be identified prior to the start of the studies in order to facilitate the coordination of different aspects of the nonclinical studies [21]. The Study Director is a professional of appropriate education, training, and experience and has overall responsibility for the technical conduct, including interpretation, analysis, documentation, and result reporting. He or she is the single point of study control, assures protocol is approved and experiments follow active SOPs [21]. He or she also has the responsibility of approving experimental data, including unanticipated responses are accurately recorded and verified and unforeseen circumstances which may affect the quality and/or integrity of the study. This information must be documented and include any of its corrective actions. Each facility or operation should have an onsite Quality Assurance Unit (QAU) [22]. Its function is to ensure independent monitoring of the studies and assure management that facilitates, equipment, personnel, methods, practices, records, and controls conform to GLP. Deviation of process will be reported to the Study Director, and corrective action shall be taken to avoid mistakes in the future. The QAU will also ensure that all personnel understand their roles in the organization and provide training to perform their role prior to their assignment. Sufficient personnel should be assigned to any task to complete it in a timely manner [23]. The personnel shall wear appropriate clothing, and necessary personal sanitation and health precautions must be taken for their job function to prevent contamination of test and control samples and testing systems. Individuals with illness, which may adversely impact the quality or the integrity of the study, must be excluded from direct contact of the study within the duration of the illness.

The subparts of 21 CFR 58 address organization and personnel, facilities, equipment, testing facilities operation, test and control articles, protocol for and conduct of a nonclinical laboratory study, and records and reports [16] as shown in Fig. 5.

Subpart A: General provisions

- 58.15 – Inspection of a testing facility

Subpart B: Organization and personnel

- 58.29 – Personnel
- 58.31 – Testing facility management
- 58.33 – Study director
- 58.35 – Quality assurance unit

Subpart C: Facilities

- 58.41 – General
- 58.43 – Animal care facilities
- 58.45 – Animal supply facilities

- 58.47 – Facilities for handling test and control articles
- 58.49 – Laboratory operation areas
- 58.51 – Specimen and data storage facilities

1.0	Facilities	Yes	No
1.1	Size - suitable for the conduct of tests, writing, storage of samples and documents.	☐	☐
1.2	Segregation - chemicals, pharmacological and microbiological substances are separated	☐	☐
1.3	Cleanliness – cleanable lab counters, floors, and walls.	☐	☐
1.4	HVAC – adequate HVAC to provide optimum environmental conditions for the animals.	☐	☐
1.5	Waste disposal – adequate and under bio-hazard regulations	☐	☐
1.6	Safety manual – address the employee safety and fire regulations	☐	☐
1.7	Emergency power supply – connected to essential equipment	☐	☐

2.0	Personnel	Yes	No
2.1	Job descriptions – appropriate details of all functions and CVs	☐	☐
2.2	Supervision – Scientific and technical	☐	☐
2.3	Training Program – Technical training, continuing education, safety courses, animal safety training, relevant experience	☐	☐
2.4	Adequate in number – to conduct the operations	☐	☐

3.0	Equipment	Yes	No
3.1	Calibrated and validated – all equipment in use is validated and calibrated	☐	☐
3.2	Preventive maintenance program - Regular maintenance of equipment	☐	☐
3.3	Quality control (QC) program – Defined QC schedule	☐	☐
3.4	Defective equipment program – Program with defined measures to take	☐	☐
3.5	Operational SOPs – Operational instructions	☐	☐
3.6	Installation Qualification – performed by vendor or trained staff	☐	☐

Fig. 5 GLP checklist

4.0.	Reagents	Yes	No
4.1	Qualification – qualify all regent according to the study protocol	☐	☐
4.2	Expiration dates – if not assigned by manufacturer, set up a timeline	☐	☐
4.3	Specifications – review certificate of analysis	☐	☐
4.4	Storage - properly stored as per manufacturer's instructions	☐	☐
4.5	Labeling – document expiration date and concentration	☐	☐

5.0.	Documentation	Yes	No
5.1	Quality assurance – all documents reviewed by QA	☐	☐
5.2	Control – current documents in circulation, obsolete ones archived	☐	☐
5.3	Errors – can be read and initialized	☐	☐
5.4	Process flow - self-explanatory forms and protocols	☐	☐
5.5	Hand written notes – legible	☐	☐

6.0.	Animal Facilities	Yes	No
6.1.	Animal care – adequate space, separation, quarantine, and cleanliness	☐	☐
6.2.	Animal waste – follow regulations	☐	☐
5.3.	Feed – properly stored, separated and free of contamination	☐	☐
5.4.	Health monitoring – ongoing observation	☐	☐

7.0.	Testing Procedures	Yes	No
7.1.	Standardized procedures – ensure quality, integrity and the validity	☐	☐
7.2.	Repeat testing – describe process to repeat	☐	☐

8.0.	Product Specifications	Yes	No
8.1.	Approvals – QA and study director approvals	☐	☐
8.2.	Change in specifications – revalidation	☐	☐
8.3.	Storage conditions – validated ranges	☐	☐

Reviewed by Quality Assurance: _____Date: _____

Fig. 5 (continued)

Subpart D: Equipment

- 58.61 – Equipment design
- 58.63 – Maintenance and calibration of equipment

Subpart E: Testing facility operation

- 58.81 – Standard operating procedures
- 58.83 – Reagents and solutions
- 58.90 – Animal care

Subpart F: Test and control articles

- 58.105 – Test and control article characterization
- 58.107 – Test and control article handling
- 58.113 – Mixtures of articles with carriers

Subpart G: Protocol for and conduct of a nonclinical laboratory study

- 58.120 – Protocol
- 58.130 – Conduct of a nonclinical laboratory study

Subpart J: Records and reports

- 58.185 – Reporting of nonclinical laboratory study results
- 58.190 – Storage and retrieval of records and data
- 58.195 – Retention of records

9.1 Rules

All personnel who will be handling any aspect of the experiment should be well trained in GLP regulations and in performing the established SOP to ensure the quality of the studies [23]. Personnel involving in any in vivo studies should have previous experience working with animals and familiar with IACUC regulations. Having the animal experience might be extremely helpful in identifying any abnormal activity of the animals and reported to the QAU or the organization. The facility requirements are extremely important for the welfare of the animals when conducting the in vivo studies [24]. The animals should always be housed according to regulations in order to rely on conclusion from the result of the study. Animal care facilities should have sufficient number of animal rooms or areas to assure proper separation of (1) species, (2) testing systems, (3) isolation of individual projects, (4) animals under quarantine, (5) routine and specialized animal housing, and (6) to ensure isolation from biohazardous, volatile, radioactive, or infectious agents [24]. Animal care facilities should also have separate areas for diagnosis, treatment, and control of lab animal disease and storage areas for feed, bedding, supplies, and equipment [25, 26]. Pest control materials should not interfere with the ongoing studies and shall be documented [26]. Any abnormal housing condition may affect or produce unrelated symptoms that may not be directly related to the intended clinical product.

When conducting a well-thought-out experiment, documenting each step of the way is essential in order to observe any abnormalities [27]. Standard Operating Procedures (SOPs) should be written and approved by the management and QAU to ensure quality and integrity of study data collection [29]. After the SOP is effective, any significant planned changes need to acquire written approval from the management and QAU prior to making them effective. Any deviation from the SOP should be documented in raw data and reported and authorized by the Study Director. A historical file of the SOPs and their revisions should be maintained and monitored by QAU. The active SOPs should be available to onsite personnel. The staff who will perform the functions based on the SOPs should be properly trained in their use. The SOPs for in vivo studies must include, but not limited to, (1) animal room preparation and animal care; (2) receipt, ID, storage, handling, mixing, and sampling of test and control test subjects; (3) test system observations; (4) laboratory tests; (5) handling of moribund or dead animals; (6) necropsy or postmortem examinations; (7) collection and ID of specimens; (8) histopathology; (9) data handling, storage, and retrieval; (10) maintenance and/or calibration of equipment; and (11) transfer, placement, and ID of animals [28].

9.2 Characterization and Documentation

For each experiment, samples targeted for safety testing such in mycoplasma, sterility, and endotoxin should be tested when applicable. The identity, purity, composition, and stability of the products should be evaluated following the approved SOP. The raw data will be kept and it will be used to prepare for the study report [23]. For any test to be conducted, a proper control must be maintained at all times to ensure the quality of the test results [30]. All raw data, documentation, protocols, specimens, and final reports are archived during and after the study [14]. For any given study, the QAU shall (1) maintain copies of master schedule of studies and study protocols, (2) inspect study at adequate intervals, (3) submit a study status reports to the Study Director, (4) determine whether deviations were made and appropriately documented, (5) review final report, (6) maintain written QAU responsibilities and procedures, and (7) certify upon FDA request that inspections are being implemented, performed, and documented [12, 14, 15].

10 Regulatory Strategy

The importance of early engagement of relevant regulatory agencies is imperative for acceptance of nonclinical safety data that are included in Investigational New Drug (IND) applications for biologics and small molecules or Investigational Device Exemptions (IDE). Research institutions performing nonclinical safety studies need experienced regulatory affairs professionals on staff to provide guidance for

interactions between internal researchers, external sponsors, and the regulatory agencies prior to launching nonclinical safety studies. Discussions should begin as early as possible once a potential new product is considered to be a candidate for GLP-compliant safety testing. The FDA's Center for Biologics Evaluation and Research (CBER) offers early and informal pre-IND meeting (now known as INTERACT meetings) with pharmacology and toxicology reviewers in the Office of Tissues and Advanced Therapies (OTAT) to discuss acceptability of proof-of-concept studies, appropriate selection of models of disease, comparability of investigational products from various sources, proposed dosing regimen, and any issues related to the route of test article delivery. Other opportunities for early input from the US FDA regulatory staff include "pre-IND" meetings for biologics (CBER) or drugs at the Center for Drug Evaluation and Research (CDER) or a "pre-submission" meeting for medical devices at the Center for Devices and Radiological Health (CDRH). These early meetings also include clinical and product manufacturing reviewers who can help address critical development issues such as investigational product manufacturing and testing and the proposed design of early-phase clinical trials [18–20, 31, 32].

Nonclinical safety data from GLP-compliant studies are communicated to the agency in finalized study reports, which are submitted to the relevant regulatory agency as elements of the IND or IDE application. The content of such documents is very specific and may vary among different regulatory agency divisions.

11 Conclusions

The importance of GLP regulations have played a vital role in the recent scientific progress in biomedical research to develop new therapeutic products. The need for safety and efficiency and the desire for high-quality nonclinical safety data for human clinical testing of novel therapeutics has led to the worldwide implementation of GLP principles in biopharmaceutical, advanced cell/tissue, gene therapy, and medical devices. Implementing GLP is much more than simply complying with FDA regulations. It also makes perfect business sense, because these guidelines can help prevent costly product quality problems. Most of the critical and expensive activities performed in a laboratory are worthless without a consistent and reliable regulatory system in place. A well-documented and traceable history of laboratory activities is an essential part of compliance management. It provides the objective means to demonstrate integrity of data and the overall effectiveness of a reliable research and development environment. These GLP principles are designed to assure that animal data is obtained from carefully controlled studies, thereby increasing confidence that the results are reliable and reproducible.

References

1. Good Laboratory Practice for Nonclinical Laboratory Studies. (2019). 21. C.F.R. § 58.
2. European Commission. (2004). Directive 2004/9/EC: On the inspection and verification of good laboratory practice (GLP). https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:050:0028:0043:EN:PDF. Last accessed on 10/01/2020.
3. European Commission. (2004). 2004/10/EC: On the harmonization of laws, regulations and administrative provisions relating to the application of the principles of good laboratory practice and the verification of their applications for tests on chemical substances. https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:050:0044:0059:EN:PDF. Last accessed on 10/01/2020.
4. Arnold & Porter Kaye Scholer LLP. (2016). FDA proposes important revisions to good laboratory practices regulations. https://www.lexology.com/library/detail.aspx?g=42b752f8-f746-43d1-ada4-d77fda96d406. Last accessed on 10/01/2020.
5. OECD (Organization for Co-operation and Development). (1981, revised 1997). Council decision on the mutual acceptance of data in the assessment of chemicals. http://www.oecd.org/chemicalsafety/testing/councildecisionsonglp.htm. Last accessed on 10/01/2020.
6. Parascandola, J. (1995). The public health service and the control of biologics. *Public Health Reports, 110*, 774–775.
7. Schneider K (1983) Faking it: The case against Industrial Bio-Test Laboratories. Amicus Journal. Spring:14-26.
8. Baldershwiler, A. M. (2003). History of FDA Food laboratory practices. *Quality Assurance Journal, 7*, 157–161.
9. European Commission. (2004). Good laboratory practice. http://ec.europa.eu/growth/sectors/chemicals/good-laboratory-practice_en. Last accessed on 10/01/2020.
10. Bolon, B., Baze, W., Shilling, C. J., Keatley, K. L., Patrick, D. J., & Schafer, K. A. (2018). Good laboratory practice in the academic setting: Fundamental principles for nonclinical safety assessment and GLP-compliant pathology support when developing innovative biomedical products. *ILAR Journal, 59*, 18–28.
11. OECD (Organization for Co-operation and Development). (1997). OECD series on principles of good laboratory practice and compliance monitoring. https://www.oecd-ilibrary.org/environment/oecd-series-on-principles-of-good-laboratory-practice-and-compliance-monitoring_2077785x. Last accessed on 10/01/2020.
12. FDA (U.S. Food and Drug Administration). (2020). Nonclinical laboratories inspected under good laboratory practices. https://www.fda.gov/inspections-compliance-enforcement-and-criminal-investigations/inspection-references/nonclinical-laboratories-inspected-under-good-laboratory-practices. Last accessed on 10/01/2020.
13. Good Laboratory Practice Regulations, Final rule. Food and Drug Administration (FDA) issued. 52 FR 33768, Codified at 21. C.F.R. § 58 (09/04/1987).
14. FDA published a proposal to amend the agency's regulations in 21 CFR Part 58. U.S. Food and Drug Administration issued. 49 FR 43530, amend 21. C.F.R. § 58 (10/29/1984).
15. Center for Drug Evaluation and Research issued (1981, last revised 2007) Good Laboratory Practice Regulations Questions and Answers. Docket Number: HFA-305.
16. EMA (European Medicines Agency). (2018). Good laboratory practice compliance. http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000158.jsp. Last accessed on 10/01/2020.
17. ICH (International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use) Safety Guidelines. https://www.ich.org/page/safety-guidelines. Last accessed on 10/01/2020.
18. PMDA (Pharmaceuticals and Medical Devices Agency). (2012). Ministerial ordinance on Good Laboratory Practice for nonclinical safety studies of drugs. https://www.pmda.go.jp/files/000153713.pdf. Last accessed on 10/01/2020.

19. PMDA (Pharmaceuticals and Medical Devices Agency). (2012). Ministerial ordinance on Good Laboratory Practice for nonclinical safety studies of medical devices. https://www.pmda.go.jp/files/000153066.pdf. Last accessed on 10/01/2020.
20. Center for Drug Evaluation and Research and Center for Biologics Evaluation and Research issued. (2010). M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals. Docket number: FDA-2008-D-0470 (2010).
21. Good Laboratory Practice for Nonclinical Laboratory Studies. (2019). Study Director, 21. C.F.R. § 58.33.
22. Good Laboratory Practice for Nonclinical Laboratory Studies. (2019). Quality assurance unit, 21. C.F.R. § 58.35.
23. Good Laboratory Practice for Nonclinical Laboratory Studies. (2019). Personnel, 21. C.F.R. § 58.29.
24. Good Laboratory Practice for Nonclinical Laboratory Studies. (2019). Animal care facilities, 21. C.F.R. § 58.43.
25. Good Laboratory Practice for Nonclinical Laboratory Studies. (2019). Animal supply facilities, 21. C.F.R. § 58.45.
26. Good Laboratory Practice for Nonclinical Laboratory Studies. (2019). Animal care, 21. C.F.R. § 58.90.
27. Good Laboratory Practice for Nonclinical Laboratory Studies. (2019). Conduct a nonclinical laboratory study, 21. C.F.R. § 58.130.
28. Good Laboratory Practice for Nonclinical Laboratory Studies. (2019). Standard operating procedures, 21. C.F.R. § 58.81.
29. OECD (Organization for Co-operation and Development). (2018). Mutual acceptance of data (MAD). http://www.oecd.org/env/ehs/mutualacceptanceofdatamad.htm. Last accessed on 10/01/2020.
30. Good Laboratory Practice for Nonclinical Laboratory Studies. (2019). Test and control article characterization, 21. C.F.R. § 58.105.
31. U.S. Food and Drug Administration (FDA). (2020). FDA regulations relating to good clinical practice and clinical trials. https://www.fda.gov/ScienceResearch/SpecialTopics/RunningClinicalTrials/ucm155713.htm#FDARegulations. Last accessed on 10/01/2020.
32. FDA (U.S. Food and Drug Administration). (2001). Food and drug administration compliance program guidance manual. Chapter 48: Bioresearch monitoring—Good Laboratory Practice (Nonclinical Laboratories). https://www.fda.gov/downloads/iceci/enforcementactions/bioresearchmonitoring/ucm133765.pdf. Last accessed on 10/01/2020.

Ethical Considerations in Cell Therapy

Erica C. Jonlin

1 Introduction

Cell therapy touches on a multiplicity of ethical considerations, regardless of the source of the cell product. A cell therapy may be autologous, derived from a patient by a surgical procedure, and delivered back to the patient; alternatively it may be allogeneic, derived from another person's tissue, or from a pluripotent stem cell resource such as a human embryonic stem cell line or an induced pluripotent stem cell bank. Fundamental to any product offered as a therapeutic are ethical questions regarding the source of the product, the quality of the product to be provided to the patient, and the robustness of the evidence supporting safety and efficacy of the product. Indeed, any cell product delivered to a patient should help the patient and not harm them. Outside of the cell product itself are market forces with conflicting demands and desires: entities, including businesses and hospitals, offering the cell therapy; patients needing and sometimes demanding the cell therapy; and regulators seeking to ensure that the cell therapy will meet a standard of safety and efficacy. Amidst this dynamic tension, ethical challenges emerge.

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2 Operating Outside the Regulations

2.1 Finding the Loopholes, Promoting the Product

Prior to 1906, when Theodore Roosevelt signed into law the Pure Food and Drug Act, predecessor to the law that established the modern US Food and Drug Administration (FDA), any individual could concoct a product, claim that it would heal any range of illnesses and ailments, and sell it. There were no standards for manufacturing, no requirements to disclose what was in the product, and no requirements to test it for safety or for effectiveness prior to sale. Whether the product sold was a matter of how convincing the advertising was, and how desperate the patients were. Congress was, for the most part, of the firm belief that the free market should not be restricted; in fact, they felt that the free market and consumer demand and customer discretion were adequate and appropriate controls for the sale of products, including putative medical products. Eventually, Congress could no longer ignore the dangers of the free-wheeling food and drug marketplace when Sinclair Lewis's *The Jungle* was published, describing the filth of the meat-packing industry; and multiple exposés by muckraking journalists (in the so-called women's magazines) revealed the harms that the commonly sold remedies had inflicted on consumers. Public outrage reached a pitch, and members of Congress recognized that Federal government regulation was necessary to help ensure the safety and healthfulness of food and medical products. Hence, the 1906 Pure Food and Drug Act was passed [1, 2]. Ushering in the beginning of the era of government regulation of consumer products, it perhaps inevitably initiated the "search for loopholes" by those entrepreneurs seeking to avoid regulation.

Like the medical products of the nineteenth century, certain cell therapy products, in particular those hawked by clinics calling themselves "regenerative medicine clinics" or "stem cell clinics," are today's products that fall through the cracks of government regulation in the USA and elsewhere. Again, fundamental to the success of these regenerative medicine clinics are consumer demand and the clinics' ability to advertise directly to the public. These stem cell clinics offer something that a segment of the population wants to buy; in fact, many consumers insist that they have access to these clinics.

The 1906 Food and Drug Act gave the government the clout to seize products that the government could prove were "misbranded" (i.e., the label was false or misleading and lacked listing of dangerous ingredients – including alcohol, heroin, and cocaine, common ingredients at the time) and/or "adulterated" (containing "a poisonous or deleterious substance that may render it injurious to health"). Unfortunately, this initial law was weak, and it limited what the government could seize to only those products that fit the narrow definitions of "misbranded" or "adulterated." Until proven otherwise, drug manufacturers who could get around the definitions of "adulterated" and "misbranded" could sell products with impunity. Notably, the onus was on the government to prove a product was under its purview and out of compliance.

Many regenerative medicine clinics similarly take advantage of loopholes in the current law. Relevant to the FDA's present-day regulation of cell therapies are the FDA's definitions of "minimal manipulation" and "homologous use" [3]. As described in another chapter of this book, cell products meeting these definitions (e.g., cell products that are only minimally manipulated) are deemed not FDA-regulated. Enterprising individuals and businesses seeking to offer "stem cell therapies" (also called "regenerative medicine therapies") seek and utilize loopholes in the definitions of "minimal manipulation" and "homologous use." The so-called stem cell therapies involve the use of easily obtained and tissues and cell products, including umbilical cord blood and placental products, as well as autologous cell products (typically, a patient's own fat cells), to "treat" a wide variety of diseases and conditions, often by physicians (and nonphysicians) who are not specifically trained in the disease areas for which they are ostensibly treating patients. Clinics marketing these products have argued that the products have been "minimally manipulated": they state that the products have been subjected to minor handling only, such as purification, centrifugation, washing, preservation, or storage (which the FDA allows), and key structural or biological characteristics have not been altered. Likewise, the clinics have argued that their product's clinical use is congruent with the original tissue source (i.e., the product forms the same basic biological function in the recipient as they have in the original donor), and hence the use is "homologous."

Unfortunately, as in the past, the onus has been on the FDA to pursue clinics whose products have not met the FDA criteria of "minimally manipulated" and/or "homologous." Obviously, when a product is FDA-regulated, manufacturers need to demonstrate that the products meet FDA standards for safety, as well as regulatorily acceptable evidence to support claims of efficacy or effectiveness for various indications. Stem cell clinics seek to avoid the time and expense of having to conduct clinical trials to demonstrate safety and efficacy. In fact, most of the clinics cannot provide robust evidence to support the use of their products for the claimed use and instead feature testimonials of happy customers on their websites: these testimonials function as "evidence" of the safety and efficacy of the products provided. Many clinics also assert that they are engaged in the practice of medicine, which is not under the purview of the FDA.

2.2 Posing as Clinical Trials

Many stem cell/regenerative medicine clinics provide their therapies under the guise of a clinical trial, perhaps trying to fend off accusations of inappropriately selling untested products. Patients interested in trying the intervention sign up for the clinical trial, which they may have found listed on the NIH-sponsored clinical trials database, www.clinicaltrials.gov. Unfortunately, the very fact of the listing on the www.clinicaltrials.gov website effectively creates the veneer of legitimacy [4], insinuating that the trials are meritorious and may even have oversight (which they

may not). Realizing the listings may be misleading, www.clinicaltrials.gov has included a disclaimer (https://www.clinicaltrials.gov/ct2/about-site/disclaimer):

> Listing a study on this site does not mean it has been evaluated by the U.S. Federal Government. The safety and scientific validity of a study listed on ClinicalTrials.gov is the responsibility of the study sponsor and investigators. Know the risks and potential benefits of clinical studies and talk to your health care provider before participating.

In response to pressure from scientists and ethicists working in the stem cell field, including Paul Knoepfler, PhD, a UC Davis scientist who maintains the stem cell blog, "The Niche," www.clinicaltrials.gov recently instituted a pop-up box that appears when a user searches for stem cell-related clinical trials [5]. This box, while not itself a warning, states "Learn more about stem cells" and provides links to informational webpages, including a consumer-oriented FDA website, "FDA Warns About Stem Cell Therapies." Should the searcher click on the FDA link, they will see a 1-min video that, in clear and indisputable language, debunks and warns of the serious harms and illegality of many "stem cell therapies." Examples of fraudulent therapy are given.

While this latest effort by www.clinicaltrials.gov is laudable, it still remains the sole responsibility of the consumer to be proactive in evaluating the merit of cell therapy studies listed on www.clinicaltrials.gov.

2.3 Trends Leading to the Popularity of Stem Cell Clinics: Patients' Rights Movements

At the FDA's September 13, 2016, public hearing on the regulation of human cells, tissues, and cellular or tissue-based products [6], a number of patients spoke regarding their experience with autologous adult stem cells, testifying that the stem cell treatments they'd received were the only interventions, of all the interventions they'd tried, that had alleviated their conditions. One patient who was representative of many who spoke was a rheumatoid arthritis patient, Georgianna Crocker (self-described as a "professional pharmaceutical rep"). Ms. Crocker asserted:

> Thank you for allowing me this opportunity to speak with you directly about the regulation of adult stem cell treatment and how this treatment has given me my health and my life back. I am a rheumatoid arthritis patient who is currently in remission because of stem cell therapy one and a half years ago. I am a passionate patient advocate for adipose autologous stem cell therapy, or rather using my own fat tissue, and keeping this therapy available and increasing access for patients like myself who have failed other conventional and non-conventional therapies for their disease… I'm here today to request that you, the FDA, continue to allow my stem cell therapy using my own fat cells; that this will be a choice made between me, myself, and my healthcare provider.

After showing slides depicting the results of her laboratory tests, which she gave as evidence of safety and efficacy of her stem cell treatments, Ms. Crocker concluded:

> I ask that you strengthen my rights as a patient to be treated with my own stem cells and to accelerate this availability of treatment that is safe and effective, and to please not classify my own cells as a drug. They are my own cells and I ask that you respectfully treat them that way.

Conflict of interest notwithstanding, stem cell clinics echo their support of the concept of the patient's rights to access their so-called treatments. After receiving a warning letter from the FDA, the Sunrise, Florida, stem cell clinic [7], the US Stem Cells, asserted:

> USRM (US Stem Cell, Inc.) believes that the patient and physician have the right to decide whether or not to use a patient's own cells for a therapeutic purpose without federal government interference.

The assertions of patients demanding access to largely unproven treatments begs the ethical questions – what *are* a patient's rights with respect to seeking treatment? What is their freedom to choose treatments, even those that are "untested"?

2.3.1 Right to Try

Consumer demands for access to medical products without government "interference" go back decades. For example, in the 1970s terminally ill patients with cancer demanded access to laetrile, which was being promoted as an alternative cancer treatment, but which was found to be poisonous and not efficacious [8]. Years later, in the 2006–2007 lawsuit "Abigail Alliance v. von Eschenbach," the Abigail Alliance for Better Access to Developmental Drugs, a patient advocacy organization, sued the FDA, arguing that patients with cancer have a constitutional right of access to investigational cancer drugs [9] – specifically, those drugs being tested in Phase 1 clinical trials for which the patients are not eligible. Ultimately, a federal court of appeals held against the Abigail Alliance, finding that "there is no fundamental right… to experimental drugs for the terminally ill" [10].

A notable case involving patient demands to the right to receive unproven stem cell treatments occurred in Europe and addressed the government's rights to protect citizens from unsupported therapies. In 2014 in Italy, the father of a patient applied to the European Court of Human Rights (ECHR) to overturn the Italian Court's refusal to authorize an unproven mesenchymal stem cell "therapy" to treat his daughter's degenerative cerebral illness. In the case, known as Durisotto vs. Italy, the ECHR agreed with the Court's prohibition to access the therapy [11]. EHCHR The ECHR found that it was legitimate for the state to protect health and regulate access to it. As stated by Riva et al., "In other words, healthcare authorities must decide on the scientific validity and on the appropriateness of the treatment."

Back in the USA, in 2009, the FDA created a pathway called "expanded access," sometimes called "compassionate use," which may enable a patient "to gain access to an investigational medical product (drug, biologic, or medical device) for treatment outside of clinical trials when no comparable or satisfactory alternative therapy options are available" (https://www.fda.gov/news-events/public-health-focus/

expanded-access) [12]. FDA review is required to gain access, but the FDA rarely denies a request for expanded access; for example, in FY2016, FDA's Center for Biologics Evaluation and Research (CBER) approved 97% of requests for expanded access [12]. However, suspicious of the FDA, and seeking to avoid any government engagement, a patients' rights movement, known as "Right to Try" took hold. Spearheaded by the conservative Goldwater Institute, the movement aspired to "(reform) the US Food and Drug Administration's (FDA) bureaucratic drug approval process, which stands between patients and potentially lifesaving vaccines and treatments." Initially a States movement, with "Right to Try" laws passed one state at a time (Colorado being the first in 2014), the US Congress eventually passed the Federal Right to Try Act (RTA) in May 2018 [13]. The RTA circumvents the FDA by removing its authority over the administration of investigational medications to eligible ill patients. Under Right to Try, a patient and their doctor can ask a company directly for an investigational drug, including during Phase 1 testing when very little is known about the drug. The company does not have to provide the drug, and the manufacturer and an Institutional Review Board (IRB) still need to approve the use of the experimental drug, but the FDA is not involved. While not addressing regenerative medicine products specifically, the Right to Try movement reflects an important patients' rights trend in the USA, exemplifying the feeling of a powerful sector of the US population that they have a right to access medical products without government "interference" if they themselves (perhaps with their doctor) have decided that the medical product could help them.

Patients' rights movements, from Right to Try to demands for access to stem cell "therapies," can be viewed as manifestations of the therapeutic misconception [14] – the patient's (and sometimes the patient's doctors), presumption that an investigational/alternative intervention is akin to treatment and that the patient can expect to benefit from receiving it (and not suffer worse harm). While some proponents of the use of these products have stipulated that patients have "nothing to lose," it is of ethical concern when the probability of benefit is very low, and the risk of worsening the patient's condition, and perhaps hastening of death of very sick patients, is very real. With respect to the Right to Try, and equally relevant to the use of unproven stem cell and regenerative medicine "therapies," in their 2018 New England Journal Medicine perspectives piece, Joffe et al. state [15] "Are we prepared to abandon the FDA's gatekeeping role in favor of unfettered patient autonomy and market forces, risking precisely the problems that prompted Congress to grant the FDA its present authority?"

2.4 What's the Harm of Unregulated Cell Therapies?

2.4.1 Physical Harm

Receiving unproven stem cell treatments may cause no improvement in a patient's condition. In the worst case scenario, the treatments can cause irreversible harm. One of the most spectacular examples of harm was the blinding of patients with

age-related macular degeneration (AMD) who underwent intravitreal injection of autologous adipose tissue-derived stem cells in both of their eyes (on the same day) at a stem cell clinic in Florida [16]. Patients, who paid for the noninsurance-covered procedures, suffered "severe retinal detachments, resulting in visual acuities ranging from 20/200 to no light perception in the better eye at one year of follow-up – which included multiple surgeries for retinal detachment" [17]. Notably, the use of this stem cell product for AMD had never been studied in clinical trials and lacked sufficient safety data. According to Kuriyan et al., [16], "Experimental bilateral intravitreal injections are both atypical and unsafe."

In response to this disaster and others, the FDA developed consumer-oriented websites, (including the site to which www.clinicaltrials.gov provides a link). The FDA warns of numerous potential safety concerns for unproven stem cell-based treatments including administration site reactions, the ability of cells to move from placement sites and change into inappropriate cell types or multiply, and the growth of tumors (https://www.fda.gov/consumers/consumer-updates/fda-warns-about-stem-cell-therapies).

The absence of FDA regulation also often results in products that are not manufactured in compliance with cGMPs, exacerbating risks to recipients. As the FDA warns consumers, manipulation of cells after removal poses the risks of contamination of the cells, even for autologous cells. In a warning letter to Kristin Comella, Chief Scientific Officer of the US Stem Cell clinic, the FDA delineated numerous instances in which the clinic had failed to take the steps necessary to ensure an aseptic product. As per the FDA's warning letter, among many other violations, the clinic failed to perform appropriate laboratory testing, including sterility and endotoxin testing, to ensure that the product was free of "objectionable microorganisms" [18].

As is typical of stem cell clinics that receive inspection and warning letters from the FDA, the US Stem Cell clinic had asserted that they were not subject to FDA regulations. They were obtaining and then processing patients' adipose tissue into a stromal vascular fraction (SVF) and administering it intravenously or directly into the spinal cord of those same patients (as an autologous product), ostensibly to treat a variety of serious diseases or conditions, including Parkinson's disease, amyotrophic lateral sclerosis, chronic obstructive pulmonary disease, heart disease, and pulmonary fibrosis. In their warning letter, the FDA declared that the SVF product did *not* meet the minimal manipulation criterion set forth in the regulations. Additionally, because the SVF product was not intended to perform the same basic function or functions of adipose tissue, such as cushioning the body, the use of the SVF product for treatment of these diseases or conditions is *not* homologous use. Hence, the product was under the FDA's jurisdiction, and the FDA asserted that the product should have been subject to FDA regulations.

As an aside, in their warning letter, the FDA could not specifically address the clinic's claims that their product had efficacy against a wide variety of serious conditions – perhaps the most serious ethical breach committed by the clinic. First, the FDA had to demonstrate the clinic's products were under their purview: i.e., that the products were *not* minimally manipulated and that the uses were *not* homologous.

With those facts established, the FDA enumerated the GMP violations. With respect to efficacy claims, the FDA could only make the oblique statement:

> Please be advised that in order to lawfully market a drug that is a biological product, a valid biologics license must be in effect [21 U.S.C. 355(a); 42 U.S.C. 262(a)]. Such licenses are issued only after a showing of safety and efficacy for the product's intended use. While in the development stage, such products may be used in humans only if the sponsor has an investigational new drug application (IND) in effect as specified by FDA regulations [21 U.S.C. 355(i); 21 CFR Part 312]. Your SVF product is not the subject of an approved biologics license application (BLA) (b) [4].

Unfortunately, this method of communication perhaps does not sufficiently chastise the clinic for their outrageous and misleading claims of what their so-called treatment could provide to patients. Making health claims of efficacy to patients rendered vulnerable by their disease or condition is highly unethical.

It is worth mentioning that, in terms of physical risk, another risk of receiving an unproven cellular product, even if it does not exacerbate the patient's condition, is the continued degeneration or worsening of a patient's condition. By delaying receipt of an accepted treatment, the patient may miss the opportunity to undergo an intervention that would have been more effective had they received it at an earlier stage of their disease.

2.4.2 Deception of Vulnerable Populations

Many people who seek treatment from stem cell clinics are suffering from a chronic and debilitating condition (e.g., chronic pain, macular degeneration) and may have not been able to get relief with current, mainstream treatments. As alluded to above, many of the stem cell clinics prey on the vulnerabilities of these patients with exaggerated claims, supported by testimonials, posted on their websites, thinly masked as evidence of efficacy. While not able to remark on this problem explicitly in their warning letters, in press releases, and other public statements, FDA officials have been more forthright. For example, in the wake of the findings at the US Stem Cell clinic, described above, in which an autologous stromal vascular fraction was administered to "treat" serious neurological, autoimmune, and degenerative diseases and conditions, in a press release from the FDA, Commissioner Scott Gottlieb, MD, emphasized "Stem cell clinics that mislead vulnerable patients into believing they are being given safe, effective treatments that are in full compliance with the law are dangerously exploiting consumers and putting their health at risk" (WL Press Release 28 Aug, 2017).

2.4.3 Financial Risks

Most stem cell clinic offerings are not covered by medical insurance. Patients must pay the full price, which is typically many thousands of dollars. Even if the "treatment" is offered under the auspices of a clinical trial, the patient must pay all of the

costs. Without experience or knowledge of how clinical trials are generally run (in which patients receive most interventions at no cost to them), patients are taken advantage of.

2.4.4 Harm to the Field of Regenerative Medicine

It may be difficult, if not impossible, for the nonscientist/non-clinician to differentiate between therapies backed by years of study and scientific evidence and "therapies" supported by patient testimonials that may or may not be real. As Scott Gottlieb affirmed, "These dishonest actors exploit the sincere reports of the significant clinical potential of properly developed products as a way of deceiving patients and preying on the optimism of patients facing bad illnesses. This puts the entire field at risk. Products that are reliably and carefully developed will be harder to advance if bad actors are able to make hollow claims and market unsafe science" [19].

Unfortunately, until the FDA can step in and issue a permanent injunction to stop a stem cell clinic from marketing their products without FDA approval, these firms continue to offer their products. The FDA inspects clinics one at a time, issuing letters of observations in a step-wise manner. As bioethicist Leigh Turner told *The Washington Post*, "If you're trying to tackle this one business at a time, you're not going to make a dent. We're talking about one new warning letter when there are now hundreds of clinics out there… Why not send out 50 warning letters, 100 warning letters?" [20].

In the meantime, *caveat emptor* is the only real preventative stopping the clinics.

3 Operating Inside the Regulatory Framework

3.1 Is It Ready Yet? The Japanese Model

With Shinya Yamanaka's discovery of factors that enable derivation of induced pluripotent stem cells (iPSCs) from mature somatic cells [21, 22], followed by his award of the Nobel Prize in Physiology or Medicine in 2012, Japan catapulted to the forefront in the field of regenerative medicine. Several months after Yamanaka won the Nobel Prize, Japanese Prime Minister Shinzo Abe announced two proposed pieces of legislation to support the development of pluripotent stem cells for therapeutic purposes. Two years later, in 2014, the two regulatory acts were passed. Described in more detail in another chapter in this book, these acts were aimed at improving patient safety and speeding the delivery of regenerative medicine products to patients.

One act, attempting to address the dearth of regulations regarding "rogue stem cell clinics," such as the clinics described above, is the Act on the Safety of

Regenerative Medicine (ASRM). ASRM allows hospitals and clinics to market regenerative medicine products, with oversight by the Ministry of Health, Labour and Welfare (MHLW). For example, the clinics must be registered with the MHLW and must utilize a cell-processing facility certified by the MHLW. Plans to use the products must also be reviewed by an independent committee.

The other act, the Pharmaceuticals, Medical Devices and Other Therapeutic Products Act (PMDA), is designed to address the historically slow-paced phase of therapeutics development by allowing conditional, time-limited marketing approval for regenerative medicine products that have undergone exploratory clinical trials only. Clinical testing primarily involves measurement of safety signals. In terms of efficacy, endpoints are chosen that "predict reasonable likelihood of clinical benefit (e.g., by using a surrogate endpoint)" [23]. A firm wanting to market the product may receive conditional approval on the basis of the data from these small clinical trials, after which the product may be sold up to 7 years while additional efficacy data is being collected. Products approved under this mechanism are covered by the Japanese insurance system [24].

While these regenerative medicine regulations in Japan have received some substantial criticism, particularly from scientists outside of Japan, it is notable that other countries, including the US and European countries, are also developing their own programs designed to accelerate the translation of regenerative medicine products into the marketplace, as reviewed in some detail by Nagai [25].

3.1.1 Ethical Issues

False Impressions of Safety and Efficacy

A feature of the ASRM Japanese regulation of cell products is the "usage of GMP-type facilities/equipment and quality control requirements for cell processing facilities that manufacture cells for clinical study and medical treatment" [23]. While the requirement for GMP procedures in manufacturing is both laudable and necessary, the average consumer could interpret this as meaning the cell therapy product is "safe" (e.g., it will not cause untoward effects) and efficacious. In fact, ASRM products are essentially unproven; prior to being marketed, they do not need to have been shown to have any efficacy in ameliorating any condition or disease. Cyranoski [26] describes a clinic in Tokyo that administers, to patients with amyotrophic lateral sclerosis (ALS), infusions of their own fat-derived stem cells, ostensibly to cure or slow the progression of their disease. In that the clinic is listed on the government registry and follows GMP procedures, they can offer these "treatments" despite the lack of efficacy data other than anecdotes.

However, as compared to the USA, Japanese stem cell clinics do have some oversight and are required to comply with manufacturing regulations from the get-go. In the USA, stem cell clinics may continue to offer products and get away with not complying with GMPs until they get into trouble with the FDA. For example, US Stem Cells, Inc., of Sunrise, Florida, described above, only complied with FDA

GMP regulations after a long drawn-out process with the FDA, including multiple inspections, letters, and a lawsuit [27]. In the meantime, the products provided by US Stem Cells were at risk of contamination.

Products Are on the Market Too Soon, Presenting Safety Risks to Patients

Some critics have remarked that, in the field of regenerative medicine, economic competitiveness is being prioritized over patient welfare [28]; in the Japanese example, it has been said that Japan's conditional approval approach is too lax, prioritizing companies, who benefit from the sale of their product, and not sufficiently benefiting patients. Sipp and Sleeboom-Faulkner [28] have remarked, "To our knowledge, the Japanese case is the first instance in which the lowering of market entry standards has been targeted to a medical product on the basis of its material composition," which perhaps does not seem like a very good basis on which to ease market entry standards, as compared to the seriousness of the disease. The international team of scientists and ethicists that authored the ISSCR Clinical Translation Guidelines has expressed concerns about conditional approvals that are based on a surrogate marker, stating "regenerative medicine products approved based on early stage trial results could prove either unsafe or ineffective when tested more widely and rigorously" [29].

For example, one of the first products conditionally approved under Japan's PMDA, which provoked much criticism from other stem cell scientists, was Stemirac, a treatment for the neurological symptoms and functional disorders associated with spinal cord injury. An autologous bone marrow-derived mesenchymal stem cell product, Stemirac is infused intravenously into a patient's spine within 40 days of spinal cord injury. Conditional approval was granted on the basis of a 13 patient clinical trial, in which all of the patients received the investigational product. While 12 out of the 13 patients enrolled on the trial were said to have improved at 6 months, there was no comparator group, and the mechanism of any effect remains largely unknown and subject to scientific debate. The conditional approval allowed marketing of the product but included the requirement that data be collected from the participants over the next 7 years to show that the intervention was efficacious.

In the Japanese agency's conditional approvals for Stemirac and for two other recently conditionally approved regenerative medicine products, HeartSheet and Collategen, post-marketing randomized comparative studies were not required [25]. Critics have claimed that the approval for Stemirac was premature; that double-blind randomized studies, considered the gold standard for demonstrating safety and efficacy, ought to be performed; and that there is insufficient evidence that the treatment works [24]. Without a comparator arm in the clinical trial, it is difficult to conclude that patient improvement is not the result of natural healing. If these regenerative medicine treatments are in fact not truly efficacious, then patients receiving them will be exposed to the significant risks of very invasive surgeries for no obvious benefit. From an ethical standpoint, the risks of the intervention may therefore outweigh the benefit.

Additionally, when a new drug is approved and marketed on the basis of its apparent safety in a small number of patients, without definitive demonstration of efficacy, the risk remains that the drug will not improve the patient's condition and may even harm them. In a report published in 2017 [30], the FDA highlighted 22 case studies in which Phase 2 (relatively small trials which may or may not have a comparator group) and Phase 3 clinical trials (larger, randomized trials with a comparator group) had divergent results. Products included a wide range of experimental interventions for a wide range of indications. After Phase 3 trials were conducted and the results analyzed, a number of the products showed a lack of efficacy. For one of the products, Phase 3 trials demonstrated a lack of safety, and for the remainder of the case studies, the products demonstrated a lack of efficacy *and* a lack of safety. Similar to Japan's conditional approvals for regenerative medicine products, biomarkers were frequently employed as surrogate markers for efficacy. The FDA highlighted the case of torcetrapib, a drug intended to reduce heart attacks. The surrogate marker for efficacy was lipid levels: an increase in "good" cholesterol (HDL) and a lowering of "bad" cholesterol (LDL). In Phase 2 trials, torcetrapib did in fact increase HDL and lower LDL, yet in the post-approval randomized Phase 3 trial, which examined whether the drug actually reduced heart attacks, patients taking the drug were actually 25% more likely to suffer a major cardiac event than those in the control group [30].

Indeed, it is of concern to the safety and welfare of patients when products are approved on the basis of minimal evidence, and randomized clinical trials to demonstrate efficacy are not conducted. And, when patients and/or their insurer pay for a treatment, it could be impossible to conduct a post-approval randomized clinical trial with a placebo comparator arm because patients/insurance cannot expect to pay for a placebo arm [26]. Additionally, it has been suggested that paying for treatments can increase the likelihood that the patient will experience a placebo effect.

Sipp and Sleeboom-Faulkner [28] have made the point that in a global economy, if one country is more permissive than the other, all countries are under pressure to loosen rules to remain economically competitive. For example, both Taiwan and South Korea are said to be adopting conditional approval systems for regenerative medicines based on Japan's legislation [26].

However, the ethical problem remains: there are many patients with serious, debilitating conditions who desperately need new options. In a June 2009 commentary in "The New England Journal of Medicine," FDA Commissioner Margaret Hamburg and FDA Principal Deputy Commissioner Joshua Sharfstein [31] succinctly summarized FDA's dilemma:

> In the domain of medical products, it has been said that the FDA has just two speeds of approval— too fast and too slow. Critics concerned about haste point out, accurately, that drugs and other products are generally approved on the basis of relatively small studies and that safety problems often emerge when large populations are exposed to the products. Those worried about delay note, correctly, that people with life-threatening diseases have no time to wait.

Their summary applies equally well to any type of medical intervention – from small molecule drugs to biologics to cell therapy:

> Some benefits are not worth the risk; some risks are worth taking. Key considerations are the severity of the illness at issue, the availability of alternative treatments or preventive interventions, and the current state of knowledge about individual responses.

Indeed, what we hope for in the development of cell therapies is an approach tailored to the needs of the patients – considering their options, the seriousness of their disease, the natural history of the disease, and their degree of suffering. The benefit-risk equation is complex, and ethical considerations are different for each patient group.

3.2 The Experimental Arena: Ethical Considerations in Cell Therapy Clinical Trial Design

3.2.1 The Problem of the Comparator Group

While it is not unreasonable to expect robust efficacy data for regenerative medicine products prior to marketing, ethical questions arise when considering the design of a randomized clinical trial. In that invasive procedures are needed to implant cell therapy products, what reasonable comparator group could be employed in a clinical trial, and how could the trial be blinded? Indeed, in his response to the "*Nature*" article criticizing the Japanese conditional approval of Stemirac for spinal cord injury patients, Shinji Miyamoto, Director-General of the Japanese Pharmaceutical Safety and Environmental Health Bureau, pointed out that Stemirac is an autologous bone marrow product; patients' bone marrow is cultured externally and then returned to the patient. "A double-blind study is therefore structurally impossible, and performing a sham operation on a control group would raise ethical issues" [32]. Miyamoto also argues that the response of the paralyzed patients to Stemirac was so convincing, and that it would be "unethical to withhold approval and deny treatment" to patients in a future clinical trial.

The controversy concerning clinical trials employing sham surgery in order to test regenerative medicine products was well-illustrated by an NIH-sponsored clinical trial, conducted in the late 1990s, that tested the use of fetal stem cells for the treatment of Parkinson's Disease. Thirty-four patients with advanced Parkinson's disease were enrolled in a three-arm trial. All of the patients underwent surgical procedures: two groups received fetal nigral cells bilaterally transplanted into the post-commissural putamen; patients randomized to the placebo group received identical procedures except that "they received partial burr holes that did not penetrate the inner table of the skull, needles were not inserted into the brain, and no tissue was implanted" [33]. Only the surgeon knew which patients got which intervention; the patients and clinical evaluators were blinded as to patient assignment. All patients, including the patients in the placebo arm, received immunosuppression for 6 months. The study was IRB reviewed and approved, and the patients gave informed consent and knew that one group would undergo sham surgery and not

receive fetal cells. The analysis of the results of the 2-year follow-up study showed no benefits of the fetal transplants [33].

While the scientific advantages of conducting a controlled study are evident, several ethical concerns emerge: a subset of patients may receive sham surgery and immunosuppressive drugs, both of which pose risks. Additionally, depending on the length of the trial, patients enrolled in such a trial may not be able to receive other treatments (experimental or otherwise) during the period of follow-up. Cell therapies involving the use of autologous cells obviate the problem of exposing patients to immunosuppressive drugs. However, even for autologous cell therapy, the risks of surgery remain (e.g., the risks inherent in obtaining the tissue, and the risks of sham surgery, if it is used for one of the patient groups). Questions also remain as to what control product to use.

As for any clinical trial, rigorous independent scientific and ethical review and robust informed consent must be employed in any cell therapy trial. Toward this end, the Parkinson's disease community has created an international initiative to bring together experts in the field for in-depth discussions of patient criteria, endpoints, and other study design elements when testing pluripotent stem cells for the treatment of Parkinson's disease [34]. The need for a sham surgery arm is among those elements that the group is addressing.

3.2.2 Study Conduct and Withdrawal from Participation

Because research study participation is voluntary, a key tenet of human subjects' protection is that a participant may withdraw at any time, without penalty. In the case of cell therapy, under what circumstances may a participant withdraw, without placing themselves at risk? From a safety and an ethical standpoint, a balance must be reached between allowing the participant to withdraw and ensuring that by withdrawing they will not be harmed. When they enroll in a clinical trial involving receipt of a cell therapy, it is advisable that research participants be informed that if they should choose to withdraw after they receive the cell product, a certain amount of follow-up, with the research team and/or with their doctor, is advisable for their safety.

4 Stem Cell Products: The Human Embryonic Stem Cell Vs. Induced Pluripotent Stem Cell Divide

Countless articles have been written proclaiming the "ethical advantages" of induced pluripotent stem cells (iPSCs) over human embryonic stem cells (hESCs) as a source of pluripotent stem cell material for therapeutic development, proclaiming that iPSCs "avoid the ethical problems" of hESCs. While it is true that human embryonic stem cell-derived therapies are made possible because a human embryo

was destroyed (unless the hESC line originated from a single blastomere), it is also true that embryos used to develop therapies are donated to research with the full informed consent of the embryo donors, in compliance with human subjects protection guidelines [35]. By NIH policy, all of the human embryonic stem cell lines on the NIH stem cell registry (https://stemcells.nih.gov/policy/2009-guidelines.htm) were derived from frozen embryos that were produced for reproductive purposes, that were no longer needed or wanted for reproductive purposes, and which were donated in accordance with NIH requirements for informed consent. In the USA, the parents/legal guardians of excess frozen embryos created for reproductive purposes are given the right, by law, to decide on the disposition of their leftover embryos. Depending on the state, disposition can include research. There has been little discussion in the literature of the rights of fertility clinic patients to decide that they do not want to attempt a pregnancy for every embryo that has been created for them by in vitro fertilization. With informed consent for research, which includes receiving information that their embryos will be destroyed, fertility clinic clients may decide to donate leftover embryos to research, and many do so gladly, relieved that the embryos will be used for what they consider a useful purpose (e.g., scientific discovery) rather than be thrown away [35], which for many individuals is the only other option.

Interestingly, the first patient to receive an experimental hESC-derived cell therapy was a religious person, 21-year-old Timothy Atchison. Paralyzed from the chest down as a result of a serious car accident, Atchison was initially stunned when he learned that the neural progenitor cells that he could receive in the Geron clinical trial were derived by destruction of a human embryo. A devout Baptist who opposes abortion, Atchison ultimately felt that enrollment in the study was acceptable after he learned that the cells did not come from an aborted fetus and that the original embryo had been destined to be thrown away. According to an interview with *The Washington Post*, he said he felt that it was "part of God's plan" for him to receive the cells [36]. Indeed, assumptions cannot be made with respect to whether any given person would or would not believe an hESC-derived therapeutic to be ethically acceptable for themselves.

At the same time, the ethical issues with respect to iPSCs are not insignificant. iPSC-derived cell therapies will have originated from a tissue donor and that donor's informed consent needs to have been obtained, not only to procure the specimen but also to utilize it as a basis for a therapy [37]. Considering the implications if a single donor's tissue forms the basis of a commercially available therapeutic that will be implanted into many other individuals, it is incumbent on the researcher who first obtains the tissue to have obtained the informed consent of the donor to utilize the tissue in this way. iPSC-derived therapies will carry the genome of the donor, something the originating donor may or may not be comfortable with, if the treatment is not used autologously, i.e., for their own personal benefit. Even if an iPSC line is used strictly for research purposes, and not therapeutic purposes, sequencing of the line can reveal disease risk for the donor and for their relatives; for actionable, disease mutations, it may be ethically imperative to inform the originating donor of the risk. Finally, even if an iPSC line or iPSC-derived therapeutic is said to be

"anonymized," it cannot truly be considered anonymous in that DNA is an identifier, and reidentification is possible [38].

Courts have determined that we do not own our own bodies or tissues that come from it [39]. By extension, any discoveries and intellectual property based on human tissues belong to the inventor and/or the institution or company in which the discovery was made, a fact that has been made painfully clear in the case of Henrietta Lacks, whose cancer cells became the basis of countless experiments and discoveries, but whose family was not eligible for compensation (e.g., see Skloot [40]). Hence, tissue donors and embryo donors need to be made aware of the possibility that their donation may result in a therapeutic of considerable economic value and that they will not benefit.

5 Conclusions

As summarized in this chapter, cell therapy poses a variety of interesting ethical questions. When is a cell therapy appropriate to administer to a human being? What amount of safety and efficacy evidence is needed to deem a cell therapy ready to be available on the market? Do patients have the right to demand and access a cell therapy if it is commercially available, even if scientific data is lacking? What if they have a serious and debilitating condition for which they have not received an effective treatment? Can they not use their own fat cells? What role does/should the government (i.e., regulatory agencies) play in overseeing cell therapies? What rights do tissue donors have, when providing source material for cell therapeutics? How do we design clinical trials to get robust data on safety and efficacy of cell therapeutics, while protecting the rights and safety of the study participants, many of whom are desperately ill patients?

In that these questions are not easily answered, to advance the field of cell therapy, it is imperative that a multiplicity of viewpoints be invited to contribute: those of scientists, clinicians, inventors, regulators, governmental agencies, industry partners, and, most importantly, the patients themselves and their families and advocates.

References

1. Hilts, P. J. (2003). *Protecting America's health: The FDA, business, and one hundred years of regulation*. Knopf.
2. US Food and Drug Administration. (2019). *FDA history: Part I: The 1906 food and drugs act and its enforcement*. https://www.fda.gov/about-fda/fdas-evolving-regulatory-powers/part-i-1906-food-and-drugs-act-and-its-enforcement. (Last updated 24 April 2019) Last accessed 6 Mar 2021.
3. US Food and Drug Administration. (2020, July). *Regulatory considerations for human cell, tissues, and cellular and tissue-based products: Minimal manipulation and homologous use, guidance for industry and FDA staff*. https://www.fda.gov/regulatory-information/search-fda-

guidance-documents/regulatory-considerations-human-cells-tissues-and-cellular-and-tissue-based-products-minimal. (Last updated 21 July 2020) Last accessed 6 Mar 2021.
4. Turner, L. (2017). ClinicalTrials.gov, stem cells and 'pay-to participate' clinical studies. *Regenerative Medicine, 12*(6), 705–719.
5. Knoepfler, P. (2021, February 28). *Regenerative reads: Brain organoids, Clinicaltrials.gov change*. The Niche: Knoepfler Lab Stem Cell Blog. https://ipscell.com/2021/02/regenerative-reads-brain-organoids-clinicaltrials-gov-change/. Last accessed 6 Mar 2021.
6. US Food and Drug Administration. (2016, September 13). Part 15 hearing: Draft guidances relating to the regulation of human cells, tissues, or cellular or tissue-based products. pp. 106–107.
7. U.S. Stem Cell. (2018, May 9). *U.S. Department of justice files lawsuit at request of FDA to stop U.S. stem cell clinic from performing autologous stem cell procedure: USRM will vigorously defend medical freedom of Americans*. http://us-stemcell.com/doj-files-lawsuit-against-usstemcellclinic/. Last accessed 6 Mar 2021.
8. Lerner, I. J. (1981). Laetrile: A lesson in cancer quackery. *CA: A Cancer Journal for Clinicians, 31*(2), 91–95.
9. Annas, G. J. (2007). Cancer and the constitution – Choice at life's end. *The New England Journal of Medicine, 357*(4), 408–413.
10. Darrow, J., Sarpatwari, J., Ameet, A., Kesselheim, J., & Aaron, S. (2015). Practical, legal, and ethical issues in expanded access to investigational drugs. *The New England Journal of Medicine, 372*(3), 279–286.
11. Riva, L., Campanozzi, L., Vitali, M., Ricci, G., & Tambone, V. (2019). Unproven stem cell therapies: Is it my right to try? *Annali dell'Istituto Superiore di Sanità, 55*(2), 179–185.
12. U.S. Food and Drug Administration. (2018, May). *Expanded access program report*. https://www.fda.gov/media/119971/download. Last accessed 6 Mar 2021.
13. Right to Try Act. https://www.fda.gov/patients/learn-about-expanded-access-and-other-treatment-options/right-try. (Current as of January 14, 2020). Last accessed 6 Mar 2021.
14. Appelbaum, P. S., Roth, L. H., Lidz, C. W., Benson, P., et al. (1987). False hopes and best data: Consent to research and the therapeutic misconception. *The Hastings Center Report, 17*(2), 20–24.
15. Joffe, S., & Fernandez, L. H. (2018). Federal right-to-try legislation – Threatening the FDA's public health mission. *The New England Journal of Medicine, 378*(8), 695–697.
16. Kuriyan, A. E., Albini, T. A., Townsend, J. H., Rodriguez, M., et al. (2017). Vision loss after intravitreal injection of autologous "stem cells" for AMD. *The New England Journal of Medicine, 376*(11), 1047–1053.
17. Ansari, Z. A., Kuriyan, A. E., & Albini, T. A. (2017). Unproven stem cell therapy for macular degeneration. *Oncotarget, 8*(52), 90636–90637.
18. US Food and Drug Administration. (2017, August 24). *Warning letter to US stem cell clinic, LLC*. https://www.fda.gov/inspections-compliance-enforcement-and-criminal-investigations/warning-letters/us-stem-cell-clinic-llc-524470-08242017. Last accessed 6 Mar 2021.
19. US Food and Drug Administration. (2017, August 28). *Statement from FDA Commissioner Scott Gottlieb, M.D. On the FDA's new policy steps and enforcement efforts to ensure proper oversight of stem cell therapies and regenerative medicine*. https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm573443.htm. Last accessed 6 Mar 2021.
20. Wan, W., & McGinley, L. (2019, April 4). *FDA, N.Y. attorney general take steps to police stem cell industry*. Washington Post.
21. Takahashi, K., & Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell, 126*, 663–676.
22. Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., et al. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell, 131*, 861–872.
23. Konomi, K., Tobita, M., Kumura, K., & Sato, D. (2015). New Japanese initiatives on stem cell therapies. *Cell Stem Cell, 16*, 350–352.

24. Cyranoski, D. (2019). Stem-cell therapy raises concerns. Independent researchers warn that approval is premature. *Nature, 565*, 544–545.
25. Nagai, S. (2019). Flexible and expedited regulatory review processes for innovative medicines and regenerative medical products in the US, the EU, and Japan. *International Journal of Molecular Sciences, 20*(15), 3801.
26. Cyranoski, D. (2019). Stem cells 2 go: Japan has turned regenerative medicine into a regulatory free-for-all. Patients across the world could pay the price. *Nature, 573*, 482–485.
27. Knoepfler, P. (2019, June 3). *Groundbreaking defeat for US stem cell, fat stem cell clinic industry in federal court*. The Niche: Knoepfler Lab Stem Cell Blog. https://ipscell.com/2019/06/groundbreaking-defeat-for-us-stem-cell-fat-stem-cell-clinic-industry-in-federal-court/. Last accessed 6 Mar 2021.
28. Sipp, D., & Sleeboom-Faulkner, M. (2019). Downgrading of regulation in regenerative medicine. *Science, 366*, 644–647.
29. Daley, G. Q., Insoo Hyun, I., Apperley, J. F., Barker, R. A., et al. (2016). Setting global standards for stem cell research and clinical translation: The 2016 ISSCR guidelines. *Stem Cell Reports, 6*, 787–797.
30. U.S. Food and Drug Administration. (2017, January). *22 case studies where phase 2 and phase 3 trials had divergent results*. https://www.fda.gov/about-fda/reports/22-case-studies-where-phase-2-and-phase-3-trials-had-divergent-results. Last accessed 6 Mar 2021.
31. Hamburg, M. A., & Sharfstein, J. M. (2009). The FDA as a public health agency. *The New England Journal of Medicine, 360*(24), 2493–2495.
32. Miyamoto, S. (2019). Stem-cell treatment: Japan responds. *Nature, 569*, 40.
33. Olanow, C. W., Goetz, C. G., Kordower, J. H., Stoessl, A. J., et al. (2003). A double-blind controlled trial of bilateral fetal nigral transplantation in Parkinson's disease. *Annals of Neurology, 54*, 403–414.
34. Barker, R. A., & TRANSNEURO consortium. (2019). Designing stem-cell-based dopamine cell replacement trials for Parkinson's disease. *Nature Medicine, 25*, 1045–1053.
35. Jonlin, E. C. (2015). The voices of the embryo donors. *Trends in Molecular Medicine, 21*(2), 55–57.
36. Stein, R. (2011, April 15). *Stem cells were God's will says the first recipient of treatment*. https://www.washingtonpost.com/national/stem-cells-were-gods-will-says-first-recipient-of-treatment/2011/04/14/AFxgKIjD_story.html. Last accessed 6 Mar 2021.
37. Jonlin, E. C. (2015). Consent for pluripotent cell use for therapy. *Current Stem Cell Reports, 1*(2), 92–101.
38. Gymrek, M., McGuire, A. L., Golan, D., Halperin, E., et al. (2013). Identifying personal genomes by surname inference. *Science, 339*, 321–324.
39. Charo, A. (2006). Body of research – Ownership and use of human tissue. *The New England Journal of Medicine, 355*(15), 1517–1519.
40. Skloot, R. (2010). *The immortal life of Henrietta Lacks*. Crown Publishing Group.

Investigational New Drug Applications for Cell Therapy Products

Bambi Grilley

1 Key Concepts

1. The FDA is the federal agency that decides which drugs, biologics, and medical devices are safe and efficacious, determinations upon which the agency decides if a product can be marketed in the USA.
2. The drug approval process in the USA is standardized by FDA review. It consists of preclinical testing and Phases I through IV of clinical testing.
3. The Investigational New Drug (IND) application is submitted by the study sponsor to the FDA to begin clinical trials in humans.
4. The IND should be amended as necessary. There are four types of documents used to amend the IND:
   - Clinical trial amendments.
   - Information amendments.
   - IND safety reports.
   - IND annual reports.
5. After sufficient evidence is obtained regarding the drug's safety and effectiveness, the sponsor will submit a New Drug application/Biologics Licensing Application to the FDA requesting approval of the agent for marketing.
6. An orphan drug is one that is used for the treatment of a rare disease, affecting fewer than 200,000 people in the USA, or one that will not generate enough revenue to justify the cost of research and development.
7. In addition, to review by the FDA, research clinical trials also require a review from Institutional Review Boards and as appropriate, Institutional Biosafety Committees.

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2 Introduction

The FDA monitors the manufacture, import, transport, storage, and sale of 25% of all goods purchased in the USA annually. The centers of the FDA involved in regulating biologics, drugs, and medical devices used in humans are as follows [1]:

- Center for Biologics Evaluation and Research (CBER).
- Center for Drug Evaluation and Research (CDER).
- Center for Devices and Radiological Health (CDRH) [2].

Since this chapter focuses on cellular therapies, we will focus on CBER which is where the majority of cellular therapies are reviewed. The FDA definition of cellular therapy products includes cellular immunotherapies, cancer vaccines, and both autologous and allogeneic cells used for therapeutic indications [3]. To date, although there is significant research in the use of cellular products as therapeutic treatments, less than 20 cellular products have been approved by the FDA [4]. Interestingly, a large number of cellular product INDs are submitted annually, and, in fact, in 2015, the number approached 250 IND submissions. Also interesting is that unlike drugs, where the vast majority of submissions are made by Biopharmaceutical companies, the majority of INDs for cellular therapies are submitted by noncommercial entities, primarily academic investigators and institutions [5].

3 History of Drug Development Regulation in the USA

For more than a century after the Declaration of Independence, drug products were not regulated in the USA. Available drugs were often ineffective, addictive, toxic, or even lethal. During this same period, physicians were not licensed and nearly anyone could practice medicine. The public was, for the most part, responsible for their own well-being when evaluating which products they would use.

The evolution of drug regulations in the USA is a study in human tragedy with medicinal crises resulting in the development of many of the laws regulating drug development, preparation, and distribution. Not until 1962, with the passage of the Kefauver-Harris Drug Amendment was there a requirement that a manufacturer had to demonstrate proof of efficacy, as well as safety, prior to marketing any new drug. [6]. Based on this and other laws, the FDA has assumed a large role in assessing the safety and efficacy of drug products prior to their distribution in the USA [7].

4 The Drug Approval Process

4.1 Preclinical Testing

The drug approval process in the USA is standardized by FDA review. It consists of preclinical testing and Phases I through IV of clinical testing. The first step in the process is preclinical testing [8]. This testing is conducted either in vitro (in a test tube or culture dish; outside of a living organism) or in vivo (within a living organism). Before filing an IND for an investigational new drug, preclinical studies should be conducted to establish feasibility of the use of the product and as possible to establish a safety profile supporting the use of the product in clinical studies. There are limitations to preclinical studies conducted in cellular therapies that relate rather specifically to difficulties identifying the appropriate animal species and animal models for cellular therapies. This makes traditional pharmacokinetic (PK) studies not feasible for cellular therapy products [9]. The FDA has provided specific guidance in this area entitled: Preclinical Assessment of Investigational Cellular and Gene Therapy Products [10].

4.2 Investigational New Drug Application

After the preclinical testing is completed, the sponsor will file an Investigational New Drug (IND) application with the FDA. The IND is the application by the study sponsor to the FDA to begin clinical trials in humans. As noted, the sponsor can be a biopharmaceutical company, but frequently in cellular therapy clinical trials, the sponsor will be an individual investigator who will file an IND and serve as a sponsor-investigator [5]. A commercial IND is one for which the sponsor is usually either a biopharmaceutical company or one of the institutes of the NIH. In addition, the FDA may designate any IND as commercial if it is clear that the sponsor intends the product to be commercialized at a later date. A sponsor-investigator IND is submitted when an investigator plans to use an approved drug for a new indication (i.e., one that is outside the package labeling) or an unapproved product in the context of a clinical trial. The IND requirements for the sponsor-investigator are generally the same as those for any other sponsor [11]. This caveat for commercial INDs relates to one of the information technology initiatives that the FDA has adopted in an attempt to facilitate the regulatory review process. Specifically, an important initiative of the FDA has been the development of systems allowing for electronic submission, management, and review of regulatory information [12]. The FDA mandated that all NDA, Biologics Licensing Applications (BLA), Abbreviated New Drug Application (ANDA), and Drug Master Files (DMF) be in the Electronic Common Technical Document (eCTD) format by May 2017 and all commercial Investigational New Drug Applications (INDs) be in eCTD format by May 2018 [13, 14]. This is a significant difference not only in how submissions are sent to the

FDA but also the format of those submissions. In this chapter we will focus on non-electronic submissions submitted in what is referred to as the ten-point format as specified in 21CFR312.23.

An IND is not required if the drug to be studied is marketed in the USA and all of the following requirements are met:

1. The clinical trial is not to be reported to the FDA in support of a new indication.
2. The clinical trial does not involve a different dose, route, or patient population that increases the risk to patients.
3. IRB approval and informed consent are secured.
4. The clinical trial will not be used to promote the drug's effectiveness for a new indication.

The FDA has developed a guidance document specifically to assist in determining whether or not an IND is required. However, in situations where it is unclear whether an IND is required or not, a call to the FDA is the best way to determine the appropriate way to proceed [15].

In recent years, there have been several therapeutic products including cellular therapies developed that depend on the use of an in vitro companion diagnostic device (or test) for their safe and effective use. It is important to note that in this situation, the in vitro device should be approved or cleared concurrently by FDA for the use indicated in the therapeutic product labeling. To be clear, this might require the clinical trial of the diagnostic device under an Investigational Device Exemption (IDE), while the therapeutic product is being studied under an IND. If the diagnostic device and therapeutic product are to be studied together to support their respective approvals (or clearance in the case of a device), both products can be studied in the same investigational clinical trial if the clinical trial has been developed and conducted in a manner that meets both IND and IDE regulations [16]. One other interesting issue related to devices is the use of a mobile app (i.e., a software application on a mobile platform such as an iPhone or Android) for the diagnosis, cure, mitigation, treatment, or prevention of disease or to affect the structure or function of the body. In these situations, the mobile app can be considered to be a medical device subject to IDE regulations [17]. IDE regulations and components will not be further discussed in this chapter; however, the applicable regulations can be found in 21CFR 812, and the FDA has extensive guidance regarding these products and their development [18].

4.3 Contents of IND

As specified in 21CFR212.23, an IND application needs to contain the following information:

- **Cover sheet:** Form 1571 (available at the FDA website under Forms, http://www.fda.gov/AboutFDA/ReportsManualsForms/Forms/default.htm). This form

identifies the sponsor, documents that the sponsor agrees to follow appropriate regulations, and any involved clinical research organization (CRO). This is a legal document.

- **Table of contents.**
- **Introductory statement:** States the name, structure, pharmacologic class, dosage form, and all active ingredients in the investigational drug; the objectives and planned duration of the investigation should be stated here.
- **General investigational plan:** Describes the rationale, indications, and general approach for evaluating the drug, the types of trials to be conducted, the projected number of patients that will be treated, and any potential safety concerns; the purpose of this section is to give FDA reviewers a general overview of the plan to study the drug.
- **Investigator's brochure:** An information packet containing all available information on the drug including its formula, pharmacologic and toxicologic effects, pharmacokinetics, and any information regarding the safety and risks associated with the drug. It is important that this brochure be kept current and comprehensive; therefore, it should be amended as necessary. The investigator's brochure may be used by the investigator or other healthcare professionals as a reference during the conduct of the research clinical trial.
- **Clinical trial.**
  - *Objectives and purpose:* A description of the purpose of the trial (a typical Phase I objective would be to determine the maximum tolerated dose of the investigational drug, whereas a typical Phase III objective would be to compare the safety and efficacy of the investigational drug to placebo or standard therapy).
  - *Investigator data:* Provides qualifications and demographic data of the investigators involved in the clinical trial (may be presented on Form 1572 (available at the FDA website under Forms, http://www.fda.gov/AboutFDA/ReportsManualsForms/Forms/default.htm).
  - *Patient selection:* Describes the characteristics of patients that are eligible for enrollment in the trial and states factors that would exclude the patient.
  - *Clinical trial design***:** Describes how the clinical trial will be completed; if the clinical trial is to be randomized, this will be described here with a description of the alternate therapy including a description of the control group [19].
  - *Dose determination:* Describes the dose (with possible adjustments) and route of administration of the investigational drug; if retreatment or maintenance therapy of patients is allowed, it will be detailed in this section.
  - *Observations*: Describes how the objectives stated earlier in the clinical trial are to be assessed.
  - *Clinical procedures*: Describes all laboratory tests or clinical procedures that will be used to monitor the effects of the drug in the patient; the collection of this data is intended to minimize the risk to the patients.
  - *IRB approval for clinical trial:* Documentation of this approval is not required as part of the IND application process; however, Form 1571 does state that an

IRB will review and approve each clinical trial in the proposed clinical investigation before allowing initiation of those studies [2].

- **Chemistry, Manufacturing, and Control Data.**
  - *Drug substance*: Describes the drug substance including its name, biological, physical, and chemical characteristics; the address of the manufacturer; the method of synthesis or preparation; and the analytical methods used to assure purity, identity, and the substance's stability [20].
  - *Drug product:* Describes the drug product, including all of its components; the address of the manufacturer; the analytical methods used to ensure identity, quality, purity, and strength of the product; and the product's stability.
  - *Composition, manufacture, and control of any placebo used in the trial*: The FDA does not require that the placebo be identical to the investigational drug; however, it wants to ensure that the lack of similarity does not jeopardize the trial.
  - *Labeling*: Copies of all labels and labeling used for the drug substance or product and packages as it will be provided to each investigator. Labels in this context is the information affixed to the product and used to identify the contents, while labeling in this context relates to product information including prescribing information.
  - *Environmental assessment*: Presents a claim for categorical exclusion from the requirement for an environmental assessment (a statement that the amount of waste expected to reach the environment may reasonably be expected to be nontoxic).
- **Pharmacology and Toxicology Data.**
  - *Pharmacology and drug disposition*: Describes the pharmacology, mechanism of action, absorption, distribution, metabolism, and excretion of the drug in animals and in vitro.
  - *Toxicology*: Describes the toxicology in animals and in vitro.
  - A statement that all nonclinical laboratories involved in the research adhered to Good Laboratory Practice (GLP) regulations.

The Letter of Authorization (LOA) to cross reference a DMF, IND, or NDA (referred to in item 9 on page 1 of Form 1571) is required when the investigational product (or some component of the investigational product) being used in the research is being supplied by a manufacturer other than the study sponsor. The original holder of the IND/NDA/DMF prepares the LOA. A LOA is frequently required when two companies are working together toward the development of a product.

Finally, proof of compliance with the requirements of ClinicalTrials.gov through submission of Form 3674 (available at the FDA website under Forms: http://www.fda.gov/AboutFDA/ReportsManualsForms/Forms/default.htm) is required as part of the IND [21].

4.4 Amendment of IND

The IND should be amended as necessary. There are four types of documents used to amend the IND:

5. Clinical trial amendments: submitted when a sponsor wants to change a previously submitted clinical trial or add a new clinical trial to an existing IND [22].
6. Information amendments: submitted when information becomes available that would not be presented using a clinical trial amendment, IND safety report, or annual report (e.g., new chemistry data) [23].
7. IND safety reports: Reports clinical and animal adverse reactions; reporting requirements depend on the nature, severity, and frequency of the experience. The following definitions are used to help evaluate adverse reactions.

- *Suspected adverse reaction:* An adverse reaction for which there is evidence to suggest a causal relationship between the drug and the adverse event.
- *Serious adverse event or serious suspected adverse reaction:* An event that results in any of the following outcomes: death, a life-threatening adverse drug experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious adverse drug experiences when, based on appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.
- *Unexpected adverse event or unexpected suspected adverse reaction:* An adverse reaction that is not listed in the current labeling for the drug product. This includes events that may be symptomatically and pathophysiologically related to an event listed in the labeling but differs from the event because of greater severity or specificity.
- For serious and unexpected suspected adverse reactions, the sponsor must report the event to the FDA in writing within 15 calendar days. Those events that are serious and unexpected require notification of the FDA within 7 calendar days. The written reports should describe the current adverse event and identify all previously filed safety reports concerning similar adverse events. The written report may be submitted as a narrative or as Form 3500A (available at http://www.fda.gov/safety/medwatch/howtoreport/downloadforms/default.htm) [24, 25].

8. Annual Reports.

These are submitted within 60 days of the annual effective date of an IND. It should describe the progress of the investigation including information on the individual studies, summary information of the IND (summary of adverse experiences, IND safety reports, preclinical studies completed in the last year), relevant developments in foreign markets, and changes in the investigator's brochure [26].

Each submission to a specific IND is required to be numbered sequentially (starting with 000). A total of three sets (the original and two copies) of all submissions to an IND file (whether a new IND or revisions to an existing IND) are sent to the FDA [8].

4.5 IND Submission

Once submitted to the FDA, the IND will be forwarded to the appropriate review division based on the therapeutic category of the product. Examples of the different divisions include oncology products, hematology products, anti-infective products, and medical imaging products. Following submission, the IND and clinical trial will be assigned to a review team that includes the following individuals:

- The regulatory project manager (RPM): Contact information for the RPM is provided in the letter sent to the applicant acknowledging receipt of the application. This will be the sponsor's (see below) primary FDA contact person. Each application that is submitted is assigned an RPM. If the RPM is changed during the course of the review, the applicant is notified by the new RPM.
- A chemistry, manufacturing, and control (CMC) reviewer.
- A nonclinical pharmacology/toxicology reviewer.
- A clinical reviewer.
- Other reviewers as needed (e.g., statisticians, epidemiologists, site inspectors, patient representatives) [27].

The FDA has 30 days after receipt of an IND to respond to the sponsor. The sponsor may begin clinical trials if there is no response from the FDA within 30 days [28]. The FDA delays initiation of a new clinical trial or discontinues an ongoing clinical trial by issuing a clinical hold. Clinical holds are most often used when the FDA identifies an issue (through initial review or through later submissions) that the agency poses a significant risk to the subjects. After this issue has been satisfactorily resolved, the clinical hold can be removed, and the investigations can be initiated or resumed [29].

4.6 Inactivation, Withdrawal, and Termination of an IND

A sponsor may withdraw an IND at any time. To do so all clinical investigations under the IND must be stopped, all investigators must be notified, and all investigational product supplies must be returned to the sponsor or destroyed. If the IND was withdrawn for safety reasons, the FDA, all IRBs, and all investigators must be notified of the reason for the withdrawal [30].

An IND may be inactivated by the sponsor or by the FDA if no subjects are entered into the related clinical trials for 2 years or more. If an IND is placed on

inactive study, all investigators should be notified and all stocks of drug supplies should be returned to the sponsor or discarded. The sponsor will not be required to submit annual reports which an IND is on inactive status. The IND can be reactivated by the sponsor by submitting an IND amendment outlining the plans for the IND and any relevant protocols [31].

An IND may be terminated by the FDA for IND deficiencies, deficiencies in the conduct of an IND, or the IND remains on IND inactive status for 5 years or more. The FDA will notify the sponsor of the plan to terminate an IND, and the sponsor will have 30 days to provide a written explanation or provide a correction that allows the IND to remain in active or inactive status [32].

4.7 Phases of Clinical Trial

There are four phases of clinical trials. Clinical studies generally begin cautiously. As experience with the agent grows, the dose and duration of exposure to the agent may also increase. The number of patients treated at each phase of clinical trial, and the duration of the studies, can vary significantly depending on statistical considerations, the prevalence of patients affected by the disease, and the importance of the new drug. However, some general guidelines regarding the four phases of clinical testing are presented below.

4.7.1 Phase I

A Phase I trial is the first use of the agent in humans. As such, these studies are usually initiated with cautious (low) doses and in a small numbers of subjects. Doses may be increased as safety is established. A Phase I clinical trial will usually include 20–80 subjects who receive the investigational product. Phase I trials last an average of 6 months to 1 year. The purpose of a Phase I trial is to determine the safety and toxicity of the agent. Frequently these trials include a pharmacokinetic portion. These trials assist in identifying the preferred route of administration and a safe dosage range. When possible, these trials are initiated in normal, healthy volunteers. This allows for the evaluation of the effect of the drug on a subject who does not have any preexisting conditions. In situations in which this is not practical, such as oncology drugs, in which the drug itself can be highly toxic, these drugs are usually reserved for patients who have exhausted all conventional options.

4.7.2 Phase II

A Phase II trial is one in which the drug is used in a small number of subjects who suffer from the disease or condition that the drug is proposed to treat. The purpose of a Phase II trial is to evaluate the efficacy of the agent. Data from the Phase I trial,

in vitro testing, and animal testing may be used to identify which group of patients is most likely to benefit from therapy with this agent. Phase II trials usually treat between 100 and 200 patients and will average about 2 years in duration. Following Phase II trials, study sponsors will frequently assess these preliminary results and predicted marketability of the product prior to initiating the larger and more expensive Phase III trials.

4.7.3 Phase III

Phase III trials build on the experience gained during the Phase II trials. The purpose of a Phase III clinical trial is to further define the efficacy and safety of the agent. Frequently, in Phase III studies, the new agent is compared to current therapy. These trials are usually multicenter studies, generally treat from several hundred to 3000 patients, and the clinical trial will usually last about 3 years (although an individual subjects participation may be significantly shorter). Usually, some of the Phase III trials will be considered pivotal studies and will serve as the basis for the NDA/BLA for a medicinal product's marketing approval [33].

4.8 Biological License Application/New Drug Application

After sufficient evidence is obtained regarding the drug's safety and effectiveness, and Phase III trials have been completed, the sponsor will submit a BLA (in the case of drugs, it is referred to as an NDA) to the FDA requesting approval of the medicinal product for marketing. Except as noted for products being developed using accelerated approval pathways, the FDA requires the completion of two well-designed, controlled clinical trials prior to submission to the FDA. However, the sponsor will include information gathered from all of the clinical trials to show that the medicinal product is safe and effective and to describe the pharmacology and pharmacokinetics of the drug. The BLA/NDA will include all preclinical data, clinical data, manufacturing methods, product quality assurance, relevant foreign clinical testing (or marketing experience), and all published reports of experience with the medicinal agent (whether sponsored by the company or not). A proposed package insert will be supplied as well [34].

4.8.1 Review of New Drug Application

The BLA/NDA will be distributed to the same FDA review division assigned, while the product was under IND status. As noted, these divisions are based on the therapeutic group of the medicinal agent. The same reviewer may be assigned to review the IND and the BLA/NDA [10].

The speed at which the BLA/NDA will be processed is to some extent determined by the classification the drug receives during its initial review. Each agent is rated with a number-letter designation that evaluates two separate aspects of the agent. The number portion of the rating is associated with the uniqueness of the drug product (ranging from 1 for a NME to 7 for a drug that has already been marketed, but without an approved BLA/NDA), and the letter portion of the rating is associated with the therapeutic potential of the medicinal agent. The P (priority review) designation is given to drugs that represent a therapeutic advance with respect to available therapy, whereas an S (standard review) is given to drugs that have little or no therapeutic gain over previously available drugs. BLA prioritization is slightly simplified but similar [35].

During the review process, the FDA may utilize one of its prescription drug advisory committees to help review the NDA. These committees are composed of experts who provide the agency with independent, nonbinding advice and recommendations regarding the NDA. Currently the FDA has 31 advisory committees, many of which are composed of various panels. Examples of such committees include the allergenic products advisory committee and the cellular, tissue, and gene therapies advisory committee [36]. Within 180 days of receipt of an NDA, the FDA will review the application and send the applicant an approval letter or a complete response letter [37]. When an approval letter is sent, the drug is considered approved as of the date of the letter [38]. A complete response letter is issued to let the sponsor know that the review period for the drug is complete but that the application is not yet ready for approval. It will describe specific deficiencies and when possible, identify recommended actions that the sponsor might take to address those deficiencies. In response to the complete response letter, the sponsor amends the NDA, withdraws the NDA, or requests a hearing with the FDA to clarify whether grounds exist for denying the approval of the application [39].

4.9 Expanded Access to Investigational Drugs for Treatment Use

The FDA has historically received considerable criticism relative to the time taken for product review. They have implemented many initiatives to address these criticisms. The most recent initiative implemented by the FDA is referred to as "expanded access to investigational drugs for treatment use." The expanded access rule clarifies existing regulations and adds new types of expanded access for treatment use. Specifically, the rule allows for investigational drugs to be used for treatment in patients with serious or life-threatening diseases where there is no other comparable or satisfactory alternative therapy. The FDA defines immediately life-threatening conditions as those where death is likely to occur within a matter of months or in which premature death is likely without early treatment. Serious conditions are defined as those associated with morbidity that has substantial impact on day-to-day

functioning [40]. The rules specify different requirements for expanded access for individual patients in emergencies; intermediate-sized patient populations; and larger populations under a treatment clinical trial or treatment IND [41].

The FDA must determine that, in addition to the patient having a serious or immediately life-threatening disease for which there is no satisfactory alternative therapy, the potential patient benefit must outweigh the risk and that the requested use will not interfere with clinical investigations that could support marketing approval of the expanded access use. In all cases, an expanded access submission to the FDA is required. The submission may be a new IND or a clinical trial amendment to an existing IND (see the above explanation). Except as justified by emergency use guidelines further discussed in this chapter, all other regulations governing new INDs and clinical trial amendments, including regulations regarding clinical trial initiation, adverse reaction reporting, and annual reports, are identical to that described for standard INDs and described elsewhere in this chapter [42].

For individual patients, submission requirements must include information adequate for the FDA to determine that the risk to the person from the investigational drug is not greater than the probable risk from the disease and that the patient cannot obtain the drug under another type of IND. Treatment is generally limited to a single course of therapy for a specified duration unless the FDA expressly authorizes multiple courses or chronic therapy. Individual patient expanded access submissions can be made in accordance with the standard submission requirements for an IND as outlined elsewhere in this chapter, or they may be submitted utilizing Form 3926. In this type of submission, the FDA does allow for emergency procedures if the patient must be treated before a written submission can be made. In that situation, the FDA may authorize the emergency use by telephone. The sponsor must agree to submit an expanded access submission within 15 business days of the FDA's authorization of the use [43]. In addition, although the FDA must authorize emergency use of a test article (investigational drug), 21CFR56.104 allows for treatment to occur without prospective IRB approval in situations where there is insufficient time to obtain such review. In such situations the emergency use must be reported to the IRB within 5 days after the treatment. Newer guidance from the FDA allows an investigator submitting an individual patient expanded access IND to request a waiver from full IRB review under 21CFR56.105 when the investigator obtains concurrence by the IRB chairperson or another designated IRB member before treatment use begins [44].

For intermediate patient populations, there must be sufficient evidence that the drug is safe at the dose and duration proposed for treatment and that there is at least preliminary clinical evidence of the effectiveness of the drug. The sponsor must also indicate whether the drug is being developed and define the patient population. If the drug is being studied in a clinical trial, the sponsor must explain why the expanded access patient population cannot be enrolled in the clinical trial and under what circumstances the sponsor would conduct a clinical trial in those patients [45].

The treatment IND, or treatment clinical trial, is a way the FDA has allowed for increased accessibility of experimental drugs for widespread treatment use. The drug must be investigated in a clinical trial under an IND designed to support a

marketing application for the expanded access use, or if all clinical trials have been completed, the sponsor must be actively pursuing marketing approval of the drug for the expanded access use. When the expanded access use is for an immediately life-threatening disease, the available scientific evidence (usually clinical data from Phase II or Phase III trials) must provide reasonable assurance that the drug may be effective for the expanded access use and would not expose patients to significant risk [46].

5 Right to Try

The Right to Try Act was signed into law May 30, 2018. This law is another way for patients with life-threatening conditions without other alternatives, and unable to participate in a clinical trial, to obtain access to certain unapproved treatments. Right to Try treatments are those that are defined as eligible investigational drugs according to the following criteria:

- A Phase I trial has been completed.
- The product is not FDA approved for any use.
- An application has been filed with the FDA that will form the basis for a claim of effectiveness, and an IND has been submitted to the FDA.
- Active development of the product is ongoing and has not been discontinued by the manufacturer or placed on hold by the FDA.

Neither the FDA nor an IRB reviews the Right to Try Act uses. A physician working with a patient will contact the sponsor of the investigational product to determine if it is an eligible investigational drug under the Right to Try Act. The Right to Try Act does not require a sponsor to provide an eligible investigational drug to an eligible patient [47, 48].

6 Cost of Using Investigational Drugs

The FDA allows the manufacturer to charge for an investigational drug under certain conditions. The sponsor must obtain prior written authorization from FDA to charge for an investigational drug. In order to charge for an investigational drug, the sponsor must provide evidence that the drug has a potential clinical benefit that would provide a significant advantage over available products, demonstrate that the data to be obtained from the clinical trial would be essential to establishing that the drug is effective or safe, and demonstrate that the clinical trial could not be conducted without charging because the cost of the drug is extraordinary to the sponsor. The sponsor may only charge recovery costs for direct cost attributable to making the investigational drug, including raw materials, labor, nonreusable supplies, and equipment used to manufacture the drug or costs to acquire the drug from another

manufacturer or to ship and handle the drug. In addition, for expanded access studies for intermediate-sized patient populations or treatment IND/clinical trials, a sponsor may recover the cost of monitoring the expanded access IND or clinical trial, complying with IND reporting requirements, and other administrative costs directly associated with the expanded access IND [49–51].

7 Expedited Review for New Drugs

As previously noted, the FDA has received considerable criticism for slow review times. Over the past two decades, the FDA and the federal government have initiated many reforms and initiatives designed to address these criticisms. Included in these reform acts are the Prescription Drug User Fee Acts (PDUFA); the Food and Drug Administration Modernization Act (FDAMA) of 1997; the Food and Drug Administration Amendments Act (FDAAA) of 2007, the Food and Drug Administration Safety and Innovation Act (FDASIA) of 2012 signed into law on July 9, 2012; and most recently the twenty-first Century Cures Act, signed into law in December 2016 [52–56]. These acts and other initiatives have resulted in expedited review pathways as follows:

9. Priority review: The FDA determines a drug will potentially provide a significant advance in medical care and sets a target to review the drug within 6 months instead of the standard 10 months.
10. Fast-track review: The FDA determines that a drug can treat unmet medical needs. Fast-track speeds new drug reviews, for instance, by increasing the level of communication the FDA allocates to developers and by enabling developers to use a rolling review process such that portions of an application can be reviewed ahead of the submission of the full application.
11. Breakthrough therapy: Allows for expedited development and review of drugs which are intended to treat serious conditions and which may demonstrate a substantial improvement over available therapy.
12. Regenerative Medicine Advanced Therapy (RMAT) designation: The CBER gives this designation to certain human gene therapies and xenogeneic cell products if it determines being that the product is intended for use in serious or life-threatening illness and preliminary clinical evidence indicates that the drug has the potential to address an unmet medical need for such disease or condition. RMAT designation includes all the benefits of the fast-track and breakthrough therapy designation programs [57].

These designations relate directly to an IND and become an integral part of the review of the product being studied under the IND.

Aside from looking at review times, the FDA has also been concerned about the increasing difficulty in drug and biologic development. To attempt to address this issue, the FDA launched a new initiative in March 2004 called the Critical Path Initiative. Having identified an increasingly large gap between laboratory

discoveries and new treatments for patients with serious diseases such as diabetes, cancer, and Alzheimer disease, the Critical Path Initiative is the FDA's attempt to facilitate modernization of the sciences and improve regulatory decision-making. The FDA has been working with the public, the pharmaceutical industry, other regulatory agencies, and academia to identify projects that they feel are most likely to help the drug development process from test tube to bedside [58].

More recently, as a result of FDASIA, the FDA has increased their focus on patient engagement [55]. A notable example of this initiative is a patient advocacy-initiated draft guidance submitted to the FDA to help accelerate the development and review of potential therapies for Duchenne syndrome. A major point of emphasis was that the parents of children affected by Duchenne syndrome were willing to accept a higher-risk profile for potential therapies even those in that may improve patient quality of life without prolonging life [59, 60].

Overall, the goal of all of the abovementioned initiatives is to review priority drugs in 6 months and standard drugs within 10 months, with an emphasis on improving consistency and transparency of the review process [61].

8 Phase IV Post-Marketing Surveillance

After the drug has been approved, post-marketing studies may be initiated. They are conducted for the approved indication but may evaluate different doses, the effects of extended therapy, or the drug's safety in patient populations that were not represented in premarketing clinical trials. The final phase of clinical trial is referred to as Phase IV trials. These Phase IV trials may be requested by the FDA, or they may be initiated by the sponsor in an attempt to gather more data on the safety and efficacy of the drug or to identify a competitive advantage of the drug over other available therapies.

9 Risk Evaluation and Mitigation Strategies (REMS)

In some situations, the FDA may actually approve a product with restrictions limiting use to certain facilities or providers or limiting the patient population to only those who have demonstrated certain performance on specified medical procedures [62, 63]. Specifically, the FDA has started to utilize Risk Evaluation and Mitigation Strategies (REMS) when they determine that safety measures are needed beyond the labeling to ensure that a drug's benefits outweigh its risks. REMS can be required before or after a drug is approved. REMS are developed by drug sponsors; however, the FDA reviews and approves them. Factors that are considered in determining the need for a REMS include the following [64]:

- The seriousness of any known or potential adverse events that may be related to the drug and the background incidence of such events in the population likely to use the drug.
- The expected benefit of the drug with respect to the disease or condition.
- The seriousness of the disease or condition that is to be treated with the drug.
- Whether the drug is an NME.
- The expected or actual duration of treatment with the drug.
- The estimated size of the population likely to use the drug.

The REMS may include the following components: a medication guide (patient package insert) and a communication plan (for providing key information to health care providers). The FDA may also require elements to assure safe use (ETASU) if the drug has been shown to be effective but is associated with a specific serious risk. Sample ETASU components include required training or certifications for healthcare providers, limitations on healthcare settings where the drug can be infused, dispensing the drug only with evidence of safe conditions (e.g., specific laboratory results), monitoring of drug use by the patient, and enrollment of the patient on a registry. The REMS must be assessed for adequacy at least by 18 months, 3 years, and 7 years after approval [65]. As an example, the product Axicabtagene Ciloleucel (trade name YESCARTA) was approved to treat certain types of non-Hodgkin lymphoma. The product however may cause side effects that are life-threatening and require intensive interventions including treatment with the product tocilizumab. Due to these risks, the drug was approved with a requirement for a REMS. The goal of the FDA-mandated REMS is to ensure that individuals who prescribe, dispense, and administer YESCARTA are aware of how to manage the toxicities, ensuring that hospitals and their associated clinics that dispense the product are certified and have onsite access to tocilizumab. The REMS therefore requires that hospitals and their associated clinics must be enrolled in the YESCARTA REMS program to be able to dispense YESCARTA. Additionally, all relevant staff involved in the prescribing, dispensing, or administering of YESCARTA must be trained on the REMS program requirements and must successfully complete a YESCARTA REMS program knowledge assessment [66, 67].

10 The Orphan Drug Act

The Orphan Drug Act was passed in 1983 and provides incentives for manufacturers to develop orphan drugs. An orphan drug is one used for the treatment of a rare disease, affecting fewer than 200,000 people in the USA, or one that will not generate enough revenue to justify the cost of research and development. There are currently more than 7000 rare diseases impacting approximately 30 million Americans [68].

The Orphan Drug Act is administered by the FDA's Office of Orphan Products, and the related program has enabled the development and marketing of over 600

drugs and biologic products for rare diseases since its inception in 1983 [69]. Of note, although to qualify for consideration as an orphan drug, a product must be under evaluation as part of an IND; the review and approval process for an orphan drug designation is separate from that of the IND. The orphan drug designation (also known as the orphan status) is awarded only if both the drug and the disease meet certain qualifications.

The orphan drug designation provides the following incentives:

- **Tax incentives***:* The sponsor is eligible to receive a tax credit money spent on research and development of an orphan drug.
- **Waive filing fees:** The sponsor is eligible to file for a waiver from the application fee associated with the review of an NDA.
- **Clinical trial assistance***:* If a sponsor can show that a drug will be used for a rare disease, the FDA will provide assistance developing the preclinical and clinical plan for the product.
- **Grants and contracts***:* The FDA budget may allot money for grants and contracts to be used in developing orphan drugs. The current annual budget for orphan drug grants is $15 million with $ten million for ongoing noncompete renewals and $five million to fund new projects annually. The orphan grants process has been used to bring more than 60 products to marketing approval [70].
- **Marketing exclusivity***:* The first sponsor to obtain marketing approval for a designated orphan drug is allowed 7 years of marketing exclusivity for that indication, but identical versions of the same product marketed by another manufacturer may be approved for other indications.

The Orphan Drug Act does not provide advantages for the drug approval process. Sponsors seeking approval for drugs that will be designated as orphan drugs must still provide the same safety and efficacy data as all other drugs evaluated by the FDA. Exceptions to the rules governing the number of patients that should be treated in the clinical trials may be made based on the scarcity of patients with the condition. Additionally, because in many cases there are no alternative therapies for the disease, the drug may be given a high review priority during the NDA process [71, 72].

11 Transparency of Drug Development

First initiated in response to components of FDAMA, the National Institutes of Health (NIH) developed a web-based system that offers information about ongoing clinical trials for a wide range of diseases and conditions. The site is available at http://clinicaltrials.gov. Initially intended as a system to provide a registry of clinical trials, it allows potential study subjects to search for studies for particular diseases and identify treatment centers that offer enrollment into those studies. The FDA requires that for studies being conducted under an IND, the sponsor verifies that the study is posted to the system through submission of Form 3674 to the IND

[73]. Requirements for postings have become increasingly more stringent over time. In 2016, both the FDA and NIH implemented initiatives intended to further enhance the availability of clinical trial information. Both entities require registration of all applicable studies in the system within 21 days after enrollment of the first participant. The difference is in the scope being related to the definition of an applicable study. The FDA defines an applicable clinical trial as any clinical trial, including an FDA-regulated product, but excludes Phase 1 or small feasibility device studies. In contrast, the NIH defines an applicable clinical trial as all clinical trials funded by NIH, including only behavioral interventions. Both the NIH and FDA require reporting of results from applicable studies no later than 12 months after the primary completion date (the date that the last participant but reached the primary objective). Results reporting includes not only information about the subjects (demographics and participant flow) but also information about adverse events, outcomes, and statistical analyses [74, 75].

12 Institutional Reviews

In additional to review of the investigational product by the FDA, institutions will have required reviews as well. The review processes may include a review by an Institutional Review Board (IRB), Institutional Biosafety Committee (IBC), and a Scientific Review Committee. Review by these committees are considered to be complementary processes within institutions and approval as required by all of them before a clinical trial can be activated.

12.1 Institutional Review Board/Institutional Ethics Committee

The Institutional Review Board (IRB), known outside of the USA as the Institutional Ethics Committee, is a committee formed to review proposed clinical trials and the progress of such studies to ensure that the rights and welfare of human subjects are protected. The US regulations governing the protection of human subjects include Title 45 Part 46 of the Code of Federal Regulations (CFR), which was designed to make uniform the protection of human subjects in all federal agencies; Title 21 Part 50 of the CFR sets forth FDA guidelines for appropriate informed consent; and Title 21 Part 56 of the CFR sets forth FDA guidelines for the IRB [76–78]. IRB review can be accomplished in two ways, either review by a locally constituted (usually institutionally based) IRB or reliance on a single IRB. The IRB must contain at least one member who has specialized knowledge in a scientific area (in situations where drugs and biologics are being reviewed, this is usually a physician) and at least one board member who has a specialty in a nonscientific area such as law, ethics, or

religion. Additionally, the IRB must contain at least one individual who is not affiliated with the institution where the research is being conducted. The membership of the IRB varies between institutions. Common members of IRBs include physicians, pharmacists, nurses, lawyers, clergy, and laypeople. Scientific membership of a locally constituted IRB can vary between institutions but will generally reflect the expertise of the scientific community at the institution. An institution that focuses only on cancer may have an IRB composed only of oncologists. However, a more general hospital will have membership inclusive of multiple disciplines and specialties. Locally constituted IRBs are generally utilized to review internally initiated clinical trials, although their scope is not limited by law. Reliance on a single IRB is more likely to occur in situations where a clinical trial will be conducted at more than one institution or when a study has an external sponsor. In this situation, the institution can enter into an agreement stating that they will rely on the review of a clinical trial as provided by another IRB. This is called *reliance* on a single IRB of record. Current law requires a single IRB review of many federally supported clinical trials including those being conducted by the National Cancer Institute (NCI). The single IRB can be for profit, or they can be constituted by other organizations including the Federal government, but in any case, although the specialties and expertise represented on the committee may closely mirror that of a locally constituted IRB, their members are more likely to be from different geographic areas. As with locally constituted IRBs, single IRBs can also be developed specifically to review certain types of research, and as an example, COG clinical trials are reviewed by the National Cancer Institute (NCI) IRB. The intent of reliance on a single IRB of record is to decrease duplicative review processes at individual institutions while still maintaining human subject protections. However, frequently institutions require administrative review of the clinical trial by the IRB or the Office of Research including a review of the consent form and a requirement for standard, institution-required verbiage to be included.

The IRB reviews new studies, amendments to existing studies, and serious adverse events that occur during the conduct of a study and provides an annual review of all studies that are active at the institution. The IRB reviews these submissions to ensure that the following requirements are met:

- The risks to subjects are minimal.
- The expected risk/anticipated benefit ratio must be reasonable.
- Equitable subject selection is utilized.
- Informed consent must be received from each participant (or his or her legally authorized representative).
- Informed consent must be documented in writing unless a modified approach is justified according to very specific criteria spelled out in the regulations.
- Data must be monitored to ensure subject safety.
- Patient confidentiality must be maintained.
- If appropriate, additional safeguards against coercion must be included in studies that include vulnerable subjects (children, prisoners, pregnant women, mentally disabled people, or economically or educationally disadvantaged persons).

- IRBs are also involved in evaluating research conflict of interest (COI). Although this is a shared responsibility for the institution, the IRB, and the investigator (and staff), the IRB has the responsibility for evaluating whether or not financial interests may impact the protection of human subjects. The IRB will have a mechanism for reporting COI, policies for managing or eliminating such COI, and as deemed necessary require that such conflicts be provided to potential study subjects as part of the informed consent process [79].

12.2 Scientific Review

Some institutions divide their review of proposed clinical research into two separate processes, IRB review as described above and scientific review. Scientific review committees are not mandated by law but are required by some funding agencies including the NCI [80]. As a result, this type of committee review is common in institutions that conduct a high volume of interventional clinical trials and is a common component of review at cancer centers. Timing of scientific review is not mandated; however, most institutions require scientific review prior to review by an IRB as this optimizes the content of the study prior to submission to the IRB. Scientific review committees include experts knowledgeable in the types of research being conducted and will generally include not only physicians but also surgeons, radiologists, statisticians, pharmacists, and nurses who work in areas of research. The typical committee usually includes about 20 scientists and specialists. Patient advocates/laypeople are frequently asked to serve on these committees as well to provide insight into logistical issues that may impact the desire of the patient to participate or the feasibility of the study.

12.3 Institutional Biosafety Committee

For clinical trials that include gene therapy (also called gene transfer), review by a local *Institutional Biosafety Committee* (*IBC*) is also required as specified in the NIH guidelines [81]. Gene therapy clinical trials use techniques that are novel and include potentially irreversible risks to the subject and their progeny (in cases of germline [egg or sperm] alteration). Gene therapy products also include the risk of inadvertent transfer to other individuals including healthcare workers, family members, and even the general public. For this reason, IBC review is intended to provide a local review of all research that includes gene transfer into any human. The use of a single IBC to cover research across multiple institutions is uncommon. IBC review is highly scientific in nature, and the requirements for submission of a project to the IBC will vary among institutions; however, in all cases the IBC is required to review:

- The source of the genes being used.
- The nature of the genes being used.
- The way in which the gene might be introduced into the cells of the patient.
- The mechanisms used for gene containment.
- The training plan for personnel involved in the project.
- The plans that exist for handling accidental spills and personnel contamination resulting from contact with the product.

Each of these components of a clinical trial including the actual gene transfer has a risk profile that requires consideration by scientific experts through the IBC review process. IRB and IBC reviews are considered to be complementary processes within institutions, and approval is required by both entities before a gene therapy clinical trial can be activated.

13 Conclusions

Clinical trials that utilize drugs, biologics, and devices in the USA are largely controlled by the FDA and by local IRBs although additional reviews may be required. A thorough understanding of the regulations governing those entities is extremely helpful when conducting clinical trials as failure to comply with the regulations can result in serious consequences including loss of funding, inability to publish, and restrictions in the ability to engage in further clinical research.

References

1. U.S. Food and Drug Administration (2018) *Executive summary: Strategic plan for regulatory science*. Updated through 2018 March. https://www.fda.gov/science-research/advancing-regulatory-science/executive-summary-strategic-plan-regulatory-science . Last accessed 17 Aug 2020.
2. U.S. Food and Drug Administration (2019) *FDA organization chart*. Updated through 2019 October. https://www.fda.gov/about-fda/fda-organization-charts/fda-overview-organization-chart . Last accessed 17 Aug 2020.
3. U.S. Food and Drug Administration (2019) *Cellular and gene therapy products*. Updated 2019 June. https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products . Last accessed 17 Aug 2020.
4. U.S. Food and Drug Administration (2019) *Cellular and gene therapy products*. Updated 2019 March. https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/approved-cellular-and-gene-therapy-products . Last accessed 17 Aug 2020.
5. Puri, R (2016) *FDA, center for biologics evaluation & research: Office of cellular, tissue and gene therapies overview*. Presented 2016 September. https://www.fda.gov/media/100476/download . Last accessed 17 Aug 2020.
6. Meadows M (2019) *Promoting Safe and effective drugs for 100 years*. FDA Consum [Internet]. Last updated 2019 April. https://www.fda.gov/about-fda/histories-product-regulation/promoting-safe-effective-drugs-100-years. Last accessed 17 Aug 2020.

7. Meadows M. Promoting Safe and effective drugs for 100 years. FDA Consumer https://www.fda.gov/about-fda/histories-product-regulation/promoting-safe-effective-drugs-100-years. Last accessed 17 Aug 2020.
8. U.S. Food and Drug Administration Code of Federal Regulations. 21CFR Part 312.23. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=312.23. Last accessed 17 Aug 2020.
9. U.S. Food and Drug Administration (2015) *Guidance for industry: Considerations for the design of early-phase clinical trials of cellular and gene therapy products*. 2015 June. https://www.fda.gov/media/106369/download. Last accessed 17 Aug 2020.
10. U.S. Food and Drug Administration (2013) *Guidance for industry: Preclinical assessment of investigational cellular and gene therapy products*. 2013 November. https://www.fda.gov/media/87564/download. Last accessed 17 Aug 2020.
11. U.S. Food and Drug Administration (2018) *CDER. MAPP 6030.1: IND Process and Review procedures*. Updated 2018 February. https://www.fda.gov/media/72751/download. Last accessed 17 Aug 2020.
12. U.S. Food and Drug Administration (2019) *Electronic regulatory submissions and review*. Updated 2019 August. http://www.fda.gov/Drugs/DevelopmentApprovalProcess/FormsSubmissionRequirements/ElectronicSubmissions/default.htm. Last accessed 17 Aug 2020.
13. U.S. Food and Drug Administration (2019) *Electronic Common Technical Document (eCTD)*. Updated 2019 September [cited 2019 October]. http://www.fda.gov/Drugs/DevelopmentApprovalProcess/FormsSubmissionRequirements/ElectronicSubmissions/ucm153574.htm. Last accessed 17 Aug 2020.
14. U.S. Food and Drug Administration (2015) *CDER Guideline for Drug Master Files*. Updated 2015 November. http://www.fda.gov/Drugs/DevelopmentApprovalProcess/FormsSubmissionRequirements/DrugMasterFilesDMFs/ucm073164.htm. Last accessed 17 Aug 2020.
15. U.S. Food and Drug Administration (2013) *CDER/CBER. Guidance for industry: Investigational New Drug Applications (INDs) – Determining whether human research studies can be conducted without an IND*. 2013 September. http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm229175.pdf. Last accessed 17 Aug 2020.
16. U.S. Food and Drug Administration (2014) *CDER/CBER/CDRH. Guidance for industry and Food and Drug Administration staff: In Vitro Companion Diagnostic Devices*. 2014 August. http://www.fda.gov/downloads/medicaldevices/deviceregulationandguidance/guidancedocuments/ucm262327.pdf. Last accessed 17 Aug 2020.
17. U.S. Food and Drug Administration (2019) *CBER/CDRH. Policy for device software functions and mobile medical applications*. 2019 September. http://www.fda.gov/downloads/medicaldevices/deviceregulationandguidance/guidancedocuments/ucm263366.pdf. Last accessed 17 Aug 2020.
18. United States Federal Government Code of Federal Regulations. 21CFR Part 812. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=812&showFR=1&subpartNode=21:8.0.1.1.9.2. Last accessed 17 Aug 2020.
19. International Conference on Harmonization (ICH) Guidance for Industry: E10 Choice of Control Group and Related Issues in Clinical Trials. 2001 Jan. https://www.ema.europa.eu/en/documents/scientific-guideline/ich-e-10-choice-control-group-clinical-trials-step-5_en.pdf. Last accessed 17 Aug 2020.
20. United States Federal Government Code of Federal Regulations. 21CFR Part 314.3. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=314.3. Last accessed 17 Aug 2020.
21. U.S. Food and Drug Administration. Guidance for Sponsors, Industry, Researchers, Investigators, and Food and Drug Administration Staff: Form FDA 3674 - Certifications to

Accompany Drug, Biological Product, and Device Applications/Submissions 2017 June. https://www.fda.gov/media/105405/download. Last accessed 17 Aug 2020.
22. Code of Federal Regulations 21CFR Part 312.30. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=312.30. Last accessed 17 Aug 2020. Last accessed 17 Aug 2020.
23. Code of Federal Regulations. 21CFR Part 312.31. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=312.31. Last accessed 17 Aug 2020.
24. Code of Federal Regulations. 21CFR Part 312.32. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRsearch.cfm?CFRPart=312. Last accessed 17 Aug 2020.
25. U.S. Food and Drug Administration (2012) *CDER/CBER. Guidance for industry and investigators: Safety Reporting Requirements for INDs and BA/BE Studies*. 2012 December. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM227351.pdf. Last accessed 17 Aug 2020.
26. Code of Federal Regulations. 21CFR Part 312.33. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRsearch.cfm?CFRPart=312. Last accessed 17 Aug 2020.
27. Yager, J. A., & Kallgren, D. L. (2000). Roles of regulatory project managers in the U.S. Food and Drug Administration's Center for Drug Evaluation and Research. *Drug Information Journal, 34*, 289–293.
28. Code of Federal Regulations. 21CFR Part 312.40. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=312.40. Last accessed 17 Aug 2020.
29. Code of Federal Regulations. 21CFR Part 312.42. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=312.42. Last accessed 17 Aug 2020.
30. Code of Federal Regulations. 21CFR Part 312.38. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=312.38. Last accessed 17 Aug 2020.
31. Code of Federal Regulations. 21CFR Part 312.45. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=312.45. Last accessed 17 Aug 2020.
32. Code of Federal Regulations. 21CFR Part 312.44. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=312.44. Last accessed 17 Aug 2020.
33. U.S. Food and Drug Administration. The FDA's Drug Review Process: Ensuring Drugs are Safe and Effective. http://www.fda.gov/Drugs/ResourcesForYou/Consumers/ucm143534.htm. Last accessed 17 Aug 2020.
34. Code of Federal Regulations. 21CFR Part 314.50 https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=314.50. Last accessed 17 Aug 2020.
35. U.S. Food and Drug Administration (2015) *Drug development and review definitions*. 2015 August. https://www.fda.gov/drugs/investigational-new-drug-ind-application/drug-development-and-review-definitions. Last accessed 17 Aug 2020.
36. 21CFR Part 14: Public Hearing before a public advisory committee. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=14. Last accessed 17 Aug 2020.
37. Code of Federal Regulations. 21CFR Part 314.100. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=314.100. Last accessed 17 Aug 2020.
38. Code of Federal Regulations. 21CFR Part 314.105. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=314.105\. Last accessed 17 Aug 2020.
39. Code of Federal Regulations. 21CFR Part 314.110. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=314.110. Last accessed 17 Aug 2020.
40. Code of Federal Regulations. 21CFR Part 312.300. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=312.300. Last accessed 17 Aug 2020.
41. U.S. Food and Drug Administration (2017) *CDER/CBER. Expanded access to investigational drugs for treatment use – Questions and answers*. 2017 October. http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm351261.pdf. Last accessed 17 Aug 2020. Last accessed 17 Aug 2020.
42. Code of Federal Regulations. 21CFR312.Part 305. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=312.305. Last accessed 17 Aug 2020.

43. Code of Federal Regulations 21CFR Part 312.310. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=312.310. Last accessed 17 Aug 2020.
44. Code of Federal Regulations. 21CFR Part 56.105. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=56.105. Last accessed 17 Aug 2020.
45. Code of Federal Regulations. 21CFR Part 312.315. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=312.315. Last accessed 17 Aug 2020.
46. Code of Federal Regulations. 21CFR Part 312.320. https://www.fda.gov/media/75414/download. Last accessed 17 Aug 2020.
47. Right to Try. 2019 May Food and Drug Administration https://www.fda.gov/patients/learn-about-expanded-access-and-other-treatment-options/right-try. Last accessed 17 Aug 2020.
48. United States Congress (2018) *Right to Try Act, Public Law 115–176*. 2018 January. https://www.congress.gov/115/bills/s204/BILLS-115s204enr.pdf. Last accessed 17 Aug 2020.
49. U.S. Food and Drug Administration (2019) *CDER/CBER. Charging for Investigational Drugs under an IND – Questions and Answers*. 2019 April. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/charging-investigational-drugs-under-ind-questions-and-answers. Last accessed 17 Aug 2020.
50. Code of Federal Regulations. 21CFR Part 312.7. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=312.7. Last accessed 17 Aug 2020.
51. Code of Federal Regulations. 21CFR Part 312.8. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=312.8. Last accessed 17 Aug 2020.
52. U.S. Food and Drug Administration (2019) *PDUFA VI: Fiscal Years 2018–2022*. Last updated 2019 May. https://www.fda.gov/industry/prescription-drug-user-fee-amendments/pdufa-vi-fiscal-years-2018-2022. Last accessed 17 Aug 2020.
53. U.S. Food and Drug Administration (2018) *Milestones in U.S. Food and drug law history*. Updated 2018 January. https://www.fda.gov/about-fda/fdas-evolving-regulatory-powers/milestones-us-food-and-drug-law-history. Last accessed 17 Aug 2020.
54. U.S. Food and Drug Administration (2018) *Food and drug administration amendments act of 2007*. Updated 2018 March. https://www.fda.gov/regulatory-information/selected-amendments-fdc-act/food-and-drug-administration-amendments-act-fdaaa-2007. Last accessed 17 Aug 2020.
55. U.S. Food and Drug Administration (2018) *Food and Drug Administration Safety and Innovation Act (FDASIA)*. Updated 2018 March. https://www.fda.gov/regulatory-information/selected-amendments-fdc-act/food-and-drug-administration-safety-and-innovation-act-fdasia. Last accessed 17 Aug 2020.
56. U.S. Food and Drug Administration (2018) *21st Century Cures Act*. Updated 2018 March. https://www.fda.gov/regulatory-information/selected-amendments-fdc-act/21st-century-cures-act. Last accessed 17 Aug 2020.
57. U.S. Food and Drug Administration (2019) *Guidance for industry: Expedited programs for serious conditions*. 2019 February. https://www.fda.gov/media/120267/download/. Last accessed 17 Aug 2020.
58. U.S. Food and Drug Administration (2018) *Critical path initiative*. Updated 2018 April. https://www.fda.gov/science-research/science-and-research-special-topics/critical-path-initiative. Last accessed 17 Aug 2020.
59. U.S. Food and Drug Administration (2018) *CDER. Duchenne Muscular dystrophy and related dystrophinopathies: Developing drugs for treatment: Guidance for industry*. 2018 April. http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/UCM450229.pdf. Last accessed 17 Aug 2020.
60. U.S. Food and Drug Administration (2019) *FDA draft guidance on Duchenne*. EndDuchenne.org [Internet]. 2019 June. http://www.parentprojectmd.org/site/PageServer?pagename=Advocate_fdaguidance. Last accessed 17 Aug 2020.
61. U.S. Food and Drug Administration (2016) *Assessment of the program for enhanced review transparency and communication for NME NDAs and Original BLAs in PDUFA V*. 2016 November. https://www.fda.gov/media/104487/download/. Last accessed 17 Aug 2020.

62. U.S. Food and Drug Administration (2014) *Phase IV Commitment Category*. 2014 December. http://www.fda.gov/drugs/developmentapprovalprocess/formssubmissionrequirements/electronicsubmissions/datastandardsmanualmonographs/ucm071716.htm. Last accessed 17 Aug 2020.
63. Code of Federal Regulations. 21CFR Part 314.520. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=314.520. Last accessed 17 Aug 2020.
64. U.S. Food and Drug Administration (2019) *Guidance for industry: REMS: FDA's Application of statutory factors in determining when a REMS Is necessary 2019 April*. https://www.fda.gov/media/100307/download. Last accessed 17 Aug 2020.
65. U.S. Food and Drug Administration (2018) *Draft guidance for industry: Format and content of a REMS document*. 2018 February. https://www.fda.gov/media/77846/download. Last accessed 17 Aug 2020.
66. U.S. Food and Drug Administration (2019) *Approved Risk Evaluation and Mitigation Strategies (REMS): Yescarta*. 2019 November. https://www.accessdata.fda.gov/scripts/cder/rems/index.cfm?event=IndvRemsDetails.page&REMS=375. Last accessed 17 Aug 2020.
67. Kite Pharma website (2020) *What is the YESCARTA and TECARTUS REMS Program?* https://www.yescartatecartusrems.com/. Last accessed 17 Aug 2020.
68. U.S. Food and Drug Administration (2018) *About orphan products natural history grants*. 2018 October. https://www.fda.gov/industry/orphan-products-natural-history-grants?abougt-orphan-products-natural-history-grants. Last accessed 17 Aug 2020.
69. U.S. Food and Drug Administration (2018) *Developing products for rare diseases & conditions*. 2018 December. https://www.fda.gov/industry/developing-products-rare-diseases-conditions. Last accessed 17 Aug 2020.
70. U.S. Food and Drug Administration (2017) *FAQ concerning the orphan products clinical trials grants program*. 2017 December. https://www.fda.gov/industry/orphan-products-clinical-trials-grants-program/faq-concerning-orphan-products-clinical-trials-grants-program. Last accessed 17 Aug 2020.
71. Code of Federal Regulations. 21CFR Part 316. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=316. Last accessed 17 Aug 2020.
72. U.S. Food and Drug Administration (2019) *Draft guidance for industry: Rare Diseases: Common issues in drug development*. 2019 February. https://www.fda.gov/media/120091/download\. Last accessed 17 Aug 2020.
73. U.S. Food and Drug Administration (2019) *FDA's Role: ClinicalTrials.gov Information*. 2019 September. http://www.fda.gov/scienceresearch/specialtopics/runningclinicaltrials/fdasrole-clinicaltrials.govinformation/default.htm. Last accessed 17 Aug 2020.
74. ClinicalTrials.gov (2018) *History, policies, and law*. Last reviewed 2018 August. https://clinicaltrials.gov/ct2/about-site/history. Last accessed 17 Aug 2020.
75. National Institutes of Health (2016) *Summary table of HHS/NIH initiatives to enhance availability of clinical trial information*. Updated 2016. https://www.nih.gov/news-events/summary-table-hhs-nih-initiatives-enhance-availability-clinical-trial-information. Last accessed 17 Aug 2020.
76. Code of Federal Regulations. 45CFR Part 46. https://www.hhs.gov/ohrp/regulations-and-policy/regulations/45-cfr-46/index.html. Last accessed 17 Aug 2020.
77. Code of Federal Regulations. 21CFR Part 50. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=50. Last accessed 17 Aug 2020.
78. Code of Federal Regulations. 21CFR Part 56. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=56. Last accessed 17 Aug 2020.
79. Department of Health and Human Services (2004) *Financial Conflict of Interest: HHS Guidance*. 2004 May. http://www.hhs.gov/ohrp/regulations-and-policy/guidance/financial-conflict-of-interest/#. Last accessed 17 Aug 2020.
80. Department of Health and Human Services (2019) *Cancer Center Support Grants (CCSGs) for NCI-designated Cancer Centers (P30 Clinical Trial Optional)*. 2019 November. https://grants.nih.gov/grants/guide/pa-files/par-20-043.html. Last accessed 17 Aug 2020.
81. NIH Office of Science Policy (2019) *NIH guidelines for research involving recombinant or synthetic nucleic acid molecules (NIH Guidelines)*. 2019 April.

FDA Inspections

Adrian P. Gee

1 FDA Inspection Program

1.1 Inspection of Type 361 Product Manufacturing Establishments (cGTP)

The inspection program for HCT/P is under the Center for Biologics Evaluation and Research (CBER) at the FDA. The recent history of the inspection program is shown in Fig. 1.

The average time taken per inspection was between 34 and 41 h. The agency generally identifies establishments to be inspected from its listing of registered establishments. This list is compiled from an annual registration procedure that is described in Title 21 of the Code of Federal Regulations Part 1271 Subpart B [1]. The registration form is completed online (FDA Form 3356), and full instruction for registration can be found at: https://www.fda.gov/media/109160/download

The HCT/P inspection program covers only products that are minimally manipulated, intended for homologous use, are not combined with another article **and** do not have a systemic effect or are dependent upon the metabolic activity of other cells for primary function **and** are for autologous use or allogenic use in a first- or second-degree relative, or are for reproductive use [2]. Cells not meeting these specifications may be regulated as biological drugs or medical devices and are inspected under different regulations described later. If the HCT/Ps were recovered before May 25, 2005, the inspections are performed according to the Inspection of Tissue Establishment regulations [2].

The frequency of HCT/P establishment inspection is not predetermined but is based upon potential risk, whether the establishment previously received an official action finding and whether the FDA has received information about potential

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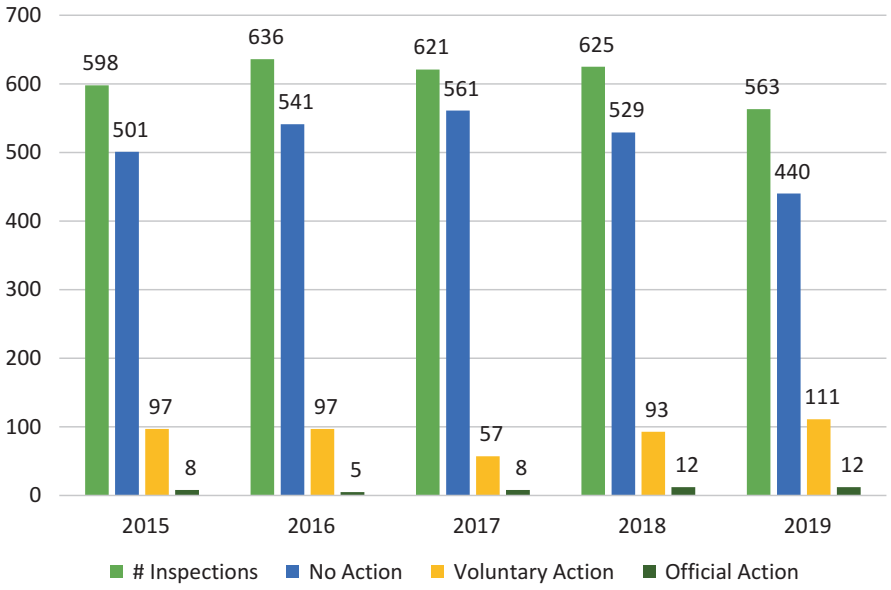

Fig. 1 Number of FDA HCT/P establishment inspections by year and actions taken

violations. Inspections are usually unannounced with the exception of those relating to medical devices, inspections under the bioresearch monitoring program (unless being performed for cause), and pre-licensing biologics inspections.

At the start of the inspection, the inspector will issue the establishment with a Form FDA-482 "Notice of Inspection" and ask to see the most responsible person at the establishment. They will identify themselves and show their official credentials. The purpose of the inspection will be explained, and they will inform you as to which records they will want to see, whom they may want to interview, and which procedures they may wish to observe.

The inspection will be based on the elements of the current Good Tissue Practice regulations shown in Tables 1a, 1b and 1c.

There must be a quality program in place. Procedures must exist for receiving, investigating, evaluating, and documenting information related to core GTP requirements. Any corrective actions that are performed must be documented and their efficacy verified and, if appropriate, both short-term and long-term actions taken to prevent recurrence included. The program must include a system to ensure that all staff are properly trained and educated to perform their tasks. There must also be evidence of environmental control showing that systems are established and appropriately maintained. All deviations related to HCT/Ps must be investigated and documented. Investigations should include a review and evaluation of the deviation, efforts to determine the cause, and any corrective actions.

The inspector will check to ensure that reporting requirements have been met. These include reporting within 15 days adverse reactions to the FDA that are fatal,

Table 1a Donor eligibility inspection of HCT/P establishments shown by regulations and major inspection elements

Regulation (21CFR Part 1271)	Inspection element
Donor requirements	Determine procedures in place to perform donor screening
Donor eligibility	Determine if each donor has a separate and complete record of all relevant medical records and that these are available for review. Verify adequacy of screening records
Donor screening	Determine if results and interpretation are in compliance with regulations
Donor testing	Check for documentation of responsible individual performing screening
	Determine whether all the required tests are being performed
	Are FDA-licensed, approved, or cleared donor screening tests being used?
	Is the testing laboratory CLIA certified or has met equivalent requirements?
	Are the results interpreted according to the manufacturer's instructions?
	Are adequate samples used for testing and do they meet the requirements specified in the product insert?
	Observe testing or verify through record review that appropriate controls are used and the testing procedure is followed properly.
	Verify equipment maintenance is per manufacturer's directions and establishment's SOP and that all equipment is appropriately qualified, calibrated, and maintained
	Confirm that testing problems are investigated, resolved, and documented
	Verify that positive or reactive tests are handled appropriately and that those from ineligible donors are handled in accordance with 21CFR Part 1271.65

life-threatening, result in permanent damage or impairment of body functions, or which necessitate medical or surgical intervention, including hospitalization. Any HCT/P deviations which meets all of the following criteria must also be reported: deviations related to distribution and core cGTP and is related to the prevention of communicable disease transmission or HCT/P contamination. These reports can be made using Form 3486 or submitted electronically.

The inspection findings are documented by the inspectors using Form 483 "Inspectional Observation" at the conclusion of the inspection. There will be a formal report on the inspections (see Establishment Inspection Report) with a classification of the findings. Actions may be taken based on these findings as shown in Table 2

Table 1b Labeling inspection of HCT/P establishments shown by regulations and major inspection elements

Regulation (21CFR Part 1271)	Inspection element
Labeling control requirements	Are there established and maintained procedures to control labeling?
	Are there appropriate labels in use for shipment of products from ineligible donors and in the case of urgent medical need?
	Is donor name and other personal information not used on labels except for autologous products and those from first- and second-degree relatives?
	Determine:
	All labels contain identification code, description of product, expiration date, and, if applicable, any required warning statements
	The name and address of the establishment that determined release criteria were met and made product available for distribution, storage temperature, appropriate warning statements, instructions related to the spread of communicable disease, and statement if donor is eligible or ineligible
	Establish that the summary of records accompanies the HCT/P including that testing performed by CLIA-certified laboratory, list of all testing performed, name and address of establishment that made donor eligibility determination, reason donor was ineligible if appropriate
	Additional items apply if the product was shipped under quarantine, if it was made available under the urgent medical use provision, and if the donor was ineligible, or the product is for nonclinical use

1.2 Inspection of Type 351 Product Manufacturing Establishments (GMPs)

Human cell and gene therapy product manufacturers are usually inspected under a Level 1 cGMP [2, 3]. This consists of an in-depth audit of three critical elements in at least four of the key systems (Table 3). In addition to the audit of the quality system, the Level 1 inspection should also include an audit of the production system [3].

cGMP inspections are normally conducted on a biennial schedule, or more often if warranted by circumstances.

The FDA has indicated that manufacturing for Phase 1 clinical trials does not have to fully conform to cGMP regulations [4]. In a guidance document, they recommend a comprehensive and systematic evaluation of the manufacturing setting to identify potential hazards and appropriate actions to eliminate or mitigate them. These can include the use of disposable equipment and process aids; use of commercial and prepackaged materials, e.g., water for injection; and use of closed systems for manufacturing. The basic requirements, however, are similar in terms of adequate facilities, trained personnel, quality program, maintenance of records, etc. It is advisable to review this guidance [4] before an inspection takes place.

In a GMP inspection, a major element will be the use of written approved standard operating procedures (SOPs) and associated records. This may include

Table 1c Inspection of other components of HCT/P establishments shown by regulations and major inspection elements

Regulation (21CFR Part 1271)	Inspection element
Facilities	Is the facility suitable for all functions that are being performed?
	Is the facility in good state of repair?
	Are manufacturing areas maintained in a clean, sanitary, and orderly manner?
Environmental controls	Are there procedures in place for control of:
	Temperature and humidity?
	Ventilation and air filtration?
	Cleaning and disinfecting of rooms and equipment?
	Maintenance of equipment used to control conditions for aseptic processing?
Equipment requirements	Determine whether equipment is appropriately designed, located, installed, maintained, and cleaned to prevent introduction and transmission of communicable diseases
	Is equipment capable of producing valid results?
Supplies and reagents requirements	Have all supplies and reagents been verified to meet specifications designed to prevent conditions that increase introduction and transmission of communicable diseases?
	Reagents must be sterile where appropriate
Recovery requirements	Has each HCT/P been recovered in a way that prevents introduction and transmission of communicable diseases?
Processing and process control requirements	Are there appropriate processing and process controls to prevent introduction and transmission of communicable diseases?
	Confirm if there is no pooling of cells from two or more donors
	Is sampling of HCT/Ps representative of the material to be evaluated?
	Determine:
	Which procedures have been validated?
	The review process for validations and verifications
	If sterility testing is contracted out how the sampling and testing methods were validated and review the documentation If performed internally review documentation
	How changes are made to validated processes; are they documented, signed, and dated; was the procedure revalidated; was the change approved by the appropriate individual?

(continued)

Table 1c (continued)

Regulation (21CFR Part 1271)	Inspection element
Storage requirements	Do storage conditions prevent mix-up, contamination, and cross-contamination of HCT/Ps, supplies, and reagents?
	Is there a proper quarantine area?
	Are HCT/Ps stored at the correct temperature?
	Have expiration dates been assigned if appropriate?
	Are there corrective actions when proper storage conditions are not met?
Receipt, pre-distribution, shipment, and distribution requirements	How does the establishment receive and evaluate incoming HCT/Ps?
	How is pre-distribution of HCT/Ps accomplished within the institution and to outside establishment?
	How is the documentation achieved that:
	HCT/Ps have met release criteria?
	Are HCT/Ps packaged and shipped to prevent contamination?
Records	Does the establishment have equipment logs, labeling records, and packaging records?
	Are records maintained, well-organized, and readily available?
	If stored electronically how are they backed-up?
	Does record identify person doing the work?
	Are entries signed and dated?
	Do records provide complete history of work?
	Can record be related to the particular HCT/P?
	Are donor eligibility records complete?
	Review procedures for preparing the summary of records
	Are records accurate, indelible, and legible?
	If donor is ineligible does use meet requirement for the first- or second-degree blood relative, or urgent medical need?
	Records must be maintained concurrently with the performance of each step

observation that these are being followed. Training records will be reviewed to ensure that staff have the appropriate educational background, training, and experience and that they are adequate in number.

The inspector will also verify through observation whether the establishment is adhering to applicable regulations. One way to help determine this is to look at documented deficiencies as an indicator of the state of control. Other elements of the inspection are shown in Tables 4a, 4b, 4c, 4d, 4e and 4f, together with examples of the types of deficiencies that may be reported. There are additional elements for specific types of products, for example, vaccines and allergens. The most relevant regulations for the cellular therapy community pertain to master and working viral seed banks. There should be a complete history, including passaging and testing profiles. The storage must be secure, and it should be at multiple locations with adequate control to prevent unauthorized access and materials loss due to equipment

Table 2 Types of FDA HCT/P inspection findings

Finding	Explanation
Untitled letter	Violations do not meet the threshold of regulatory significance for a warning letter; however, regulatory concerns exist that cannot be addressed through other means
Warning letter	Violations of regulatory significance suggesting systemic problems exist within one or more areas of operations
Order of retention, recall, and destruction	Significant deviations suggesting that HCT/P was manufactured in violation of the regulations
	Conditions of manufacture do not provide adequate protection against risks of communicable disease transmission, or the HCT/P is contaminated
Order of cessation of manufacturing	HCT/P is manufactured in violation of regulations, and there is not adequate protection against the risk of transmission of communicable disease, or the HCT/P is contaminated, or there are reasonable grounds to believe that a danger to health exists
Prosecution	Gross, flagrant, or intentional violations; fraud, danger to health or continued; or repeated course of violative conduct

Table 3 Key systems and critical elements of a cGMP inspection

Key systems	Critical elements
Quality system	Standard operating procedures
Facilities and equipment system	Training
Materials system	Records
Production system	
Packaging and labeling system	
Laboratory control system	
Donor eligibility system (only for certain HCT/Ps)	

failure. There must be a complete inventory which correlates with the amount of material on hand, and the storage locations should be fitted with an alarm system. Similar regulations apply to master and working cell banks, with additional attention to the passage numbers at which the cells are used. Establishment of new working cell banks from the master bank should be documented in the annual report to the FDA.

For aseptic processing emphasis is put on ensuring that all transfers, transports, and storage stages are carefully controlled to maintain sterility. Wherever possible closed systems should be used. If this is not possible, the product must be handled in a unidirectional Class 100 (ISO 5) environment located in a Class 10,000 (ISO 7), or better, surrounding room. Monitoring activities should include obtaining the identity of detected microorganisms. There must be microbial surface monitoring at the end of production before cleaning and also personnel monitoring. There should be a process simulation performed to demonstrate that process controls are adequate to protect the product [5]. These should model the worst case scenario, e.g.,

Table 4a GMP inspection: Elements of quality system and deficiencies

Element	Deficiencies
Quality system	Employees not trained, experienced, sufficiently educated, or sufficient in number
Component and in-process materials release	Failed to review records at least annually to evaluate quality
Change control	Procedures for production/process control not drafted, reviewed, and approved
Batch release	Quality audits not performed
Record review	Complaint procedures not drafted or followed and no documentation of findings
Validation protocols	Failure to conduct investigations into unexplained discrepancies. Failures to meet specifications not documented did not include conclusions, did not examine other batches, or did not extend to other products with associated discrepancies
Evaluation of biological product deviations	Out of specification procedure, deviations not recorded or justified and failure to conduct investigations
Complaint handling	Change control procedures did not approve or reject procedures/ specifications impacting product strength, quality, and purity and did not draft/review/approve written procedures
Evaluation of returned/ salvaged products	Stability investigations not performed and/or failure to review records and ensure appropriate investigation if appropriate
	Quarantine procedures not written or followed and rejected components, closures, and containers not identified and controlled to prevent use
	Finished product distribution records not established or implemented to facilitate product recall if necessary
	Adverse event reports not reported to CBER
	Significant manufacturing changes implemented before CBER approval

maximum number of open operations. The inspector may ask to observe aseptic technique.

At the conclusion of the inspection, a Form 483 will be issued that lists significant findings that relate to observed or potential problems detected at the establishment. The most critical observations are listed first. It may include deficiencies from prior inspections that have not been corrected.

1.3 Establishment Inspection Reports (EIR)

The establishment will receive an EIR after the inspection. This provides documentation of what the inspector(s) did from the time at the establishment until the issuance of the Form 483. It includes a summary of the findings, a history of the

Table 4b GMP inspection: Elements of facilities and equipment system and deficiencies

Element	Deficiencies
Facilities and equipment system	Buildings not in good state of repair, not of suitable size and construction to facilitate cleaning, maintenance, and proper operations
Appropriateness of buildings and facilities, including maintenance, equipment qualification and maintenance, cleaning and validation of cleaning, prevention of contamination, and cross-contamination including contaminants on product contact equipment	Inadequate ventilation and no equipment to adequately control air pressure, microorganisms, dust, humidity, and temperature air filtration not used where appropriate
	Inadequate space to prevent mix-ups and/or contamination
	No separate or defined areas or control systems
	Building not maintained in a clean and sanitary condition, not free of pest infiltration, no pest control written procedures, and not designed to prevent contamination
	Cleaning records not retained for 3 years
	Equipment not of appropriate design, size, or suitably located for cleaning and maintenance
	Equipment surfaces not constructed to prevent changes to product and/or free of contaminants. Equipment lubricants, coolants, etc. in contact with product
	No cleaning or maintenance logs. Improper or insufficient cleaning
	No written procedures for equipment cleaning and/or maintenance
	Improper or no calibration or inspection
	Equipment not properly identified

establishment, a listing of individual's responsibilities and persons interviewed, a discussion of the quality operation and training program, manufacturing design and operations, product testing, recall procedures, objectionable conditions and responses, and a description of general discussion with the management.

It will state whether the facility is found to be acceptable and provide classification of the findings. These are official action indicated (OAI), voluntary action indicated (VAI), or no action indicated (NAI). A VAI indicates that re-inspection is required within 12–24 months. An OAI indicates that a warning letter will be issued until the observations have been addresses and have been verified by the FDA through an inspection.

A warning letter may be issued subsequently that lists violations of regulatory significance that cause one or more systems not to be a state of control. For licensed

Table 4c GMP inspection: Elements of materials system and deficiencies

Element	Deficiencies
Material system	Procedure not written or followed for receipt, identification, sampling, testing, and approval of components, product containers, and closures
Validation of computerized inventory systems, storage and distribution controls, detection and prevention of counterfeiting	Items not stored to prevent contamination or cross-contamination or held under quarantine until approved and released
Monitoring of utility systems	Representative sample of each component not collected for testing or examination
Review of calibration and maintenance and verification of following manufacturer's recommendations and/or user manuals	Tests not conducted to verify the identity of each product component
Addition of or modifications to equipment	Containers and closures not inspected visually
	No written specifications for components, containers, or closures and failure to reject those not meeting specifications
	Items not retested after prolonged storage or quarantined if specifications not met
	Inadequate containers and/or closures, e.g., no written cleaning methods; not shown to be nonreactive, additive, or absorptive; sealing not performed to maintain integrity; etc.
	Cell cultures and lines not properly stored to prevent contamination and deterioration, not identified by lot number and date of preparation, no records maintained on periodic verification, and freedom from contaminants

product manufacturing other actions may include license revocation or suspension, seizure of products, injunctions in the case of the existence of a current health hazard, and finally prosecution. Deficiencies are listed by the systems shown in Tables 4a, 4b, 4c, 4d, 4e and 4f.

2 EU Inspections

cGMP in Europe is regulated by the European Medicines Agency [6, 7], and inspections are performed by the appropriate national competent authority (NCA). Manufacturing sites outside the EU are inspected by the NCA of the Member State where the EU importer is located, unless a mutual recognition agreement is in place between the EU and the country concerned. If an MRA applies, the authorities

Table 4d GMP inspection: Elements of production system and deficiencies

Element	Deficiencies
Production system	Deficiencies in written procedures for production and/or process control
Written procedures	Deviations not recorded
Equipment identification	No identification of compounding and storage containers, major equipment, etc.
Yield calculations	Yields and percentage of yield not calculated at each appropriate phase of manufacturing and packaging
Batch production and control records	No batch or control records
Time limits for phases of manufacturing	No established time lines for each phase of production
Use of in-process controls, tests, and examinations	No established or followed written procedures for in-process controls, tests, and examinations
Consistency of in-process and final specifications	In-process specifications inconsistent with final specifications
Prevention of contamination	No written procedures for the prevention of contamination
Production and control records	Processing procedures and deviations not recorded or documented at the time of performance
Storage temperatures	HCT/Ps not stored at appropriate temperatures and temperatures not periodically reviewed or maintained. No recommended temperatures for performance at each step of manufacturing to inhibit contamination
Record retention	Records not retained for appropriate times
	HCT/Ps pooled during manufacturing

mutually rely on each other's inspections. The results of national cGMP inspections and details of nonconformances are reported on the EudraGMDP website [8].

3 Behavior During a Regulatory Inspection

Establishments should pre-designate one or more people to facilitate inspections. It is also a good idea to have a written SOP for regulatory inspections. This will contain the procedure to be followed, the people to be notified, the names of additional contact people, where the meeting will be held, how requests for documents will be handled, and behavior to follow during the inspection. All staff and relevant ancillary people must be trained on this SOP. It is critical that the staff are already familiar with the relevant regulations upon which the inspection will be based and have ready the appropriate guidance documents.

Table 4e GMP inspection: Elements of packaging and labeling system and deficiencies

Element	Deficiencies
Packaging and labeling system	No written labeling procedure or procedure not followed
Acceptance operations for packaging and labeling systems	Labels not sampled or tested on receipt and/or did not meet specifications. Labels for different doses not stored separately
Control of label issue, examination of issued labels, and reconciliation of used labels	Labeling issue not controlled and labels not examined for identity or conformance
Line clearance for packaging and labeling	Label quantities not reconciled
Accompanying records	No destruction of excess labels
Expiration dating	No procedure to ensure correct labels are used and no procedure to prevent mix-ups
Examination of labeled finished products	Lot or control numbers not used for products
Labeling control	HCT/Ps do not have code to relate to donor and product records, no tracking system, and no procedure to relate old and new codes
	Shipping conditions not established for HCT/Ps and packaging and shipping containers not designed to protect from contamination
	Accompanying records for HCT/Ps not adequate
	No expiration dates used or related to storage conditions on label
	No examination or documentation of examination of labeled finished products
	Inadequate labeling records

A sign-in sheet should be used for all those attending the inspection meeting, and the inspectors should complete the visitor log when entering the facility. The meeting should take place in a room with adequate space and sufficient chairs for the inspection team and establishment staff. The locations of toilets and water fountains and beverages should be provided. An offer should be made to bring in lunch for the team (which will be paid for by them) or to provide the location of nearby eating facilities. Normally, after initial introductions and an explanation of the purpose of the visit, the team will indicate when they want to meet with establishment staff and when they wish to be alone. They should be provided with contact information for the establishment representative.

They should be accompanied at all times (except when they ask to meet alone) by a facility representative(s) who takes meeting notes and designates who should meet with the team when information is requested. It is a good idea to have a current table of contents available to enable the establishment staff to rapidly find and provide copies of requested procedures. A list should be maintained of all documents provided to the inspection team, and there should be a designated person available

Table 4f GMP inspection: Elements of laboratory control system and deficiencies

Element	Deficiencies
Laboratory control system	No specifications, standards, sampling plans, test procedures, or control systems available or reviewed by a quality program
Written procedures and control systems	Procedures not written or followed for instrument calibration
Calibration and maintenance of analytical instruments and equipment	No written procedures to describe sampling methods or number of units from each batch to be tested
Adherence to and validation/ verification of written analytical methods	Accuracy, sensitivity, specificity, and reproducibility of test methods not established or documented
Testing and release for distribution	Laboratory testing does not determine conformance to final specifications
Specification, standards, and representative sampling plans	No testing for objectionable microorganisms
Stability testing program	Controls did not include sound and appropriate specifications, standards, sampling plans, and test procedures
Special testing requirements	Stability testing programs did not include sample size and test intervals or storage conditions for samples
Adequate reserve samples	Adequate reserve samples not retained or stored under appropriate storage conditions per product label and not examined visually at least annually
Required testing performed on correct samples	Samples not representative or adequately identified
Laboratory records	Laboratory records did not include full testing documentation including calculations performed

to make copies. Ideally there should be a person available to retrieve requested documentation from the files or to locate it electronically. Only documents specifically requested by the team should be provided. The speed with which documents are provided indicates familiarity with the quality system and facility operations. Requests for information should be answered directly and specifically without offering supplementary details.

Staff should be familiar with how to interact with inspectors. During observation of procedures, it is acceptable to tell an inspector to wait until questions can be asked or answered. If the staff member does not know the answer, they should indicate this and tell the inspector that they will find out the answer or refer him/her to another staff member. They should not be evasive. Again, the staff member should not volunteer supplementary information, but should directly answer the question posed.

Toward the end of the day, the team will usually indicate how they wish to close out the day's activities. This may include meeting with certain establishment staff and requests for additional information to be provided on the following day and/or the agenda for the next day. If deficiencies have been detected during the day, it is

usually acceptable for the establishment to try to correct these and to provide the team with evidence of their correction on the following day.

On the final day of the inspection, an exit interview will normally be held, after the team has met together to plan their findings. Usually the establishment representative can decide who will attend this meeting. The team will normally thank the establishment for facilitating the inspection, and FDA inspectors will then present their findings and the Form 483. There may be an opportunity for the establishment to seek clarification of the findings and ask to supplementary questions. The team should be thanked for performing the inspection. In the United States responses to deficiencies may be made at the meeting or should be submitted to the District Office within 15 days of the inspection. A formal EIR will be provided by CBER after the inspection (see Establishment Inspection Report). Actions to be taken will be documented.

4 Complaints About FDA Inspections

If you have complaints about the inspectors or the conduct of the inspection, these should not be raised during the inspections itself. The District Office should be contacted in writing after the inspection.

5 FDA Pre-inspection Opportunities

In the United States, the FDA offers a number of opportunities for pre-operational inspection of the manufacturing facility [9]. These are of four types:

5.1 Design Review

A design review usually involves a review of conceptual drawings, proposed plant layouts, and flow diagrams for the entire facility including critical systems and areas. Such reviews provide an opportunity to emphasize the importance of the fundamental principles of good design as outlined in the cGMPs. As a result, extensive changes in design can be made with little cost and very minor delay to the design and construction cycle. The FDA expects the facility to prepare complete final plans and to identify, if possible, specific questions regarding how the facility will meet cGMPs or areas where the FDA's comments are specifically desired. When the review includes a meeting with the facility, advance delivery of the package of documents to the District Office is recommended. This type of review is particularly valuable for academic cGMP facilities.

5.2 Pre-construction Review

A pre-construction review involves a study of the plan, elevation, and isometric drawings for all manufacturing areas and utility and process systems for the plant; i.e., drainage and water systems; product systems; compressed air systems; heating ventilation and air-conditioning (HVAC) systems; and all equipment, layouts, and piping in the manufacturing and laboratory areas. Further examples, where applicable, include zones of positive air pressure; HEPA filtration and laminar flow; air locks; protective clothing; change rooms; toilet and washup facilities; pedestrian traffic patterns; raw materials and components, and similar considerations.

The various packages of prints, specifications, design standards, and vendors' descriptions should be supplied in advance to permit meaningful review and comment prior to any meeting.

5.3 Construction/Equipment Installation and Qualification Review

Facilities may request an FDA on-site review of specific portions of the plant, while construction is in progress. This is an excellent opportunity to review piping systems and methods of construction before they are concealed by walls, floors, and ceilings. These reviews or site visits may be done in phases. The final inspectional review of validation and control data from production runs can then be accomplished quickly and more efficiently.

5.4 Pre-production Review

At the pre-production stage, the review will normally be an inspection. Additionally, facilities may request investigators to visit new buildings or production areas during inspections on the same campus. Investigators conducting the review should provide the facility with general feedback and can provide examples of what they have seen at similar facilities.

6 Conclusions

Regulatory inspections are a component of GMP/GTP compliance. They may be random or based upon specific issues. In either case it is important to have (i) an understanding of the regulations against which compliance is being evaluated and (ii) procedures in place on how your facility will deal with the inspection. These

include keeping good documentation of what happens, knowing how to deal with requests for information, behavior of staff, and ensuring effective and timely follow-up. Attention to such details will ultimately result in a smooth and effective inspection.

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References

1. Code of Federal Regulations. *Drug good manufacturing practices (GMP)*. Title 21, Parts 210 and 211. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=210https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=211. Last accessed 13 July 2020.
2. Code of Federal Regulations. *Human cells, tissues, and cellular and tissue-based products*, Title 21 Part 1271. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=127. Last accessed 13 July 2020.
3. CBER. (2010). *Compliance program guidance manual chapter – 45 biological drug products inspection of biological drug products 7345.848*. https://www.fda.gov/media/73834/download. Last accessed 13 July 2020.
4. Federal Drug Administrations. (2008). *Guidance for industry: CGMP for phase 1 investigational drugs*. https://www.fda.gov/media/70975/download. Last accessed 13 July 2020.
5. U.S. Department of Health and Human Services Food and Drug Administration. (2004). *Guidance for industry: Sterile drug products produced by aseptic processing—Current good manufacturing practice*. September 2004. https://www.fda.gov/media/71026/download. Last accessed 13 July 2020.
6. European Medicines Agency. *Human regulatory, good manufacturing practice.* https://www.ema.europa.eu/en/human-regulatory/research-development/compliance/good-manufacturing-practice. Last accessed 13 July 2020.
7. European Commission. (2017). *Good manufacturing practice guidelines on good manufacturing practice specific to advanced therapy medicinal products*. https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-4/2017_11_22_guidelines_gmp_for_atmps.pdf. Last accessed 13 July 2020.
8. European Medicines Agency. *Eudra GMDP Website. Compliance with good manufacturing practice*. http://eudragmdp.ema.europa.eu/inspections/gmpc/index.do. Last accessed 13 July 2020.
9. Food and Drug Administration. *Pre-operational reviews of manufacturing facilities*. https://www.fda.gov/inspections-compliance-enforcement-and-criminal-investigations/field-management-directives/pre-operational-reviews-manufacturing-facilities. Last accessed 13 July 2020.

Commercialization of Investigational Cell Therapy Products

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1 Introduction

In the field of regenerative medicine, cellular therapy is defined as the therapeutic application of cells, irrespective of cell type or clinical indication, used to heal or cure medical problems [1]. These therapies use multiple types of cells, including hematopoietic stem cells (HSCs), mesenchymal stromal cells (MSCs), human embryonic stem cells (ESCs), and induced pluripotent stem cells (iPSCs). Adoptive cellular therapies involve the transfer of modified T cells, such as virus-specific T cells (VSTs) and chimeric antigen receptor (CAR) T cells, to patients. Despite the rush to expand clinical use of cellular therapies for a variety of disorders in clinical trials [2], only a few have actually made it to market in the United States (Table 1) [3]. Between 1997 and 2017, the US Food and Drug Administration (FDA) has approved only 21 biologics license applications (BLA) for cellular therapies [4]. While safety and efficacy (albeit modest for some products) have been demonstrated, the field faces structural, commercial, and economic challenges that need to be addressed in order to increase the commercial success and availability of these potentially curative cellular therapies [5, 6]. Given the significant time and financial investment required to successfully bring these products from bench to bedside, fostering commercial relationships is necessary to realize their potential and deliver on promised results for patients [7]. These requirements have only been heightened by the recent approval of two commercial CAR-T cell products.

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Table 1 Examples of cellular therapies currently approved in the United States

Product	Cell source	Clinical indication	Year	Manufacturer	Nature of approval
Epicel	Skin; autologous	Deep dermal burns	1988, 2007	Vericel	Unregulated devicehumanitarian use device
Carticel	Cartilage; autologous	Cartilage defects	1997	Vericel	PHS act
Prochymal	MSCs; allogeneic	Graft vs host disease	2005	Mesoblast	Compassionate use
Sipuleucel-T	APCs; autologous	Metastatic prostate cancer	2010	Valeant	PHS act
Tisagenlecleucel	T-cells; autologous	B-ALL	2017	Novartis	PHS act
		B-NHL	2018		
Axicabtagene ciloleucel	T-cells; autologous	B-NHL	2017	Gilead/Kite pharma	PHS act

Key: *APCs* antigen-presenting cells, *B-ALL* B-cell acute lymphoblastic leukemia, *B-NHL* B-cell non-Hodgkin lymphoma, *MSCs* mesenchymal stromal/stem cells, *PHS* public health service

2 Beginnings of Cell Therapy Commercialization

2.1 Epicel

While many cellular therapy products have been tested in early phase I/II studies, few have made it to the market. Epicel was the first cell-based product for tissue repair commercialized in the United States. Epicel is an autologous epidermal autograft used to treat adult and pediatric patients with deep dermal or full-thickness burns involving greater than or equal to 30% body service area [8, 9]. The product was initially created in 1975 based on work by Howard Green at Massachusetts Institute of Technology [9]. In 1980, the first two patients were treated with Epicel for third-degree burns and showed successful engraftment [10]. A few years later, two young brothers with burns over 97% of their bodies, of which >80% were third-degree burns, were successfully treated with Epicel [10]. Both boys survived and lived for more than 20 years following therapy. In 1986, Dr. Green founded Biosurface Technology to produce these skin grafts and commercialized the product in 1988 under the trade name Epicel [11, 12]. At that time, the FDA did not regulate cell therapies, so the product was an "unregulated device" until 1995. Biosurface Technology was subsequently acquired by Genzyme, and Epicel was designated by the FDA as a medical device in 1998. Later that year, the graft was reclassified as a humanitarian use device (HUD), which is a medical device used for the diagnosis or treatment of a rare medical disease or conditions affecting fewer than 4000 (later increased to ≤8000) individuals per year [13, 14]. Genzyme sought a humanitarian use device exemption from the FDA in 1999, which allows a HUD to go to market without results of efficacy from clinical investigations as long as the company

demonstrates that the device does not pose significant risk of illness or injury and there is probable benefit to health outweighing the risk of injury or illness from its use [14]. The application was approved in 2007, granting Epicel market access [12]. In 2011, Genzyme was bought out by Sanofi, which in 2014 was bought by Aastrom (now known as Vericel), which continues to successfully market the product worldwide [12].

2.2 Carticel

Around the same time that Genzyme acquired Biosurface Technology in 1994, Carticel was being developed by Biosurface. Carticel is an autologous chondrocyte product used to treat cartilage injuries of the knee that have not adequately responded to arthroscopic or surgical repair [12]. The cell therapy was initially marketed in 1995 as an unregulated device before Genzyme filed a biologics license application and received accelerated FDA approval in August 1997 in compliance with the newly instituted cell therapy guidelines. Carticel thus became the first FDA-approved cell therapy. In 2007, Genzyme demonstrated the safety and efficacy of Carticel in a prospective observational study (STAR Trial) enrolling patients from 29 centers throughout the United States. This study fulfilled the post-marketing confirmatory studies required by the FDA [15]. A next-generation product, known as MACI (autologous cultured chondrocytes on porcine collagen membrane), was developed simultaneously in Germany in 1998 and in 2013 became the first tissue-engineered Advanced Therapy and Medicinal product approved by the European Medicines Agency. In 2016, MACI became the first FDA-approved product utilizing tissue engineering to grow cells on scaffolds from patient's own healthy cartilage [16]. The product continues to be produced and marketed by Vericel after undergoing the same acquisition patterns as described above for Epicel.

2.3 Sipuleucel-T

Sipuleucel-T (Provenge™) is an autologous cellular immunotherapy for the treatment of metastatic hormone refractory prostate cancer. Sipuleucel-T is produced by ex vivo activation of patient-derived antigen-presenting cells (APCs), specifically dendritic cells, by a recombinant human protein prostatic acid phosphatase (PAP) linked to granulocyte-macrophage colony-stimulating factor (GM-CSF), an immune cell activator [17]. This APC-based therapy was hailed as the first so-called cancer vaccine to be FDA approved and was seen as a major stepping-stone for the approval of other therapies that stimulate the immune system and fight cancer. Based on technology developed at Stanford University, a company called Activated Cell Therapy began the commercialization process for Sipuleucel-T in 1992 [18], before ultimately becoming Dendreon under the leadership of Christopher Henney. In

1999, Dendreon established a partnership with Kirin Brewery to develop dendritic cell-based immunotherapies for cancer. Approval of sipuleucel-T was based on three pivotal phase III studies. The initial phase III randomized, double-blind, placebo-controlled clinical trial (D9901) included 127 subjects randomly assigned in a 2:1 ratio to receive sipuleucel-T (n = 82 or placebo (n = 45)) [19]. The study failed to reach its primary endpoint of time to progression (TTP), and investors backed out of the company [18]. However, a subgroup analysis of patients with a Gleason score of 7 or less showed a significant difference in TTP and median overall survival, noting a difference of 4.5 months (p = 0.01) for sipuleucel-T compared to placebo [19].

At the same time, a separate two-part double-blind, placebo-controlled study (D9902A) was being done in patients with metastatic, asymptomatic hormone refractory prostate cancer. The study was stopped early based on findings from the D9901 study. The protocol was subsequently amended to focus on patients with Gleason scores $\leq$7 in the second part of the study, D9902B (also called IMPACT) which was a randomized, double-blind, placebo-controlled study of 512 subjects with metastatic hormone refractory prostate cancer [20]. In a combined analysis of data from D9901 and D9902A, the authors showed improvement in median overall survival of 4.5 months in Sipuleucel-T-treated patients compared to those who received placebo [21]. In 2003, the FDA designated sipuleucel-T a fast track development program for treatment of asymptomatic, metastatic, hormone-independent prostate cancer. Dendreon also received a Special Protocol Assessment provision for D9902B, allowing Sipuleucel-T to serve as the basis for a BLA. The FDA granted fast track status in November 2005. A year later, Dendreon submitted a BLA requesting approval for Sipuleucel-T based on the survival benefit observed in the D9901 trial [18]. The FDA Cellular, Tissue, and Gene Therapies Advisory Panel recommended approval in March 2007. In an unusual turn of events, the FDA denied the application and requested additional evidence of efficacy from the ongoing D9902B trial. The study confirmed a survival benefit of 4.1 months at 3 years for subjects who received Sipuleucel-T, leading to final FDA approval in 2010. Due to a number of hurdles, including cost, manufacturing, and marketing challenges, Dendreon filed for bankruptcy in 2014 [12] and was purchased by Valeant Pharmaceuticals in February 2015. The company was sold again in January 2017 to China's Sanpower group, which continues to market sipuleucel-T in the United States.

2.4 Remestemcel-L

Osiris Therapeutics was founded in 1992 by Arnold Caplan at Case Western Reserve University to commercialize research on human MSCs [12]. MSCs are non-HSCs present in the bone marrow that have the ability to differentiate into multiple mesodermal cell lineages including osteoblasts, chondrocytes, and endothelial cells. MSCs were first evaluated as an autologous cellular therapy in 1995 to improve

hematopoietic recovery in patients with hematologic malignancies [22]. Osiris Therapeutics developed Remestemcel-L (Prochymal), an allogeneic off-the-shelf MSC product derived from the bone marrow of healthy donors, and has studied the drug in multiple clinical trials for a variety of indications including cardiac disease [23, 24], multiple sclerosis [25], Crohn's disease [26], diabetes [27–29], and graft versus host disease (GVHD) [30–32]. Remestemcel-L received fast track designations for use in Crohn's disease and GVHD, and in 2005 received approval for GVHD under the FDA compassionate use program [12]. Unfortunately, the MSC product failed in three clinical studies in 2009: two phase III studies in patients with steroid refractory acute GVHD and one study for chronic obstructive pulmonary disease.

Kebriaei et al. reported results of a randomized, phase II study of Remestemcel-L in combination with corticosteroids for newly diagnosed acute GVHD [33]. Thirty-two patients aged 18–70 were enrolled, but only 31 patients were treated. The response rate was 94% (77% complete response rate), with a majority of responses maintained for at least 90 days. The response rate in gastrointestinal GVHD was 82%. No significant toxicities were noted. A phase III randomized, placebo-controlled study of Remestemcel-L in steroid refractory acute GVHD assigned patients in a 2:1 ratio to an accepted second-line treatment plus Remestemcel-L or placebo. The primary endpoint was durable complete response (≥28 days), which in the intent to treat population was not different between the two groups (35% vs 30%, $p = 0.3$) [34, 35]. An open-label, single-arm, prospective multicenter study evaluated the use of Remestemcel-L for severe refractory aGVHD in pediatric patients ($n = 75$) ages 2 months to 17 years [36]. The day 28 ORR was 61%, with 26% of patients with GI involvement achieving a complete response by day 28.

In May 2012, Osiris received conditional marketing approval for Remestemcel-L (Prochymal) in Canada for treatment of steroid refractory acute GVHD in children, which was followed shortly after by approval from the New Zealand Medicines and Medical Devices Safety Authority, making it the first agent to receive approval for treatment of steroid refractory GVHD [37]. In 2013, Osiris sold Remestemcel-L to Mesoblast for approximately $100 million [12]. Mesoblast is continuing efforts to commercialize Remestemcel-L (which will now be known as Ryoncil) in the United States. Based on data aggregated from three separate trials demonstrating consistent safety and efficacy in children with steroid refractory GVHD, the FDA accepted the BLA for Remestemcel-L for priority review [35, 38–40]. Results presented at the 2020 Transplantation and Cellular Therapy Meeting demonstrated 66% of patients ($n = 204$) achieved an overall response at day 28 (CR = 18%; PR = 48%), consistent across all grades of GVHD [41]. Day 28 responders were more than twice as likely to survive as nonresponders (84% vs 39% at day 100). There are multiple phase I/II studies evaluating the use of Remestemcel-L in chronic GVHD, refractory Crohn's disease, advanced heart failure, rheumatoid arthritis, and diabetic nephropathy [42].

2.5 Chimeric Antigen Receptor T Cells (CAR-T Cells)

The concept of T-cell engineering was first introduced in the 1980s and included the use of retroviral vectors as a genetic engineering tools to introduce genes into T cells to augment antitumor activity. Several groups were also simultaneously working on developing "chimeric receptors" on T cells [43, 44]. Eshhar and colleagues fused a single-chain variable region domain (scFv) of an antibody (targeted to a specific tumor antigen) to the CD3-zeta (CD3ζ) signaling domain of the T-cell receptor (TCR) [45] to create what ultimately became known as first-generation CARs. However, these CARs were unable to sufficiently activate resting T cells, and their limited expansion and persistence due to incomplete T-cell activation led to minimal clinical efficacy [46–48]. Subsequently, several groups demonstrated that the addition of a co-stimulatory molecule (such as CD28, 4-1BB, or OX-40) into the CAR enhanced survival and expansion of genetically modified T cells [49–53]. These second-generation CARs showed sustained T-cell proliferation and improved antitumor activity [47, 50, 54–56]. Second-generation CARs targeting the CD19 antigen in B-cell acute lymphoblastic leukemia (B-ALL) and non-Hodgkin lymphoma (NHL) showed significant preclinical activity, ultimately leading to clinical translation [57–59]. Promising clinical responses in small pilot/phase I studies for relapsed/refractory B-ALL and NHL patients [60–64] served as the basis for larger phase to II trials, and the ultimate FDA approval of two CD19-specific commercial CAR-T cell products.

Kochenderfer et al. at the National Cancer Institute (NCI) published the first report of a clinical response in NHL using CD19 CAR-T cell therapy in a patient with multiply relapsed follicular lymphoma (FL) [65]. Shortly after, in 2012, Kite Pharma partnered with the NCI to develop novel cellular therapy clinical products [66]. The authors later reported that patients with chemotherapy refractory diffuse large B-cell lymphoma (DLBCL; n = 2), and primary mediastinal B-cell lymphoma (PMBCL; n = 2) achieved complete remissions, of which three were durable [64]. In the ZUMA-1 multicenter phase I study evaluating KTE-C19 (CD19-CD28 second-generation CAR T cells) in combination with low-dose conditioning chemotherapy for patients with refractory DLBCL, five of seven patients treated had ORR (71%) with four attaining a CR (57%) [67]. A larger phase I study in 22 patients, 19 of whom had DLBCL, demonstrated an overall response rate (ORR) of 68% with a complete remission (CR) rate of 47% among patients with DLBCL [68]. The CRs were durable with 11 of 12 ongoing for 7–24 months. For all participants, 12-month progression-free survival (PFS) was 63% at the time of study publication. The phase II portion of ZUMA-1 followed, which evaluated the safety and efficacy of axicabtagene ciloleucel (formerly KTE-C19), in patients with relapsed/refractory DLBCL, PMBCL, or transformed FL after two or more lines of therapy [69]. Of 111 patients enrolled, 101 were treated with an ORR of 82%. Of these patients, 54% achieved complete responses. The median duration of response was 8.1 months with an 18-month survival rate of 52%.

Meanwhile, researchers at the University of Pennsylvania opened a pilot/phase I study (NCT01029366) of their second-generation CD19 CAR T cell (expressing the co-stimulatory receptor 4-1BB) for adult patients with relapsed/refractory CD19-positive leukemia or lymphoma. The treatment was successful in the first three patients treated, all of whom had chronic lymphocytic leukemia (CLL). Two patients achieved CRs, and one patient experienced a partial remission (PR), all lasting greater than 8 months at the time of publication [60, 70]. While subsequent responses in an additional 11 CLL patients yielded disappointing results [71], in August 2012, Novartis obtained an exclusive worldwide license to further study and commercialize the CAR T technologies developed by the University of Pennsylvania. In return, Novartis helped establish the Center for Advanced Cellular Therapies (CACT) on the UPenn campus to develop and manufacture novel cellular immunotherapies. Due to lackluster results in CLL with the initial strategies, the team shifted focus to pediatric B-ALL (NCT01626495). The first two B-ALL patients treated with this product, called CTL019, achieved CRs, with one lasting 11 months [63]. In a larger cohort, CTL019 showed a 90% complete response rate in heavily treated relapsed/refractory B-ALL [72], which led to its accelerated development. A phase I/II study of 59 pediatric patients with B-ALL showed an impressive CR rate of 93% [73], leading to the pivotal phase 2 multicenter study (ELIANA) sponsored by Novartis at 25 sites in 11 countries including North America, Europe, Asia, and Australia.

ELIANA enrolled 92 subjects up to 30 years old, with 75 receiving CTL019 infusion. This trial showed a 3-month ORR of 81%, and overall survival/event-free survival at 6 and 12 months were 73% and 50%, respectively, with median duration of remission not reached [74]. These data established the potential durability of remissions in the B-ALL cohort. CTL019 was also evaluated in a phase II trial for relapsed/refractory DLBCL, FL, and mantle cell lymphoma (MCL). Complete remission was achieved in 43% of DLBCL (n = 14) and 71% of FL (n = 14) patients, and more importantly all subjects who obtained a CR by 6 months remained in remission for a median follow-up of 29.3 months [75]. Subsequently, Novartis led a national multicenter phase II trial (JULIET) that enrolled 93 patients with relapsed/refractory DLBCL who were ineligible for or had disease progression after autologous hematopoietic stem cell transplantation. The overall response rate in this cohort was 52%, with 40% of patients achieving a CR and 12% of patients achieving a partial response. The median duration of response had not been reached at the time of publication; however, it was estimated that 79% of patients who had a CR would remain relapse free at 12 months [76].

2.6 Race to Regulatory Approval for Commercial CAR-T Therapies

In July 2014, Novartis received breakthrough therapy designation for CTL019 from the FDA for use in patients with relapsed/refractory B-ALL [77]. This designation is designed to expedite development and review of drugs for serious conditions that have preliminary clinical evidence of significant improvement over available therapies. CTL019 was the first cellular therapy to receive this status as a treatment for cancer. In early 2017, with results from the ELIANA trial, Novartis filed a biologics license application with the FDA, which was granted priority review in March 2017. The FDA Oncologic Drugs Advisory Committee, which is responsible for reviewing and recommending investigational human drug products for cancer treatment, voted unanimously to recommend CTL019 for treatment of relapsed/refractory in pediatric and young adult B-ALL. On August 30, 2017, the FDA approved Tisagenlecleucel (Kymriah; formerly CTL019) for this pediatric patient population, thus making it the first FDA approved gene therapy. Following results from the pivotal JULIET trial, in April 2017 the FDA granted breakthrough therapy designation for the use of CTL019 in relapsed/refractory DLBCL, which was approved in May 2018 [78]. Tisagenlecleucel subsequently received approval for treatment of both relapsed/refractory B-ALL and DLBCL in the European Union (EU), Canada, Switzerland, Australia and Japan.

Nearly simultaneously, Kite Pharma received breakthrough therapy designation for KTE-C19 for the treatment of relapsed/refractory DLBCL. In August 2017, Kite was acquired by Gilead Sciences for $12 billion. Shortly thereafter, based on results from the ZUMA-1 trial, the FDA approved Axicabtagene ciloleucel (Yescarta; formerly KTE-C19) for the treatment of adults with relapsed/refractory B-cell lymphomas who fail at least two prior therapies. Axicabtagene ciloleucel now also has approval in the EU, Canada, and Switzerland. The FDA's approval of these pioneering CD19 CAR-T therapies propelled research and development of CAR-T products and clinical trials for both hematologic and solid cancers, with over 500 clinical trials currently underway.

3 Key Aspects of Cellular Therapy Commercialization

Cell- and gene therapy-related research and development in the United States continues to grow rapidly, with a number of products advancing in clinical development. Commercialization is a valuable and necessary tool in translating cell therapy research into clinical products, in that it accelerates translation of research into clinical products, increases revenue for research and development, broadens dissemination, and, of course, provides profit (Table 2). However, commercialization of cellular therapies is more complicated than of small molecules or biologics due to their complicated manufacturing, distribution, and reimbursement. The FDA

Table 2 Pros and cons of commercialization of cell therapy products

Benefits of commercialization	Challenges of commercialization
Increased funding for research and development	Lengthy developmental timelines
Faster product production	Navigating the regulatory environment
Earlier knowledge translation	Encouraging adoption
Increased profit	Obtaining reimbursement
	Scaling up production

Center for Biologics Evaluation and Research (CBER)/Office of Cellular, Tissue, and Gene Therapies (OCTGT) provides sponsors and individuals guidance on multiple aspects of the approval process including (1) design and implementation of preclinical studies, (2) design of early-phase clinical trials and investigational new drug applications (IND), (3) BLA applications, (4) pre-market reviews, and (5) monitoring of safety and efficacy both pre- and post-marketing.

The sponsor's primary goal during preclinical development is to determine if the product is reasonably safe for initial use in humans, and if the agent exhibits pharmacological activity that justifies commercial development. Once the product is identified as a potential candidate for further development, the sponsor should focus on early-stage clinical trials to collect data and establish that the product will not expose humans to unreasonable risks. The FDA's role in the development of a new drug begins when the drug's sponsor (usually the manufacturer or potential marketer), having screened the new agent for pharmacological activity and acute toxicity in animals, wants to test its diagnostic or therapeutic potential in humans. At that point, the molecule or biologic changes in legal status under the Federal Food, Drug, and Cosmetic Act and becomes a new drug subject to specific requirements of the drug regulatory system.

While there are certainly benefits to commercialization, in the field of cell therapy research there are specific concerns that commercialization could have negative effects. For instance, a focus on commercialization could pressure scientists to concentrate on potentially marketable therapies at the expense of "curiosity-driven" research, skewing of academic research agendas, and creating conflicts of interest that might affect research [79, 80]. In addition, it remains a concern that industry sponsorship might affect research outcomes and quality [81, 82].

4 Challenges of Cellular Therapy Commercialization

The challenges of commercialization for any drug can be divided into three phases: pre-marketing, post-marketing, and manufacturing. The main pre-market challenges in the field of cell therapy are primarily related to the amount of time required to develop these products and dealing with their intense regulatory oversight to get them approved for marketing. As described above, it has taken between 10 and

20 years after the founding of a company to successfully bring cell therapy products to market. In some cases, the length of time required for development may be directly related to the regulatory requirements that must be satisfied. This issue was more significant when cell therapies were first emerging, as there was little guidance from the FDA. While clear guidance and regulations have since been instituted, the lengthy development of cell and gene therapies significantly impacts the financial cost of commercializing them, and companies must be prepared and able to generate sufficient capital to provide stable funding to survive the long and often uncertain journey to market. Several companies have gone bankrupt in the pre-marketing phase. While Provenge eventually made it to market, due to a number of manufacturing and post-marketing challenges including delayed reimbursement and newer, less costly competitive therapies, Dendreon filed for bankruptcy in 2014 just a few years after gaining marketing approval [83]. Thus, venture capital and pharmaceutical firms were initially hesitant to enter the cellular therapy space [12].

Once a cell therapy product receives marketing approval, the next phase of hurdles must be overcome. Obtaining reasonable and prompt reimbursement is one of the most significant post-marketing challenges. Payers must be convinced to pay the often exorbitant cost of these therapies; otherwise, providers and patients will not use them. The reimbursement must also be sufficient not only to cover the cost of production but should exceed that cost in order for the product to be profitable. Even after successful market entry, changes in reimbursement policies by the US Centers for Medicare and Medicaid Services (CMS) significantly impact the success of cell therapy products. The recent commercialization of two CAR-T therapies is a prime example. There has been intense discussion regarding their reimbursement, and recently CMS released fiscal year (FY) 2021 Inpatient Prospective Payment System (IPPS) proposed rules with changes that would provide a significant increase in Medicare reimbursement for hospitals caring for patients receiving CAR-T treatments.

Buy-in or adoption of new cellular therapies by providers and patients is as important as proper reimbursement. Given that products such as CAR-T cells may be administered only at a limited number of centers, which require physicians trained in administration and management of potential toxicities, proximity of these centers to local physicians' offices is one barrier. Another is that physicians may be unwilling to refer patients if they are unaware of the treatment, unsure how to identify eligible patients, or do not believe in the product. Patients must also be capable and willing to travel, sometimes long distances, in order to receive these therapies. Thus, targeting the proper physician/patient population has a significant impact on the success of cell therapies.

Other factors also influence the adoption hurdle, including the nature of the therapy, other available therapies with similar outcomes, treatment indications, and convenience for patients. Early physician involvement and mapping out how to best target both physicians and patients early in the development of cellular therapies may help companies achieve faster adoption of these treatments.

Finally, the impact of post-marketing challenges such as short shelf lives, stringent storage and shipping conditions, and the need for traceability systems [84]

cannot be overlooked. These concerns present an ongoing challenge to the successful development and marketing of cellular therapies. Production and distribution issues often arise early in clinical development and continue throughout the lifespan of the product. Scale-up of production to meet demand is another major challenge, particularly for autologous products. This personalization increases the lead time, introduces variances in the starting material, and complicates scaling to meet commercial demand. Moving to commercial-grade production must be carefully considered to avoid making changes in the manufacturing process that might potentially affect the final product. Scaling up is not just about being able to grow cells in large numbers, but companies must also ensure that the final products have the same characteristics and efficacy as the products developed in early clinical studies.

Production of cells used for clinical trials and subsequently approved by FDA must comply with good manufacturing practice (GMP). Implementing GMP to manufacture cells significantly increases cost but is required to ensure patient safety and product quality. Compliance with GMP regulations covers all aspects of production, including trained personnel with appropriate experience, quality control (QC) plans, adequate space and equipment, procedures for handling of the components, manufacturing, laboratory controls, packaging, labeling and distributing procedures, as well monitoring and recordkeeping systems [85]. Once expanded in the GMP, cell products must be checked for several standard release criteria, including purity, sterility, potency, stability/tumorigenicity, and viability [86]. While traditional drug products usually consist of pure chemical substances that are easily analyzed after manufacture, it often is difficult to identify the clinically active component(s) of a complex biological product. Thus, cell therapies are often defined by their manufacturing processes instead. Changes in the manufacturing process, equipment, or facilities could therefore alter the biological product itself, requiring additional clinical studies to demonstrate the product's safety, identity, purity, and potency. This process also can have significant financial implications, and companies must be able to meet demand at a cost that still allows room for profit.

Just as scale-up procedures can have a significant cost, so can distribution processes. As cellular therapies are living products, they must be stored and transported under very strict conditions to maintain their viability. These requirements add additional complexity and logistical challenges, which are particularly important for autologous products. To generate autologous cell therapies, cells are often collected and shipped to a cell processing/manufacturing facility where they undergo processing before being shipped back to the treatment center. Stringent chain of custody practices must be in place to ensure the correct product is delivered and administered to the correct patient. The need for cryopreservation may add additional overhead to shipping. There is also the potential that distribution processes may affect the final product outcome, but this is very difficult to determine. Thus, companies should conduct detailed analyses of the end-to-end delivery at commercial scale to identify potential barriers and determine possible solutions. These solutions may include offering training and certification of medical personnel, helping physicians/patients identify treatment centers, and supporting establishment of reimbursement systems. The Foundation for Accreditation for Cell Therapy (FACT) has established

standards for immune effector cell to ensure that products are administered safely (http://www.factwebsite.org/iecstandards/).

While the initial success of a cellular therapy product depends on its safety and efficacy in clinical trials, its ultimate financial success depends on overall costs and pricing. However, pricing for cell therapies is often determined by reimbursement policies and decisions of government entities. Most current reimbursement models do not accommodate many of the unique factors common to cellular therapies, including small patient populations, short treatment windows, high upfront cost, and potential for durable cures. In addition, these products often require complex administration and patient monitoring requirements. Payers may struggle with the financial risks associated with a high upfront cost product of unknown durability due to the current lack of long-term outcomes. In addition to payers, physicians and patients must feel comfortable with the cost of treatment. Companies should therefore carefully consider and integrate their approach to pricing and reimbursement and may need to consider options to share the risk such as outcome-based payments or pay-by-installment options. The cellular therapy market is poised to expand dramatically in the coming years, both in terms of the number of products available and the targeted patient population, and it is not yet clear how sustainable the current reimbursement model is. Developing the best payment model will require input from all stakeholders including manufactures, payers, physicians, and patient advocacy groups.

Each of these individual challenges, when combined with those associated with the scientific aspects of development, complicates the successful commercialization of cellular therapies. In order to prevent the demise of an otherwise promising cell product, these hurdles must be overcome simultaneously given that actions taken to address one may impact or exacerbate another. Both scientific and policy changes regarding manufacturing and reimbursement, respectively, can create significant financial challenges, as demonstrated in the above examples of commercialized products. However, the past few years have seen significant improvement in commercializing cell therapies, and, with more time, perhaps some of these remaining obstacles will be overcome.

5 Conclusions

As demonstrated in the timelines of the above products, the development of cellular therapies from discovery to authorization is a lengthy, costly, highly regulated, and high-risk process. Transformation of research into clinical practice requires strong collaboration between universities/academic institutions, hospitals, payers, and the biotech and pharmaceutical industries. To capitalize on the economic, scientific, and medical potential of cellular therapy research, it is increasingly apparent that commercialization, including productive partnerships with industry, is both necessary and inevitable as pharmaceutical companies have the enormous resources necessary to get products to market. The field of cell therapy has historically attracted little

interest from pharma due to high production costs, rigorous regulatory climate, logistical complexity, and long-term investment revenues. However, the current excitement around CAR-T cell therapies has shifted this climate, as is evidenced by the astronomical rise in the number of individual products under development and the number of clinical trials currently underway. Commercialization of cellular therapies should be a focus early on in their development, as it requires input from both internal and external stakeholders, to provide insight that may impact market access after product launch and support decisions related to pricing. Discussions should include strategies to maintain a strong pipeline along with planned growth of intellectual property and investment in a broader portfolio. Companies should also critically assess whether they have found the right partner with the right capabilities for the product they are hoping to deliver. New business models will need to be explored to ensure that novel cellular therapies will be both commercially viable and widely available. Pricing of cellular therapies will depend to a great extent on their perceived value related to level of innovation, durable clinical benefit, and impact on health systems. As the market for cell therapies is just now starting to gain footing, companies have the opportunity to establish best practices and technologies, which have not yet been established, in order to build for long-term success. One fundamental approach to improve commercialization of cellular therapies will be transitioning from patient-specific products to mass-produced products. This transition would reduce lead times and potentially lower production costs, and work in this area is currently ongoing throughout the field.

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References

1. Mason, C., & Dunnill, P. (2008). A brief definition of regenerative medicine. *Regenerative Medicine, 3*(1), 1–5.
2. Tang, J., Hubbard-Lucey, V. M., Pearce, L., et al. (2018). The global landscape of cancer cell therapy. *Nature Reviews. Drug Discovery, 17*(7), 465–466.
3. Li, M. D., Atkins, H., & Bubela, T. (2014). The global landscape of stem cell clinical trials. *Regenerative Medicine, 9*(1), 27–39.

4. Yano, K., Speidel, A. T., & Yamato, M. (2018). Four Food and Drug Administration draft guidance documents and the REGROW Act: A litmus test for future changes in human cell- and tissue-based products regulatory policy in the United States? *Journal of Tissue Engineering and Regenerative Medicine, 12*(7), 1579–1593.
5. Mason, C., Brindley, D. A., Culme-Seymour, E. J., & Davie, N. L. (2011). Cell therapy industry: Billion dollar global business with unlimited potential. *Regenerative Medicine, 6*(3), 265–272.
6. Rao, M. S. (2011). Funding translational work in cell-based therapy. *Cell Stem Cell, 9*(1), 7–10.
7. Mason, C. (2009). ISSCR 2009 industry panel session: Promoting translation and commercialization. *Cell Stem Cell, 5*(4), 379–384.
8. Epicel. (2020). *Epicel 2020*. https://www.epicel.com/. Last accessed 3 Sept 2020.
9. Rheinwald, J. G., & Green, H. (1975). Serial cultivation of strains of human epidermal keratinocytes: The formation of keratinizing colonies from single cells. *Cell, 6*(3), 331–343.
10. Green, H. (2008). The birth of therapy with cultured cells. *BioEssays, 30*(9), 897–903.
11. De Bie, C. (2007). Genzyme: 15 years of cell and gene therapy research. *Regenerative Medicine, 2*(1), 95–97.
12. Dodson, B. P., & Levine, A. D. (2015). Challenges in the translation and commercialization of cell therapies. *BMC Biotechnology, 15*, 70.
13. Administration USFaD. (2016). *Guidance for Industry and Food and Drug Administration Staff – Humanitarian Use Device (HUD) Designations*. https://www.fda.gov/media/85356/download. Last accessed 3 Sept 2020.
14. Administration USFaD. (2019). *Humanitarian device exemption*. Updated September 05, 2019. https://www.fda.gov/medical-devices/premarket-submissions/humanitarian-device-exemption#definitions. Last accessed 3 Sept 2020.
15. Zaslav, K., Cole, B., Brewster, R., DeBerardino, T., et al. (2009). Investigators SSP. A prospective study of autologous chondrocyte implantation in patients with failed prior treatment for articular cartilage defect of the knee: Results of the Study of the Treatment of Articular Repair (STAR) clinical trial. *The American Journal of Sports Medicine, 37*(1), 42–55.
16. Administration USFaD. (2019). *MACI package insert June 2019*. https://www.maci.com/patients/?carticel. Last accessed 3 Sept 2020.
17. Administration USFaD. (2010). *PROVENGE package insert* 2010. https://www.fda.gov/media/78511/download. Last accessed 3 Sept 2020.
18. Timmerman, L. (2009). *Dendreon may not survive its success: Q&A with founder Chris Henney, Part 1*. https://xconomy.com/seattle/2009/07/27/dendreon-may-not-survive-its-success-qa-with-founder-chris-henney-part-1/. Last accessed 3 Sept 2020.
19. Small, E. J., Schellhammer, P. F., Higano, C. S., Redfern, C. H., et al. (2006). Placebo-controlled phase III trial of immunologic therapy with sipuleucel-T (APC8015) in patients with metastatic, asymptomatic hormone refractory prostate cancer. *Journal of Clinical Oncology, 24*(19), 3089–3094.
20. Kantoff, P. W., Higano, C. S., Shore, N. D., Berger, E. R., et al. (2010). Investigators IS. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. *The New England Journal of Medicine, 363*(5), 411–422.
21. Higano, C. S., Small, E. J., Schellhammer, P., Yasothan, U., et al. (2010). Sipuleucel-T. *Nature Reviews Drug Discovery, 9*(7), 513–514.
22. Lazarus, H. M., Haynesworth, S. E., & Gerson, S. L. (1995). Ex vivo expansion and subsequent infusion of human bone marrow-derived stromal progenitor cells (mesenchymal progenitor cells): Implications for therapeutic use. *Bone Marrow Transplantation, 16*(4), 557–564.
23. Pittenger, M. F., & Martin, B. J. (2004). Mesenchymal stem cells and their potential as cardiac therapeutics. *Circulation Research, 95*(1), 9–20.
24. Kuci, S., Henschler, R., & Muller, I. (2012). Basic biology and clinical application of multipotent mesenchymal stromal cells: From bench to bedside. *Stem Cells International, 2012*, 185943.

25. Karussis, D., Karageorgiou, C., Vaknin-Dembinsky, A., Gowda-Kurkalli, B., et al. (2010). Immunological effects of mesenchymal stem cell transplantation in patients with multiple sclerosis and amyotrophic lateral sclerosis. *Archives of Neurology, 67*(10), 1187–1194.
26. Garcia-Olmo, D., Garcia-Arranz, M., Herreros, D., Pascual, I., et al. (2005). A phase I clinical trial of the treatment of Crohn's fistula by adipose mesenchymal stem cell transplantation. *Diseases of the Colon and Rectum, 48*(7), 1416–1423.
27. Wu, H., & Mahato, R. I. (2014). Mesenchymal stem cell-based therapy for type 1 diabetes. *Discovery Medicine, 17*(93), 139–143.
28. Katuchova, J., Harvanova, D., Spakova, T., Kalanin, R., et al. (2015). Mesenchymal stem cells in the treatment of type 1 diabetes mellitus. *Endocrine Pathology, 26*(2), 95–103.
29. Davies, L. C., Alm, J. J., Heldring, N., Moll, G., et al. (2016). Type 1 diabetes mellitus donor mesenchymal stromal cells exhibit comparable potency to healthy controls in vitro. *Stem Cells Translational Medicine, 5*(11), 1485–1495.
30. Ringden, O., Uzunel, M., Rasmusson, I., Remberger, M., et al. (2006). Mesenchymal stem cells for treatment of therapy-resistant graft-versus-host disease. *Transplantation, 81*(10), 1390–1397.
31. von Bonin, M., Stolzel, F., Goedecke, A., Richter, K., et al. (2009). Treatment of refractory acute GVHD with third-party MSC expanded in platelet lysate-containing medium. *Bone Marrow Transplantation, 43*(3), 245–251.
32. Kebriaei, P., & Robinson, S. (2011). Treatment of graft-versus-host-disease with mesenchymal stromal cells. *Cytotherapy, 13*(3), 262–268.
33. Kebriaei, P., Isola, L., Bahceci, E., Holland, K., et al. (2009). Adult human mesenchymal stem cells added to corticosteroid therapy for the treatment of acute graft-versus-host disease. *Biology of Blood and Marrow Transplantation, 15*(7), 804–811.
34. Martin, P. J., Uberti, J., & Soiffer, R. J. (2010). Prochymal improves response rates in patients with steroid-refractory acute graft versus host disease (SR-GVHD) involving the liver and gut: Results of a randomized, placebo-controlled, multicenter phase III trial in GVHD. *Biol Blood Marrow Transplant, 16*(2 (Suppl 2)), S169–S170.
35. Kebriaei, P., Hayes, J., Daly, A., Uberti, J., et al. (2020). Phase 3 randomized study of Remestemcel-L versus placebo added to second-line therapy in patients with steroid-refractory acute graft-versus-host disease. *Biology of Blood and Marrow Transplantation, 26*(5), 835–844.
36. Kurtzberg, J., Prockop, S., Teira, P., Bittencourt, H., et al. (2014). Allogeneic human mesenchymal stem cell therapy (remestemcel-L, Prochymal) as a rescue agent for severe refractory acute graft-versus-host disease in pediatric patients. *Biology of Blood and Marrow Transplantation, 20*(2), 229–235.
37. Daly, A. (2012). Remestemcel-L, the first cellular therapy product for the treatment of graft-versus-host disease. *Drugs Today (Barc), 48*(12), 773–783.
38. Kurtzberg, J., Abdel-Azim, H., Carpenter, P., Chaudhury, S., et al. (2020). A phase 3, single-arm, prospective study of Remestemcel-L, ex vivo culture-expanded adult human mesenchymal stromal cells for the treatment of pediatric patients who failed to respond to steroid treatment for acute graft-versus-host disease. *Biology of Blood and Marrow Transplantation, 26*(5), 845–854.
39. Kurtzberg, J., Prockop, S., Chaudhury, S., Horn, B., et al. (2020). Group MSBS. Study 275: Updated expanded access program for Remestemcel-L in steroid-refractory acute graft-versus-host disease in children. *Biology of Blood and Marrow Transplantation, 26*(5), 855–864.
40. Astor, L. (2020). *FDA priority review granted to Remestemcel-L for pediatric steroid-refractory acute GVHD* https://www.targetedonc.com/view/fda-priority-review-granted-to-remestemcell-for-pediatric-steroidrefractory-acute-gvhd. Last accessed 3 Sept 2020.
41. Kurtzberg, J., Martin, P. J., Prockop, S., Burke, E., Segal, K., & editors. (2020). *Aggregate results of Remestemcel-L treatment of steroid-refractory acute graft-versus-host-disease in pediatric patients*. Transplantation and Cellular Therapy Meeting; Orlando, Florida, 19–23 February 2020.

42. Mesoblast. http://mesoblast.com/product-candidates/product-candidates-overview. Last accessed 3 Sept 2020.
43. Kuwana, Y., Asakura, Y., Utsunomiya, N., Nakanishi, M., et al. (1987). Expression of chimeric receptor composed of immunoglobulin-derived V regions and T-cell receptor-derived C regions. *Biochemical and Biophysical Research Communications, 149*(3), 960–968.
44. Gross, G., Waks, T., & Eshhar, Z. (1989). Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity. *Proceedings of the National Academy of Sciences of the United States of America, 86*(24), 10024–10028.
45. Eshhar, Z., Waks, T., Gross, G., & Schindler, D. G. (1993). Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors. *Proceedings of the National Academy of Sciences of the United States of America, 90*(2), 720–724.
46. Brocker, T., & Karjalainen, K. (1995). Signals through T cell receptor-zeta chain alone are insufficient to prime resting T lymphocytes. *The Journal of Experimental Medicine, 181*(5), 1653–1659.
47. Gong, M. C., Latouche, J. B., Krause, A., Heston, W. D., et al. (1999). Cancer patient T cells genetically targeted to prostate-specific membrane antigen specifically lyse prostate cancer cells and release cytokines in response to prostate-specific membrane antigen. *Neoplasia (New York, NY)., 1*(2), 123–127.
48. Kershaw, M. H., Westwood, J. A., Parker, L. L., Wang, G., et al. (2006). A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. *Clinical Cancer Research, 12*(20 Pt 1), 6106–6115. https://doi.org/10.1158/1078-0432.CCR-06-1183. Epub 2006/10/26. PubMed PMID: 17062687; PMCID: PMC2154351. Last accessed 3 Sept 2020.
49. Krause, A., Guo, H. F., & Latouche, J. B. (1998). Antigen-dependent CD28 signaling selectively enhances survival and proliferation in genetically modified activated human primary T lymphocytes. *The Journal of Experimental Medicine, 188*(4), 619–626.
50. Brentjens, R. J., Latouche, J. B., Santos, E., Marti, F., et al. (2003). Eradication of systemic B-cell tumors by genetically targeted human T lymphocytes co-stimulated by CD80 and interleukin-15. *Nature Medicine, 9*(3), 279–286.
51. Finney, H. M., Lawson, A. D., Bebbington, C. R., & Weir, A. N. (1998). Chimeric receptors providing both primary and costimulatory signaling in T cells from a single gene product. *Journal of Immunology, 161*(6), 2791–2797.
52. Finney, H. M., Akbar, A. N., & Lawson, A. D. (2004). Activation of resting human primary T cells with chimeric receptors: Costimulation from CD28, inducible costimulator, CD134, and CD137 in series with signals from the TCR zeta chain. *Journal of Immunology, 172*(1), 104–113.
53. Imai, C., Mihara, K., Andreansky, M., Nicholson, I. C., et al. (2004). Chimeric receptors with 4-1BB signaling capacity provoke potent cytotoxicity against acute lymphoblastic leukemia. *Leukemia, 18*(4), 676–684.
54. Maher, J., Brentjens, R. J., & Gunset, G. (2002). Human T-lymphocyte cytotoxicity and proliferation directed by a single chimeric TCRzeta /CD28 receptor. *Nature Biotechnology, 20*(1), 70–75.
55. Kowolik, C. M., Topp, M. S., Gonzalez, S., Pfeiffer, T., et al. (2006). CD28 costimulation provided through a CD19-specific chimeric antigen receptor enhances in vivo persistence and antitumor efficacy of adoptively transferred T cells. *Cancer Research, 66*(22), 10995–11004.
56. Pule, M. A., Savoldo, B., Myers, G. D., Rossig, C., et al. (2008). Virus-specific T cells engineered to coexpress tumor-specific receptors: Persistence and antitumor activity in individuals with neuroblastoma. *Nature Medicine, 14*(11), 1264–1270. Epub 2008/11/04. https://doi.org/10.1038/nm.1882
57. Brentjens, R. J., Santos, E., Nikhamin, Y., Yeh, R., et al. (2007). Genetically targeted T cells eradicate systemic acute lymphoblastic leukemia xenografts. *Clinical Cancer Research, 13*(18 Pt 1), 5426–5435.

58. Milone, M. C., Fish, J. D., Carpenito, C., Carroll, R. G., et al. (2009). Chimeric receptors containing CD137 signal transduction domains mediate enhanced survival of T cells and increased antileukemic efficacy in vivo. *Molecular Therapy, 17*(8), 1453–1464.
59. Kochenderfer, J. N., Yu, Z., & Frasheri, D. (2010). Adoptive transfer of syngeneic T cells transduced with a chimeric antigen receptor that recognizes murine CD19 can eradicate lymphoma and normal B cells. *Blood, 116*(19), 3875–3886.
60. Porter, D. L., Levine, B. L., & Kalos, M. (2011). Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. *The New England Journal of Medicine, 365*(8), 725–733.
61. Kochenderfer, J. N., Dudley, M. E., Feldman, S. A., Wilson, W. H., et al. (2012). B-cell depletion and remissions of malignancy along with cytokine-associated toxicity in a clinical trial of anti-CD19 chimeric-antigen-receptor-transduced T cells. *Blood, 119*(12), 2709–2720.
62. Brentjens, R. J., Davila, M. L., Riviere, I., Park, J., et al. (2013). CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. *Science Translational Medicine, 5*(177), 177ra38.
63. Grupp, S. A., Kalos, M., Barrett, D., Aplenc, R., et al. (2013). Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *New England Journal of Medicine, 368*(16), 1509–1518.
64. Kochenderfer, J. N., Dudley, M. E., Kassim, S. H., Somerville, R. P., et al. (2015). Chemotherapy-refractory diffuse large B-cell lymphoma and indolent B-cell malignancies can be effectively treated with autologous T cells expressing an anti-CD19 chimeric antigen receptor. *Journal of Clinical Oncology, 33*(6), 540–549.
65. Kochenderfer, J. N., Wilson, W. H., Janik, J. E., Dudley, M. E., et al. (2010). Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19. *Blood, 116*(20), 4099–4102.
66. Gilead. (2012). *Kite Pharma partners with the National Cancer Institute to develop novel cellular immunotherapy clinical products*. https://www.gilead.com/news-and-press/press-room/press-releases/2012/10/kite-pharma-part-ners-with-the-national-cancer-institute-to-develop--novel-cellular-immuno-therapy-clinical-products. Last accessed 3 Sept 2020.
67. Locke, F. L., Neelapu, S. S., Bartlett, N. L., Siddiqi, T., et al. (2017). Phase 1 results of ZUMA-1: A multicenter study of KTE-C19 anti-CD19 CAR T cell therapy in refractory aggressive lymphoma. *Molecular Therapy, 25*(1), 285–295.
68. Kochenderfer, J. N., Somerville, R. P. T., Lu, T., Shi, V., et al. (2017). Lymphoma remissions caused by anti-CD19 chimeric antigen receptor T cells are associated with high serum Interleukin-15 levels. *Journal of Clinical Oncology, 35*(16), 1803–1813.
69. Neelapu, S. S., Locke, F. L., Bartlett, N. L., Lekakis, L. J., et al. (2017). Axicabtagene Ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. *The New England Journal of Medicine, 377*(26), 2531–2544.
70. Kalos, M., Levine, B. L., Porter, D. L., Katz, S., et al. (2011). T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. *Science Translational Medicine, 3*(95), 95ra73.
71. Porter, D. L., Hwang, W. T., Frey, N. V., Lacey, S. F., et al. (2015). Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia. *Science Translational Medicine, 7*(303), 303ra139.
72. Maude, S. L., Frey, N., Shaw, P. A., Aplenc, R., et al. (2014). Chimeric antigen receptor T cells for sustained remissions in leukemia. *The New England Journal of Medicine, 371*(16), 1507–1517.
73. Maude, S. L., Teachey, D. T., Rheingold, S. R., Shaw, P. A., et al. (2016). Sustained remissions with CD19-specific chimeric antigen receptor (CAR)-modified T cells in children with relapsed/refractory ALL. *Journal of Clinical Oncology, 34*(15 suppl).
74. Maude, S. L., Laetsch, T. W., Buechner, J., Rives, S., et al. (2018). Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. *The New England Journal of Medicine, 378*(5), 439–448.

75. Schuster, S. J., Svoboda, J., Chong, E. A., Nasta, S. D., et al. (2017). Chimeric antigen receptor T cells in refractory B-cell lymphomas. *The New England Journal of Medicine, 377*(26), 2545–2554.
76. Schuster, S. J., Bishop, M. R., Tam, C. S., Waller, E. K., Borchmann, P., et al. (2019). Tisagenlecleucel in adult relapsed or refractory diffuse large B-cell lymphoma. *The New England Journal of Medicine, 380*(1), 45–56.
77. Penn Medicine News. (2014). *University of Pennsylvania's personalized cellular therapy for Leukemia receives FDA's breakthrough therapy designation*. https://www.pennmedicine.org/news/news-releases/2014/july/university-of-pennsylvanias-pe. Last accessed 3 Sept 2020.
78. Administration USFaD. (2018). *FDA approves tisagenlecleucel for adults with relapsed or refractory large B-cell lymphoma*. https://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm606540.htm. Last accessed 3 Sept 2020.
79. Slaughter, S., & Rhoades, G. (1996). The emergence of a competitiveness research and development policy coalition and the commercialization of academic science and technology. *Science Technology and Human Values, 21*(3), 303–339. https://doi.org/10.1177/016224399602100303
80. Lathyris, D. N., Patsopoulos, N. A., Salanti, G., & Ioannidis, J. P. (2010). Industry sponsorship and selection of comparators in randomized clinical trials. *European Journal of Clinical Investigation, 40*(2), 172–182.
81. Lexchin, J., Bero, L. A., Djulbegovic, B., & Clark, O. (2003). Pharmaceutical industry sponsorship and research outcome and quality: Systematic review. *BMJ, 326*(7400), 1167–1170.
82. Patsopoulos, N. A., Ioannidis, J. P., & Analatos, A. A. (2006). Origin and funding of the most frequently cited papers in medicine: Database analysis. *BMJ, 332*(7549), 1061–1064.
83. Grunbaum, R. *Biotech's pitfalls tripped up Dendreon, led it to bankruptcy 2014*. Available from: https://www.seattletimes.com/business/biotechrsquos-pitfalls-tripped-up-dendreon-led-it-to-bankruptcy/. Last accessed 3 Sept 2020.
84. Levine, B. L., Miskin, J., Wonnacott, K., & Keir, C. (2017). Global manufacturing of CAR T cell therapy. *Molecular Therapy Methods & Clinical Development, 4*, 92–101. https://doi.org/10.1016/j.omtm.2016.12.006. Epub 2017/03/28. Last accessed 3 Sept 2020.
85. Gee, A. P. (2018). GMP CAR-T cell production. *Best Practice & Research Clinical Haematology, 31*(2), 126–134. https://doi.org/10.1016/j.beha.2018.01.002. Epub 2018/06/19. Last accessed 3 Sept 2020.
86. Cuende, N., & Izeta, A. (2010). Clinical translation of stem cell therapies: A bridgeable gap. *Cell Stem Cell, 6*(6), 508–512. https://doi.org/10.1016/j.stem.2010.05.005. Epub 2010/06/24. Last accessed 3 Sept 2020.

Part II
Quality Systems

The Meaning of Quality

J. Wade Atkins

1 Quality Principles and Concepts

Quality as a noun is defined as simply as how good or bad something is [1] when compared to an expectation. Quality is determined by the perception of the receiver of goods or services. Within a healthcare setting, the expectations are defined by many customers or stakeholders. These may be, but are not limited to, patients, treating physicians, peers, accrediting agencies, and regulators. Cellular therapy quality characteristics for both products and services are defined within regulations and community standards developed by scientific experts.

Robust quality programs are comprised of several elements. Most commonly a quality system will incorporate elements from quality management, quality control, quality assurance and process improvement. Sometimes this is collectively referred to as total quality management [2] or may also be referred to as quality systems management [1, 3].

2 Quality Management

To think about quality systems, we have to think about quality. What is quality? How do we know it when we get it? Quality may also be defined as the features and characteristics which determine the extent to which outputs satisfy the customer's needs [1]. In other words, are we providing what the customer wants, can we exceed their expectations? Implementing a quality system and managing it begins with a soundly formulated and written quality policy.

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The quality policy is the "overall intentions and directions of an organization related to quality as formally expressed by top management" [4]. This policy should be broad in concept and express corporate core values and beliefs. It should be approved and adopted by senior staff as the corporate commitment to quality [4]. Upper management must decide the direction and commitment about quality and state it clearly in a written policy statement [5]. This action step will create a true investment in the success of quality initiatives and should ensure the understanding by senior management of the role of quality management personnel.

The quality management personnel are charged with ensuring that there is documented evidence that the quality policy is being fulfilled. The quality policy should define the quality objectives [6]. Quality objectives should be directly related back to the quality policy and should be stated in concrete and measurable terms. The quality team must develop and implement the policy and objectives in a manner that provides clear direction to all staff. Staff must be able to understand the intent of the quality objectives and their role in accomplishing them. The objectives should provide a clear and specific direction for the organization with regard to quality. These clearly defined specifications and objectives are usually the body of the quality plan.

Typically, the quality plan will at minimum define the organizational policy and practices for essential systems. These universal concepts are embedded across all disciplines and industries that seek to systematically deliver high quality. These systems include but are not limited to:

- Organizational structure.
- Resource selection.
- Equipment.
- Suppliers.
- Process control.
- Documents and records.
- Error management.
- Assessments.
- Process improvement.
- Safety.

There should be a written policy statement that addresses these crucial factors. The goals of these specific policies along with the overarching quality policy and its measurable objectives are the foundation of a quality plan and can be monitored by the quality management staff.

Implementing, managing, and maintaining the quality system essentials are the actualization of the quality policies and plan. A widely accepted method is plan/do/check (study)/act model [7]. The individual elements are the following:

2.1 Plan

The plan includes the activities that go into the design and development of products or services. Hazards, probability, and detectability should be identified. A risk assessment should be conducted during this phase, and risk mitigation strategies should be developed for any unacceptable risks. Policies and procedures are developed in this phase to consistently and reproducibly deliver outputs. These procedures or processes should be validated or verified for intended outcomes.

2.2 Do

Once the policies and procedures are vetted, the staff should be trained to competently perform or complete them. Records following good manufacturing and documentation practices should be created.

2.3 Check/Study

Periodic performance checks such as quality control checkpoints should be developed and implemented that can verify that specifications and attributes are achieved. Audits may be used to verify expected outcomes as well as compliance with policies and procedures. Data analysis and trends should be used to determine systems are working as designed.

2.4 Act

If the checks or studies indicate shortcomings, then alterations are changes that should be planned, and the cycle starts over.

There are various management models that can be used to structure the overall approach to quality, but all incorporate these four concepts. Although the quality policy statement does not have to specifically address every aspect that ensures that quality happens, developing each policy and procedure with the quality policy plan in mind as the compass will drive the organization into a quality mindset and create an environment where quality and compliance come first.

3 Regulations and Standards

Within CT programs, quality expectations for products and services are based on public health laws and defined and enforced through federal regulations such as 21 CFR 1271 and 21 CFR 211 [8, 9]. The regulations also refer to other regulations that may apply for specific products or manufacturing processes. The overarching regulation for the manufacturing and distribution of cellular therapy products comes under the umbrella of human cells and tissue products (HCTP). The HCTP regulations provide for the pathway for compliance requirements. Cell and tissue products that pose lower risks to the recipient such as unmanipulated autologous progenitor cells must comply with only the requirements found in section 1271, especially the core good tissue practices (cGTPs) [8]. If there are manipulations to the product, such as gene insertion or cultured expansion, more stringent regulations apply. Products not solely regulated under 21 CFR 1271, sometimes referred to as cGMP products or 361 products [8], the cellular therapy production lab must also adhere to the requirements in 21CFR 211.28-208 [9].

The HCT/P regulations require that the facility designates a responsible party to ensure that the core requirements have been met. An essential core requirement is the establishment of a quality unit. Common quality concepts are included as requirements in 21 CFR 1271.150 [8]. These requirements are very similar to those found in the 21 CFR 211 pharmaceutical cGMPs [9]; however, the language is more specific for the core GTPs [8].

Quality management system elements are also outlined in 211.22 and calls for the creation of a "quality control unit" [9]. The fundamental requirement is for the empowerment of a non-biased group with the responsibility to ensure that all components and elements from raw material to final, labeled finished pharmaceutical, have been manufactured without error. These regulations are also the basis for the standards published by accrediting agencies, such as AABB Cellular Therapy Standards [10] and FACT Standards [11] as the requirements for accreditation. After a peer review for documented compliance, the professional society may grant accreditation as a sign that compliance with standards has been met.

Federal regulations and peer accrediting association standards are very clear in their expectations for a fully implemented, functional, and effective quality system. There is also the expectation that the quality systems will be continuously managed and assessed for effectiveness on a regular basis.

The quality management system must be annually evaluated for success by reviewing measurable data for each quality objectives to determine in a timely manner if the objectives have been or are continuously being met or exceeded. If the objectives have not been met, then corrective action must be implemented.

4 Quality Control

Quality control is the combination of all the efforts to determine adequate achievement of the pre-defined quality specifications. There are many activities that can help make this determination. The most common and often first thought of is analytical testing or assays. These laboratory tests determine the presence, absence, or quantity of one or more components. They may be requirements for determining if the product is capable of delivering the expected results and may be referred to as release criteria. Analytical or lab testing is but one of the many actions that can be used to determine quality.

Other methods may be the use of direct observation that critical steps or control points are performed or verified by some other means, and a second person attests they observed the step. This may be something like sanitizations steps for aseptic processing or verification of an amount of reagent delivered at a specific time or production step. Often these steps are incorporated directly in the manufacturing records. In biologics manufacturer, this is often referred to as the batch record that is created currently with the performance of manufacturing activities.

The creation of the batch record allows for secondary, peer, or quality assurance review before the final product is released from manufacturing. This step may also be delayed and performed at the time the product is being released for final labeling or distribution.

The key element here is that a review independent from the performer is carried out prior to final release of the lot. In cellular therapy, each product is initiated from a human source and is unique to other manufacturing if the product is being manufactured for an identified, directed recipient. This results in each product and manufacturing run to result in a single lot, and the batch release review must be done for each individual product.

A key element in quality control is also directly related to another quality systems, error/incident, or accident management. If an error has occurred during product manufacturing, quality control, lot release, labeling, storage, or distribution, the quality unit must fully investigate, and this group is charged with approving or rejecting all production batches prior to final release [9].

There may be occurrences when the error has potentially impacted the purity, potency, or efficacy of the product. If this is determined to be the case, then there must be policies and procedures to address actions to be taken on products that are still in inventory or that have been distributed. If the cellular therapy product is still available, then a decision must be made about disposition. If there is an acceptable alternative in inventory, then disposal should be strongly considered. If there is no equivalent or suitable product available, then responsible clinicians and the manufacturing facility's medical director can discuss risk versus benefits and determine if the patient should still receive the product. These discussions and decisions should be meticulously documented. If the product has been distributed and is an FDA-licensed product, then agency notification may be warranted. If the product was not

licensed, then the incident may have to be reported to the Institutional Review Board and documented in the Investigational New Drug annual report.

5 Quality Assurance

The quality policy and the quality objectives form the structure for a quality assurance plan. Quality assurance includes the planned, formalized activities intended to provide confidence that the output will meet the required quality levels and there is compliance with all policies and procedures assuming they are written to comply with regulations [7].

The quality assurance plan should specifically acknowledge the factors that determine quality, organizational structure, resource selection, equipment, suppliers, process control, documents and records, error management, assessments, process improvement, and safety. Assessments of compliance with policies and procedures should be conducted periodically and reported to senior management. If there are shortcomings, then corrections must be made.

Once the policies, procedures, processes, and monitoring systems are in place, a system of checkups can be developed. These elements create the audit criteria, or performance expectations.

5.1 Audits

Auditing is the action of inspecting or examining a process or quality system to ensure compliance with requirements. An audit is an assessment performed by a qualified person to determine if defined steps outlined in the policies, procedures, and policies are performed or met. The concept of the audit is crucial to healthcare delivery. It is often impossible to inspect every quality expectation of a cellular therapy product. While 100% inspection of parts or widgets is possible in some industries, it is not possible in cellular therapy product manufacturing. It is not possible to conduct a laboratory test that detects adequate assessment of donor eligibility and suitability. When dealing with lot sizes of one, individual products per patient, random sampling is not an acceptable method to determine compliance. Each product must be developed with quality built in as it is manufactured. Testing alone should not be relied on to ensure quality. Compliance with procedures and policies designed to ensure quality is built in is documented through recordkeeping practices. This is often referred to as the “batch record.” An audit of records in real time prior to distribution to determine if all steps and requirements are complete, accurate, and fulfilled can serve as a surrogate to demonstrate that the required action steps were taken and therefore quality expectations are met. If the audit finds a lack of compliance, then corrective action can be taken to prevent an unfortunate

outcome. If an investigation of a problem-prone area detects an area of weakness where a problem might occur, preventative action may be taken to clarify expectations before an error occurs.

As with all other procedures, there should be a written document to explain the audit process and elements. While the quality plan addresses the need to audit and management's desire for audits, an audit plan should be developed so that everyone in the organization can understand the process. The plan should cover the elements of auditing: types of audits, what aspects will be audited, who will audit, the audit schedule, how audit findings will be reported, to whom findings will be reported, and what will be done with the findings.

5.1.1 Types of Audits

There are three types of audits, first-party, second-party, and third-party audits. Each type of audit has specific uses and outcomes. First-party audits are internal audits authorized by management and conducted by employees of the same organization. These are probably the most thorough audits because the audit is very familiar with internal processes, procedures, policies, and employees. Second-party audits are external audits conducted by an agent outside the organization but are also requested by management. The value of these audits is that the operation is evaluated by someone with a different perspective of the same objective. These auditors are usually paid consultants who may be recognized as experts in their fields. The intrinsic value of the external viewpoint may be offset by the time it takes for the auditor to fully understand the operation. Whereas the first two types of auditors are working on behalf or at the request of management, third-party audits are conducted by an outside agent to determine compliance with regulations or standards for accreditation.

For first- and second-party audits, the scope of the plan can be devised and agreed to by management. An agreement is developed as to the elements, processes, or records that will be reviewed. Therefore, the internal audits may be focused, process, or system based.

5.1.2 Focused Audits

Focused audits usually look at a specific step, procedure, or record to determine compliance with the written directions to staff. Examples of focused audits include such things as reviewing temperature monitoring records for completeness, donor eligibility, and suitability records for completeness, or review of critical calculations for accuracy. Just as the name suggests, these audits focus on a single, measurable element. These audits are usually the most straightforward and least controversial.

5.1.3 Process Audits

Process audits look at the results of putting several independent steps or procedures together to obtain the desired outcome. Process audits evaluate the consistency of obtaining the expected result. Often these audits cross into many independent areas of the organization and reviews how well the process is controlled when more than one manager is responsible for the final result. An example of a process audit may be to review the effectiveness of finding suitable, eligible, and compatible donors, harvesting enough cells during collection for a therapeutic dose for transplant, and processing the cells in a manner that allows viable cells to engraft in a timely manner.

5.1.4 System Audits

System audits are even more comprehensive and complex. Systems are all the elements needed to ensure that processes and procedures have been adequately and consistently set up for success. There are many required systems to ensure outcome quality. The system audit should evaluate if written policies and procedures are in place that direct what must be done to ensure quality. The auditor then should look for objective evidence that the policies and procedures are met. For example, a system audit might be to look at how vendors of critical supplies and services are evaluated to determine if the vendor has established and implemented systems to ensure the quality of the elements that you do not have control over. A system audit may be performed to determine if the credentials and references for each new hire are reviewed prior to employment if these are requirements for job placement. These types of audits make sure that quality is being brought into the system and ensure that it keeps functioning at a level that maintains or exceeds the customer's expectations.

Developing an audit schedule is essential to the success of the overall audit program. The schedule will allow managers, supervisors, employees, and auditors to be prepared for the process. Unannounced audits should not be conducted unless everyone agrees in advance to the practice. The audit schedule allows all parties to be able to manage their workload and responsibilities. The auditor will need to plan for time to review policies and procedures, develop an audit tool, conduct the audit, write a report of findings, and deliver the report to senior management. After that report is published, the auditor may also be responsible to verify that appropriate follow-up has been completed. The audited party will need to ensure that the workflow is not interrupted and that all critical processes are not interrupted by the audit process. These distractions may lead to irreversible errors, so it must be managed in a way to prevent such outcomes. A published audit schedule also allows a way to audit the compliance of the quality unit for compliance with their own written policies and procedures.

Thoughtful selection of internal auditors is critical. The internal auditor must not only understand the organization, policies, processes, and procedures but also have a firm foundation in the principles of auditing. The key principle is that the auditor

must be fair, non-biased, and not involved in the management of the aspect being reviewed [12, 13]. Ownership of the process or responsibility for corrective action on the part of the auditor may lead to bias in reporting findings. The auditor must also have strong written and verbal communication skills as well as the ability to put people at ease so that open-ended questions can be readily answered. The auditor must also be prepared for the pressure of delivering unfavorable findings. These meetings may become contentious, and the auditor will have future interactions with their fellow employees. Professionalism and tact are the keys to surviving as an internal auditor.

The interactions of the auditor with staff are pivotal to the success of the program. The audit must not distract from the work process. Much of the audit can be performed at a desk away from the work space. Procedures, policies, and records can be reviewed before work process observations or interviews.

After the auditor has developed an understanding of the work process and final product, an audit tool or checklist of the critical steps should be made so that the audit can stay on track and develop a working list of potential findings. A list of open-ended questions should be developed. Written interview questions ensure that the same questions will be asked to multiple staff members. After the tools have been developed, then direct observation can be performed to determine if the procedures and policies are being followed consistently by all staff. These observations should be performed from a point that allows a clear view of the process but does not impede, distract, or intimidate the employee performing the function. After the direct observations have been completed, then the staff interviews can be performed. Once again, it is very important that the interviews be conducted away from the actual work process so that the potential for error is not increased by the ancillary auditing process. The staff responses will document compliance or failures. It is important for the integrity and confidentiality of the interviews and findings so that the auditor maintains the respect of the client and becomes a trusted part of success and not seen as an enemy.

Qualified and competent auditors will verbally report suspected findings at the time of detection, and many will allow the auditee to review a draft of the report before publishing the final report to management. This practice may alleviate some of the tension that may be created. All initial findings must be corroborated before they are reported. Confirmation may be from another staff member giving the same response to the same question or multiple examples of the same record deficiency. The wording of the audit findings must be fact based and not opinion based. The most effective written audit reports will cite the regulation, standard or internal policy, or procedures that have not been met.

The purpose of internal audits should be to find areas for improvement. After the written report has been delivered, it is the responsibility of the process owner to develop a response to the audit. Information may be submitted to clarify a finding or to refute a finding, but more often a corrective action or prevention plan will have to be written so that the nonconformance can be corrected.

5.2 Monitors and Indicators

Just like technical processes and procedures, the quality management processes and procedures must be monitored for effectiveness. To understand how well you are doing at meeting your goals and objectives, there must be a way to measure them [4]. It is imperative that the management goals be written so that they can be measured. These objective measures are often referred to as monitors or indicators. The monitors can be process or outcome based. Processes should be measured in a manner that demonstrates that there is consistency or reproducibility. Outcomes should measure if the desired effect has occurred [14].

The monitors should also take into account that the goal or threshold is obtainable, economically feasible, organizationally valuable, and straightforward [4]. Development of these monitors must consider the capabilities and the limitations of the organization. Setting an obtainable goal sounds simple, but it is human nature to wish for absolute perfection and to be overly optimistic. Reasonable thresholds should be developed that meet the customer's and management's expectations. It is not effective time and resource management to measure the items that are not candidates for improvement. Often, in order to set obtainable thresholds, the objective must be measured over a period of time to determine consistent performance. It is acceptable to monitor the objective and collect data before setting a realistic but obtainable threshold. When creating monitors, one should also consider the cost to perform the measure. There are many elaborate and expensive measurements that can be performed to characterize or define the quality of cellular therapy products. A balance between safety, purity, and potency along with time to results, skilled labor requirements, and cost must all be weighed to develop effective quality monitors. Selecting a monitor that has meaning to the organization will also help in garnishing support and buy-in from all factions of the organization. If everyone values the desired outcome, then there is increased drive to obtain the goal. Success is measured by output so there should be less resistance to measuring to see if the goal has been obtained. These monitors are often associated with the core values of the organization. The monitor should also be straightforward so that the outcome can be measured in a way that demonstrates that the goal has been met. There may have to be several steps or calculations, but the final result should be obtainable by everyone that is familiar with the process.

6 Improvement

Reporting findings from the various quality data inputs; quality control results, audit findings needing corrective action, quality indicator trends, error management; and customer complaints are the lynch pin that holds the quality improvement process together. The final summary report of these findings must be written and be fact based.

6.1 Error Management

Another important aspect of the quality assurance plan is the error management system. Even with thoroughly written SOPs and effective training programs, there will be times when errors occur. How these errors are identified, reported, investigated, and used for process improvement is the basis for an effective error management system. As with any other system, there must be written procedures and policies that direct the actions that need to be taken when an error occurs. When an error is detected, it should be reported to management as soon as possible. These errors should be carefully investigated to determine the real cause of the problem. This investigation is referred to as a root cause analysis. Error reports should be aggregated and trended over time to look for patterns. These patterns may show trends related to the time of day that they occur, who is involved, at what step they happen in the process, if they happen on certain shifts or days, or only on certain protocols. Looking for these patterns can help in discovering ways to eliminate the cause. Once the error is fully understood, corrective action can be taken. A true corrective action will fix the root cause. Once the corrective action has been decided, staff should be made aware of the error and trained on any changes that impact the process. Even after actions have been completed, the issue should still be monitored to ensure that it is effective and sustainable and that the change has not disrupted another part of the process that was working. Learning from errors through systematic investigation and implementing change designed to prevent reoccurrence is imperative to quality improvement.

By definition, quality is pleasing the customer. In order to understand customer concerns, there must be a mechanism to document relevant customer issues. An unhappy customer equals poor quality delivery. For regulated cellular therapy products, these issues should be documented in a complaint file. The complaint file should be reserved for issues concerning patient safety and purity, potency, or efficacy of the product. Issues surrounding unexpected disease transmission or failure to engraft with expected time frames are examples of issues that are included in complaint files. These comments should be treated in the same manner as errors. There should be a complete investigation to determine the root cause, and if possible, corrective action should be implemented as soon as possible. If the complaint is potentially a threat to public health, then consideration must be given to halting the cellular therapy program until the issue is resolved to the satisfaction of all involved parties. As with all other systems, there must be plan of action determined by senior management. This action plan can serve as a foundation for the development of policies and procedures. When policies and procedures are in place that define the quality system, the attention of the quality section personnel can turn to focusing on continuous improvement. Quality improvement projects demonstrate management's intent to achieve the quality goals. As with all critical processes, there needs to be a written policy and procedure to address how to select, develop, track progress, and document improvement projects. Quality improvement projects can be very resource intensive both in personnel time and financially. Careful attention to

the performance for quality indicator data, findings from audits and complaint file entries are all sources to identify opportunities for improvement. It is very easy to try to turn every incident into an improvement project. Some events will be addressed by a specific corrective or preventive action. Quality improvement projects should be reserved for multiple stakeholder problems.

6.2 Root Cause Analysis

Understanding the root cause of a problem prone process will allow the development of a project plan. Effective root cause investigation may reveal common issues across more than reported issue. Correcting a common, root issue may help resolve these identified issues. Each stakeholder needs to be represented during the problem-solving and decision-making sessions so that all aspects of the issue will be considered. Once the required changes have been decided, then an action plan should be created so the efforts of the cross-functional team can stay the course. Quality management employees or a project leader should plan periodic team meetings to track progress and keep the momentum moving forward.

There are several reasons to document the success of a project improvement team. Some include demonstrating commitment to succeeding in the quality goals but also many accrediting organizations require documentation for accreditation. The improvement can be documented in a narrative format or pictorial format. A narrative would include a written summary report that includes the baseline data that identified the problem, the actions of the team, and the data summary that demonstrates improvement. The pictorial, sometimes referred to as a story board, would show the same results but with visual presentations such as graphs or charts to demonstrate the improvement. Product and service quality will differentiate organizations and those that succeed in exceeding their customer's expectation will thrive.

Process improvement is the practice of ensuring that processes to detect error-prone issues are identified, their cause determined, changes made to eliminate the problem, and the successful implementation of a corrective or preventive action.

A significant element of successful quality management is a periodic, critical evaluation of the program. This review should be documented in some form as an annual review report. The objective evidence and data gathered from the individual elements of the quality plan should be compared to define expected performance. Areas of strength should be noted, but more importantly, deficient areas should be brought to the attention of senior management along with proposals that may effectively correct identified deficiencies.

7 Conclusions

Many cellular therapy programs are finding that no matter the size of the program, there should be dedicated personnel that focus on just quality activities. The issues and solutions can be very complex and time consuming but are critical to the sustained success of any cellular therapy program.

References

1. Macmillan Dictionary. https://www.macmillandictionary.com. Last accessed 14 Aug 2020.
2. Kiran, D. R. (2017). *Total quality management an overview in total quality management*. Elsevier. https://doi.org/10.1016/B978-0-12-811035-5.00001-5. Last accessed 14 Aug 2020.
3. American Society for Quality. https://asq.org/quality-resources/quality-management-system. Last accessed 5 Apr 2020.
4. Badley, J. K. (2004). Do you have any plans? In L. M. Walters (Ed.), *Introducing the big q: A practical quality primer* (p. 100). AABB Press.
5. Hammer, M. *How to write good quality objectives*. https://advisera.com/9001academy/knowledgebase/how-to-write-good-quality-objectives/. Last accessed 14 Aug 2020.
6. Arnold, K. L. (1994). *The Manager's guide to ISO 9000* (p. 21). The Free Press.
7. Johnson, P. L. (1993). *ISO 9000: Meeting the new international standards* (p. 23). McGraw-Hill.
8. Code of Federal Regulations. (2020). *Title 21 CFR part 1271*. US Government Printing Office. April 2, 2020 https://www.ecfr.gov. Last accessed 4 Apr 2020.
9. Code of Federal Regulations. (2020). *Title 21 CFR part 211*. US Government Printing Office. April 2, 2020. https://www.ecfr.gov. Last accessed 4 Apr 2020.
10. AABB. (2019, July). *Standards for cellular therapy services* (9th ed.). AABB. http://standards.aabb.org. Last accessed 14 Aug 2020.
11. Foundation for the Accreditation of Cellular Therapy. (2019, March). *Common standards for cellular therapies* (2nd ed.). University of Nebraska Medical Center. http://CommonStandardsSecondEdition. Last accessed 14 Aug 2020 http://www.factweb.org/forms/store/ProductFormPublic/second-edition-fact-common-standards-for-cellular-therapies-free-download?ticket=ST-1622286809-3oTs9qXpJDqM9er2RtFIILbhN5fsZ1Yy.
12. Grovenstein, A. R. (2004). You must have been a beautiful baby…. In L. M. Walters (Ed.), *Introducing the big q: A practical quality primer* (p. 38). AABB Press.
13. ISO 9001:2015-Quality management systems. *Sub clause 5.2*. https://www.iso.org/standard/62085.html. Last accessed 14 Aug 2020.
14. Cohn, C. S. (Ed.). (2020). *Technical manual* (20th ed., p. 4). AABB Press.

Development and Maintenance of a Quality Program

Adrian P. Gee

1 Major Regulatory Requirements of a Quality Program

The Food and Drug Administration's (FDA) concept of quality is based on a number of systems [1–6]. These are shown in Table 1, which also details the elements of each system. The European Medicines Agency has published quality information as part of its guideline on human cell-based medicinal products [7]. This requires the use of release criteria, stability testing, and special requirements for cells that have been genetically modified and for combination products.

The FDA regulations indicate that the quality program must be under a member of management who will, irrespective of his/her other duties, establish and maintain quality system requirements, or the quality plan (QP), and who will report its performance to senior management. Documented reviews of the QP must be at defined intervals, e.g., annually. The plan should define quality practices, resources, and activities and indicate how these requirements will be met and what documentation practices will be used. In addition, it must contain a provision for audits to ensure that the quality system is in compliance and to determine its efficacy. The audit program should include a requirement for documentation of corrective actions and reaudits to determine their effectiveness.

Other components include requirements for sufficient staff with the required education, training, and experience to ensure that all activities are correctly performed. There must be established training procedures, and training must be documented. There must be a system to control documentation, and documents must be reviewed for adequacy before issue. This review will be documented by the date and signature of the individual responsible for review. Approved documents must be readily available, and obsolete documents must be removed from use. Similarly, all

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Table 1 FDA concept of modern quality systems

Parameter	Elements
Quality	Product identity, strength, purity required for safety and efficacy
Quality by design	Product consistently attains pre-defined quality, by design appropriate manufacturing processes
Quality risk management	Helps guide setting of specifications and process parameters for manufacturing and assesses and mitigates the risk of changing a process and/or specification
Corrective and preventative action (CAPA)	Investigation, understanding, and correction of discrepancies while attempting to prevent recurrence
Change control	Managing change to prevent unintended consequences
The quality unit	Usually responsible for the quality control (QC) and quality assurance (QA) units. **QC** assesses suitability of incoming products; evaluates performance of manufacturing to ensure adherence to specifications, and determines acceptability of each product for release **QA** reviews and approves all procedures related to product, associated records, and audits and performs trend analysis
Six System Inspection Model	Consists of production, facilities and equipment, laboratory control, materials, and packaging and labeling

changes to a document must be reviewed, and the changes communicated to the staff. Records of changes must be documented.

There should be procedures in place to ensure that all suppliers conform to specified requirements. This procedure should include an evaluation and selection procedure for vendors. There should be a provision for vendors to inform the manufacturer of product changes.

There must be written standard operating procedures (SOPs) in place to define and control the methods of production. The environment must be adequately controlled, and the control systems must be inspected to verify that system(s) are functioning properly. The facility must also establish requirements for health, cleanliness, personal practices, and clothing of staff. Procedures must be in place to prevent contamination of equipment or products.

Buildings must be of suitable design to perform the proposed operations, prevent mix-ups, and ensure orderly handling. Equipment must meet specified requirements, and there must be maintenance schedules and periodic inspections. Equipment must be calibrated, and calibration procedures must include directions for the calibration procedure and limits of accuracy and precision. Calibration standards that are used must be traceable to national or international standards, and the calibration must be documented.

Procedures must be validated, and validation records must be signed and dated by the reviewing individual. Validated procedures must be performed by qualified individuals who must document their performance of that procedure. When changes are made, the procedure should be revalidated.

There must be acceptance criteria for products and for incoming supplies. Acceptance or rejection must be documented. Product acceptance records must include a description of the activities performed, the dates of those activities, and the signature of the individual performing these activities. There must be procedures for dealing with nonconforming products. An investigation should be performed for nonconformance and actions documented.

There must be procedure to control labeling activities, and labels must remain legible and affixed to products during all stages of manufacturing, distribution, and use. Labels must be examined before use for accuracy, and there must be a system to prevent mix-ups. Packaging must be designed to protect the product, and it must be stored under conditions that prevent mix-ups, damage, and deterioration. There must be a system to document distribution.

All documents and records must be maintained using a system that is readily accessible to the facility and to FDA inspectors. If stored electronically, there must be a backup system. There must be a system to handle complaints and procedures for their receipt, review, and evaluation. There must be a system to evaluate whether or not an investigation must be performed.

The International Standards Organization (ISO) [8] is an independent, nongovernmental international organization that sets standards. It has 164 national standard bodies as members. The ISO 9001 standard [9] deals with quality management. It addresses customer focus, leadership, engagement of people, process approach, quality improvement, evidence-based decision-making, and relationship to management. ISO does not provide certification. This is done through accredited certification bodies after an extensive audit of the company's quality management system. The audit is performed annually. The components required for ISO 9001 certification are shown in Table 2 [10].

The International Council of Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) has the mission of achieving global harmonization to ensure that safe, effective, and high-quality medicines are developed, registered, and maintained while meeting the highest standards. It publishes an extensive series of guidelines for quality, safety, and efficacy and seeks buy-in from national organizations. One of these is the ICH guidance Q10 on pharmaceutical quality systems [7]. It is largely based upon the ICH Q7 guideline "Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients" [11] and the ISO quality management system (see above). It augments these by describing specific quality system elements and management responsibilities. The guideline largely conforms to the FDA and ISO proposals in structure but also includes an annex on potential opportunities to enhance science and risk-based regulatory approaches [12]. These opportunities include compliance with GMP and demonstration of (1) an effective quality system (including the use of risk-based management principles), (2) product and process understanding, and (3) pharmaceutical quality systems.

Table 2 Components of ISO 9001 certification

Element	Component
Process approach: a process is the set of work steps that transform inputs into a more complete form – the product	Controls and checkpoints: Source of inputs Inputs – materials, information, and resources required to produce product Actions performed to create products Outputs – quality of the deliverable Receivers of outputs – customers for products
Risk-based thinking	Identify plan and implement actions to address potential risks and rewards Should be applied to product requirements review, contract negotiations, operations management, design and development, purchasing and work transfer
Leadership	Commitment to the QP Leadership defines measurable quality objectives and delegates tasks and assigns adequate resources
Planning of quality management system	Actions should be proportionate to the risk and the impact on product Planning should be results-driven All actions must be documented
Support and resources	Provide sufficient human resources and ensure competency through training Effective methods for awareness of QP and communication of changes in relevant documentation Maintain needed infrastructure, e.g., equipment and facilities Maintain and calibrate equipment
Customer focus	Monitor customer perception of the degree to which their needs and expectations have been met QP should address customer, statutory and regulatory requirements Establish processes to protect customer from receiving nonconforming products Specify objectives for product quality and delivery times Utilize customer feedback
Operations control	Plan, implement, and control processes needed for provision of product Control planned changes and review the consequences of unintended changes
Key business processes	Control product development steps and consider obsolescence Control external providers Control release and delivery of products Provide post-delivery support

(continued)

Table 2 (continued)

Element	Component
Performance evaluation improvement	Monitor, measure, analyze, and evaluate processes Use trained internal auditors to maintain QP Implement new systems for continued improvement

2 Development of a Quality Program

2.1 Initial Activities

The major aims of a QP are continual improvement of processes, lowest overall cost, and maximizing customer satisfaction. The plan is usually developed in phases which include the architecture of the QP, an analysis of the current state of quality at the facility, preparation of the required documentation, implementation of the plan, and post-follow-up [13–15]. The stages of development of a QP are shown in Table 3.

It is important that upper management is seen as fully committed to quality, by actively participating in the QP design, implementation, and monitoring. They should also be advocates of quality improvement and should commit adequate resources to these activities. There must be a documented structure to the QP with clearly assigned responsibilities and authorities. When designing the QP, it is also important to determine the requirements of what needs to be documented and controlled and how policies and procedures will be organized and managed.

The next step is to perform a current state analysis. This identifies and maps the core products, as well as identifying the gaps in various processes and policies. It should include the prioritization for new or redesigned processes and any updated project plans. This should result in the development of the quality policy and its objectives and writing of the QP and related procedures. In the QP, the organizational structure should be defined and presented in the form of an organizational chart. Staff responsible for overseeing quality should not be involved in manufacturing activities, or, in the case of very small facilities, there may be external quality review by an institutional representative, or an internal review with a separation of time between manufacturing and records review. The individual responsible for overseeing the QP and reporting its activities to upper management must be identified. This individual (and designees) should also have sign-off authority to changes in processes, documents audits, etc.

The next stage is to write the SOPs that will comprise all activities performed by the facility, including those performed by the quality staff. SOPs must be written so that they provide adequate instructions for a staff member with relevant education and experience (see the chapter on SOP writing).

Table 3 Major steps in development and maintenance of a quality program

2.2 Quality Plan SOPs

It is suggested that there should be specific quality SOPs. These should include the SOP for writing SOPs, role of the quality assurance and quality control groups, document control and retention, risk management, deviation reporting, annual quality report, validation and qualification procedures, product accessioning and management, product release and distribution, environmental monitoring, management of external inspections, audits and CAPA, quality improvement, facility accreditation and registration, complaints, recalls, out-of-specification results, disaster and emergency plans, training, proficiency and competency assessment, vendor management and materials management, calibration, cleaning, pest control, and waste management.

Responsibility for defining the facility and equipment requirements falls to technical experts who understand the pharmaceutical science, risk factors, and manufacturing procedures. The quality unit should review and approve all of the initial facility design criteria and procedures pertaining to facilities and equipment.

Material management SOPs should include either testing or the use of a certificate of analysis plus an identity analysis of incoming materials. Identity analysis is not required for products made for phase 1 clinical trials [16]. Vendors should be periodically audited based on a risk assessment. Where appropriate, materials must be obtained from qualified sources. Changes to materials should be implemented through a change control system.

Training SOPs should include the following: evaluation of training needs, provision of training to meet these needs, evaluation of effectiveness of training, and documentation of training and retraining. Training should include both on specific job functions and related cGMP/cGTP regulatory requirements.

Control over the product from its design to its delivery must be defined and approved. Documentation of this control must include the resources and facilities used, procedures used to carry out the process, identification of the investigator who will maintain and update the process as needed, identification and control of important variable, quality control measures, data collection, monitoring and controls for the product and process, validation activities including acceptance criteria, and effects or related process, functions, or personnel.

One method to evaluate quality on an ongoing basis is the use of quality indicators for each operational unit. These provide a series of parameters that can be monitored on a regular basis. Examples of indicators are product sterility, accidents, number of deviations, turnaround times, corrected test reports, failure to meet release specifications, etc.

2.3 Procedure SOPs

Procedure-specific SOPs, which can be written by manufacturing/testing group managers, should include those for testing procedures, product manufacturing, release testing, cryopreservation and storage, transportation and shipment, records assembly, and specific equipment use. Procedures should be subject to (1) risk analysis to identify process weaknesses and to (2) scale-up to demonstrate that the design is fundamentally sound. The SOPs should provide an expected outcome for the procedure, and this should also be validated. The need for a change to a procedure should be based on a review and evaluation of records. All procedure SOPs must be reviewed as part of the QP and released through a documented procedure. There must then be documentation of training of relevant staff members.

3 The Quality Manual

Table 4 shows the major elements that are suggested for inclusion in the quality manual.

Table 4 Suggested major sections in a quality policy manual

Section	Purpose
Introduction	The *introduction* of the quality manual introduces you to both the standards that will be met and the manual itself
Quality management principles	The *quality management principles* section covers the core principles that drive compliance with the standards, in addition to your quality management system
References and definitions	Provides a glossary of terms, definitions, and abbreviations that will be used throughout the manual
Context of the organization	Provides information discussing various types of issues that may arise while implementing or updating the QP and features strategies that can help overcome such issues. These include both internal and external issues that may be identified by the use of a strengths, weaknesses, and opportunities (SWOT) analysis
Leadership	Indicates who is responsible for making sure that the development and implementation of the policies regarding the QP are going according to plan, and who make sure that resources for QP implementation are allocated
Management system planning	Evaluation of internal and external connections, risks/issues, successes, and opportunities that may arise
Support	The goal of *support* is to ensure improvements are made in some of the following areas: Customer satisfaction Employee satisfaction Human resources Financial resources Working area
Operations	Includes: Objectives and requirements for the product or service Verification, validation, monitoring, inspection, and test requirements Documented information to demonstrate conformity Related risks and opportunities Documented information to demonstrate conformity and control of nonconforming products Necessary resources or outsourced processes and their controls Criteria for process performance and product/service acceptance Potential consequences and mitigation to change affecting input requirements Resources necessary to support the ongoing operation and maintenance of the product
Performance evaluation	Routine review of performance to know which aspects of the QP are working correctly – and which are not, so that improvements can be made
Improvement	Analysis of data that is relevant to the QP and relates to both short-term and long-term improvement. Data can include supplier performance, internal and external audit results, and evaluation of risks and opportunities

4 Maintaining the Quality Program

Once the QP has been developed and implemented, it is important to monitor the data that are collected and to act upon the information obtained. This process is part of continuous quality improvement (Fig. 1). The following sources of information can be used:

4.1 Deviations

Deviations from procedures must be documented to provide a history of facility activities. The deviations may be planned or unplanned. Planned deviations should be cleared by QA prior to implementation. Staff must be encouraged to use the

Fig. 1 Maintenance of quality program by continuous quality improvement

deviation system routinely to report any unintended change to a procedure. This should be documented, describing what happened, potential implications, and any corrective actions taken at the time. This report should be forwarded to QA who will assign it a degree of seriousness, review it for implications, and suggest corrective actions. These findings must be communicated to the staff involved in the deviation for review and for implementation of the corrective action. There must be follow-up by QA to determine the efficacy of the corrective actions and to make formal changes to the SOP if required. Deviations should be tracked as a quality indicator.

4.2 Audits and CAPA

Internal audits should be performed at sufficient frequency to enable the prompt detection of problems. Some professional standards require that there should be a calendar available detailing the audits to be performed annually. An audit should consist of a formal, planned check of the elements of the system being audited, following written audit procedures. Deficiencies and determination of whether CAPA is required should be taken in consultation with management. There should be a written description of the CAPA process and the process by which data is input. Issues to be addressed by the CAPA system can include individual or multiple procedure deviations or adverse trends detected in quality indicators. If necessary, statistical analysis should be used to detect such trends. The CAPA investigation is ultimately aimed at determining the root cause of a problem and development of corrective or preventative actions. These actions should be, where necessary, validated and should not adversely affect other product indicators. The efficacy of CAPA activities can be determined in a subsequent reaudit, and, if effective, the changes made must be disseminated to the staff, and retraining performed.

Audits should be performed by staff experienced in the procedure to be audited using a written copy of the SOP and relevant worksheets during observation of the procedure. Other audits may consist of an evaluation of systems, e.g., discard of outdated materials, recording of cleaning procedures, presence of calibration stickers on equipment, etc. These do not require the presence of facility staff during the audit. Audit findings must be documented and summarized and include a list of the issues detected. This report is returned to the audited staff, and they should address each problem with a corrective action and document it on the audit report. The report is then returned to QA for evaluation and potential closure with a scheduled follow-up to determine the efficacy of the actions taken.

4.3 Quality Meetings

Formal quality meetings should be held at regular intervals, usually quarterly, with the facility staff. There should be an attendance list and an agenda. At this meeting, it is normal to report findings for the previous quarter on the selected quality indicators, a review of actions taken as a result of the previous meeting, the numbers of products made and released, upcoming events that may affect the facility, and any other issues raised by the audience. The proceedings must be minuted, and the minutes made generally available to the staff and upper management.

4.4 Quality Agreements

It is increasingly common for facilities to use quality agreements both to audit potential vendors and in response to implementing contracts to provide manufacturing or testing services to a third party. These agreements usually define the work to be performed, the specifications to be met, timelines, etc. Central to the agreement is a questionnaire that requests information on the quality management system and indicates which party is responsible for performing specified actions. These agreements help as an additional assessment of the QP. Another source of this information can be obtained by review of complaints from customers and any recalls that were necessary.

4.5 Quality Reports

Regulatory authorities generally require that an annual quality report be submitted to upper management (usually the director of the business or academic unit). This should summarize the work of the QP during the previous year, critical findings, trends, and future plans. This report can be based on the minutes of the quarterly quality meetings supplemented with additional information. Management should acknowledge in writing receipt of the report and offered the opportunity to make comments or provide feedback. Annual quality reports are frequently requested at external audits.

4.6 Accreditation

Many facilities apply for accreditation by professional organizations. This provides an evaluation of the quality system as part of its inspection procedure. For general accreditation of the QP, ISO 9001 certification can be sought from a number of third-party organizations, which can be found online.

In the case of cell and gene therapies, accreditation can be performed by the Foundation for the Accreditation of Cellular Therapy (FACT) (predominantly in North and South America, with some additional countries), the Joint Accreditation Committee of the International Society for Cell and Gene Therapy, and the European Bone Marrow Transplant Group (JACIE) (predominantly in Europe and some additional countries) or the AABB (international). These organizations perform on-site inspections to evaluate the facility's compliance with their written standards. Other organizations, such as the College of American Pathologists, perform focused inspections on specific activities, e.g., flow cytometry, and provide accreditation in that specialty. Facilities are encouraged to participate in these programs to provide an additional information on the quality of their operations.

5 Problems Developing and Maintaining a Quality System

The following section is based on the issues confronted by the Center for Cell and Gene Therapy when developing its cGTP and cGMP Quality Systems. The first was managing writing the required SOPs. It rapidly became apparent that production of one SOP resulted in a requirement for multiple others, and a decision had to be made as to when there was a sufficient number to start operation of the facility. The number of documents produced resulted quickly in the need for a clear document control policy and the development of procedures for ensuring that staff were properly trained. The first documents to be written include the QP, the SOP for writing SOPs, and document control. Staff training probably comes next.

Initial training will be on gowning and behavior in the facility and aseptic technique. For new facilities, a facility qualification will be required with associated SOPs on facility cleaning and environmental monitoring. Once the facility is qualified, it is necessary to source order and manage reagents and materials. This will be followed by SOPs for equipment operation, maintenance, and calibration. Equipment will require qualification SOPs to ensure that it operates properly for its intended use. Manufacturing SOPs will then be developed in parallel with those for release testing and assembly of records. The development of these procedures, in turn, required preparation of a validation procedure that would meet regulatory requirements.

Manual documentation of training is extremely time consuming since the training forms have to be circulated to many people and will often sit on an individual's desk for some time, causing often undetected delays. This can be resolved by

implementing an electronic system for documenting training or adapting the e-mail system to provide a somewhat simplistic alternative.

After QP implementation, it is important to monitor the effectiveness of the plan (Table 3), perform audits, and address any detected deficiencies. The review of the QP after implementation should include the appropriateness of the quality policy, the results of audits and inspections, customer feedback and complaints, trend analysis of data, status of actions to prevent a potential problem, and follow-up of actions from previous management reviews. The review should result in improvements to the QP, to manufacturing processes and products, and realignment of resources. Initially, we performed a minimal number of audits, due to a lack of QA staff. This situation has improved, and we now have a staff of seven, with a resulting improvement in QA activities.

An area with which we have struggled is CAPA. In many cases, the deviations which are reported are classified as of low impact and appear to be the result of an isolated simple human error. We have found it difficult to perform an in-depth CAPA review and assign a root cause to such problems. We have now implemented the CAPA element of the Q-pulse quality assurance software. This guides the user through the CAPA procedure and, therefore, provides a more uniform report.

6 Conclusions

The QP provides management and regulatory authorities with the assurance that activities follow the relevant policies and procedures and result in safe and effective products of assured quality. Quality must be seen as a communal activity involving not only all facility staff but also a number of external groups and organizations that provide sources of both input and oversight. It must be seen as an activity that is mutually beneficial rather than punitive.

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References

1. Food and Drug Administration. (1999). *Guide to inspections of quality systems*. https://www.fda.gov/files/Guide-to-Inspections-of-Quality-Systems.pdf. Last accessed 13 July 2020.
2. Food and Drug Administration. (2006). *Guidance for industry. Quality systems approach to pharmaceutical cGMP regulations*. https://www.fda.gov/media/71023/download. Last accessed 13 July 2020.
3. Food and Drug Administration. *cGMP in manufacturing, processing, packing, or holding of drugs and finished pharmaceuticals*. Title 21 Code of Federal Regulations. Parts

210 & 211. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=210&showFR=1 and https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=211. Last accessed 13 July 2020.
4. Food and Drug Administration. *Human cells, tissues, and cellular and tissue-based products*. Title 21 Code of Federal Regulations. Part 1271. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=121. Last accessed 13 July 2020.
5. Wilbon, T. (2018). *Overview of the quality system regulation*. https://www.fda.gov/media/118197/download. Last accessed 13 July 2020.
6. Tartal, J. (2014). *Quality system regulation overview*. https://www.fda.gov/files/drugs/published/Overview-of-Quality-System-Regulation.pdf. Last accessed 13 July 2020
7. European Medicines Agency. (2008). *Guideline on human cell-based medicinal products*. https://www.ema.europa.eu/en/documents/scientific-guideline/international-conference-harmonisation-technical-requirements-registration-pharmaceuticals-human_en.pdf. Last accessed 13 July 2020.
8. The International Standards Organization. https://www.iso.org/about-us.html. Last accessed 13 July 2020.
9. ISO 9000 Family Quality Standards. https://www.iso.org/iso-9001-quality-management.html. Last accessed 13 July 2020.
10. Keen, R. (2019). *ISO 9001 quality manual – Everything you need to know*. https://www.iso-9001-checklist.co.uk/quality-manual.htm. Last accessed 13 July 2020.
11. ICH Q7 Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients. https://www.gmp-compliance.org/guidelines/gmp-guideline/ich-q7-good-manufacturing-practice-guide-for-active-pharmaceutical-ingredients. Last accessed 13 July 2020.
12. European Medicines Agency. (2015). *ICH guideline Q9 on quality risk management*. https://www.ema.europa.eu/en/documents/scientific-guideline/international-conference-harmonisation-technical-requirements-registration-pharmaceuticals-human-use_en-3.pdf. Last accessed 13 July 2020.
13. Medcalf, N., Hourd, P., Chandra, A., & Williams, D. J. (2019). *Quality assurance and GMP in the manufacture of cell-based therapeutics*. https://www.stembook.org/node/8571. Last accessed 13 July 2020.
14. King, K. (2017). *Building a quality program from the ground up*. https://www.leanmaryland.com/resources/Documents/112-King_Quality_Program_from_the_Ground_Up.pdf. Last accessed 13 July 2020.
15. Longelin, J. (2018). *8 steps to create a quality assurance program from scratch*. https://blog.playvox.com/8-steps-to-building-a-qa-program-for-your-support-team-from-scratch. Last accessed 13 July 2020.
16. CGMP Guidance for Phase 1 Investigational Drugs. Guidance for Industry. July 2008. fda.gov/media/70975/download accessioned June 1, 2021

Quality Control of Cellular Therapy Products and Viral Vectors

Adrian P. Gee

1 Introduction

Quality control is defined as "a system for verifying and maintaining a desired level of quality in an existing product or service by careful planning, use of proper equipment, continued inspection, and corrective action as required" [1]. In contrast, quality assurance has a broader definition as "a system for ensuring a desired level of quality in the development, production, or delivery of products and services" [1]. Regulatory authorities place a great deal of importance on product quality, and in its Guidance on the Quality Systems Approach to Pharmaceutical CGMP Regulations [2], the FDA has stated that "Every pharmaceutical product has established identity, strength, purity, and other quality characteristics designed to ensure the required levels of safety and effectiveness. For the purposes of this guidance document, the phrase achieving quality means achieving these characteristics for a product."

The testing required for cellular therapy and gene therapy products has been described in two guidance documents for FDA reviewers and sponsors. The first "Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Somatic Cell Therapy Investigational New Drug Applications (INDs)" [3] provides a guide to FDA reviewers on what to expect in the Chemistry, Manufacturing and Control (CMC) section of an Investigational New Drug (IND) application to perform a clinical trial using a cellular therapy product. The second "Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs) [4] provides similar information for gene therapy products. These guidances form the basis of this chapter.

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2 Quality Control (QC) of Somatic Cell Therapy Products

The somatic cell therapy CMC guidance [3] contains sections on product manufacturing and product testing. The testing section recommends that this should include, but not be limited to, microbiological testing and assessments of other product characteristics, such as identity, purity, and potency.

2.1 Microbiological Testing

2.1.1 Sterility

It is recommended that microbiological testing should be performed on cell banks, in-process intermediates, and the final product(s). The FDA indicates that the 21 Code of Federal Regulations (CFR) 610.12 method should be used [5]. This method is described in the United States Pharmacopoeia (USP) <71> [6]. If an alternative method is employed, its suitability should be determined and must be equal or greater than the assurance provided by the recommended method. The two most popular rapid methods are the Bactec system from Becton Dickinson [7] and the BacT/ALERT system from bioMérieux [8]. In 2008, the FDA initially published a guidance on the validation of rapid sterility testing methods, but this was withdrawn in 2015 [9]. Our facility has performed an internal validation of the use of the Becton Dickinson Bactec rapid sterility testing method, but this has not been submitted to the FDA (the Agency). In spite of this, the agency has allowed us to use this method for sterility testing of cell therapy products. We do, however, include a modification of the CFR method in our sterility testing. We incubate the aerobic and anaerobic Bactec cultures for 14 days and the fungal cultures for 28 days. We do have some INDs where the FDA has allowed us to release the products after 7 days of incubation. Comparisons of the various methods have been published [10, 11]. The FDA is now encouraging the use of rapid test systems [4].

If antibiotics are used during manufacturing, there should be documentation that these were removed before sterility was tested. If they cannot be removed, then a bacteriostasis/fungistasis assay should be performed as described in USP <71> [6].

Sterility tests should also be performed at critical points during manufacturing. For cryopreserved products which are not manipulated before administration, the testing should be performed prior to cryopreservation. If the products are thawed and manipulated, e.g., washed, the sterility testing should be repeated. These should include a Gram stain to provide immediate results before administration, and a 14-day culture, with provision to report the results to the recipient's physician as soon as they are obtained, and a plan to deal with positive results from the 14-day culture. The plan should also address the results of an investigation into the positive result, corrective actions. An information amendment should be submitted to the IND within 30 calendar days after receipt of the initial positive result [3]. If the

recipient experiences any serious or unexpected adverse reactions to product administration, the FDA should be notified not more than 15 days after receipt of the information in an IND safety report [3].

In other cases where the product is administered before the results of the culture test are available, it is recommended that a sample be sent for sterility testing 48–72 hours before the final harvest or after the last feeding of the cultured cells. This should be combined with a Gram stain on the final formulated products and the routine 14-day culture test [3].

2.1.2 Mycoplasma

The FDA identified the sources of mycoplasma infections as any animal serum used during manufacturing and the culture environment, particularly if open systems are used [3]. They recommend testing at a manufacturing stage when contamination is most likely to be detected, e.g., after pooling cultures but before cell washing.

The test should be performed on both the cells and the supernatant. Where there is insufficient time to perform the culture-based test [12], they suggest the use of a PCR-based or rapid detection assay. There are several approved PCR assays. These include MycoSEQ™ [13] from Thermo Fisher and MycoTOOL™ from Roche [14]. If a non-approved method is used, a validation should be performed to show that the method is comparable to the culture technique in terms of both sensitivity and specificity. An alternative to the PCR assays is the MycoAlert™ assay system from Lonza [15]. This is a biochemical test that exploits the activity of mycoplasmal enzymes which are found in all six main mycoplasma cell culture contaminants.

Viable mycoplasma in the test sample is lyzed, and the enzymes react with a test substrate that catalyzes the conversion of ADP to ATP. This is then transferred to a light signal via the luciferase enzyme, which is detected using a luminometer. By measuring the level of ATP in the sample before and after the addition of the substrate, a ratio is obtained that indicates the presence or absence of mycoplasma. Since this test has not received full regulatory approval, a validation against the culture method is recommended. Recently, bioMérieux announced the launch of their BIOFIRE®MYCOPLASMA assay [16].

In cases where there may be interference with the mycoplasma assay by constituents of the final product, a mycoplasmastasis assay should also be performed [17].

In 2019, the FDA issued a proposed rule to remove the testing method for mycoplasma detection in virus harvest pools and control fluid pools of live and inactivated virus vaccines produced from in vitro living cell cultures, as currently required by 21 CFR 610.30 [18]. This indicates a possible flexibility by the agency in considering other test methods.

2.2 Adventitious Agent Testing

The FDA advises consultation of their "Points to Consider in the Characterization of Cell Lines used to Produce Biologicals" [19] and the International Conference on Harmonization (ICH) Guidance Q5A "Guidance of Viral Safety Evaluation of Biotechnology Products derived from cell Lines of Human and Animal Origin" in determining what testing may be required for other adventitious agents [20]. It is generally true to say that viral testing is not usually required for cell therapy products derived from autologous or allogeneic lines derived from donors who have undergone donor eligibility testing [21], unless these are used to derive continuous cell lines, e.g., mesenchymal stromal cells. If a cell line is produced, it is sometimes possible, if the line is used for a very small clinical trial, to perform only in vitro adventitious virus testing. More commonly, however, the line must be tested for a panel of specific viruses by PCR, in addition to in vitro and in vivo adventitious viral testing [3].

3 Identity Testing

Identity testing is performed to verify that the product is the correct one and to distinguish it from other products manufactured in the facility. This may be accomplished using a variety of tests. The most commonly used is immunophenotyping by flow cytometry. In most cases, the immunophenotype of the final product should meet specifications for the expression, or lack of expression, of particular CD markers. During early-phase clinical trials, the expression levels may be relatively generous, e.g., <2% CD19, >70% CD3, but as the trials progress, tighter expression levels will be expected. It is normal to incorporate a number of both positive and negative markers for the analysis, and these should be reviewed by the regulatory agency. Immunophenotyping is also used to determine the relative cell purity in the final product. These assays should be performed by an accredited flow cytometry laboratory.

Another assay that may be used to establish product identity is typing for genetic polymorphisms, such as blood type, which may be included [3], although this is not highly specific. Instead HLA identity between the cell donor and the final product is preferable for cells expressing HLA antigens.

4 Purity

Purity is defined as relative freedom from extraneous material in the final products, whether or not that material is harmful to the intended recipient or deleterious to the product [22]. Impurities consist of endotoxin, residual proteins, or peptides used to

pulse or stimulate cells, reagents/components used during manufacturing, e.g., cytokines, antibodies and serum, and unintended cellular phenotypes.

4.1 Residual Contaminants

The final product should be tested for residual proteins and peptides used during manufacturing and reagents used during culturing and purification, e.g., cytokines, growth factors, antibodies, beads, and serum. The final product should also be tested for cell debris and other immunophenotypes. The assays to be used and the specifications for product release must be described in the IND [3].

4.2 Endotoxin

The traditional method for testing for pyrogenicity was the rabbit pyrogen test [23]. This, however, has been largely replaced by the limulus amebocyte lysate test. This in turn has been automated into a rapid assay using an FDA-approved device – the Endosafe® nexgen-PTS™ system with FDA-licensed Endosafe® LAL cartridges [24]. The specification normally approved is <5.0 EU/kg body weight/hr. for the administered product. For intrathecally administered product, the limit is 0.2 EU/kg body weight/hour [3]. The use of the Endosafe system provides a rapid turnaround time that is suitable for all products.

5 Potency

Potency assays should be performed on the product at all stages of clinical trials; however, by the start of Phase 3, the assay should consist of in vivo or in vitro tests that measure appropriate biological activity. This assay must be validated prior to product licensure. If it is not possible to develop a quantitative biological assay, then a quantitative physical assay can be used, if it is performed in conjunction with a qualitative biological assay and correlates with it [3].

For early-phase assays, where the mechanism of action of the product in vivo may not be clear, it is normal to use an assay which quantitates possible effector mechanisms, e.g., cytokine release, cytotoxicity assays, etc. These should be discussed with the regulatory agency at the time of protocol submission.

6 Other Assays

6.1 General Safety Assay

Cellular therapy products are not required by the FDA to undergo general safety testing [3].

6.2 Viability

There should be a specified release criterion for cell viability. The FDA usually requires a benchmark of 70% [3]. If this cannot be achieved, then it should be demonstrated that the dead cells do not adversely affect safe administration of the product or its therapeutic effect.

A number of assays can be used to determine cell viability. Commonly used are the dye exclusion tests, e.g., trypan blue exclusion [25]; however, this is subject to variability by the observer and does not always detect sublethal damage that may occur subsequently. Assays using flow cytometry, e.g., staining with 7 aminoactinomycin D (7-AAD), provide a more reproducible assessment of viability [26]. Apoptosis can also be measured by staining for annexin [27]. Devices are now available for performing automated cell counts and viability measurements, e.g., the Cellometer Auto 1000 Bright Field Cell Counter from Nexcelom [28] and the NucleoCounter® NC-202™ from Chemometec [29]. These should be validated before use.

The FDA usually bases the viability release criteria on the viability at cryopreservation. It should be confirmed that this criterion can be met after thawing by validating the method used for freezing.

6.3 Cell Dose

The minimum number of viable and functional cells to be administered should be specified. It is recommended that the FDA be informed of the maximum dose that has been established based on preclinical experiments.

7 Product Stability

The stability of the product must be evaluated during the early phases of the clinical trial to determine that it will be stable over the entire trial period. The ICH has published guidelines to help with these studies: “ICH Guideline Q1E ‘Stability Testing

of Biotechnological/Biological Products' [30], and Guideline Q1A(R2) 'Stability Testing of New Drugs and Products'" [31].

The FDA requires stability testing at all phases of the IND. The test protocol should include measures of sterility, identity, purity, quality, and potency [3]. The test methods must be described, together with the sampling time points (including a time zero point), the test temperature, and any other appropriate information. The sterility should be tested at zero time, the end of the stability study, and at one intermediate time point [3].

7.1 In-Process Stability Testing

The stability of frozen products should be regularly assessed.

7.2 Final Product Stability Testing

The stability of the final product between the time of product formulation and administration should also be established. This should be done at the appropriate temperatures and at time points consistent with the anticipated holding times. It should include stability during shipment of the product to other sites, preferable under stressed conditions [3].

8 Quality Control for Manufacture of Viral Vectors

Due to the complexity of manufacturing viral vectors, the quality control testing required is more extensive. The FDA requirements are outlined in the guidance "Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications" [4]. This guidance clearly outlines the requirements for manufacturing and testing manufacturing intermediates, e.g., master and working cell banks as well as the final drug product. The following sections describe the testing to be performed:

8.1 Master Cell Banks (MCB)

Qualification of an MCB should include testing for sterility, mycoplasma, and adventitious viral agents, in addition to testing for retroviral contamination using reverse transcriptase assays and transmission electron microscopy. The MCB should be tested vigorously for adventitious viral agents (Table 1 for retroviral and

Table 1 Quality control testing performed on cellular therapy products

Testing parameter	Test performed
Microbiological testing	*Sterility* 21 CFR part 610.12 USP <71> ICH Q4B annex 8 sterility test Bactec rapid test (Becton Dickinson) BacTALERT rapid test (BioMerieux) Gram stain *Mycoplasma* Culture methods 21 CFR 510.30; USP <63> WHO nucleic acid test PCR tests MycoTOOL (Roche) MycoSEQ (Thermo Fisher) Other. MycoAlert (Lonza)
Purity	*Residual contaminants* Specific assay, e.g., for residual antibiotics, cytokines, etc. *Pyrogenicity/endotoxin* 21 CFR 610.13(b): rabbit pyrogen test USP <85>: limulus amebocyte lysate assay, ICH guideline Q4B: Limulus amebocyte lysate assay Endosafe: automated limulus amebocyte lysate assay
Identity	Immunophenotype. Genetic polymorphisms.
Potency	In vivo and/or in vitro assay for functionality. Required to correlate with biological activity by Phase 3 trial.
Viability	Dye exclusion assay Stain + Flow cytometry Automated systems
Dose	Manual cell count Automated cell count
General safety	Not required
Stability	Test for sterility, identity, purity, quality, and potency over storage period and after preparation for administration

adenoviral vectors). This may necessitate the use of viral clearance studies to remove and inactivate adventitious agents. Cell lines that have been exposed to bovine (serum) and porcine (trypsin) components should be tested fort the relevant adventitious viruses [4].

8.2 Working Cell Banks (WCB)

The WCB is derived from the MCB, and its testing is less extensive. Generally testing for sterility, mycoplasma, identity, and in vitro adventitious virus is sufficient (Table 2 for retroviral and adenoviral vectors) [4].

8.3 Master Viral Banks (MVB)

MVB should be tested for the absence of contamination, e.g., sterility, mycoplasma, and in vivo and in vitro adventitious viral agents. It should be tested for the presence of replication-competent virus in replication-incompetent vectors. The viral titer or concentration should be determined and the presence of transgene activity. The identity of the vector and the therapeutic transgene should be tested, and the correct genetic sequence confirmed (Table 2 for retroviral and adenoviral vectors) [4].

8.4 Working Viral Banks (WVB)

The WVB is derived from the MVB, and testing is generally limited to sterility, mycoplasma, identity, and in vitro adventitious agents (Table 2 for retroviral and adenoviral vectors) [4].

8.5 Final Vector Product

8.5.1 Process-Related Impurities

The final vector (drug substance) should be tested for manufacturing impurities, e.g., residual cesium chloride for adenoviral vectors, host cell DNA, cytokines, growth factors, etc. The manufacturing process should be designed to reduce non-vector DNA contamination. It is difficult to find an FDA specification for residual DNA, but the World Health Organization recommends a limit of 10 ng/dose [32]. The size of the DNA should preferably be below that of a functional gene to minimize its biological activity. Host cell protein must also be assayed [33].

Table 2 Suggested testing during manufacture of retroviral and adenoviral vectors

Master cell bank	Working cell bank derived from tested master cell bank	Master viral banks	Working viral bank derived from tested master viral bank
Sterility	Sterility	Sterility	Sterility
Mycoplasma	Mycoplasma	Mycoplasma	Mycoplasma
Endotoxin	Endotoxin	Endotoxin	Endotoxin
Identity	Identity	In vivo adventitious virus	Identity
Cytomegalovirus	In vitro adventitious virus	In vitro adventitious virus	In vitro adventitious virus
HIV-1 and 2		Replication-competent virus	Replication-competent virus
HTLV-1 and 2		Viral titer or concentration	Viral titer or concentration
Human herpes virus 6, 7, and 8		Transgene activity	Transgene activity
JC virus		Identification of viral vector and therapeutic transgene, e.g., by southern blot or restriction endonuclease	Identification of viral vector and therapeutic transgene, e.g., by southern blot or restriction endonuclease
BK virus		Correct genetic sequence, e.g., by full sequencing (for 40 kb or smaller), annotated sequence analysis For >40 kb, sequence analysis including testing by restriction endonuclease analysis. Sequence analysis of gene insert, flanking regions, and any regions modified or deleted and susceptible to recombination For integrating vectors, DNA sequencing on integrated vector	
Epstein-Barr virus			
Human parvovirus B19			
Human papilloma virus			
Hepatitis C			
Bovine/porcine virus if appropriate	Bovine/porcine virus if appropriate		
Identity by genetic analysis (e.g., STR)			

(continued)

Table 2 (continued)

Master cell bank	Working cell bank derived from tested master cell bank	Master viral banks	Working viral bank derived from tested master viral bank
Reverse transcriptase			
Transmission electron microscopy			
Additional tests Stability over time Tumorigenicity	Stability over time	Stability over time	Stability over time

8.5.2 Product-Related Impurities

Product-related impurities consist of noninfectious particles, empty capsid particles and replication-competent virus contaminants, etc. For genetically modified cells, impurities would include unmodified target cells and nontarget cells. Where possible, their presence should be enumerated.

8.5.3 Testing of Vector Product [4]

Testing on the final vector product should include microbiological testing, such as bioburden or sterility testing as appropriate, mycoplasma and adventitious viral agent testing. Rapid tests for mycoplasma and sterility should be qualified/validated to ensure their suitability. Replication competence should be tested for nonreplicating vectors at various points during the manufacturing procedure. The assay results should be supported by data demonstrating the accuracy, reproducibility, sensitivity, and specificity of the test method, and the assay should include the appropriate controls.

8.5.4 Testing of Genetically Modified Cells

In general, the testing of genetically modified cells closely follows that of nonmodified cells; however, some additional testing is normally required. This includes the vector copy number in the transduced cell population. A general specification of <5 copies per cell is normally acceptable. There should also be some test for evidence of satisfactory modification. These may include expression of the transgene detected by flow cytometry and/or evidence of functionality of the gene in a potency assay, e.g., confirmation of production of a cytokine in response to specific stimulation. The acceptability of the proposed test should be cleared with the regulatory authority.

9 Stability Testing on Vector Intermediates and Final Products

Stability testing should be performed on all of the various intermediates involved in the manufacture of a genetically modified therapeutic cell product. These include the master and working cell banks, the master and working viral banks, the final vector product, and the genetically modified cells. These will include stability of the cells or vector during long-term storage and between thawing and administration of the vector or gene-modified cells. The test protocol should include measures of sterility, identity, purity, quality, and potency [3, 4].

10 Release of Products for Administration

Products that are prepared using more-than-minimal manipulation (e.g., cultured, genetically modified, activated, etc.) are released under a Certificate of Analysis (CofA) [2, 34], the contents of which have been approved by the regulatory authority that has cleared the clinical trial. The CofA contains the appropriate testing specifications for microbiological testing, purity, identity, potency, and viability, together with information of the tests used (preferably including their specificity and sensitivity), and the results obtained. This information is assembled by the quality control laboratory and transferred to the quality unit, which reviews it and generates the CofA, which is signed by the quality director (or designee) and a Laboratory Medical Director. At this stage, the product may be made available for distribution and administration.

Products that are minimally manipulated still undergo basic quality testing, e.g., sterility, endotoxin, identity, viability, etc., but are not usually released under a CofA.

11 Other Quality Control (QC) Responsibilities

In addition to product testing, the QC laboratory usually has other responsibilities [35]. These may include performing environmental monitoring of the facility, managing cleaning schedules and disinfectant rotation, selecting external testing vendors, shipment of samples, and collation of results. The QC unit must consistently evaluate new testing technologies and introduce them where appropriate.

12 Conclusions

The quality control unit plays an essential role in the release of therapeutic products by performing in-process and final product testing. Information that they provide can also help identify improvements in manufacturing procedures and identify weak points during operations. As such, they play a vital role in GMP operations.

This chapter represents a point in time, and investigators are always encouraged to contact the appropriate regulatory authority to determine the current regulations for the release of cellular therapy and viral vectors for clinical use.

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References

1. Dictionary.com website. (2020). www.dictionary.com. Last accessed 3 Aug 2020.
2. U.S. Food and Drug Administration. (2006, September). Center for Drug Evaluation and Research (CDER) Center for Biologics Evaluation and Research (CBER) Guidance for Industry. Quality Systems Approach to Pharmaceutical CGMP Regulations. https://www.fda.gov/media/71023/download. Last accessed 3 Aug 2020.
3. U.S. Food and Drug Administration. (2008, April). Center for Biologics Evaluation and Research. 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). https://www.fda.gov/media/73624/download. Last accessed 3 Aug 2020.
4. U.S. Food and Drug Administration. (2020, January). Center for Biologics Evaluation and Research. Guidance for Industry: Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs). https://www.fda.gov/media/113760/download. Last accessed 3 Aug 2020.
5. U.S. Food and Drug Administration. *Sterility. Code of Federal regulations Title 21 Part 610.12*. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=610.1. Last accessed 3 Aug 2020.
6. Sterility Tests. *United States Pharmacopoeia <71>*. http://www.triphasepharmasolutions.com/Private/USP%2071%20STERILITY%20TESTS.pdf. Last accessed 3 Aug 2020.
7. BD BACTEC™ FX Blood Culture System. https://www.bd.com/en-us/offerings/capabilities/microbiology-solutions/blood-culture/blood-culture-instrumentation/bd-bactec-fx-blood-culture-system. Last accessed 3 Aug 2020.
8. BACT/ALERT® 3D. https://www.biomerieux-usa.com/bactalert3d/automatedsystem. Last accessed 3 Aug 2020.
9. Center for Biologics Evaluation and Research. (2008, February). *Testing of cellular and gene therapy products. Food and drug administration*. http://rapidmicromethods.com/references/Validation%20of%20Growth-Based%20Rapid%20Microbiological%20Methods%20for%20Sterility%20Testing%202011.pdf. Last accessed 3 Aug 2020.
10. England, M. R., Stock, F., Gebo, J. E. T., Frank, K. M., et al. (2019). Comprehensive evaluation of compendial USP<71>, BacT/Alert dual-T, and Bactec FX for detection of product

sterility testing contaminants. *Journal of Clinical Microbiology, 57*(2), e01548-18. https://jcm.asm.org/content/jcm/57/2/e01548-18.full.pdf. Last accessed 3 Aug 2020
11. Khuu, H. M., Stock, F., McGann, M., et al. (2004) Comparison of automated culture systems with a CFR/USP-compliant method for sterility testing of cell-therapy products. *Cytotherapy, 6*, 183–195.
12. Code of Federal regulations Mycoplasma Subpart D. *Title 21 Part 610.30*. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=610.30. Last accessed 3 Aug 2020.
13. ThermoFisher Scientific website. *MycoSEQ™ Mycoplasma Detection Kit*. https://www.thermofisher.com/order/catalog/product/4460623#/4460623. Last accessed 3 Aug 2020.
14. Roche website. *MycoTOOL Mycoplasma Real-Time PCR Kit*. https://custombiotech.roche.com/home/Product_Details/3_5_3_19_3_2.product-classes%5CTestKits.html. Last accessed 3 Aug 2020.
15. Lonza website. *MycoAlert™ Mycoplasma Detection Kits, Lonza*. https://us.vwr.com/store/product/21346689/mycoalerttm-mycoplasma-detection-kits-lonza. Last accessed 3 Aug 2020.
16. bioMérieux website. (2020). *Launch of BIOFIRE® MYCOPLASMA test for mycoplasma detection in biopharmaceutical products*. https://www.biomerieux.com/en/launch-biofirer-mycoplasma-test-mycoplasma-detection-biopharmaceutical-products. Last accessed 3 Aug 2020.
17. Russell, C. M., Brenton, M., LaBombard, S., Schieving, A., et al. (2014). *Regulatory aspects of mycoplasma testing for cell therapy products*. http://www.stem-art.com/Library/Miscellaneous/Regulatory%20Aspects%20of%20Mycoplasma%20Testing%20for%20Cell%20Therapy%20Products.pdf. Last accessed 3 Aug 2020.
18. Economics Staff, Office of Planning. (2019). Elimination of 21 CFR 610.30 Test for Mycoplasma. Docket No. FDA-2018-N-4757. https://www.fda.gov/media/122863/download. Last accessed 3 Aug 2020.
19. Draft of Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals. (1993). https://www.fda.gov/media/76255/download. Last accessed 3 Aug 2020.
20. European Medicines Agency website. *ICH Q5A (R1) Quality of biotechnological products: viral safety evaluation of biotechnology products derived from cell lines of human or animal origin*. https://www.ema.europa.eu/en/ich-q5a-r1-quality-biotechnological-products-viral-safety-evaluation-biotechnology-products-derived. Last accessed 3 Aug 2020.
21. U.S. Food and Drug Administration. *Subpart C – donor eligibility*. Title 21 Code of Federal Regulations Part 1271. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=1271&showFR=1&subpartNode=21:8.0.1.5.60.3. Last accessed 3 Aug 2020.
22. U.S. Food and Drug Administration. 610.13 Purity. Title 21 Code of Federal Regulations Part 610 General Biological Product Standards. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=610.13. Last accessed 3 Aug 2020.
23. U.S Food and Drug Administration. (2012). Center for Drug Evaluation and Research (CDER) Center for Biologics Evaluation and Research (CBER) Center for Veterinary Medicine (CVM) Center for Devices and Radiological Health (CDRH) Pyrogen and Endotoxins Testing: Questions and Answers. Guidance for industry. https://www.fda.gov/media/83477/download. Last accessed 3 Aug 2020.
24. Charles River website. Endosafe® nexgen-PTS™. https://www.criver.com/products-services/qc-microbial-solutions/endotoxin-testing/endotoxin-testing-systems/endosafe-nexgen-pts?region=3611. Last accessed 3 Aug 2020.
25. Strober, W. (2015, November 2). Trypan blue exclusion test of cell viability. *Current Protocols in Immunology, 111*, A3.B.1–A3.B.3. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6716531/. Last accessed 3 Aug 2020.
26. R&D Systems website. *7-amino actinomycin D (7-AAD) cell viability flow cytometry protocol*. https://www.rndsystems.com/resources/protocols/analysis-cell-viability-using-7-amino-actinomycin-d-7-aad. Last accessed 3 Aug 2020.

27. Lakshmanan, I., & Batra, S. K. (2013, March 20). Protocol for apoptosis assay by flow cytometry using Annexin V staining method. *Bio-Protocol, 3*(6), e374. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4943750/. Last accessed 3 Aug 2020.
28. Nexelom website. *Automated cell counters, image cytometers and reagents*. https://www.nexcelom.com/nexcelom-products/?gclid=EAIaIQobChMIuabU99fw6gIVzsDACh1skAp9EAAYASAAEgJBFvD_BwE. Last accessed 3 Aug 2020.
29. Chemometec website. *NucleoCounter® NC-202™ automated cell counter*. https://chemometec.com/cell-counters/nc-202-automated-cell-counter/. Last accessed 3 Aug 2020.
30. European Medicines Agency website. *ICH Topic Q 1 E evaluation of stability data*. https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-1-e-evaluation-stability-data-step-5_en.pdf. Last accessed 3 Aug 2020.
31. ICH Harmonized Tripartite Guideline. (2003). *Stability testing of new drug substances and products Q1A (R2)*. http://academy.gmp-compliance.org/guidemgr/files/Q1A(R2)%20Step4%20(2).pdf. Last accessed 3 Aug 2020.
32. Yang, H. (2013). Establishing acceptable limits of residual DNA. *PDA Journal of Pharmaceutical Science and Technology, 67*(2), 155–163.
33. U.S. Pharmacopeia. *Residual host cell protein measurement in biopharmaceuticals*. USP <1132>. https://www.usp.org/sites/default/files/usp/document/our-work/biologics/USPNF810G-GC-1132-2017-01.pdf. Last accessed 3 Aug 2020.
34. U.S. Food and Drug Administration. (2016, September). Center for Drug Evaluation and Research (CDER) Center for Biologics Evaluation and Research (CBER) Q7 Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients. Guidance for Industry. https://www.fda.gov/media/71518/download. Last accessed 3 Aug 2020.
35. Parry, D. (2012). *Duties of key personnel in GMP – Part 1 (production, QC and the QP)*. Inspired Pharma Training website. https://www.inspiredpharma.com/2012/04/03/duties-of-key-personnel-in-gmp-part-1-production-qc-and-the-qp/. Last accessed 3 Aug 2020.

Quality Management Software: Q-Pulse

Natalia Lapteva

1 Implementation of Q-Pulse

The cell processing facility, vector production facility, quality control, and flow cytometry laboratories of the center for cell and gene therapy (CAGT) at Baylor College of Medicine operate under one quality assurance (QA) department. Our QA department ensures the quality, safety, and regulatory compliance of all the cell and gene therapy products we manufacture, test, and release for administration at the clinical sites. Some of the Q-Pulse modules are also shared with research laboratories and with two clinical sites, i.e., bone marrow transplant units in Houston Methodist and Texas Children's Hospitals. The Q-Pulse application is installed throughout these units and hosted remotely by Ideagen. This software allows us to source the data from the remote server and for all the units to operate as one clinical program, which is accredited by the Foundation for Accreditation of Cellular Therapy (FACT). Q-Pulse brought significant benefits to our organization by improving the overall quality system and its efficiency in the manufacturing facilities, for the clinical services we provide, and also for the clinical research program.

2 Q-Pulse 21 CFR Part 11 Compliance

We successfully implemented Q-Pulse as our primary quality assurance software in 2016. The software is an electronic closed system, as defined by 21CFR part 11. This means that it is an environment in which system access is controlled by persons who are responsible for the content of electronic records that are on that system. The

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quality assurance leadership at CAGT are the system administrators and responsible for maintenance of the Q-Pulse system. Q-Pulse's 21 Code of Federal Regulations (CFR) Part 11 compliance for electronic records includes (i) limited system access to authorized and trained individuals; (ii) a validated system which ensures accurate, reliable, and consistent performance; (iii) the ability to generate complete copies of records in both human readable and electronic form for inspection or review; (iv) protection of records to enable their accurate and ready retrieval; (v) availability of a secure, computer-generated, and time-stamped audit trail to document date and times of entries and various actions; (vi) the use of built-in system checks to enforce appropriate sequence of steps; (vii) the use of authority checks to ensure that only authorized personnel can perform certain actions, such as electronic signature alteration and printing of records. Q-Pulse provides intruder lockout and notifies system manager of any locked-out accounts. It also has appropriate controls over system documentation such as controlling the distribution of documents, revisions, and change control processes for electronic documentation.

3 Electronic Signatures

Electronic signatures in Q-Pulse are unique to each staff member and cannot be reassigned. They employ unique user names and passwords, and the latter are periodically revised. Signed electronic records contain the printed name of the signer, the date and time of signature execution, the meaning or purpose of the signature (e.g., approval, completion, or review). To ensure users' understanding of the legal binding status of electronic signatures, we have configured Q-Pulse to state the following message at the time of electronic signature: "By performing this action I understand that according to 21 CFR Part 11 Sect. 11, that my electronic signature is the legally binding equivalent to my hand written signature."

4 Q-Pulse Modules

Q-Pulse electronic quality management systems consist of several customized modules, which are linked through the common database. These modules include "Document Control"; "Audits; Corrective and Preventative Actions" (named Deviations/Incidents at CAGT); "Training Courses" (for training documentation); "People" (for documentation of training plans, training certificates, or competencies); "Assets" (for equipment management), "Vendors" (to store vendor contact information and vendor-specific compliance management), "Analysis" (for analysis of nonconformances), and "Administration" (for system administration, security, and system configuration). The following sections describe some of these Q-Pulse modules which are extensively used in our facility.

4.1 Document Control Module

Document control is an essential component of any quality management program. It is required by the US FDA, and by other applicable accreditation agencies such as FACT and College of American Pathologists (CAP), etc., to assure product quality. We have customized and adapted the document control module to follow both FACT and CAP standards, as well as National Committee for Clinical Laboratory Standards guideline GP2-A2. Currently, at CAGT, we manage about 1600 electronic documents in Q-Pulse, including over 330 standard operating procedures (SOPs). The controlled critical documents managed include the SOPs, worksheets, production batch records, forms, and labels. Documented details include assigned alphanumeric identifier, revision number, document author, document owner, active (effective) date, scheduled review date, keywords, distribution list with names of individuals, date and time of distribution, associated change requests (with details and statuses), review history with all the dates and outcomes of the review, and revision history (including the list of all revisions and their statuses and active dates). Each controlled document has an approval history with the names of the approvers, responses with date and time of the approvals, action items, such as notification of the manager that the training is required, completion of the training, and the update to the hard copy of the SOP manual. Multiple documents can be linked to each other, e.g., SOPs and related worksheets, forms, or labels, and this linkage makes it easier for the users to find the required information and documents. As has been mentioned above, Q-Pulse is used as a closed QA-controlled system in our facility; therefore, the GMP user group is configured not to be able to upload any documents into the system or access obsolete and archived documents.

A new SOP is uploaded into Q-Pulse by QA as a draft document (Fig. 1). QA determines which operational section in the facility uses that specific SOP and assigns the approvers accordingly. The technical SOPs then go through the process of electronic approval by Technical Laboratory Director, a Medical Director, and GMP Facility and QA Director. General SOPs for facilities are approved by the Quality Assurance Staff, a Medical Director, and the GMP Facility QA Director.

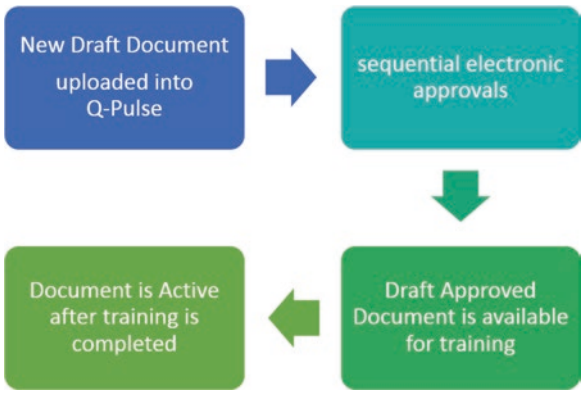

Fig. 1 New controlled document implementation in Q-Pulse

Clinical SOPs are approved by the Quality Assurance Staff, Medical Directors of BMT/Immunotherapy Units at both hospitals and the CAGT Program Director.

The approval process follows the sequential chain in which all approvers must approve in a specific sequence. A request to approve is received by the next approver after the previous approval is issued, e.g., Technical Directors approve first, followed by the Medical Director and the last approval is done by the GMP Facility and QA Director. Each approval is documented with an electronic signature stamped with the date and time. This sequence clearly guides the approval process from start to end. As Technical Directors may have many changes, they approve first. This workflow can be easily customized on case-by-case basis.

After the approvals are issued, the QA and Facility Director assigns the SOPs a "Draft Approved" status and creates actions, such as create a training event for the approved SOP, document completion of the training and update to the hard copy of the SOP manual, for QA Staff to be fulfilled within a month. After the training event is created and has been completed in the Training Module of Q-Pulse, the Draft Approved SOP is activated by QA Staff. Q-Pulse notifies the users trained on the SOP that the new revision is active. CAGT QA maintains electronic versions of all the controlled documents in a secure, password-protected folder on a backed-up server. In case of electronic system outages, CAGT QA continues to maintain a paper-based manual with all critical controlled documents.

4.2 Document Revision

Revision of documents is performed after a "Change Request" has been raised by the user(s). All users have rights to place change requests. These requests are received and reviewed by QA Department. If revision is required, QA assigns "Draft Status" to the document, at which point a new revision number is automatically created by Q-Pulse. QA then formally reviews the "Change Request" raised by the user, and, in most cases, the documents are revised by the Technical Directors or QA Staff. When these revisions are completed, QA Staff initiates the approval process, followed by "Draft Approved" status change, training, and activation of the document. When a new revision becomes "Active," the previous revision becomes obsolete and is no longer available to the users (Fig. 2).

4.3 Review of Document Control Module

The advantage of document management in Q-Pulse is that it brings an improved regulatory compliance and reduced document storage space. Centralized location of the documents promotes collaboration between clinical sites and the manufacturing facility. All the documents are efficiently managed, revised, readily available, and easily retrievable from the system during audits. Obsolete documents can be

Fig. 2 Document revision steps and actions in Q-Pulse The steps are repeated for every revision

digitally archived and stored indefinitely. In addition, the remote real-time electronic approval is very helpful when dealing with the busy schedules of the Medical and Facility Directors.

There are some downsides with the introduction of an electronic system, such as security of the data and the network. These can be addressed by staff training on electronic data security and implementation of firewalls. Potential electronic and power outages may interrupt access to the controlled documents; therefore, keeping both hard copies of active versions of electronic documents on secure, regularly backed-up servers may be helpful.

4.4 Training Module

The training module of Q-Pulse is tightly connected to the document control module and allows CAGT QA to oversee all of the required training on SOPs and other courses. New employees involved in product manufacturing or testing are required to undergo training on general GMP policies and procedures, as well as technical SOPs. Training on general SOPs requires passing the associated quizzes. These are provided as links in the training events generated by the training module by CAGT QA. When the new employee passes the quiz, they document this in the system's training event. CAGT QA Staff grades the quizzes, issues training certificates, and completes the training events.

Training on technical SOPs requires hands-on training for which paper-based training forms are submitted to QA. These forms document reading, explanation, and discussion of the SOP with the supervisor and record two observations and

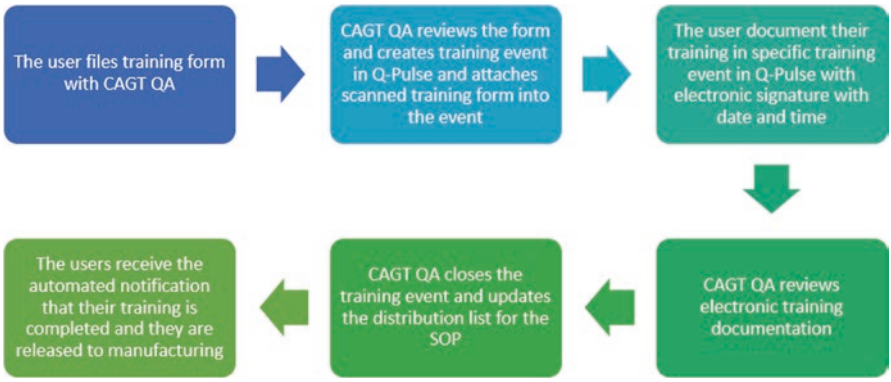

Fig. 3 Training workflow in Q-Pulse

two performances of the technical procedure under observation. These forms are reviewed by CAGT QA Staff and uploaded into the Q-Pulse training event designated for the specific SOP. When a new event is created, the users receive an automated Q-Pulse notification and document their training electronically in the system. CAGT QA Staff completes and closes the training event and adds the specific user to the SOP notification list. The overall training workflow is outlined in Fig. 3.

4.5 Review of Training Module

There are many advantages of electronic training documentation. Centralized storage of training documents allows these records to be easily accessible to the users, QA Staff, and auditors. The remote access allows everyone to document their training on time even if the staff member is out of office. The system sends reminders to the users to complete training and also notifications when the training is complete, so the users are aware when they are released for manufacturing procedures using a new or revised SOP. The electronic system allows us to maintain efficiently training compliance for 100+ users on over 330 SOPs.

Electronic training documentation can be a somewhat tedious process during the initial stages, because many paper-based forms have to be scanned and uploaded into Q-Pulse. However, after the initial hurdle, training on revised SOPs becomes very efficient because all the trained users can be pooled into the training event and notified about any pending new training automatically by Q-Pulse.

4.6 Corrective and Preventative Action Module

Corrective and preventative (CAPA) actions are an essential process managed by quality assurance. This module of Q-Pulse in CAGT is named as "Deviations/ Incidents." Planned deviations are defined as those in which an intention to deviate from an SOP was pre-approved by QA prior to the procedure. Unplanned deviations are those that occur unexpectedly during the procedure, or when procedure produced unexpected results, even if the SOP was followed. We also document incidents in the CAPA Module. These are defined as matters that are outside of the direct control of CAGT quality system but can have a potential impact on CAGT operations, product manufacturing, and quality, e.g., loss of a test sample by an external testing facility.

Q-Pulse allows us to electronically capture and manage all the nonconformances, such as planned and unplanned deviations and incidents. CAPA is a QA-led sequential process, which goes through reporting, planning, and executing short-term corrective actions, investigation, root cause analysis, implementation of long-term corrective actions, and follow-up on effectiveness of these actions to ensure that the nonconformance will not happen in the future. CAPAs are usually initiated by users through the Q-Pulse WIZARD-driven nonconformance report. The WIZARD generates a specific automated workflow to ensure robust and complete data entry into the nonconformance report. CAGT QA Staff are immediately notified through an automated e-mail when a new nonconformance is filed. The sequential process is fully managed by QA Staff, and each stage of CAPA starts when the previous stage has been completed. Action dates are tracked, and when the actions are overdue, escalations are automatically sent to the users. At the end of the CAPA process, the electronic approvals are executed as assigned by CAGT QA Staff. All additional electronic and scanned documents related to nonconformances can be attached to the CAPA report.

4.7 Review of the Corrective and Preventative Action Module

The advantages of using electronic management of CAPA are that the automated process is very efficient and saves the effort of searching for people to sign paper-based documents. This promotes improved communication and quality management. The remote electronic approvals are also very helpful for staff with busy schedules. Furthermore, because of integration of different Q-Pulse modules, specifically Analysis and CAPA modules, trending and analysis of the CAPA module data promotes higher compliance and reduces risks of recurrence.

4.8 Audit Module

At CAGT, we manage and document both external and internal audits and inspections using the "Audit" module. Only QA Staff are authorized to initiate the audits in Q-Pulse. For internal audits, QA leadership electronically schedules all the audits required for the prospective year and assign QA Staff to their performance. Q-Pulse sends reminders to the responsible staff to perform the audits and escalations when audits are overdue. Each audit in Q-Pulse includes the names of auditors and auditees. The findings are summarized, and observations are documented. Nonconformances can be raised directly from individual audit and those with nonconformances can be closed only after all the associated nonconformances are closed. Electronic documents can be pre-attached to the scope of the audit, e.g., the SOP being audited, and the audit can be conducted paperlessly if electronic documents are made available to the auditors or inspectors. Customized checklists can be created and used in Q-Pulse, for example, we have created a customized room checklist, which can guide the auditor at the time of the clean room audit (Fig. 4). Additional electronic or scanned paper documents and pictures could be attached to document findings during the audit.

The advantages of using electronic management are that the audits can be conducted without paper, and all the documents used during the audit, including findings, can be retained.

#	Section Header/Question
1	**General Room Appearance**
1.1	Visually inspect the room upon entering and record any observations. Is the room tidy and uncluttered? There should not be too many opened packages or loose paperwork present.
2	**Structural Integrity, Cleanliness**
2.1	Is the appearance of walls, doors and floors satisfactory?
2.2	Is the appearance of work benches satisfactory?
2.3	Is the appearance of the Biological Safety Cabinet satisfactory?
2.4	Is the appearance of the equipment satisfactory?
3	**Equipment Monitroing and Calibration**
3.1	Are the equipment calibration stickers apparent, in sight and in date?
3.2	Is equipment monitoring complete and up to date?
3.3	Are current versions of the equipment forms being used?
4	**Barcodes**
4.1	Are there barcodes on all applicable equipment?
4.2	Do the barcode labels on equipment match with barcode scansheet?
5	**Reagents/Supplies**
5.1	Are there barcodes on applicable supplies and within expiration?
5.2	Are all in-house reagents labeled?
5.3	Are the reagent labels complete?
5.4	Are there any expired reagents in the room?
6	**Safety**
6.1	Are biohazard materials being disposed appropriately?
6.2	Are sharps being disposed appropriately in a sharps container?
6.3	Ensure biohazard and sharps containers are not overflowing
7	**Other**
7.1	There should not be any Post-It notes present in the room.
7.2	There should not be any uncontrolled copied of worksheets present.
8	Room Number:

Fig. 4 Electronic checklist used for room audit by CAGT QA Staff

5 Q-Pulse Validation

In order to fully comply with 21CFR part 11, we have validated Q-Pulse prior to its implementation. Q-Pulse customization, system validation, and risk assessment were performed by an external company – ECL2 Quality Solution, which is specialized in quality and safety management. The validation plan was approved by both parties prior to execution of installation qualification (IQ), operational qualification (OQ), and performance qualification (PQ). ECL2 documented the requirements, developed the protocols, executed them, documented the deviations, and provided CAGT with the validation report. Validation activities took about 3 months.

6 Q-Pulse Implementation

Our organization started Q-Pulse implementation in 2016 in stages (Fig. 5). First, we implemented the "Document" and "Audit" modules. It took several months to convert all the paper-based controlled documents into Q-Pulse format and upload them into the system. After that, all the controlled documents were electronically approved and activated in the system. We then trained all the initial users in the GMP Facilities and clinical sites on the use of Q-Pulse, with the help of ECL2 Quality Solutions. QA Staff then continued to train the rest of the users after the initial launch. Currently, our organization has 440+ users of Q-Pulse. After about 1 year of using the system, we launched its "Training" module for the GMP users. In 2019, we implemented the "CAPA" module in stages for different groups. Implementation has been conducted in stages to allow each group to perform training hands-on and to give them a solid grasp of the system.

Fig. 5 Stages of Q-Pulse implementation into CAGT quality system

7 Conclusions

The drawbacks of Q-Pulse are that it is relatively costly to purchase, and time-consuming to configure, customize, validate, implement, and maintain the system an academic setting. This was made all the more difficult when there are restrictive hospital information services' requirements and when there is preexisting paper documentation with piles of records to upload electronically. Overall, our organization significantly benefited from the implementation of an electronic system for quality management. It helped to unite our manufacturing and clinical facilities, allowed cross-organizational uniformity, and brought us to a higher regulatory compliance. Help from the ECL2 Quality Solutions team was pivotal to set up and customize the new configuration and to make it similar to the existing paper-based documentation.

Selection of Contract Manufacturing and Testing Organizations

Adrian P. Gee and Deborah Lyon

1 Alternative Cell Processing Options

If an institution has made the decision not to build a cell processing facility, an option is to contract out manufacturing and/or testing of the cell therapy products. The two most available options are to (i) use an academic current good manufacturing practices (cGMP) facility or (ii) use a commercial manufacturing organization (CMO). A major consideration will be the phase of the clinical trial in which the product will be used. Most academic cGMPs can prepare cells for Phase I/early Phase II trials, where the expectations for cGMP compliance are somewhat less rigorous and have been outlined by the United States Food and Drug Administration (FDA) [1].

2 Contracts with Academic GMP Facilities

The first step in investigating the option of using an academic GMP facility is to find one that is capable of making your product. The best option is to select several from lists of facilities that have received accreditation from the professional accreditation organizations, e.g., AABB, the Foundation for the Accreditation of Cell Therapy (FACT), the Joint Accreditation Committee in Europe (JACIE), etc. These are usually listed on the organization's website. This ensures that the facility has been inspected using standards that are generally in compliance with the regulations in the country in which the facility is located.

The next step is to contact the facility to find out whether (i) they perform contract manufacturing and (ii) whether they have the experience and capability to

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make the specific product in the quantity required and by the expected timelines. If the answer is in the affirmative, the next stage is usually to sign a nondisclosure agreement (NDA) between the parties to allow the exchange of confidential information on the proposed project. Generally, both organizations will have template NDAs that can be exchanged and one selected and adapted to meet common needs.

Issues that must be addressed when negotiations begin include (i) the status of the manufacturing procedure – is it a finalized and validated procedure, or does it need additional translational and validation procedures to be performed?; (ii) the source of the incoming cells for manufacturing, the informed consent process, donor screening procedures, and shipment of the cells to the manufacturing facility; (iii) technology transfer of the manufacturing procedure to the contracted facility, how will staff training be performed, plans for validation of the technology transfer, manufacturing timelines (Fig. 1), cell holding and storage options, etc.; (iv) product testing – what tests are required, who will perform them, where will the quality review of product records and testing results be performed?; (v) product release, distribution, and administration – who will be responsible for each procedure?; (vi) shipment of the product – how will this be performed, e.g., by a third party?; (vii) the quality program – how will this be organized, will there be a formal agreement, performance of audits, resolution of issues, etc.; and (viii) regulatory status of the Investigational New Drug Application (IND) – have there been any meetings with the regulatory agency, have manufacturing and testing

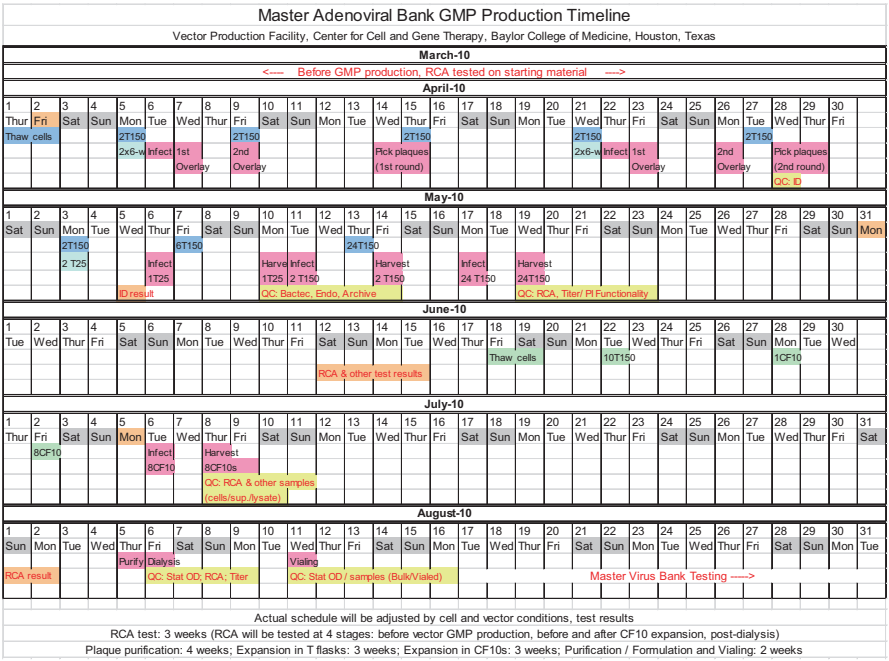

Fig. 1 Timeline for adenoviral vector manufacturing

procedures been approved, etc.? There will be discussion about whether the facility has received any Form 483s as a result of an FDA inspections and performance of an on-site audit by the contractor or a third party.

It is common these days to establish a quality agreement between both parties [2]. This outlines all phases of the project and assigns responsibilities for each component item. It addresses what is to be done in cases of disputes between the parties, termination of the contract, involvement of each party during regulatory audits, frequency of progress reports, etc. It should also define points of contact between both parties and lines of communication that are to be followed. This avoids duplication of effort and resulting delays to contract performance. The major elements of a quality agreement are shown in Tables 1 and 2.

These negotiations are likely to be prolonged as issues of many different types emerge. The contract facility will also be required to develop a budget relatively early in the course of discussion to determine whether the costs can be met by the contractee. It is probably fair to say, however, that most academic facilities will not

Table 1 Major elements of a quality agreement

Section	Description
	Signature page
1.0	Purpose
2.0	Scope
3.0	Policy statements
4.0	Definitions
5.0	Regulatory authorizations and communications
6.0	Equipment and testing facilities
7.0	Documentation
8.0	Materials receipt and storage
9.0	Reference standard management
10.0	Analytical methods and testing
11.0	Deviations, discrepancies, OOS, and investigations
12.0	Change control
13.0	Data acceptance and disposition
14.0	Sample and record retention
15.0	Complaints and recalls
16.0	Inspections and audits
17.0	Annual product review (commercial)
18.0	Subcontracting
19.0	Revision history
20.0	Communication contacts
	Attachment A: Events requiring client company notification
	Attachment B: Documents requiring client company signature

Table 2 Detail from a quality agreement showing items and assigned responsibility

	Responsibilities	Client	Contractor
11.2.5	Shall communicate if in agreement with the proposed plan within two (2) working days of receipt from contractor. If in agreement, the plan will ha approved by client.	X	
11.2.6	Investigations shall be approved within thirty (30) calendar days of the date of discovery.		X
11.2.7	If an extension is required for the investigation, a written request for extension shall be submitted to client for approval prior to the due dale.		X
11.2.8	Shall approve (or deny) due data extension requests within one (1) working day of receipt.	X	
11.2.9	Shall submit a written interim report to client within three (3) working days of the end of the 30 day timeframe.		X
11.2.10	Shall ensure that all corrective actions are closed within the assigned due dale.		X
11.2.11	If an extension is required for corrective actions, a written request for extension shall be submitted to client for approval prior to the due date.		
11.2.12	Shall approve (or deny) due date extension requests within one (1) working day of receipt.	X	
11.2.13	Shall approve all investigations.		X
11.2.14	Contractor will furnish client a copy of all approved investigation reports.		X
12.0	**change control**		
12.1	Shall have established procedures for managing control of changes to facility, equipment, critical computer systems, Neukoplast batch records, specifications, and test methods.		X
12.2	May propose change requests related to Neukoplast for review, which shall be managed through the Contractor's change control process.	X	X
12.3	Changes proposed by client will be forwarded to the regulatory/ quality assurance unit at contractor.	X	
12.4	Changes proposed by contractor will be forwarded to Client's regulatory/quality assurance unit for approval.		X
12.5	Shall obtain all necessary and appropriate regulatory and quality approvals, as required, prior to authorizing any changes.	X	
12.6	Upon approval from client, contractor will initiate the changes, as defined by internal contractor SOPs.		X
12.7	Ensures that only valid/current versions of the documents are available for use.		X
12.8	Ensures that only valid/current versions of the documents are used.		X

be as expensive as a commercial vendor. The choice to use an academic manufacturing facility will depend on the contractee's evaluation of their capabilities, proficiency, ability to comply with regulatory requirements, budget, etc. This can be addressed by performance of a facility audit, preferable by a third-party auditor for the contractee.

A major delay in the contracting procedure can be legal review of the draft contract by both parties. Issues such as indemnity and liability are often points for prolonged discussion. In fact, the legal review process may take longer than the development of the manufacturing and testing agreement.

3 Contracts with Commercial Manufacturing Companies

In recent years, the number of commercial entities providing cellular therapy/vector manufacturing services has increased. There is now a database of cell and gene therapy contract manufacturing organizations (CMOs) [3]. This includes 128 commercial and 28 academic manufacturing facilities. Some of these are companies that have expanded from testing products to their manufacture. A number are direct outgrowths from the development of specific products and manufacture only that product, while others are available for a variety of contract projects – contract manufacturing organizations (CMOs). Among the latter are Lonza, Cognate, TaKaRa, Hitachi, Merck, Cytiva, and the not-for-profit Canadian, public-private consortium, the Center for Commercialization of Regenerative Medicine (CCRM) for cell therapy products [4], and VGXI, Aldevron, Puresyn, Cognate, BioReliance, and WuXi AppTec for plasmids and viral vectors. Several of these companies manufacture both types of products. The activity and number of these organizations are expected to increase in the coming years [5–7].

When selecting a CMO, consideration must be given to their level of experience (Table 3) [8]. What types of products have they manufactured and in how many clinical trials have these been used? Additional information should be requested on interactions/problems with regulatory agencies, ability to meet release criteria,

Table 3 Factors influencing the selection of a CMO

Selection criterion	Evidence of compliance
Ability to source starting material	Meets exacting specifications Retrieves cells from FDA-compliant sources following FDA regulations
Availability of established procedures	Feeder-free and animal component-free methods for cell handling
GMP compliance	Evidence of consistently meeting cGMP regulations
Familiarity with current regulatory status	Incorporation of latest regulatory expectations in procedures
Reputation for good working partnerships	History of previous productive relationships
Ability to provide customized solutions	Experience in providing specialized solutions at all stages of the development process
Availability of additional services	Ability to perform testing, ship materials, process development services

Adapted from: *https://www.takarabio.com/about/bioview-blog/stem-cell-research/choosing-a--cmo-partner-for-stem-cell-therapy-manufacturing*

manufacturing volumes and deadlines, and product recalls. Their experience in technology transfer, and their ability to bring manufacturing advances (process development), e.g., use of closed systems and automation of the procedure, should also be considered. Availability of ancillary services, such as automated finish and fill, shipping capabilities, and testing services, can also be an advantage.

4 Selection of Commercial Testing Companies

A critical component of selecting a commercial testing company is their familiarity with regulatory expectations for product testing, whether it be a cell therapy product or a viral vector. These change over time, and it is vital that current requirements are met. In parallel, their understanding of the approved test methods is essential. There is some flexibility at regulatory agencies as to what tests may be used for product release, and to a great extent, these depend on the phase of the clinical trial the product will be used. For example, validated potency assays are not formally required by the FDA until the start of the Phase III trial but are recommended even in Phase I studies. In most cases, however, the approved test must be used, and, if there is any doubt, regulatory preapproval should be obtained for an alternative. Representatives of both CMO and testing companies should be encouraged to participate in Initial Targeted Engagement for Regulatory Advice on CBER Products (INTERACT) meetings [9] (the new version of pre-pre-IND meetings) with the FDA to ensure that they fully understand the regulatory requirements for the specific product.

It is beneficial to select a company that is able to provide a wide range of testing services. This centralizes interactions and may reduce costs by minimizing shipping costs and obtaining discounts for the multiple services provided. In some cases, large testing companies contract out some test procedures to other entities, and it may be worth dealing directly with these to reduce both cost and turnaround time.

Before selecting a testing company, it is useful to check on their interactions with the appropriate regulatory agency, e.g., have they received warning letters, etc., to determine whether there have been previous issues. This should be followed by requesting the summaries of the testing procedures. These are frequently not the standard operating procedures, which are proprietary. Instead they provide a summary of the procedure that should be kept on file by the contractee. Where possible, it is advisable to audit a potential testing company. This can be most efficiently achieved using a third-party auditor with experience in testing procedures, as most contractees do not have sufficient experience to perform a thorough audit of multiple tests. The types of testing services for a viral vector drug product and a cell therapy final product are shown in Tables 4 and 5, respectively.

Major issues with commercial testing companies are cost and turnaround time. Most tests are considerably more expensive than the equivalent performed at an academic institution, often by multiple fold. The decision must be made as to whether the most expensive tests should be brought “in-house” and what this would involve in terms of development time and validation, etc. The rapid expansion of

Table 4 Recommended testing for viral vector drug products [10]

Required test	Example of suitable test
Microbiological testing	
• Sterility	21 CFR 610.12 / USP <71>/ Rapid test (with approval)
• Mycoplasma	USP <63> / 1993 Points to Consider / EP 2.6.7
• Viruses	• In vitro testing for adventitious agents • In vivo testing for adventitious virus • Tests for specific pathogens originating from the cell line used to make the master virus Bank* • Tests for replication-competent virus • Viral titer • Transmission electron microscopy on-end-of production cells
Purity	
• Residual contaminants	Assay for residual contaminants
• Endotoxin.	EU 2.6.32 / JP 4.01 / USP <85> Endosafe
Identity	• Southern blot • Restriction endonuclease Sequence (full with annotation of discrepancies)
Potency	• Detection of transgene by flow cytometry Assessment of transgene function

aThe specific pathogens to be tested should be confirmed with the regulatory agency. Additional testing is required on the cell lines

Table 5 Recommended tests for cell therapy final products [11]

Required test	Example of suitable test
Microbiological testing	
• Sterility.	• Gram stain for fresh products with accompanying 14 day test +/− pre-harvest test • 21 CFR 610.12/USP <71>/Ph. Eur. 2.6.1. and 5.1.6/JP 4.06/ICH Q4B
• Mycoplasma.	USP <63>/1993 Points to Consider/Ph. Eur. 2.6.7/21CFR 610.30
Purity	
• Residual contaminants.	Assay for residual contaminants, e.g., cytokines
• Endotoxin.	EU 2.6.32/JP 4.01/USP <85> Endosafe
Identity	Cell surface markers Genetic polymorphisms
Potency	Describe and justify assay *Recommended from phase I* *Mandatory by start of phase III*
Multiplicity of infection for gene- modified cells	<5–7 copies/cell

cellular and gene therapies has, additionally, dramatically increased the workload of both CMO and testing companies, with a resulting increase in the turnaround times [12], with some now as long as 6 months. Sometimes, this can be resolved by outsourcing these tests to smaller or specialized companies or those in another country. Care must be taken when using a foreign company that they are very familiar with the regulatory requirements of the home country and that their facility has been approved/audited by the relevant regulatory agency.

5 Conclusions

The use of contract manufacturing and testing organizations can provide a method for obtaining cellular/gene therapy products for use in clinical trials by organizations who do not wish to build and operate their own manufacturing facilities. Services provided by academic GMP facilities are generally less expensive than those furnished by commercial equivalents. They may, however, have less experience and been unable to provide services for all phases of a clinical trial. In both cases, there must be careful selection procedures to evaluate the capabilities of the service provider, their experience, and familiarity with regulatory requirements.

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References

1. U.S. Food and Drug Administration (2008) *Current good manufacturing practice for phase 1 investigational drugs*. Guidance for Industry. July 2008. https://www.fda.gov/media/70975/download. Last accessed 13 July 2020.
2. U.S. Food and Drug Administration (2016) *Contract manufacturing arrangements for drugs: Quality agreements guidance for industry*. November 2016. https://www.fda.gov/media/86193/download. Last accessed 13 July 2020.
3. Cell Trials Data Website. *Cell and gene therapy CMO and CDMO facilities*. https://celltrials.org/public-cells-data/cell-and-gene-therapy-cmo-and-cdmo-facilities/72. Last accessed 13 July 2020.
4. Center for Commercialization of Regenerative Medicine website. https://www.ccrm.ca/. Last accessed 13 July 2020.
5. Biopharm International website. *The Outlook for CMO Outsourcing in 2019*. http://www.biopharminternational.com/outlook-cmo-outsourcing-2019. Last accessed 13 July 2020.
6. Businesswire website. *Biopharmaceutical CMO & CRO Market Size Analysis Report By Source, By Service Type (Contract Manufacturing, Contract Research), By Product (Biologics, Biosimilars), and Segment Forecasts, 2019–2025″*. https://www.businesswire.com/news/home/20190626005691/en/Global-Biopharmaceutical-CMO-CRO-Markets-2019-2025-Service. Last accessed 13 July 2020.

7. Businesswire website. *Stem cell therapy contract manufacturing market, 2030 – ResearchAndMarkets,com*. https://www.businesswire.com/news/home/20191203005513/en/Stem-Cell-Therapy-Contract-Manufacturing-Market-2030 Last accessed 13 July 2020.
8. Boyds Consultants website. *The critical role of a CMO in advanced therapy clinical and commercial product manufacture*. https://www.boydconsultants.com/role-of-cmo-in-atmp-manufacture/. Last accessed 13 July 2020.
9. U.S. Food and Drug Administration. *INTERACT Meetings (Initial Targeted Engagement for Regulatory Advice on CBER products).* https://www.fda.gov/vaccines-blood-biologics/industry-biologics/interact-meetings-initial-targeted-engagement-regulatory-advice-cber-products. Last accessed 13 July 2020.
10. U.S. Food and Drug Administration (2020) *Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs) Guidance for Industry*. January 2020. https://www.fda.gov/media/113760/download. Last accessed 13 July 2020.
11. U.S. Food and Drug Administration (2008) *Content and review of chemistry, Manufacturing, and Control (CMC) Information for Human Somatic Cell Therapy Investigational New Drug Applications (INDs)*. Guidance for FDA Reviewers and Sponsors. April 2008. https://www.fda.gov/media/73624/download. Last accessed 13 July 2020.
12. Pharmaceutical Technology website (2019) *Gene-ie in a bottle' gene therapy opportunity is a treasure trove but CMOs lack capacity*. https://www.pharmaceutical-technology.com/comment/gene-therapy-cmo-capacity/. Last accessed 13 July 2020.

Part III
Facility Design

Introduction: Facility Design

Adrian P. Gee

1 Design of a GTP Facility

The American GTP facility regulations provide little information on the design of GTP manufacturing facilities [1]. They indicate that the facility must be of "suitable size, construction and location to prevent contamination of human cells, tissues or cellular or tissue-based products (HCT/Ps), and to ensure orderly handling of HCT/Ps without mix-ups." The facility must be divided into separate or defined areas of adequate size for each operation that takes place in the facility to prevent improper labeling, mix-ups, contamination, cross-contamination, and accidental exposure to communicable diseases. It also states that environmental conditions must be adequately controlled to prevent contamination or cross-contamination of HCT/Ps, or equipment, or their accidental exposure to communicable disease agents.

Many institutions have interpreted these regulations to mean that HCT/P manufacture can be carried out in an unclassified environment in which the products are manufactured in Class 100/ISO 5 biological safety cabinets (BSC). This requires the appropriate compliance with all other GTP regulations and monitoring of the environment within the BSC. Consideration must be given to the placement of the BSCs within the room to prevent improper airflow between individual cabinets. Where possible, the room(s) should comply with GMP facility recommendations, e.g., sealed flooring, walls, and ceilings that can be disinfected, etc., and staff traffic should be limited to avoid disruption of airflow.

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2 Design of a GMP Facility

It is probably fair to say that, these days, GMP manufacturing requires the use of a cleanroom environment [2]. The classification for cleanroom environments is shown in Table 1. For cell/gene therapy manufacturing, an ISO 5/Class 100 environment is preferred. In Europe, this should be located within (or contiguous to) either an ISO 5 or 6 environment [3]. In the United States, it may be located within an ISO 7 environment. For each of these environments, the maximum number of particles/m^3 is shown in Table 1 [4], together with the type of activity recommended for each classification. Table 2 [5] shows the maximum incident of contamination for each type of environment. These recommendations replace the older limits of viable colony-forming units for each classification. The incident rates should be based on actual monitoring data and should be re-tabulated monthly. Action levels should be based upon actual empirical process capability. When contamination recovery rates are observed that exceed the recommendations in the table, or are greater than established process capability, corrective actions should be taken.

To achieve these definitions, the air changes per hour for each cleanroom classification should achieve those shown in Table 3.

Consideration should next be given to the type of gowning to be used. In some facilities, gowning is performed once at the entrance to the cleanroom. In more modern and complex manufacturing facilities, there may be multiple phases of gowning (Fig. 1). The first level occurs at the primary entrance to the facility. This allows the staff member to enter the corridors of the facility. Located at the entrance to the manufacturing suite(s) is a secondary gowning room. In this room, the operator puts on specific additional gowning for the particular manufacturing procedure. This is then removed in an ancillary degowning room located at the exit to the manufacturing suite. This is used to remove the supplementary gowning before

Table 1 Cleanroom classifications, activities and maximum particle levels [4]

Grade	ISO classification	Class classification	Activity	Maximum particles/m^3 at rest	Maximum particles/m^3 in use
A	ISO 5	Class 100	Aseptic preparation and filling of sterile products	3500	3500
B	ISO 5	Class 100	Background environment for grade A zone operations, when needed for transfers and other less-critical tasks	3500	3500
C	ISO 7	Class 10,000	Preparation of solutions that need to be sterile filtered	350,000	350,000
D	ISO 8	Class 100,000	Handling of components after washing	3500,00	3500,00

Adapted from: *https://high-techconversions.com/gmp-eu-cleanroom-classifications-a-b-c-d/*

Table 2 Maximum incidences of cleanroom contamination for different types of monitoring [5]

Classification		Incident Contamination Rate			
ISO classification	Class classification	Active air sample	Settle plate (9 cm) 4 hr. exposure	Contact plate	Gown or glove
ISO 5 BSC	100	<0.1%	<0.1%	<0.1%	<0.1%
ISO 5 background	100	<1.0%	<1.0%	<1.0%	<1.0%
ISO 6	1000	<3.0%	<3.0%	<3.0%	<3.0%
ISO 7	10,000	<5.0%	<5.0%	<5.0%	<5.0%
ISO 8	100,000	<10.0%	<10.0%	<10.0%	<10.0%

Adapted from: *https://www.pda.org/docs/default-source/website-document-library/chapters/presentations/new-england/usp-guidances-on-environmental-control-including-related-usp-fda--emea-pda-activities.pdf?sfvrsn=6*

Table 3 Average number of air changes/hour for different cleanroom classifications [6]

ISO class	Average number of air changes/hour
ISO 5	240–360 (unidirectional flow)
ISO 6	90–180
ISO 7	30–60
ISO 8	10–25
Unclassified	2–4

Adapted from: *https://www.mecart-cleanrooms.com/learning-center/cleanroom-classifications-iso-8-iso--7-iso-6-iso-5/*

exiting to the main degowning area, where all remaining gowning items are removed. If this latter scenario is selected, this will require the location of multiple supplementary gowning and degowning areas at the entrance and exit to each manufacturing suite. It will also utilize unidirectional flow of staff, reagents, products, and waste. While this provides a more protective environment for manufacturing, it also uses up considerably more floor space than a multidirectional flow system. Approximately half of the number of rooms can be located in a fixed amount of space if a unidirectional flow path is selected. Guidance should be sought from the regulatory agency as to which design is optimal for the product(s) being manufactured. Gowning areas are frequently selected as areas in which to sacrifice space. In reality, this can be a bad idea, since they potentially pose the highest risk for contamination and require storage of large amounts of material. The degowning areas must house sufficient waste containers for discarded disposable gowning, and/or laundry hampers for discard of reusable gowning. The recommended level of gowning is shown in Table 4 for different cleanroom classifications.

Most cleanroom facilities will manufacture multiple products, and the decision has to be made whether each type should be prepared in an individual suite or whether multiple products can be prepared in a single space containing multiple BSCs. Manufacturing in a single room increases the risk of a shutdown of manufacturing if a contamination is discovered. It also requires strictly enforced changeover or line clearance procedures. The choice of configuration should be discussed with the appropriate regulatory agency at the time of planning the facility.

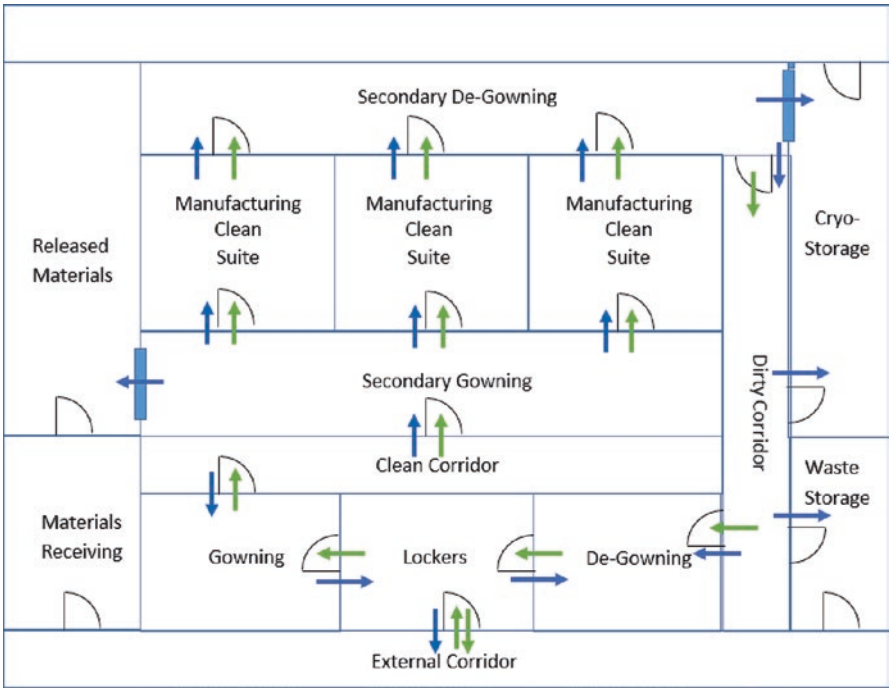

Fig. 1 Suggested design of a high-level GMP facility floor plan (partial)
The floor plan shows an arrangement of three manufacturing cleanrooms that are used by staff requiring secondary gowning. The staff directions of travel are shown by the green arrows and the relative pressure relationships by the blue arrows. The shaded blue boxes are pass-**throughs**

Table 4 Recommended gowning levels for different cleanroom classifications [7]

Classification	Face mask	Beard cover	Booties	Gown or Tyvek® suit	Gloves	Hair cover	Hood	Coverall
ISO 5	X	X	X	X	X	X	X	
ISO 6		X	X	X	X	X		
ISO 7		X				X		X
ISO 8		X				X		X

Adapted from: *https://high-techconversions.com/cleanroom-gowning-requirements/*

3 Design Features of Manufacturing Suites

The pressurization of manufacturing suites must be considered**.** In multidirectional facilities, the suites are at positive pressure to the corridors, to protect the cell therapy product. The central corridor is at positive pressure to the gowning and degowning rooms. In unidirectional facilities, the primary gowning room is at negative

pressure with respect to the clean corridor, which is at positive pressure with respect to the secondary gowning room. The manufacturing suite is at negative pressure with respect to the secondary gowning room and at positive pressure to the primary degowning room (Fig. 1).

The ceilings of cleanroom suites should be solid, with an access panel, if necessary. The lighting fixtures, supply HEPA filters, and other ceiling-mounted fixtures, e.g., fire alarms, should be sealed to the ceiling. The return HEPA filters should be located at floor level at the opposite end of the room to the supply filter(s). Although windows are frequently omitted from commercial cGMP facilities, a number of academic cGMP facilities have them. Where installed they should be sealed, flush to the walls, and located for easy cleaning. The floors should be seamless and coved to the walls. Cabinetry should be metallic with stainless steel working services. The tops should be angled to the walls to facilitate cleaning as should the frames of any glass panels. Ideally, cabinetry should be mounted off the ground to facilitate cleaning underneath. It should also be moveable to allow for different configurations. Walls should be seamless and coated with an easily cleanable surface, such as epoxy paint or plastic laminate. Anything that penetrates a wall, e.g., electrical or gas outlets, should be sealed to the wall surface. Cleanroom phones are available, but well-designed regular phones can be used, as long as they are cleaned regularly. Central gas/vacuum supplies should be considered so that the working parts, e.g., gas tanks, can be located outside the cleanroom environment. The doors must be wide enough to accommodate the entry and removal of large pieces of equipment. Recirculating biological safety cabinets can be used, but for critical applications, e.g., viral vector manufacturing ducted cabinets, should be considered. Depending on the nature of the viral vectors, it is possible to include high temperature decontaminating apparatus in the exit air ducting. There should be no sinks or floor drains within the cGMP manufacturing areas.

Manufacturing suites should be wired for networked computer workstations. An alarm/monitoring system should be provided in the cGMP facility to record critical operating parameters related to equipment performance and environmental conditions. These systems are available in both wired and wireless versions, both of which have some associated wiring. A location should be found for the computer providing output, unless access is available to desk computers through the internet or network.

Emergency power should be available for critical equipment and air handling systems. In most cases, a whole-building system is appropriate. Some equipment may require an uninterruptable power supply for continuous operation.

Safety equipment should be provided, including fire alarms, eyewash stations, and emergency showers.

4 Ancillary Areas

4.1 Materials Supply Rooms

There should be a receiving and unpacking area located outside the controlled area. It is difficult to overestimate the space that this requires in a busy facility. There should be an adequate area to receive and unpack incoming items together with a desk area for dealing with the accompanying paperwork. There needs to be a shelving area to store supplies before they are transferred to the manufacturing space. This should discriminate between released and non-released supplies. Rolling shelves fixed to a framework can be used to maximize storage space. There also needs to be low-temperature storage devices within the receiving area. A pass-through box, of adequate size, should connect the exterior released supply area to the storage area for released supplies within the GMP controlled areas.

4.2 Product Storage Areas

Low and ultralow temperature storage facilities are required for cell and gene therapy manufacturing facilities. For cell therapy products, there must also be an area for the controlled rate freezing of the products prior to long-term storage. We would recommend storage facilities both inside and outside the controlled areas and connected by a pass-through. This allows products to be transferred for freezing and short-term storage within the controlled area and then transferred later to the outside area for longer-term storage. The outside area also contains all of the liquid nitrogen supply tanks and exchange manifolds, so that they do not have to be taken into the controlled space. The carbon dioxide supply tanks for the incubators, the associated manifold, and the carts to take products over to the hospital for administration are also stored in this area. Both storage areas are also equipped with oxygen sensors and concrete or poured epoxy floors. Electrical freezers are also located within both areas.

4.3 Janitor's Closet

There should be a janitor's closet for use by the facility cleaning staff for storage and preparation of disinfectants. It should have sufficient space to store the cleaning agents and apparatus, e.g., buckets, mops, etc. It may also house a specialized water supply, equipment for the automated dilution of cleaning agents, and bags and boxes for the removal of waste.

4.4 Waste Area

Facilities may require an area in which to store biohazardous waste and routine garbage outside the controlled area, before it is removed from the premises.

4.5 Quality Control (QC) Area

Most facilities will perform some quality testing and environmental monitoring on site. There should be laboratory space available for these activities. In addition, the QC laboratory may be required to hold samples taken for testing and must have the correct temperature refrigerators and freezers for this purpose.

4.6 Quality Assurance (QA) Area

An office area should be available for QA staff to handle and review documentation. This may be supplemented by a high-density storage area for all of the paperwork generated for review.

4.7 Document Storage

GMP facilities generate huge quantities of documentation. While some of this can be in an electronic format, there is usually still a large amount of paper. This should be stored in a centralized area equipped with high-density storage systems. It must also be secure from unauthorized entry. If possible, it should be fireproof. If a large enough area cannot be provided, there must be an arrangement in place for secure off-site storage.

4.8 Desk Space

GMP staff should not be expected to work continuously inside the facility. They should have access to desk space outside the controlled area. This should be large enough to work on processing records and other paperwork. Computer access should be provided. There should be lockable storage space to temporarily store confidential paperwork.

4.9 Meeting Area

There should be access to an area in which staff meetings, training, and presentations can be held. This need not be contiguous with the GMP facility but should be in a convenient location.

5 GMP Certification

In the United States, the FDA does not provide routine certification of GMP facilities. The FDA Center for Drug Evaluation and Research will provide a cGMP Declaration for a US Facility to a foreign regulator, such as the competent authorities of a European Union (EU) member state. This declaration issued by the FDA is intended to confirm the GMP US compliance status for the requesting establishment. A number of private companies in the United States offer GMP certification services.

In the EU, GMP certification is obtained by the authorized person at the facility applying to the national competent authority and performance of an inspection by this agency in accordance with the agreed European procedures for inspectorates [8]. The certificates follow the common EU format agreed upon by the European Medicine Agency (EMA) and are valid for 3 years. They are accessible on the EMA EudraGMDP database [9], on which nonconformances are also reported.

6 Conclusions

There is no doubt that planning an academic GMP facility can be a complex task, given the limitations of space and budget. The following chapters provide some examples of plans developed by different institutions. It is certainly a very good idea, once a plan has been developed, to submit it for review to the appropriate regulatory agency for comment. The FDA provides pre-operational review of facilities [10]. These can include (i) a design review, this includes a review of the layout, flow diagrams, and conceptual drawings. The FDA expects the applicant to prepare a complete final plan and to identify specific questions on how the facility will meet cGMPs or for which FDA guidance is sought; (ii) pre-construction review, this is a review of the final plan, elevation, and isometric drawings for all manufacturing areas, utility, and process systems for the facility, e.g., water systems, HVAC, etc. Guidance can be sought on air pressurization, air locks, protective clothing, gowning, and degowning areas, traffic patterns, raw component handling, etc.; and (iii) pre-production review, this is usually an inspection at which investigators visit the new facility during inspections on the same campus. Every opportunity should be taken to make use of these opportunities.

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References

1. Code of Federal Regulations. *Human cells, tissues, and cellular and tissue-based products, Part 1271*. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=1271. Last accessed 13 July 2020.
2. Code of Federal Regulations. *Drug good manufacturing practices (GMP)*. Title 21, Parts 210 and 211. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=211 Last accessed 13 July 2020.
3. European Commission (2017) *Good manufacturing practice guidelines on good manufacturing practice specific to advanced therapy medicinal products*. https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-4/2017_11_22_guidelines_gmp_for_atmps.pdf. Last accessed 13 July 2020.
4. International Standards Organization. *Cleanrooms and associated controlled environments*. International Standards ISO 14644-1 https://zoser.com.co/wp-content/uploads/2015/10/ISO%2014644-1%20Version%202015.pdf. Last accessed 13 July 2020.
5. United States Pharmacopoeia <1116> Microbiological Evaluation of Clean Rooms and Other Controlled Environments. http://ftp.uspbpep.com/v29240/usp29nf24s0_c1116.html/. Last accessed 13 July 2020.
6. Mecart Cleanrooms Website: Cleanroom Classifications (ISO8, ISO7, ISO6, ISO5). https://www.mecart-cleanrooms.com/learning-center/cleanroom-classifications-iso-8-iso-7-iso-6-iso-5/. Last accessed 13 July 2020.
7. High-Tech Conversions Website. *Cleanroom gowning requirements*. https://high-techconversions.com/cleanroom-gowning-requirements/. Last accessed 13 July 2020.
8. European Medicines Agency. *human regulatory, good manufacturing practice*. https://www.ema.europa.eu/en/human-regulatory/research-development/compliance/good-manufacturing-practice . Last accessed 13 July 2020.
9. European Medicines Agency. Eudra GMDP Website. *Compliance with good manufacturing practice*. http://eudragmdp.ema.europa.eu/inspections/gmpc/index.do. Last accessed 13 July 2020.
10. Food and Drug Administration. *Pre-operational reviews of manufacturing facilities*. https://www.fda.gov/inspections-compliance-enforcement-and-criminal-investigations/field-management-directives/pre-operational-reviews-manufacturing-facilities. Last accessed 13 July 2020.

Design and Operation of a Multiuse GMP Facility at the City of Hope

Larry Couture, David Hsu, Yasmine Shad, Mihir Vashi, Rajiv Nallu, and Joseph Gold

1 CBG Organization

The CBG, which was completed in the year 2000, is a 20,000 sq. ft. free-standing biologics manufacturing facility capable of producing viral and nonviral vectors, vaccines, recombinant proteins, cell therapeutics, bacterial products, and monoclonal antibodies. The CBG is licensed by the State of California's Food and Drug Branch and operates under the requirements of cGMP for biologics, drugs, and pharmaceuticals as set out in the Code of Federal Regulations Title 21 (21CFR). Currently, the CBG produces viral vectors, cell therapies for oncology and regenerative medicine, and GMP-grade monoclonal antibodies.

The CBG serves as both a core facility for COH investigators and as a contract manufacturing organization with clients including academics, start-up companies, and larger commercial organizations; its clientele has recently expanded to organizations outside the United States. The CBG's role is currently restricted to research stage (i.e., not yet approved) products rather than commercial biologics.

All of CBG's manufacturing activities, as well as process development and assay development, are overseen by the Senior Director of Manufacturing, who reports directly to the Chief Operating Officer of COH. In parallel, all quality activities related to biologics manufacturing and manufacturing facility maintenance are

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managed by the Director of the Office of Quality Systems (OQS), who independently reports to the COO.

CBG's manufacturing staff are organized into three teams comprising approximately 30 members (Viral Vector Production, GMP Cell Therapy Production, and Process Development; the Process Development Team also has experience in GMP productions). In addition, the assay development function (but not release testing function) of the Quality Control Group reports to the Senior Director of Manufacturing. Each team is headed by a staff scientist leader with extensive experience in GMP processes.

2 CBG Facility

2.1 Layout

The first floor of the building includes a reception area, conference room, a lunchroom, and administrative and general offices associated with the facility, along with the offices of OQS, manufacturing, quality control (QC), and associated document and record control areas.

The first floor contains areas for inspection of incoming (quarantined) materials and both process development and QC laboratories. The cryogenic storage units are also maintained on this floor. Storage is strictly segregated, and access to materials associated with projects subject to cGMP regulations is available only to authorized production or QC personnel via an electronic card system. Major equipment such as the reverse osmosis/deionized (RO/DI) water system, pure steam generator, vacuum pumps, and process gas tanks are housed on the first floor of the building and piped to the manufacturing facility on the second floor.

More than 9000 sq. ft. of classified space on the second floor was designed to meet cGMP facility requirements according to CFR 21 Section 210 (as part of the design process, a full-scale mock-up of a tissue culture room was built on campus to evaluate all materials, design, and build details). The second floor production space is divided into four zones originally designated as Cell Engineering, Cellular and Biologics, Viral Vector (VV) Production, and Fill & Finish. As initially constructed, there were 13 separate production rooms, but in recognition of the need for larger spaces for some operations, some rooms have been combined over the years, resulting in a current total of 11 rooms plus various ancillary support spaces (equipment rooms, quarantine and released materials rooms, glass wash areas, autoclave, etc.). Figure 1a shows the current layout of the second floor with specific zones and their air handling units indicated.

In order to maintain segregation of viral vector products from the other biologics, the viral vector suite has its own dedicated gowning area and exit airlock which contains a dedicated autoclave utilized for disinfection of biologic waste prior to disposal. There is also a cart-sized materials pass-through from the biologics zone

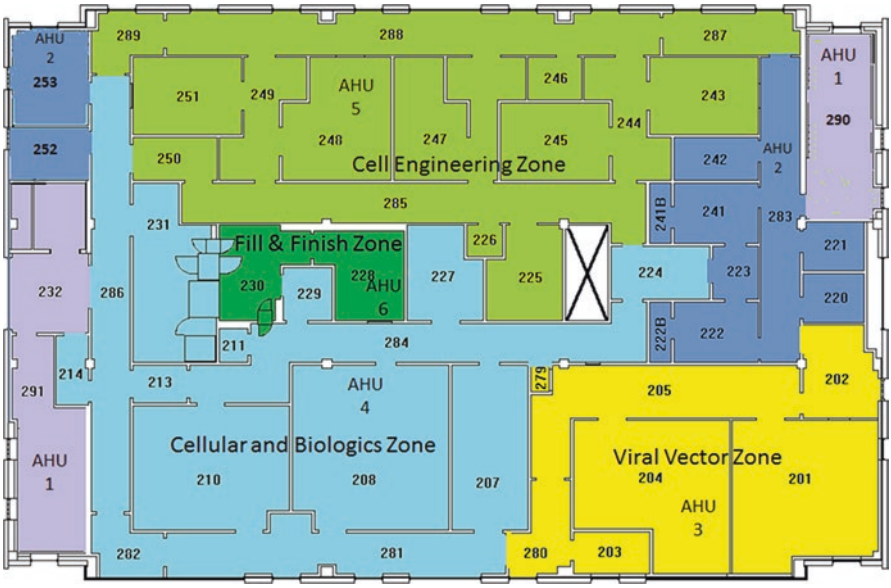

Fig. 1a Floor plans of City of Hope Center for Biomedicine and Genetics GMP facility. Individual zones are indicated; air handling units are indicated. Areas with common colors share AHUs

main "clean" corridor into the viral vector suite that is negative to both the VV and the biologics zone. This is to permit the transfer of VV materials into the biologics zone for use in cell production process.

In general, personnel must follow unidirectional flow through the various work zones (Fig. 1b). Personnel are not permitted entry to the manufacturing areas through airlocks 213, 250, 280, or through the exterior corridors. Entry to the interior corridors for the Cell Engineering, Fill & Finish and Cellular and Biologics areas is gained only through the first stage gowning rooms 223 and 224. These zones are exited through exit airlocks 213 and 250. If personnel are working in an active production room, the room can only be exited through the exterior corridors. The exceptions to these are the Fill & Finish and the Viral Vector Production zones, in which the production rooms exit into an interior corridor. For this reason, these zones are equipped with a secondary gowning/degowning areas, airlocks 229 and 280 for Fill & Finish and Viral Vector Production, respectively. When exiting an active Fill & Finish Suite, the outer layer of gowning is removed in airlock 229, and clean gloves and shoe covers are donned before entering interior corridor 284. When exiting the Viral Vector Production zone, all outer gowning is removed in airlock 280, and clean gowning is donned before entering exterior corridor 281. Entry into the VV Production zone is not permitted through airlock 280. Entry through the interior corridor into multiple production rooms within the same work zone without having to gown/degown is acceptable, provided the rooms are common areas such as equipment rooms and the production rooms accessed are inactive.

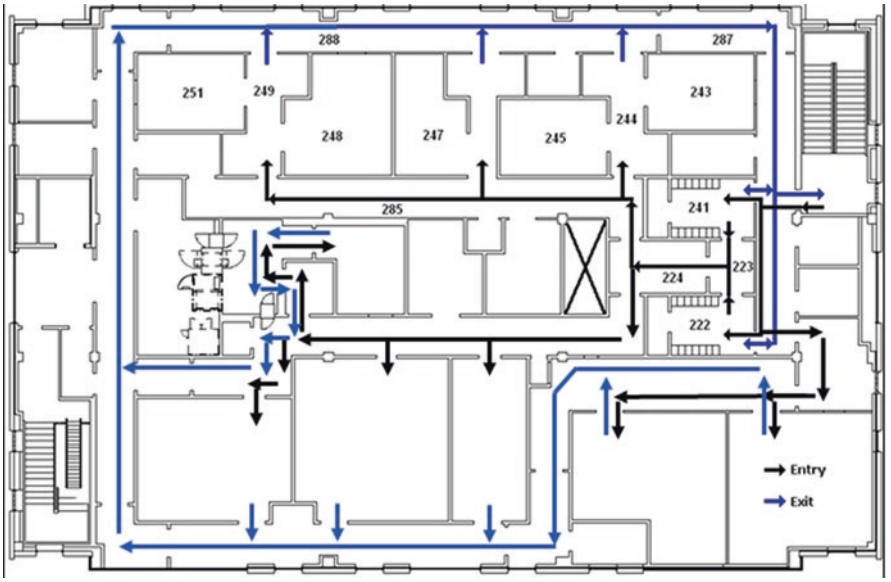

Fig. 1b Personnel flow is indicated. Black arrows: entry; blue arrows: egress Individual zones are indicated; air handling units are indicated. Areas with common colors share AHUs. (**b**) Personnel flow is indicated. Black arrows: entry; blue arrows: egress

Electronic card access systems, highly segregated air handling systems, and strict control over personnel and materials flow are combined such that distinguishable processes can be performed simultaneously in functionally isolated areas of the building. Access to and within the manufacturing space is controlled by a programmable electronic card access system; the access level granted for manufacturing and QC staff is based on their activity on a specific project in the four specialized zones, and after-hours access can be limited to the manufacturing areas and not to the OQS area, QC area, or office areas. A Master Validation Plan and SOPs are in place for qualification and operation of the facility and its systems.

2.2 Exterior Construction

The shell of the two-story building has a tilt-up concrete shell with an interior structural steel frame. The ground floor consists of a reinforced concrete slab; the second floor is concrete poured onto a corrugated, structural steel deck fixed onto the structural steel frame of the building. There is no basement or other cavity beneath the first-floor slab. “Live load” for the second floor is on the order of 125–150 lbs./sq. ft. to ensure a solid and stable platform for manufacturing operations.

The building roof is flat and supports all of the major components of the air handling systems including air handlers, exhaust fans, etc. Access to the roof by COH

facility personnel can be provided without accessing the first or second floor controlled areas.

2.3 Interior Construction

Generally, all systems are designed to use nonabsorbent, non-shedding, nonadditive, and nonreactive construction materials where they could potentially directly affect the efficacy, potency, or purity of the products being manufactured in the facility.

The construction of the facility and other support areas within the main structure of the building is noted below.

2.4 Walls

Conventional 3 5/8″ steel stud walls with 5/8″ gypsum wallboard are used throughout.

2.5 Floors

Production areas have sheet vinyl flooring with heat welded seams and an integral covered base of 5 3/4″ up all walls, with the exception of flooring in the Fill & Finish Suite, in which it extends to 6″ up the walls.

2.6 Ceilings

- The glassware wash area, the vector production suites, and the sterile staging area are equipped with solid 5/8″ gypsum board ceiling construction.
- Other ceilings are constructed with a deep section metal “T” bar grid system combined with lay-in ceiling tiles. Tiles have a washable vinyl surface and are clipped in place.
- The VV zone originally had a hard lid ceiling. However, we subsequently added tiles to the center of the ceiling for easier access to the duct work and necessary maintenance.
- The ceiling grid system in the Fill & Finish suite is a proprietary ceiling grid system that incorporates extruded metal—solid section “T” bar supported by threaded rods attached to the structure above.

2.7 Paint

Walls and doors have a final surface coat of washable epoxy/enamel paint. Pre-fabricated, pre-finished panels for equipment access are finished with baked enamel or epoxy resin surface.

2.8 Doors

There are no exterior doors opening directly into the facility on the second floor; rather there is an intervening space into which exterior doors open to provide continuous and complete air lock around the manufacturing core at all times. Paint grade metal doors are used throughout the facility. The majority of doors are equipped with large, glazed vision panels in the upper half of the door; door hardware is stainless steel.

2.9 Windows

The manufacturing areas of the facility have no exterior wall windows; the exterior corridors running the entire length of each of two sides of the facility are equipped with windows that are fixed pane, non-opening with tinted and reflective glazing. Other interior windows are fixed and, in the classified "clean" areas of the facility, are flush fitted.

2.10 Lighting

Lighting is provided by multi-tube, fluorescent fixtures that are either flush-fitted and sealed lay-in units or surface mounted with flush-fitting lenses designed to minimize penetrations and to allow for easy replacement of light tubes. In all cases, penetrations in ceilings and walls are kept to a minimum and sealed to provide an airtight finish.

2.11 Water Sprinklers

The facility is equipped with a sprinkler system that meets building safety codes and is specifically designed for a cleanroom environment. Sprinkler heads are located in the production rooms as well as within the exhaust ductwork, plenum, and

interstitial spaces above the room. Covers on sprinkler heads are made of flexible rubber skirts that form airtight seals at the ceiling tile.

3 Critical Systems

3.1 Building Monitoring

The Building Automation System (BAS), provided by Siemens Building Technologies, integrates multiple building functions including HVAC equipment supervision and control, alarm management, energy management and historical data collection. The system monitors air handling units, exhaust fans, constant air volume terminal boxes, room temperature, and room pressure. Temperature alarms, pressure alarms, flow alarms, smoke alarms, pump failure alarms, and fan failure alarms are logged by the BAS. The system is monitored by OQS and maintained and operated by COH Engineering.

3.2 Equipment Monitoring

In addition, the CBG facility is equipped with a Rees Scientific 24-h process monitoring system, used to monitor the temperature of all production-related refrigerators, freezers, and cell culture incubators in the facility. Percent CO_2 is also monitored on the cell culture incubators. Additional dry contact points are provided for the process gas banks and RO/DI water. All probes are calibrated against NIST traceable standards, and this calibration is verified annually for each point. Two central nodes collect all trend and alarm data and are backed up by battery power. Two computer screens from the Rees software depict all points, locations, and status of monitored equipment; monitoring is performed in a first floor office. For each production, selected staff are designated as primary or secondary responders to Rees callouts; these responsibilities are rotated throughout the teams involved.

3.3 Steam Supply

Central plant steam, monitored by the COH Engineering Department, is provided to the building at a pressure of approximately 110 psi and a temperature of approximately 338 °F. Before it enters the CBG, high-pressure central plant steam is converted to low pressure at 27 psi. Steam distribution piping inside the building is divided into two systems: one line serves the domestic hot water heater and the central heating hot water heater installed on the roof and operates at a line pressure

of approximately 15 psig; the other line serves all other equipment and operates at a line pressure of approximately 100 psig. Maximum total demand placed on the COH central plant steam system by the simultaneous maximum demand of all systems in the CBG building is 7074 lbs./hr. Condensate is returned to the central plant via a pumped return system.

Plant steam also supplies the CBG pure steam generator, the glassware washer, and two autoclaves. In the cGMP autoclave, central plant steam is supplied only to the outer jacket of the autoclave chamber (pure steam is supplied to the inner chamber of the autoclave). In the second floor VV area non-GMP autoclave (used for all documents and reusable materials specifically in the viral vector zone), plant steam is used for both the outer jacket and the inner chamber.

3.4 Electricity and Emergency Backup Systems

The CBG facility electrical system is designed to provide service of two types:

- A noncritical main building service that provides electricity for all administrative, building control, and noncritical process equipment.
- An emergency power system that includes all fire/life safety loads as well as the entire HVAC/mechanical systems for the building, plus specific designated outlets in the production rooms. These loads are connected to an automatic transfer switch (ATS) which switches electrical power from normal to emergency when normal power is lost. The switch remains on emergency power until normal power is restored.

  All electrical conduits to electrical boxes within the rooms are sealed**.**

3.5 Heating, Ventilation, and Air Conditioning

The CBG facility has a dedicated heating, ventilation, and air conditioning (HVAC) system that is not shared with other buildings. The system maintains differential room pressures, airflows, and temperature. An extensive array of terminal HEPA filters remove airborne particulate matter from the air supplied to the manufacturing and laboratory rooms. The HVAC system is a traditional “four-pipe” system utilizing 6 BAS computer-controlled air handling units (AHUs) equipped with supply fans, cooling and heating coils, primary and secondary filters, and outside air intake to supply conditioned air to the facility. Supply air velocity, supply air pressure, supply air temperature, cleanliness of air filters, exhaust air velocity, and pressure differential within the clean room are controlled and monitored using the BAS system.

OQS personnel oversee the air handling system and ensure appropriate air temperatures, pressures, and airflows are maintained. Air quality within the majority of

the facility is classified to meet or exceed ISO 8 requirements. All production rooms are ISO 7 with the exception of the sterile filling suite, which includes an ISO 6 fill room. Air changes for the various areas are listed in Table 1.

The main air handling system uses 100% outside "single-pass" air (i.e., air is not recirculated within the building) drawn into the system at roof level and upwind of exhaust stacks. Air entering the system is drawn first through a "rough" 30% efficient prefilter and then passes through a cooling coil that is designed to reduce the air temperature to a predetermined temperature, typically ~55 °F. Air is then drawn through a secondary bank of 95% efficient final filters immediately upstream of the fan and then moved into the distribution system of sealed, airtight, galvanized steel ductwork, passing through variable-volume air control valves followed by separately controlled reheating coils. In all of the areas with predetermined air quality classification (e.g., ISO 8, ISO 7, ISO 6), the incoming air passes through ceiling-mounted, terminal HEPA filters or ultralow penetration air (ULPA) filters immediately prior to entering the room or work area. In those areas that do not have an air quality classification, conventional ceiling registers with adjustable vanes are used in place of terminal HEPA/ULPA filters.

The section of the HVAC system serving the Fill & Finish areas of the facility allows for 90% recirculated air. However, recirculated air is returned only to the room from which it originated and is not recirculated to any other rooms or areas of the facility.

The design of the air handling system ensures that airflow is unidirectional and moves from spaces of higher classification (i.e., cleaner; e.g., ISO 7) to areas of lower classification (e.g., ISO 8). Production rooms in the Cell Engineering, Cellular and Biologics, Fill & Finish, and released materials rooms are under positive pressure, relative to the adjacent corridors. The exception to this rule is the VV Production area, which is designed to contain all materials using a net negative airflow into the production rooms. (Note: none of the viral vector area HVAC ducts cross over or under any of the ducting for the remainder of the facility – further minimizing the risk of cross contamination between virus and non-virus zones but adding considerable expense to construction costs.) While the vector production gowning and material airlocks are all under positive pressure with respect to the external corridors, VV Production rooms are under negative pressure with respect to the Vector Staging Area, resulting in a completely contained VV Production suite. A minimum differential pressure of 0.02 inches of water is maintained between all production rooms and adjacent corridors or ante-rooms.

Table 1 Air changes for classified areas

Production area	ISO classification	Air changes per hour
Fill & Finish Suite	ISO 6	>120
Production rooms/suite	ISO 7	60
Common areas	ISO 8	20

3.6 Process Gas Supply System

The compressed gas distribution system is designed to provide the proper pressure and flow of process gasses (CO_2) to incubators and other points of use located throughout GMP production areas. The system consists of two independent banks of commercially provided cylinders of each gas (USP grade); copper gas lines are run from these cylinder banks to cleanroom areas. Cylinders in each bank are connected to a high-purity switchover brass manifold with multiple station valves. Each manifold pressure is regulated by a two-stage regulator to maintain a constant downstream pressure; the system includes an automatic changeover manifold device.

3.7 Vacuum System

The vacuum system, which enables filtration and aspiration of liquids in the QC and manufacturing areas, is designed with operational redundancies (lead and lag pumps and a pressure tank) to prevent significant interruption of service. The system is located on the first floor of the CBG in a controlled access mechanical equipment room, and vacuum service is distributed to the QC and manufacturing laboratories through brazed copper lines. The total vacuum for the facility is 30 inches of mercury; the system was overbuilt with dual large industrial scale vacuum pumps capable of maintaining the designated vacuum with 50% of all point-of-use values fully open.

3.8 Storage Space

The CBG facility has dedicated storage space for materials on both floors. All materials and reagents received by the CBG are quarantined and inspected by OQS personnel on the first floor. Once released for use, materials are issued to manufacturing and QC personnel who transfer them to the appropriate locations. Materials intended for development are distinctly identified with a “Development Use Only” label and are segregated from released materials.

Only materials that are released by OQS are permitted in the second floor classified manufacturing areas. Each of the production zones on the second floor has designated materials storage areas for in-process materials, reagents, disposables, and small equipment. Materials may be stored in plastic easy-to-clean bins on shelves. Materials intended for use in QC release testing are stored at designated released materials storage locations and in designated cold storage units within the lab.

All materials, reagents, and small equipment needed for a specific campaign are transferred from the designated storage areas to the OQS-released production room.

Storage of nonessential materials for a given production within the rooms is prohibited. At the completion of a campaign, unused disposables and reagents are discarded or labeled as "For Development Use Only" and transferred to designated locations in the process development and QC labs. Small equipment is cleaned and returned to its specified storage location. Solid waste and used sharps containers from each of the zones are stored in a designated room for daily pickup by COH Environmental Services.

3.9 Cryostorage

The CBG is equipped with −80 °C, −20 °C, and 4 °C units, plus liquid nitrogen (LN_2) freezers, which are remotely monitored by the Rees system. The COH Engineering Department is responsible for the maintenance (a semiannual schedule for the −80 °C, −20 °C, and 4 °C units and annual maintenance for the liquid nitrogen freezers). The −80 °C, −20 °C, and 4 °C units in each of the production zones on the second floor and in the QC lab are used to store in-process materials and released reagents and are overseen by OQS.

OQS controls security access to the units located in the first floor cryogenic storage rooms, shipping/receiving room, and an additional room used to store quarantined or released materials. OQS also maintains an access-restricted database, which inventories all materials stored in units dedicated to GMP/GLP materials; release of materials from these tanks requires written approval.

Liquid nitrogen freezers containing GMP/GLP material automatically fill to the appropriate level of liquid nitrogen when needed; temperature and LN_2 level data are archived through the Rees system and stored on the COH network. The Rees monitoring system also alerts the staff (remotely) if any abnormal conditions occur within the freezer's chamber, such as high temperature (freezers are set to between −190 °C and − 130 °C) or low LN_2 levels.

At least two (of six) LN_2 refill supply tanks are full and ready for use at any given time to ensure a ready supply of LN_2 for cryostorage. OQS personnel routinely monitor the LN_2 supply.

In total, the CBG facility has approximately 240cu. ft. of −20 °C and approximately 270cu.ft. of −80 °C storage (a combined capacity of approximately 4.8 million 2 mL vials). The facility has capacity for more than 19,000 2 mL vials in five separate LN_2 tanks. Different systems of racks and boxes are used to accommodate different types of samples.

4 Improvements

Not surprisingly, during the course of manufacturing over the last two decades, we have identified design features that could be improved and would be incorporated into any newer facility. These include:

- Insufficient storage space for raw materials: the combination of limited space at CBG with limited storage of bulk items at the COH warehouse (which serves the entire campus) means that we are constantly searching for creative solutions.
- Lack of a staging area for equipment setup/maintenance/repair: we have had to resort to storing unused/broken equipment in the office of a (very dedicated and patient) senior director.
- Lack of a discrete area for shipment preparation: currently, we have a single area dedicated to shipping and receiving. Space is limited, and, while materials are being received, there is little space to maneuver when QC samples are being prepared for shipment (often using dry ice) to third-party sites.
- Manually operated swinging doors in the clean rooms, rather than sliding doors (to make entrance and egress easier, particularly when staff are transporting materials).
- Insufficient numbers of pass-throughs – ideally, there would be one per clean room.
- No cameras in the production rooms. These would be useful both for auditing purposes and to let visitors see the manufacturing processes without requiring gowning.
- Inadequate audio intercom system. The facility has with phone panels built into the walls of each room, but the system never worked as well as intended.
- Difficulties in tracking staff in case of emergencies. Motion sensors in the rooms or GPS staff tags would help locate missing staff during fires/earthquakes, etc.
- Limited AHU capacity with no room for expansion.
- ISO 8 corridors adjacent to ISO 7 rooms containing ISO 5 BSCs, rather than stepwise ISO classifications (i.e., ISO5 BSCs in ISO 6 rooms, with ISO 7 corridors, and ISO 8 airlocks). The discontinuous nature of the CBG's current spaces means that products made here are not suitable for use in the EU.
- The lack of control over the HVAC system by the CBG OQS/facilities staff has at times contributed to problems arising over temperature and air pressure concerns.
- Lack of digital controls for room monitoring: the CBG building was not designed to have continuous air particle monitoring in the clean rooms, and the HVAC system was not designed to have controllers for each clean room to adjust room temperatures, humidity, or airflows. Accordingly, we cannot adjust parameters for any single room without impacting the entire zone monitored by that single controller.
- Because there is a 5–10 second gap between facility power loss and emergency power, certain sensitive equipment (e.g., centrifuges, specialized bioreactors, etc.) will not continue to operate without uninterrupted power supplies (UPS).

Due to the prohibitive cost of UPS with sufficient capacity, these devices have to be manually restarted after shutdown by CBG staff.

5 Conclusions

The Center for Biomedicine and Genetics at City of Hope demonstrates the strengths and weaknesses inherent in a first-generation multiproduct facility designed for a specific niche: IND-enabling studies and early-phase clinical trials. Its limitations include an outmoded design (small production rooms; lack of storage areas; inefficient use of space; an air handling system that does not allow for expansion or isolation of most of the individual rooms and was not designed with EU requirements in mind; a centralized control system that does not allow fine tuning of individual room environments). On the other hand, its capacity as a core facility for vector and cell therapy productions has served COH investigators well over the 2+ decades of its existence by allowing the rapid transition of translational research to experimental clinical products, and its excess capacity has allowed it to serve as a small-scale CMO for external clients, thereby offsetting its expenses and allowing it to heavily subsidize work for COH investigators. In the end, its successes prove that the state of the art of a manufacturing facility is less important than the skill, determination, and dedication to the cause of the manufacturing, quality, and facilities staff who work so hard to provide hope to patients sorely in need of it.

Design of a Multiuse Acdamic GMP Facility at University of Miami

Aisha Khan, Bangon Longsomboon, and Joshua M. Hare

1 ISCI Organization

The primary goal of ISCI [1] is to support and develop basic and translational research and to advance the clinical use of stem cells and cellular regenerative therapies. To advance ISCI's goal further, the clinical research cell manufacturing program (CRCMP) [2] houses a preclinical program (PCP) and clinical program (CP). Recent advances in cell biology and genetic engineering have fueled the development of new therapies for regenerative medicine and need of facilities like CRCMP. The programs operate under good laboratory practices (GLP) [3], current GMP [4, 5], and good clinical practice (GCP) [6] standards. The PCP provides large and small animal and translational research studies, with unique access to expertise in all major medical and scientific disciplines including surgery, biomedical engineering, advanced imaging, pathology, radiography, interventional cardiology, neurology, animal behavior, chemistry, and engineering. CRCMP served as the primary production site for the National Heart, Lung and Blood Institute (NHLBI) [7] Cardiovascular Cell Therapy Research Network (CCTRN) [8] as well as a contract Product Assistance for Cellular Therapy (PACT) [9] facility for 4 years. Furthermore, the facility serves as a preclinical and translational research site for the advancement of cell manufacturing technologies from the bench to bedside. Our CRCMP

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facility works collaboratively with investigators at the University of Miami [10] and various other research organizations in this field.

2 CRCMP Facility

2.1 Mission and Vision of CRCMP

The primary focus of CRCMP is to develop research activities and provide core services in the areas of drug and biologics development, preclinical studies under GLP, and biomedical imaging. The vision of ISCI is to become the leader in translational research and provide comprehensive preclinical research services using an interdisciplinary approach to improve the effectiveness and efficacy of drug and biologics development from early-stage and into clinical trials by: (1) seamlessly integrating all aspects of product development from basic research/design to imaging and preclinical testing through human clinical trials; (2) facilitating the use of animals with naturally occurring disease as a viable research model; and (3) using unique resources to develop new tools for molecular and translational imaging.

2.2 Service Highlights

CRCMP covers the spectrum of research in drug discovery and development including the dedicated, leading-edge technology for research on cellular, molecular, and biochemical sciences.

2.3 Accreditations

The facility is registered with the Food and Drug Administration (FDA) [11] and accredited by the Foundation for the Accreditation of Cellular Therapy (FACT) [12] and the American Association of Blood Bank (AABB) [13]. CRCMP is able to provide an investigator with the infrastructure, core capabilities, and processing/manufacturing expertise to develop any project from its inception to the point of clinical implementation. CRCMP provides cell manufacturing assistance to investigators and networks to conduct their clinical trials under approved INDs. The CRCMP also manufactured cell for multicenter clinical trials.

2.4 Objectives

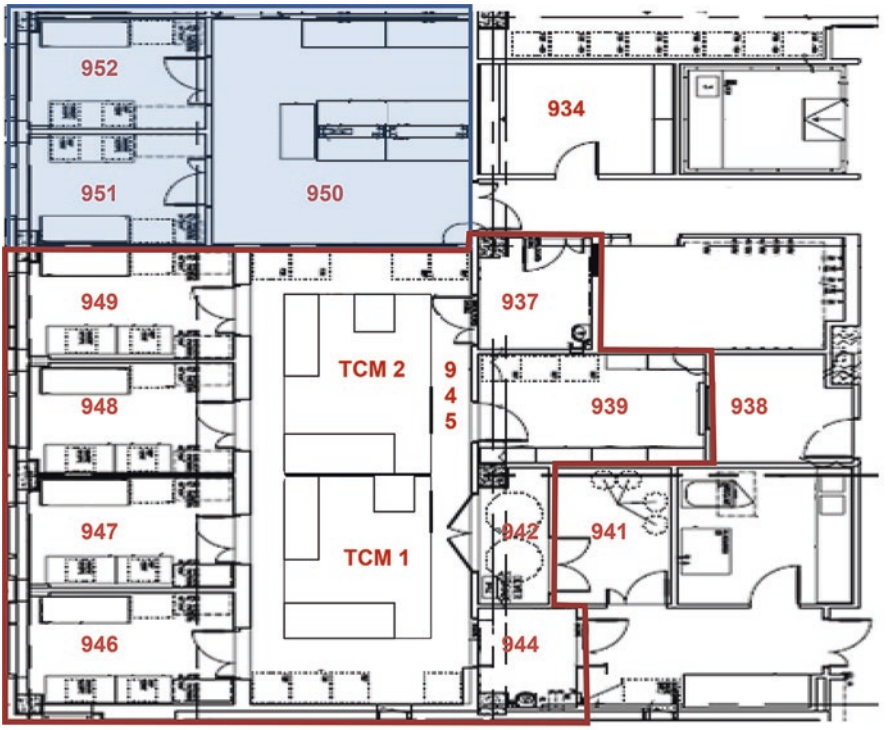
The CRCMP serves as a national resource for translational research (translating basic science ideas into clinical practice) in the field of cellular therapies utilized for structural tissue repair and regeneration (cardiac disease), hematopoietic disorders and malignancies, tolerance induction, and immunomodulation of effector cell populations (cellular-based vaccines). The facility and its infrastructure are equipped to service regional and national research and clinical efforts in reparative/regenerative medicine.

The CRCMP serves as a clinical resource and facilitates the translational efforts of basic research ideas into clinical practice. Specifically, CRCMP has the capabilities to: (1) provide regulatory and scale-up expertise to bridge the gap between basic and preclinical research efforts, which includes development of SOPs and process validation for various cell-processing applications. Expertise and targeted assistance will be provided to investigators interested in cGMP product development services, which extend from the proof-of-principle and preclinical testing phases, throughout the development and implementation of a process, and development and submission of an IND application; (2) assure the transfer of skills and training to the participating investigators in the area of cellular product manufacturing and handling of the final cellular products within the controlled cGMP/current good tissue practice (cGTP) [14] infrastructure, based on a highly collaborative, synergistic, and cost-effective platform; (3) provide handling expertise for various cellular therapy products required by investigators participating in the program. This allows providing clinical-grade cellular therapy products received, manufactured, stored, and analyzed within the framework of compliance with federal and other applicable regulations, guidelines, and requirements.

The underlying principle is to assist investigators in developing successful preclinical studies and to facilitate a smooth transition from preclinical studies to clinical trial studies. The program provides investigators with a well-structured, well-designed product development process to ensure the integrity of the scientific data, reproducibility of research findings, as well as fulfilling regulatory requirements. The investigators are assisted in managing budget to avoid overspending. The CRCMP has been very successful in numerous preclinical studies transitioning them to clinical trials.

2.5 Layout of the CRCMP Facility

The CRCMP facility is located on the 9th floor of the Biomedical Research Building (BRB) on the Medical Campus of the University of Miami. The CRCMP comprises six cell production clean rooms, a designated gown-in and gown-out area (approximately 3000 square feet) and a centralized environmental monitoring system with a commonly displaced positive pressure airflow. To ensure product integrity, each room is designated for a specific product for the entirety of cell production. The



Legend

934 – Storage Room	944 – Gowning out of the clean room	TCM 1 – Tissue Culture Module 1
937 – Gowning in to the clean room	945 – General Area	TCM 2 – Tissue Culture Module 2
938 – Storage Room	946 – Tissue Culture Room	950 – General Area of DL
939 – Clean Storage Room	947 – Tissue Culture Room	951 – DL Tissue Culture Room
941 – Cryogenic Freezer Room	948 – Tissue Culture Room	952 – DL Tissue Culture Room
942 – Cryogenic freezer Room	949 – Tissue Culture Room	

Fig. 1 Floor Plan of the Clinical Research Cell Manufacturing Facility (9th floor BRB building). *Clean room area is in red boundaries, Development Laboratory (DL) area is in shaded blue

development laboratory (DL) and preclinical laboratories (PCL) occupy approximately 5000 square feet, respectively. They are conveniently located next to each other and are used exclusively for processing human cellular and tissue products (see Fig. 1 for floor plan of CRCMP facility). All cellular products are inspected in the DL before processing and before leaving the CRCMP facility for infusion. The materials and reagents used in manufacturing and processing are inspected at the time they are received before being transferred into the dry storage or clean storage areas. The critical pieces of equipment are connected to emergency power outlets that are served by a backup generator in the event of a power failure. The laboratory space is specifically designed and equipped for human cell processing, purification,

culture, and cryopreservation. All areas have controlled access to authorized personnel by access card. The CRCMP facility is cleaned and disinfected after the end of each procedure, or weekly if not used, to ensure aseptic processing operations [15].

2.5.1 Supporting Areas

The supporting areas consist of:

- Storage room (Room# 934 & 938, refer to Fig. 1) – Rooms dedicated to store reagents and supplies that pass inspection and are ready to be transferred into room #939, the clean storage room.
- Cryogenic freezer storage room (room # 941, refer to Fig. 1) – This room is equipped with an LN_2 manifold and LN_2 supply tanks to feed cryopreservation system in the clean room and to feed LN_2 storage tanks located inside the clean room (room # 942, refer to Fig. 1). There are two oxygen sensors connected to the 24/7 central alarm monitoring system to ensure staff's safety when handling the products in both rooms, room # 941 and room # 942 (refer to Fig. 1).

2.5.2 Clean Room Area

The clean room area consists of the following classified monitored areas: gowning in, general processing area, four tissue culture rooms, and two tissue culture suites, cryogenic freezer storage room, storage area, and gowning out.

- Gowning in (room # 937, ISO 8) [16, 17]: This room is used for gowning before entering in the tissue culture area.
- General processing (room # 945, ISO 8): Area designated to general equipment – refrigerators, centrifuges, microscopes, etc.
- Tissue culture manufacturing rooms and suites (rooms # 946, 947, 948, and 949, TCM #1 and TCM #2, ISO 7) [16, 17]: These are solely dedicated to culture of various types of human cells. Each room has been designed to provide a contained area, suitable for aseptic manufacture of more than minimally manipulated products using biological safety cabinet [15].
- Clean storage area (room # 939, ISO 8): This room is dedicated to store all materials ready for use in the clean room.
- There is a pass-through between the clean storage area and the general storage area.
- Cryogenic freezer storage room (room # 942, ISO 8): Area dedicated to cryogenic freezer for cryopreserved products storage and controlled rate freezer for cryopreservation. There is an oxygen sensor connected to the 24/7 central alarm monitoring system to ensure staff's safety when handling the products in the cryogenic freezers.
- Gowning out (room # 944, ISO 8): Area used to degown. This room contains a sink, emergency shower, and eyewash.

2.5.3 Exterior Construction

The CRCMP facility is located on ninth floor. The building complies with hurricane requirements for the region.

2.5.4 Walls

The walls are nonporous and washable basic steel stud construction with gypsum board paneling coated with a non-shedding finish. The wall finish includes baked enamel of a semi-gloss type.

2.5.5 Floors

The floors are covered by epoxy resins resulting in a surface free of roughness, bumps, ridges, or irregularities. Everything is designed to resist a wide range of chemicals allowing frequent cleaning.

2.5.6 Ceilings

The ceiling is a non-porous, low moisture absorption surface with low particle emissions. The performance considerations for clean room ceilings include high fire performance, high light reflectance, durability, ease of cleaning, and inhibiting the growth of microorganisms.

2.5.7 Doors

The clean room entry doors airlock to maintain clean room pressure differentials. The exit doors are locked to exclude entry from the outside yet permit exiting from within. All doors have airtight seals. All internal doors are swinging with self-closing mechanisms.

2.5.8 Windows

The clean room area does not have exterior windows.

2.5.9 Lighting

Lighting fixtures are energy-efficient, fluorescent type with high-frequency electronic ballasts and energy-saving long-life lamps. The lamps have a color temperature of 4000 Kelvin, with a minimum power factor of 0.95. The voltage level is 277

Volts. Fluorescent fixtures used in clean areas are gasket type with a sealed body without knockouts. The fixtures have a prismatic lens mounted with the smooth side facing the clean space. The fixtures have a stainless steel mounting flange, which are sealed with silicone.

2.5.10 Fire Sprinklers

The building is fully equipped with automatic fire sprinkler system and alarms.

2.5.11 Americans with Disabilities Act (ADA) Compliance

The facility is accessible and was designed to be ADA compliant where applicable.

3 Work Flow Within the CRCMP Facility

3.1 Personnel Flow

The cleanroom laboratory – Personnel access the cleanroom laboratory only through the gowning-in area (BRB room 937). After donning appropriate coveralls, as well as head and shoe coverings, the staff enter the general area, which is connected to all of the ancillary rooms in the cleanroom lab. Personnel exit the clean room laboratory using the gowning-out area (BRB room 944). A handwashing sink is available near the exit.

The development laboratory (DL) – Personnel access to the DL is only by the door # 950. After donning appropriate laboratory coat, personnel enter the general area, which is connected to the tissue culture rooms (# 951 and # 952). The laboratory coat designated for use in the DL is not to be worn in other areas. Personnel exit the DL using only the door # 950.

Refer to Fig. 2a for personnel flow plan of the CRCMP.

3.2 Reagent/Material Flow

3.2.1 The Cleanroom Laboratory Area

There is a clean storage area in the clean room laboratory (BRB room 939). Reagents and materials enter the clean storage area using a pass-through between clean storage and the storage/transfer room (BRB 938). Bottles and packages of reagents and materials must be disinfected before entering the clean storage area using a pass-through.

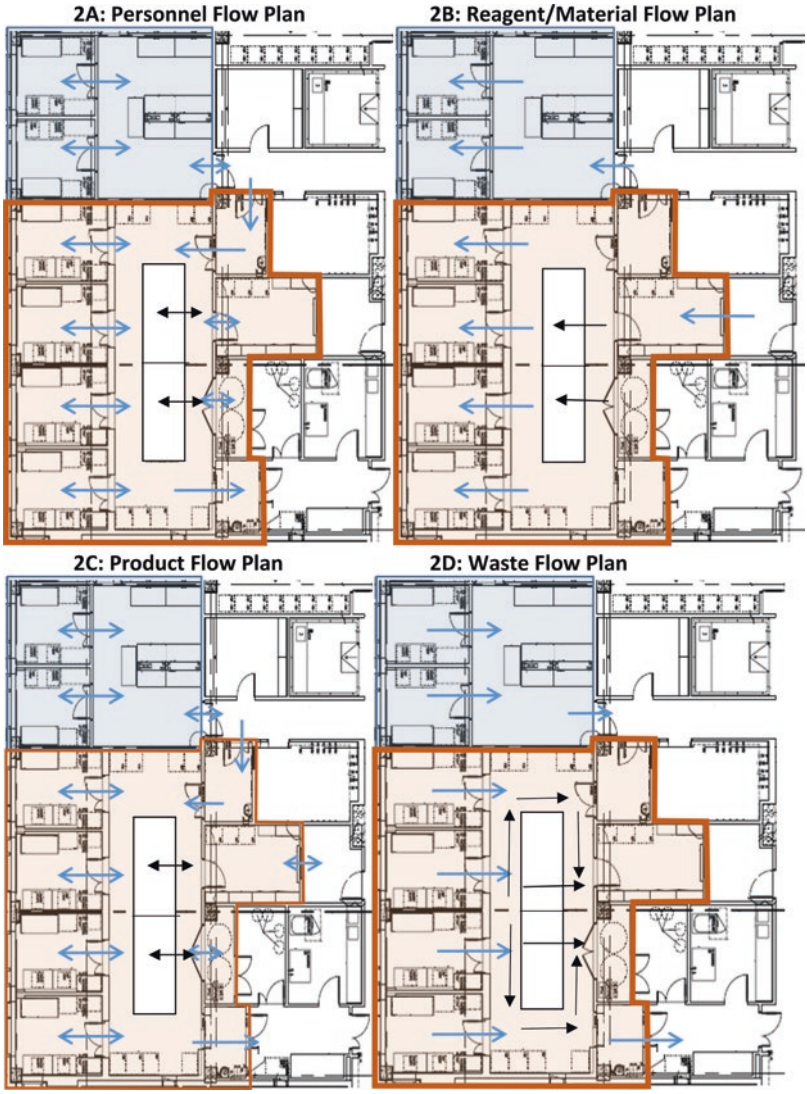


Fig. 2 Work Flow within CRCMP Facility. (**2A**) Personnel Flow Plan. (**2B**) Reagent/Material Flow Plan. (**2C**) Product Flow Plan. (**2D**) Waste Flow Plan. The Cleanroom Laboratory area is shaded in red and the Development Laboratory is shaded in blue

3.2.2 The Development Laboratory (DL)

All reagents and supplies are received in room 903 and released to room 938 or the dry storage room (room 934) after QC inspection. All clean material enters into the DL through the door # 950. Refer to Fig. 2b for reagent/material flow of the CRCMP.

3.3 Product Flow

Products arrive at the facility in a cooler or a shipping box. The product is taken to the clean room laboratory through the pass-through window. Cooler boxes or shipping containers are not allowed in the clean room area. Inner contents of the shipping box have to be wiped before entering the clean room laboratory. The final product is kept in vapor-phase liquid nitrogen storage tank until the product is released. When the final product is ready to be sent to the administration site, it goes out through the pass-through window or the gowning-out area. Refer to Fig. 2c for product flow of the CRCMP.

3.4 Waste Flow

All wastes produced in the laboratory as well as cellular products that are deemed for discard are placed in red-labeled "biohazard" bags or sharps containers. All wastes produced in the clean room area exit through the gowning out (room 944). After each procedure, the waste is taken out of the laboratory. All biohazard waste goes into a designated biohazard disposable container. Refer to Fig. 2d for waste flow of the CRCMP.

4 Critical Systems

4.1 Building Monitoring

The University of Miami Facilities and Operations Department is responsible for daily oversight of the building operations and performance of utilities distribution, all mechanical, electrical, and plumbing systems and HVAC maintenance.

4.2 Facility Monitoring

All products stored in the CRCMP facility are monitored at all times by Amega View central alarm monitoring system. The Amega View system is designed to monitor and report data that is recorded from various inputs in the CRCMP facility [18]. These inputs include, but are not limited to, temperature, % CO_2, and % O_2, and differential pressure. The areas and equipment that are monitored within the CRCMP facility include:

- Environmentally controlled clean rooms (humidity, temperature, and pressure)
- General laboratory areas
- Refrigerators and freezers

- Incubators
- LN_2 cryogenic freezer storage tanks, CO_2 tanks, and O_2 levels

In the CRCMP facility, the Amega View system monitors designated areas and equipment 24 h, 7 days a week. Each input connected to the Amega View Monitoring System logs a data point every hour during normal operation and every 15 min when it is in alarm. Monitoring data points for % O_2 occurs every 15 min during normal operation and when in alarm. If one input goes into alarm, only that input logs at a faster programmed rate, all the other inputs remain the same.

4.3 Equipment Management

The equipment used during manufacturing is cleaned, maintained, and sanitized at appropriate intervals to prevent malfunction or contamination that would alter the safety, identity, strength, quality, or purity of the final product beyond the established lot-release criteria. In addition, in order to maintain all equipment in good standing order and functioning as expected, manufacturer's instructions for cleaning, maintenance, and quality control are strictly followed by CRCMP.

4.4 Electricity and Emergency Backup Systems

The University of Miami has 14 megawatts (MW) of emergency power capacity at the central plant. The goal is to provide 100% power redundancy to all critical buildings on campus in the event of a prolonged outage due to hurricanes or other catastrophic events. The CRCMP building is currently backed up by emergency power. Backup generator powers up as soon as the power goes down and runs for 27 consecutive hours on one fuel tank. Contingencies are in place to provide priority status fuel replenishment in case of disastrous event. Two separate air handlers, as well as the power supply, are connected to an emergency generator.

4.5 Heating, Ventilation, and Air Conditioning (HVAC)

The HVAC design criteria are established to ensure compliance with the requirements defined by the US FDA for the manufacture of cell therapy products using aseptic processing [15], and the NIH's requirements for safe handling of materials categorized as biosafety level 2. The distinct manufacturing areas within the facility provide the appropriate level of control to attain the different degrees of air quality depending on the nature of the operation. The design of the HVAC system satisfies microbiological and particle criteria established in cGMP requirements and address the equipment, components, and products exposed, as well as the operational

activities conducted in the areas. Environmental room air quality conditions are based on ISO-14644-1 classification system [16, 17]. The conditions for room temperature, relative humidity, ventilation, air change rates, pressurization, and supply/exhaust systems are defined to ensure the required conditions are met.

4.5.1 Classified Areas

All rooms are provided with a separate thermostatic control due to variation in cooling loads from room to room. The primary-secondary system consists of classified space and has been provided with a custom fan-powered module. The module consists of a circulation fan, supply air plenum, and terminal supply high-efficiency particulate air (HEPA) filters. The return ductwork to the fan provides a connection to the primary makeup air system that takes care of cooling loads and pressurization requirements. The air handling unit (secondary system) provides makeup air to the fan-powered modules with a constant flow control box and hot water reheat coils for room temperature control. The unit is sized to provide air to maintain room pressurization and meet ventilation requirements and designed with the capability of 100% outdoor air economizer cycle for energy conservation. Supply and return duct systems provide with a constant flow control boxes for pressure control/monitoring.

4.5.2 Air Handling

The air provided to the clean room is HEPA filtered to meet the standard classification described above. The gowning rooms, general processing area, clean storage room, and cryogenic freezer storage room are designed to meet particle concentration limits per federal standard (FS) 209 ISO 8. An air exchange rate of 20 air change per hour (ACH) is utilized to accomplish this criterion. All tissue culture rooms are designed to meet particle concentration limits per FS 209 ISO 7. An air exchange rate of 30 ACH is utilized to accomplish this criterion [16, 17].

- The clean room has air conditioning delivered through HEPA filters 24/7. Its power supply is connected to an emergency generator.
- The positive pressure decreases from the tissue culture manufacturing rooms toward the general and gowning area. This is to prevent particles from “dirtier” areas being drawn into the cleaner areas.
- To maintain pressure and prevent contamination, the gowning-in and gowning-out areas have doors interconnected, so only one door is open at a time.

4.5.3 Process Gas Supply System

CO_2, N_2, and liquid nitrogen are supplied by a bottled gas distribution system or Dewar storage, a changeable manifold, a distribution system to processing equipment, including incubators, controlled-rate freezers, and cryogenic storage tanks.

Automated bottle/Dewar changeover is provided at the manifold. Connections to equipment are provided locally. Distribution piping is copper tubing up to the point of prefiltration, or a point before entry into a classified area, upon which the piping is transitioned from copper tubing to stainless steel. Point-of-use filters and sample valves are provided for routine monitoring of the CO_2 and N_2 systems.

4.5.4 Vacuum System

The vacuum system consists of two Nash liquid ring vacuum pumps with a receiver. This system supplies vacuum to users through a distribution piping network. Distribution piping is a copper tubing up to the point of entry into the classified area and 316L grade stainless steel once it enters the classified area.

5 Conclusions

The CRCMP facility enables researchers to take their ideas and therapies from the bench to bedside. The facility offers everything investigators need to further their research from basic sciences to product development and actual manufacturing of clinical products. It provides regulatory platform to the investigators for their translational research needs. The facility also provides assistance to commercial partners for scale-up manufacturing needs. From first-in-human, early-stage clinical trials to commercial products, CRCMP facility supports many critical stages of a product's life cycle. We fulfill our clients' manufacturing needs, complemented by innovation, development, compliance, and scale-up. With a unique operational model, CRCMP assisted preclinical and clinical production of cell therapies for hundreds of patients annually nationwide. The current design of CRCMP imposes few limitations on concurrent product workload and product variety. We currently do not have a designated space for gene editing or work requiring biosafety level 3.

References

1. University of Miami website. Interdisciplinary Stem Cell Institute. http://isci.med.miami.edu. Last accessed 25 Sept 2020.
2. University of Miami, Cellular Manufacturing Program website. Interdisciplinary Stem Cell Institute. http://isci.med.miami.edu/clinical-trials-research/cellular-manufacturing-program. Last accessed 25 Sept 2020.
3. Nonclinical Laboratory Studies; Proposed Regulations for Good Laboratory Practice Regulations; Proposed rule. 41 FR 51206. https://www.federalregister.gov/documents/2016/08/24/2016-19875/good-laboratory-practice-for-nonclinical-laboratory-studies. Last accessed 25 Sept 2020.

4. Current Good Manufacturing Practice in Manufacturing, Processing, Packing, or Holding of Drugs; General. 21 CFR Part 210. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=210 Last accessed 25 Sept 2020.
5. FDA Good Manufacturing and Good Tissue Practice regulations. Code of Federal Regulations Title 21CFR. Parts 210, 211, 1271, 600. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=210 https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=211 https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=1271 https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=600 Last accessed 25 Sept 2020.
6. FDA Good Clinical Practice Code of Federal Regulations Title 21: Parts 11, 50, 54, 56, 312 & 314 https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/ccfr/CFRSearch.cfm?CFRPart=11 https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=50 https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=54 https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=56 https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=312 https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=314 Last accessed 25 Sept 2020.
7. National Heart Lung and Blood Institute website. https://www.nhlbi.nih.gov. Last accessed 25 Sept 2020.
8. Cardiovascular Cell Therapy Research Network website. https://sph.uth.edu/research/centers/ccct/cctrn/. Last accessed 25 Sept 2020.
9. Production Assistant for Cellular Therapy website. https://www.pactgroup.net. Last accessed 25 Sept 2020.
10. University of Miami website. https://welcome.miami.edu. Last accessed 25 Sept 2020.
11. U.S. Food and Drug Administration website. https://www.fda.gov. Last accessed 25 Sept 2020.
12. FACT-Common Standards for Cellular Therapies, Current edition. Foundation for the Accreditation of Cellular Therapy, University of Nebraska, NE.
13. AABB Standards for Cellular therapy Services, Current Edition. AABB, Bethesda, MD.
14. FDA Human Cells. Tissues and Cellular and Tissue-based Products. Code of Federal Regulations Title 21, Part 1271. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=1271. Last accessed 25 Sept 2020.
15. USP <1116> Microbiological Control and Monitoring of Aseptic Processing Environments.
16. International Standard, ISO 14644-1, Clean-rooms and Associated Controlled Environments-Part 1: Classification of Air Cleanliness.
17. International Standard, ISO 14644-2, Clean-rooms and Associated Controlled Environments-Part 2: Specifications for Testing and Monitoring to prove Continued Compliance with ISO 14644-1.
18. Centralize Monitoring System User Manual, Amega Scientific Corporation. https://monitoring.mesalabs.com/amegaview/

Design of a New Academic GMP Facility for Today and Beyond at the Dana-Farber Cancer Institute

Olive Sturtevant, Sarah Nikiforow, and Jerome Ritz

1 Introduction

The FDA approval of axicabtagene ciloleucel (axi-cel; Yescarta®) [1] and tisagenlecleucel (tisa-cel; Kymriah®) [2] in 2017 ushered in a new wave of interest and investment in cellular therapies. The remarkable efficacy of these autologous T-cell products in patients with relapsed and refractory B-cell malignancies demonstrated that cells could be genetically engineered to have specific and long-lasting effects in vivo, raising the hope that further research in cellular therapies may be able to address a wide variety of unmet clinical needs in cancer, regenerative medicine, and many other therapeutic fields. Importantly, commercialization of axi-cel and tisa-cel also demonstrated that complex hurdles to manufacturing autologous genetically engineered cells could be overcome and that manufacturing individual products for individual patients was commercially feasible. Dramatically increased investments in cellular therapeutics have led to a rapid increase in early-phase clinical trials with large numbers of patients enrolled on these studies. This has in turn fueled demand for capacity to manufacture cellular products that meet strict regulatory standards. To meet this need, many new facilities are being planned and built, and existing facilities are being upgraded and expanded. While many cell manufacturing facilities are being constructed by for-profit entities to produce a limited number of cellular product types at relatively large scale, facilities at academic centers are also being expanded. Unlike commercial manufacturing facilities, academic facilities focus primarily on early-phase clinical trials and are expected to support a wide

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variety of novel cellular products manufactured for different indications. This chapter will describe our experience at the Dana-Farber Cancer Institute and the factors considered as we planned, designed, and built a new academic GMP cell-manufacturing facility. Construction of the new Connell and O'Reilly Families Cell Manipulation Core Facility (CMCF) began in January 2017. Construction was completed in February 2018, and the facility was occupied and became fully functional in August 2018.

2 General Programmatic Considerations

Facility design is critically dependent on the types and numbers of cellular products that will be manufactured. Many academic cell-processing labs are an extension of their hospital's transfusion services and primarily support processing of hematopoietic progenitor cell (HPC) products for autologous and allogenic bone marrow transplant. Requirements for these labs are very different from cell-processing labs that perform more-than-minimal manipulations. Specific considerations are needed for facilities that expand cells in vitro for prolonged periods, use viral vectors, or other genetic engineering modalities to modify cells, manufacture viral vectors, or manufacture a variety of 351-HCTP products [3]. Similarly, the scale of planned processing will impact facility design as space requirements, and equipment will be different for manufacturing autologous cells for individual patients compared to manufacturing large banks of allogeneic, off-the-shelf, cell products. Table 1 outlines some of these important programmatic considerations that impact the design and scale of cell manufacturing facilities and support functions needed.

As cell therapies continue to expand, it also becomes important to plan support for cellular products manufactured at other facilities but administered to patients at a local site. This "cell pharmacy" is often the responsibility of the academic cell-processing facility, with shipping out of apheresis collections and receiving Yescarta or Kymriah products being prime examples. Handling these products, which can be commercially approved or manufactured for a clinical trial, often requires collection, packaging, and distribution to other manufacturing facilities. Fresh final

Table 1 Different cell processing and support functions that may be included in new facilities

Cell processing activities	Support activities
Minimal processing of hematopoietic stem cells and lymphocyte products	Production of viral vectors
Extensive and complex manipulation of investigational cell products	In-process and product release testing (cell counts, viability, sterility, flow cytometry)
Genetic modification of cells with viral or nonviral vectors	Support for investigational cell products manufactured at other facilities
Production of large-scale allogeneic cell product banks	Support for FDA-approved cell products manufactured at other facilities
Short and long-term cryostorage	Internal quality assurance program

products may be received in the processing lab, the pharmacy, or directly at the patient location. If products manufactured off site (MOS) are cryopreserved and shipped to the clinical facility well in advance of administration, resources are needed for local cryostorage, inventory management, thawing, and distribution prior to administration. Need for space and workflows to accommodate these transactions and inventory will impact facility design.

Another programmatic consideration is the extent to which product testing and environmental monitoring will be carried out in the manufacturing facility or whether these functions will be outsourced to other laboratories. Assays required for release of almost all cell products include cell counts, viability, identity by flow cytometry, and sterility. Endotoxin and mycoplasma testing are also required for release of most manufactured products that are extensively manipulated. These assays can be performed by hospital clinical labs or commercial labs, but it is often more efficient to have robust testing capability within the cell processing facility. As genetic engineering becomes more commonplace, it may become necessary to devote space for PCR testing for vector copy number (VCN) or replication-competent viruses within the manufacturing facility.

3 Basic Ancillary Space Required to Support Cell Manufacturing

While not as glamorous as designing manufacturing cleanrooms, it is also necessary to provide adequate and well-designed spaces for critical functions and staff that support cell manufacturing. This includes storage for manufacturing supplies and reagents, manufactured products, product samples, and processing records. Different supplies and products may be stored at room temperature, 4 °C, −20 °C, −80 °C, or −195 °C. Administrative work areas are needed for all staff, as well as for frequent team meetings and external audits. It is also very helpful to have clear visibility into and out of cleanroom spaces wherever possible. This can be accomplished by windows set within doors, cleanrooms, or hallways as well as through video cameras or other devices that provide real-time two-way communication.

4 DFCI-Specific Strategic Considerations

Our Connell and O'Reilly Families Cell Manipulation Core Facility is accredited by the Foundation for Accreditation of Cellular Therapies (FACT) [4] to support both adult and pediatric stem cell transplant programs at the Dana-Farber Cancer Institute/Brigham and Women's Hospital and Boston Children's Hospital. We are also a shared resource of the Dana-Farber/Harvard Cancer Center, and in this capacity, we support immune effector cell programs at hospitals within this consortium,

which also includes Massachusetts General Hospital and the Beth Israel Deaconess Medical Center. We also support collaborations with the Harvard Stem Cell Institute. All of these programs are expected to grow in the next 10 years as cellular products become accepted therapies for a subset of solid tumors as well as hematologic malignancies. With improved methods for genetic manipulation and developmental reprogramming, cellular therapies will also be applied to a variety of other common diseases and regenerative medicine applications, such as hemoglobinopathies, genetic disorders leading to immune or metabolic deficiencies, autoimmune diseases, and solid organ transplantation. Genetically modified cells will also be combined with tissue engineering for treatment of Parkinson's disease, cardiovascular diseases, and many others. To meet this expected high demand for cell manufacturing, several strategic decisions outlined below affected the planning and overall design of DFCI's new facility. Facilities at other academic institutions will likely need to address many of these same issues.

- Design highly flexible manufacturing spaces capable of employing different strategies among cell selection, genetic manipulation, and cell expansion.
- Provide space for bioreactors and automated devices that may be developed for all phases of cell processing and product finishing.
- Maximize capacity for therapeutic cell manufacturing instead of viral vector production.
- Include resources needed for managing cellular products manufactured at other facilities (MOS cell pharmacy).
- Include resources needed for CLIA-certified product testing and the development of novel assays for product identity, function, and safety.
- Include dedicated non-cleanroom laboratory space for development and evaluation of new manufacturing procedures and equipment and staff training.
- Provide on-site short- and long-term storage for manufactured products and critical reagents.

5 FDA Type-C Review

For both our current facility and our previous cleanroom lab built in 2004, we asked the FDA to review our plans and requested a Type-C meeting [5]. We prepared a formal document outlining the types of products we currently manufactured and projections for the next 5 years. We included detailed designs of the proposed facility and mechanical systems for our ISO-7 cleanrooms, along with floor plans and process-flow diagrams for personnel, products (incoming and outgoing), materials, samples, air, and waste flow. We provided detailed plans for segregation of products and materials to avoid cross-contamination especially when working with genetically modified products.

Facility plans included 15 ISO-7 cleanrooms that would provide up to 36 individual workstations. We described architectural features such as finishes for floor,

walls, ceilings, work surfaces, and design features to avoid contamination in the cleanroom spaces and how we would maintain air classification standards. We also described nonclassified workspaces for receipt and distribution of products, storage of products and supplies, and our internal cell therapy testing (QC) lab. We identified equipment that would be used in and outside of cleanroom spaces and described various ancillary systems such as gases, water, HVAC, access control, and monitoring systems for airflow, pressurization, temperature, and humidity. We described our master validation plan for the commissioning and ongoing qualification of the facility, along with all the equipment and new processes that would be implemented. While preparing detailed plans can be difficult and time consuming, the Type-C meeting provided valuable feedback which led to significant design changes. In particular, the FDA suggested that all cell processing, including minimal manipulations of hematopoietic progenitor cells (HPC) and lymphocyte products, be performed within an ISO-7 cleanroom given the flexibility and range of products we were anticipating. We followed these suggestions even though most facilities at other centers do not perform these functions in ISO-7 cleanrooms.

6 Mechanical Systems

6.1 HVAC

Cleanrooms are classified based on airflow volume, differential pressures, and air quality (number of viable and nonviable particulates and lack of viable organisms). CMCF mechanical systems are primarily located on a restricted-access mechanical floor one floor above the new cleanrooms. Three air handling units on this floor supply all laboratories and support spaces with 100% outside air. One air handling unit supplies MERV-14 filtered air to the QC Laboratory, the Methods Development Laboratory and Support areas on both floors. Room air from these labs are 100% exhausted. Two air handling units supply 100% HEPA-filtered outside air to all cleanrooms. For redundancy, these units feed a common supply main that then separates into one supply main for each of our two main cleanroom areas. ISO-classified rooms are supplied with outside air changes from these units, plus additional conditioned air to meet the demand of each room's cooling load. Rooms are individually ducted to exhaust mains and heat recovery exhaust air handling units on the mechanical floor.

ISO-classified rooms maintain environmental parameters specified by ISO 14644-1:2015, including viable and nonviable particulate counts and air change rates. The increased air change rates for ISO-7/ISO-8 rooms are supplemented through individual fan-powered recirculating units. Each recirculating unit supplies ceiling-mounted HEPA modules and returns from low-wall exhaust grilles. For each ISO-classified space, the dedicated HEPA module and fan-powered unit

configuration maintain the required cleanliness and control airflow and temperature in an energy-efficient manner.

6.2 Gasses and Liquid Nitrogen

Advanced cell processing that utilizes incubators, bioreactors, automated manufacturing systems, and cryopreservation mandates access to various gasses including CO_2, nitrogen, oxygen, compressed air, and LN_2. These gasses are available in "point-of-use" tanks that can be located in individual cleanrooms or storage facilities. However, to enhance efficiency, save space, and eliminate the risk of introducing contaminants in our cleanrooms, we elected to use bulk systems with gasses delivered via piping to the various drops/connections within the cleanrooms. This avoids moving tanks in and out of cleanroom spaces and makes it much easier to provide onsite backup. To accommodate the large numbers of incubators and LN_2 storage tanks in the facility, bulk CO_2 and LN_2 tanks were located outside the building. Individual tanks for CO_2 (backup), O_2, N_2, and medical-grade compressed air were located on the mechanical floor above our cleanrooms.

6.3 Other Systems

GMP manufacturing requires continuous monitoring of critical functions, environmental systems, and equipment. This typically includes monitoring for temperature, CO_2, humidity, and LN_2 levels as well as pressure differentials, airflows, air exchanges, and nonviable particle counts. We elected to use the Rees Scientific Monitoring System to provide automated independent monitoring; logging and alarming of temperature for all rooms, incubators, refrigerators and freezers; nonviable particulate counts in each biosafety cabinet; as well as monitoring gasses and humidity where relevant.

Our DFCI Building Management System (BMS) supports the facility's environment by monitoring and controlling the HVAC system. The BMS controls and monitors the supply and exhaust airflow, temperature, relative humidity (rH), and room pressurization including the differential pressures across all doors where pressurization is required. Display screens showing the temperature, air rH, pressure differential, and airflow direction are located at the entrance to every cleanroom and all air locks. In addition to the display is a local visual and audible alarm alerting the staff if there is a pressure failure at one of the locations.

Other system controls include access controls to all entry points into the facility, including individual processing labs, QC labs, and support areas. Access is controlled by ID-badge access which makes it possible to identify all individuals who access any area. Some rooms have access restricted to a specific group of trained/approved staff. All control and monitoring systems operate 24/7 and alert

appropriate personnel if action limits are reached. In addition, preprogrammed reports are reviewed and examined for gradual changes that can indicate whether a sensor is starting to fail or if there is a potential problem that should be addressed.

A pneumatic tube system is used to transport samples between labs which may be in different floors or buildings. Pneumatic tube stations are located in product receipt and release rooms as well as the QC laboratory. Samples for testing are placed in sealed bags and loaded into a transfer tube container which is then placed in the pneumatic transfer system. Most product samples are sent to the QC laboratory for testing. Samples can also be sent to other labs or received from the apheresis center using this method.

7 Overall Design of the Connell and O'Reilly Families Cell Manipulation Core Facility: 2018

The new facility occupies all of the 12th floor and part of the 11th floor of the Smith Building at DFCI. This building primarily supports wet-lab research, and the areas that were completely renovated during CMCF construction previously supported a large small animal research facility. The 13th floor of the Smith building is entirely dedicated to mechanical support for the entire building and is where all mechanical systems supporting the new GMP facility were located. In the final design shown in Fig. 1, the 12th floor is dedicated entirely to cleanroom manufacturing and direct support for cell manufacturing. The stem cell therapy (SCT) lab (darker blue) is responsible for processing autologous and allogeneic stem cell products and

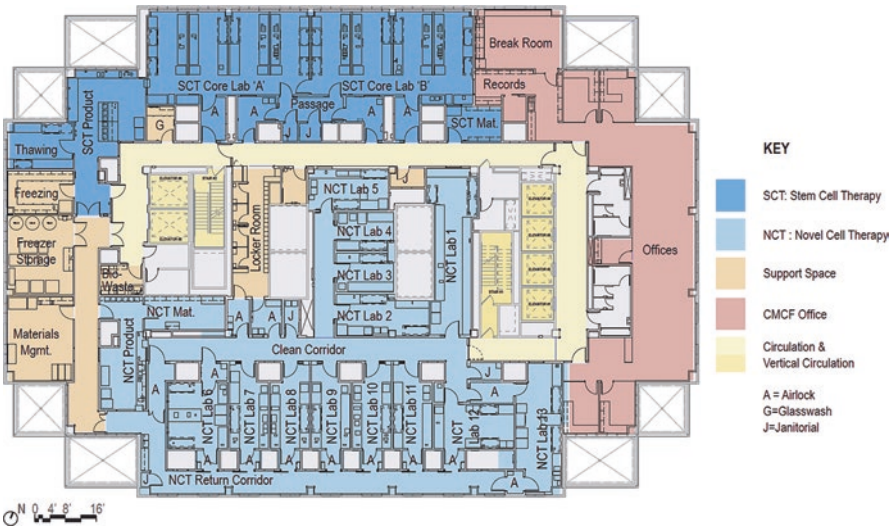

Fig. 1 Schematic design of 12th floor cleanrooms and support areas

lymphocyte infusions for adult and pediatric patients undergoing stem cell transplantation. This can include complex cell selection procedures. The novel cell therapy (NCT) lab (light blue) is responsible for complex cell manufacturing of investigational products that require genetic manipulation or more than several hours of culture/expansion. Areas shown in orange provide support functions for both SCT and NCT labs. Areas shown in pink include office space for supervisors and technical staff that work on this floor as well as administrative support and record storage.

7.1 Stem Cell Therapy (SCT) Laboratory

Work in SCT focuses on processing autologous and allogeneic hematopoietic stem cell products. This includes bone marrow, mobilized peripheral blood, cord blood, and lymphocyte collections. While autologous stem cell products are always cryopreserved, allogeneic products are usually processed fresh just prior to infusion. This changed during the COVID-19 pandemic when frequent flight delays and cancellations made it impossible to guarantee that fresh products would be available for infusion when patients completed transplant conditioning. As a result, allogeneic products were routinely cryopreserved at the transplant center before conditioning therapy was begun. While most stem cell processing requires only minimal manipulation, complex positive stem cell selection procedures or selective T-cell depletions and short-term incubations are also performed in SCT.

The SCT processing area consists of two ISO-7 classified rooms (SCT Core A and Core B) each with six workstations. Each workstation is equipped with a biosafety cabinet (BSC), undercounter refrigerator, sterile docking devices, and a computer workstation. A centrifuge is shared by two workstations and less commonly used equipment such as a Cobe cell washer and Miltenyi CliniMACS is shared by multiple workstations. As shown in Fig. 2, SCT Core A and Core B are almost identical. If necessary, this redundancy allows one room to be shut down with only minimal impact on SCT manufacturing.

As shown in Fig. 2, the two SCT cleanrooms are entered through a shared unidirectional air lock (A to passage to A). Within the central area of the air lock, two closets (labeled J) are used for janitorial supplies. One is used to store supplies, while the other contains the reverse osmosis (RO) water supply and waste drain. Both Core A and Core B have positive airflow toward the unidirectional air lock passage areas. Janitor closets were designed to be negative to the air lock passage area to keep water contaminants from entering the air lock. Both the entrance and exit air lock have positive airflow toward the common corridor. We also incorporated large material air locks that are used when equipment is moved in or out of the cleanroom space. Both equipment air locks (A2) also have positive air pressure from the cleanroom toward the common corridor.

Incoming 361 HCTP cellular products and immune effector cell (IEC) products manufactured off site are received in the SCT receipt and distribution area (labeled

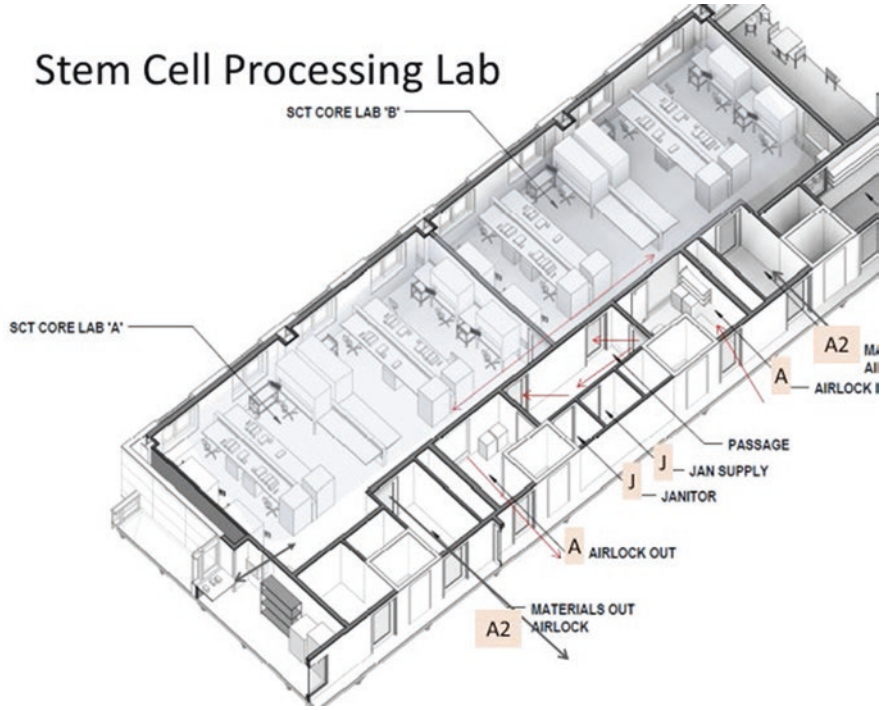

Fig. 2 Three-dimensional view of stem cell therapy cleanrooms

SCT product in Fig. 1). This area was designed to accommodate receipt of bone marrow, apheresis, or cord blood products for stem cell transplant patients as well as products that are manufactured at other facilities for treatment of patients at our center. This includes commercial products as well as products manufactured for patients enrolled on clinical research protocols. All of these products are received, inspected, and transferred to the appropriate SCT suite for the next step of processing or stored in our adjacent LN_2 tanks. This is also where released products are signed out, packaged, and shipped to other sites or taken to the patient location for infusion.

The support area also includes separate rooms designed for thawing cryopreserved products and control rate freezing (Fig. 1). A short-term cryostorage area contains both mechanical freezers and LN_2 tanks. These rooms support both SCT and the novel cell therapy (NCT) lab. A much larger area for long-term LN_2 storage is located on the ground floor of an adjacent building.

7.2 *Novel Cell Therapy Lab*

Our novel cell therapy lab (NCT) is where most of the investigational product manufacturing occurs. As an academic lab that supports a wide range of clinical and laboratory investigators, we manufacture a broad range of cellular products. These include cancer vaccines, genetically engineered T cells and hematopoietic progenitor cells, natural killer cells, regulatory T cells, mesenchymal stromal cells, limbal stem cells, induced pluripotent stem cells (IPSC), and IPSC-derived cells. Considering this wide range of cellular products and the expectation that the diversity of manufactured products will only increase in the future, our facility design emphasized flexibility of space and the ability to continually incorporate new procedures and new manufacturing technologies. The NCT laboratory (Fig. 3) has 13 separate processing labs that are all accessed from the internal NCT clean corridor. A central entry air lock is located off the locker room area. NCT labs 1–5 allow for bidirectional flow into each lab direct from the clean corridor. These cleanrooms are positively pressured with respect to the internal clean corridor. NCT lab 1 is a large room designed to support different types of equipment and bioreactors used for cell selection or cell expansion. NCT labs 7–12 are designed for unidirectional flow with exit from the cleanrooms to the NCT return corridor via dedicated exit air locks; these labs are negatively pressured with respect to the clean corridor and exit air locks. NCT Lab 6 and NCT Lab 13 are also designed for unidirectional flow but are segregated from the clean corridor by entry air locks to allow for flexible reconfiguration (between campaigns) from negative to positive pressurization with respect to the Clean Corridor. These two rooms have also been configured to support

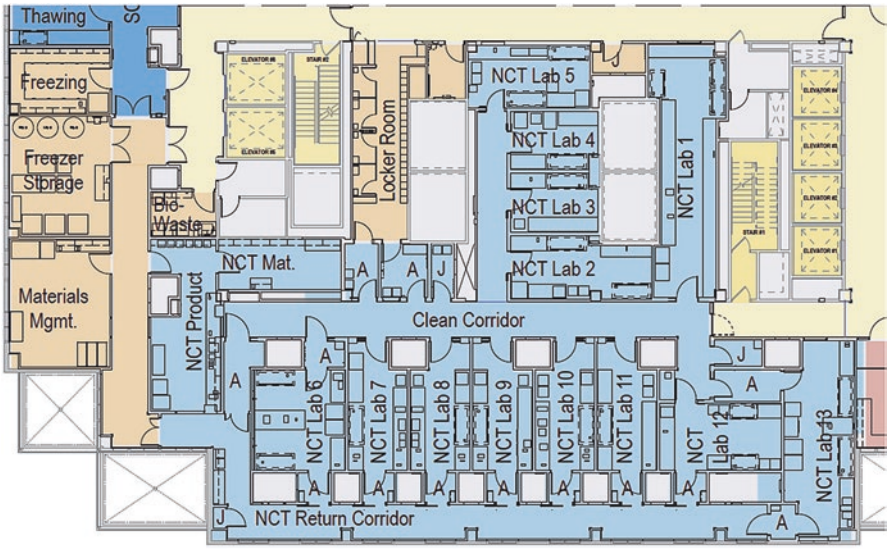

Fig. 3 Schematic Layout of Novel Cell Therapy Cleanrooms

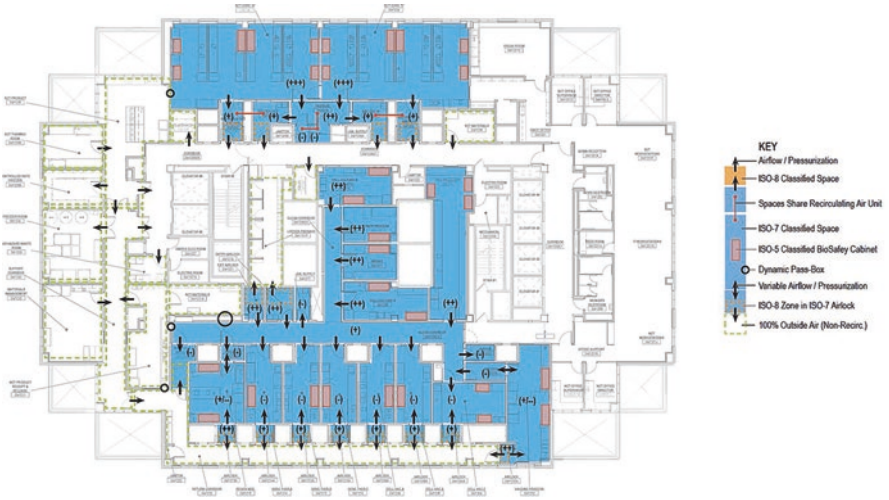

Fig. 4 ISO classification and airflow pressurization in CMCF cleanrooms

long-term culturing of cells such as iPSCs. Processing that requires viral vectors to genetically modify cells is carried out in negative-pressure cleanrooms to avoid any risk of viral cross-contamination. ISO classification and airflow in the CMCF cleanrooms are shown in Fig. 4.

Personnel flow in the NCT lab varies depending on the manufacturing process and cleanrooms that are used. Work that does not require viral vectors is carried out in positive-pressure cleanrooms with bidirectional traffic in the Clean Corridor. For these procedures, techs enter the ISO-8 entrance air lock from the changing rooms. They then enter the ISO-7 clean corridor and proceed to one of the ISO-7 positive-pressure rooms. The pressure differential of the air lock is positive to the changing room and negative to the ISO-7 clean corridor. When exiting the ISO-7 lab space, they move in the clean corridor to the ISO-8 exit air lock. The exit air lock pressure differential is also positive to the changing room and negative to the ISO-7 clean corridor.

Work with genetically modified cells and viral vectors is carried out in negative-pressure cleanrooms with unidirectional traffic flow. Once in the ISO-7 clean corridor, staff move into an ISO-7 negative-pressure room. Staff exit the cleanroom through an ISO-7 air lock into a return corridor (controlled, not classified) and degown prior to leaving the return corridor and entering a common corridor area. The air locks coming off the negative pressure rooms are negative to the ISO-7 lab space but positive to the return corridor. They are also classified as ISO-7.

A large ISO-7 air lock is used to move equipment in and out of the NCT lab. This air lock is negative to the NCT ISO-7 clean corridor and positive to the return corridor. This large air lock also houses RO water and a drain for the janitors cleaning the positive-pressure rooms. Within the clean corridor is another janitor's closet for supplies that is similar to the one in SCT. Waste and wastewater coming out of the

negative-pressure rooms leave the individual rooms through the room exiting air lock to the janitor's (J) closet located in the return corridor. Cleaners must degown before leaving the return corridor. They then need to circle back to the changing room area and go back through NCTs entering air lock to access the clean corridor to clean the next negative-pressure room.

All NCT labs are equipped with biosafety cabinets, double-stacked incubators, and centrifuges. Several were designed with additional gasses such as nitrogen, oxygen, and compressed air in addition to CO_2, to accommodate bioreactors and hypoxic incubators.

Support spaces dedicated to the NCT laboratory are located outside the core NCT laboratory. These include the NCT product receipt and release room and the NCT materials room (Fig. 3). All of the NCT support spaces are controlled but not classified and are accessed from the support corridor. Incoming cellular products for the NCT laboratory are received in the NCT product receipt and release room, inspected, and transferred to the appropriate NCT lab for the next step of processing. Similarly, outgoing patient products are transferred to the NCT released receipt room prior to transfer to patient locations. Products and supplies enter through this room via HEPA-filtered dynamic pass-throughs. There is a small pass-through for products and samples and a large pass-through to accommodate the kits that are prepared for each process.

8 Smith 11 Areas to Support GMP Cell Processing

Additional space for the CMCF is located on the 11th floor of the Smith building, one floor below the GMP cleanroom areas. Approximately one-third of this floor is used by the CMCF, and the remainder of the floor is used for wet-lab research by our collaborators. The schematic design of the CMCF areas on Smith 11 is shown in Fig. 5. Areas shown in pink include office space for senior clinical and administrative staff, quality assurance staff, and a conference room. The quality control lab is a CLIA-certified clinical laboratory that primarily supports product testing and environmental monitoring. The methods development lab (MDL) is used for process development and training.

9 Quality Control (QC): Cell Therapy Testing Lab

The QC laboratory performs a variety of assays routinely needed to characterize the safety and identify of starting cellular material, intermediate processing stages, and final cellular products. These include cell counts, viability, sterility, endotoxin, flow cytometry, enzyme-linked immunosorbent assay (ELISA), colony assays, and polymerase chain reaction (PCR). The QC laboratory contains open bench space, biosafety cabinets, sinks, temperature-controlled and humidity-modulated storage units (refrigerators, freezers, and incubators), and flammable material storage

Fig. 5 Schematic design of 11th floor support areas

cabinets. Specific electrical, telephone, and data outlets are located to support specific instruments and associated procedures. All areas are provided with computer workstations. The QC laboratory has designated space for accessioning product samples for testing via a pneumatic tube system delivering samples from cleanrooms one floor above the QC lab and the donor apheresis center located in an adjacent building. Testing equipment in the QC lab includes automated hematology analyzers, multiple flow cytometers, and ELISA readers. A separate room was designed for sterility testing using Bac-T Alerts (Millipore Filtration System) and incubators for viable environmental cultures. A designated area for DNA extraction and PCR testing was designed with a pressure-differential gradient. This area includes an air lock, separate labs for DNA extraction, and amplification and space for plate reading. The QC laboratory air supply is 100% outside air (non-recirculating) that is HEPA filtered in the sterility testing and PCR areas.

10 Methods Development Laboratory

As an academic cell processing facility, an important goal is to support the development of new, safe, and more effective cellular products, analytic testing, and manufacturing approaches. As a result, we constantly incorporate new projects and procedures to support innovative clinical research trials. The methods development lab (MDL) was created to support process development outside of the GMP cleanroom space and is used to develop new manufacturing procedures, evaluate new equipment, and train staff on new procedures. This area provides the option of working out processing variables in less costly space and allows for collaborators to participate in the tech-transfer process. The two MDL labs (Fig. 5) are entered from

a common air lock. The labs are unclassified but built to qualify as ISO-8 if needed. All the other system controls (access, monitoring, etc.), gasses, and electrical systems are similar to those in the classified ISO-7 labs.

11 Additional Shared Support Areas

Changing room and staff lockers (Fig. 1): Staff changing rooms and lockers, as well as scrub storage is located on the 12th floor in an area adjacent to the NCT Airlocks. This area includes four gender-neutral individual changing rooms. Staff arrive each day and change into low-particle shedding scrubs and shoes before working in the cleanroom areas. At the end of the day, used scrubs are placed in storage bins for pick up by an outside vendor.

Document storage and office space: like many cell processing labs, most records are stored as paper documents. A separate paper batch record is created for each product and stored on site for at least 2 years. After this period, most documents are sent off site for long-term storage. In a high-volume lab, there is never enough onsite space for document storage. Clean desk space is also needed for staff to complete documentation. We created a large area for desk space on the lab processing floor (Fig. 1), but given the large number of staff, individual spaces are shared. This became an issue when we needed to ensure appropriate distancing between staff during the COVID-19 pandemic. Our facility's processing demands did not slow down during this period, and it was necessary to find additional desk workspace in other areas.

12 Conclusions After 2 Years of Operation

While the new facility more than doubled our previous capacity to manufacture cellular products, our operational debut in 2018 coincided with dramatic growth in the cell-therapy field. Within several months of opening the new facility, we were able to initiate a variety of new cellular procedures including manufacturing of several novel CAR T-cell therapies. New staff were hired and trained to work on multiple projects. Almost all design features were stress-tested; luckily, almost all features worked as expected. One feature we found very useful was the incorporation of large windows and glass doors wherever possible. This provided a great deal of natural light and markedly enhanced visibility and communications within and between all the cleanroom areas.

Of course, we did not anticipate the pandemic that arrived in March 2020 and the need for physical distancing. This primarily impacted work outside of the cleanrooms. We were required to find additional clean desk space, and both QA and administrative staff were able to work remotely. This made it possible to maintain a full cell-processing schedule throughout the pandemic for patients who were

undergoing stem cell transplantation or were enrolled on cell therapy clinical trials. Few new trials were opened during the initial 4-month period, but the peak surge of SARS-COV-2 infections in Massachusetts began to abate by June 2020. Several new protocols that had been delayed began to accrue patients. With the new facility, we were able to continue to provide critical cell therapy support for our patients. Overall volume of work in the CMCF declined only slightly during the peak of the pandemic and rapidly recovered to a high level. This was a reminder that one can never envision all possible scenarios or demands that will arise over a 5- or 10-year period, but that building a cell processing facility with the flexibility to shift direction and bandwidth as needed within a strict regulatory and quality environment is a requirement in today's age of burgeoning cell therapy opportunities.

As the cell therapy field continues to expand and "living drugs" become a regular component of the therapeutic arsenal for many indications, new facilities will be needed to meet increased demand for cell manufacturing capacity. While large central manufacturing facilities will expand to meet this demand, it will also be necessary for local facilities to provide increased manufacturing capacity. As cell manufacturing procedures become more standardized and automated, local capacity for cell manufacturing may be used for FDA-approved products as well as for investigational products. In both cases, manufacturing expertise and experience at academic facilities will be needed to support patients at their clinical centers.

Acknowledgments This facility could not have been built without a great deal of support and dedication from many people. We would like to specifically acknowledge the support and expertise of Michael Hinchcliffe and Mollica Manandhar at Payette, our primary architects, Francesca McBride, Director of Regulatory Compliance at Jacobs Engineering, and Skanska, USA, our lead contractor as well as many others who helped us accomplish this build.

References

1. Yescarta website. https://www.yescarta.com/?gclid=CjwKCAiA8Jf-BRBEiwAWDtEGvUFhge8aGLloQknhZ1_cBlUaNuVUey7GU9VInezjWV0Vxqw33oVMhoC0dcQAvD_BwE. Last accessed 14 Dec 2020.
2. Kymriah website. https://www.hcp.novartis.com/products/kymriah/?site=KYD-1236331GK100072&utm_source=google&utm_medium=cpc&utm_campaign=2020_q4_kymriah_gateway_hcp_branded_treatment_exact%3Bs%3Bph%3Bbr%3Bonc%3Bhcp%3Bbr&utm_content=kyd-1236331&utm_term=kymriah&omap_code=KYD-1236331&gclid=CjwKCAiA8Jf-BRB-EiwAWDtEGqn0sN1ndMkGsy-gcTCu_kM0FIpimdzT2Al8BbqlNqLk_Fc4QdUMSRoC68AQAvD_BwE&gclsrc=aw.ds . Last accessed 14 Dec 2020.
3. US Food and Drug Administration. (2018). *FDA regulation of human cells, tissues, and cellular and tissue-based products (HCT/P's) product list*. https://www.fda.gov/vaccines-blood-biologics/tissue-tissue-products/fda-regulation-human-cells-tissues-and-cellular-and-tissue-based-products-hctps-product-list. Last accessed 14 Dec 2020.
4. Foundation for the Accreditation of Cellular Therapies website. http://www.factwebsite.org/. Last accessed 14 Dec 2020.
5. US Food and Drug Administration. *Formal meetings with the FDA – Type C meetings*. https://www.accessdata.fda.gov/cder/sb-navigate/topic3/topic3/da_01_03_0140.htm. Last accessed 14 Dec 2020.

Design and Licensure of an American Cord Blood Bank

Jeffrey M. Wilson, Erin N. Eaton, Krystle Pool, Donna Reioux, Mil Fontenot, David Marin, Richard Champlin, Katayoun Rezvani, Elizabeth J. Shpall, and Chitra Hosing

1 History of Regulatory Requirements

Public cord blood banking as an industry is highly regulated. Voluntary third-party accreditation for cord blood banking is available through organizations such as FACT [1] and AABB [2]. Additional accreditations for testing processes necessary for the manufacture of HPC, cord blood fall under the purview of organizations such as the American Society for Histocompatibility and Immunogenetics (ASHI) [3], College of American Pathologists (CAP) [4], and Clinical Laboratory Improvement Amendments (CLIA) [5]. Participation in accreditation, while voluntary, is impactful as third-party consumers of the HPC, cord blood products such as transplant centers and insurance companies look for accreditation to provide a level of assurance of the quality of the cord blood unit they intend to use to treat their patients.

In October 2009, HPC, cord blood became one of the first HCT/Ps to be regulated as a biologic drug when the FDA introduced a guidance for industry to help manufacturers apply for licensure of their manufacturing process of HPC, cord blood. This guidance was updated in March 2014 when the FDA published their final guidance for industry "BLA for Minimally Manipulated, Unrelated Allogeneic Placental/Umbilical Cord Blood Intended for Hematopoietic and Immunologic Reconstitution in Patients with Disorders Affecting the Hematopoietic System, Guidance for Industry" [6]. The FDA recommendations in the guidance cover minimally manipulated umbilical cord blood products intended for use in unrelated donor hematopoietic progenitor cell transplantation procedures. However, in the guidance, the FDA does:

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A. P. Gee (ed.), *Cell Therapy*, https://doi.org/10.1007/978-3-030-75537-9_19

> …encourage manufacturers of hematopoietic stem/progenitor cells from umbilical cord blood for autologous use, or use in a first- or second- degree blood relative, to also follow the recommendations concerning the manufacture of these products and how to comply with regulatory requirements, even though their products may not require premarket review [6].

HPC, cord blood for unrelated allogeneic use is regulated by the Center for Biologics Evaluation and Research (CBER) under Section 351 of the PHS Act and the FD&C Act, and as such, the applicable regulations include, but are not limited to, the following sections of the CFR:

- 21 CFR Parts 201 and 610 Subpart G – Labeling
- 21 CFR Part 202 – Prescription Drug Advertising
- 21 CFR Parts 210 and 211 – Current Good Manufacturing Practice Regulations (cGMP)
- 21 CFR Part 600 – Biological Products: General
- 21 CFR Part 610 – General Biological Products Standards

The current good tissue practice (cGTP) requirements govern the methods used in, and the facilities and controls used for, the manufacture of HCT/Ps to prevent the introduction, transmission, or spread of communicable diseases by HCT/Ps [21 CFR 1271.150(a)]. Because cord blood and HPC, Cord Blood are HCT/Ps, these provisions are applicable to both cord blood and HPC, Cord Blood.

The cGMP requirements, in 21 CFR Parts 210 and 211 [7, 8], govern the methods to be used in, and the facilities or controls to be used for, the manufacture, processing, packing, or holding of a drug to ensure that such drug meets the requirements of the FD&C Act as to safety, has the identity and strength, and meets the quality and purity characteristics that it purports or is represented to possess.

Due to the broader scope of these regulations, most of the cGMP regulations under 21 CFR Parts 210 and 211 [7, 8] would be applicable to your HPC, Cord Blood. Additionally, due to the broad scope of the regulations, for the most part, cGTP would be subsumed under the broader cGMP requirements. Compliance with these requirements would result in compliance with applicable cGTP requirements [6].

At the time of writing, the FDA recommends, but does not require, licensure for all public cord blood banks. However, with the evolution of new cellular therapies, HPC, Cord Blood is increasingly seen as a source tissue for the development of immunotherapeutics, and licensure of the bank represents a level of quality to the commercial entities developing these technologies. In the end, the intent of all of these regulatory agencies is to establish robust quality systems that ensure a high level of consistency and quality for HPC, Cord Blood manufactured for use in hematopoietic stem cell transplantation and cellular therapies.

Designing a Cord Blood Bank with the intention to apply for biologics license adds a significant operational expense to the process of cord blood banking in order to meet the regulatory requirements for licensure and the post-marketing period. This is mainly due to the increased cost for facility design, maintenance, infrastructure, quality systems, and personnel required for licensure, which is unfortunately a hurdle that many smaller or start-up banks simply do not have the capital to overcome. At this time, the FDA has not officially required biologics licensure for public cord blood banks. However, licensure is required for participation in the National

Cord Blood Inventory (NCBI) [9] project as a contracted provider for Health Resources and Services Administration (HRSA), which is a major source of funding for the US Cord Blood Banks. Many of the US Public Cord Blood Banks with the financial capability have, therefore, opted to pursue licensure in order to maintain eligibility for NCBI contracts.

2 Facility Selection and Design

Facility selection and design are a key component in the establishment of a cord blood bank and preparing to apply for FDA licensure. First and foremost, all regulatory agencies require that there be adequate space to perform the manufacturing activities safely. This includes the safety of both staff and product. Ideally, the space should allow the ability to both spatially and temporally separate multiple products, which may be manufactured in a single day, in an effort to reduce the potential for contamination/cross-contamination. If the manufacturing facility is to reside within an existing or previously established laboratory space, consideration must be given to adjacent operations that may create concerns or have the potential to impact product quality.

The cGMP requirements for facilities:

- Buildings used in the manufacture of HPC, Cord Blood must be maintained in a state of good repair [21 CFR 211.58].
- Building(s) used in the manufacture of HPC, Cord Blood must be of suitable size, construction, and location to facilitate cleaning, maintenance, and proper operations [21 CFR 211.42(a)].
- Operations must be performed within specifically defined areas of adequate size [21 CFR 211.42(c)].

Redundancy of critical systems (heating, ventilation, and air conditioning (HVAC), central monitoring) and equipment (centrifuge, biological safety cabinet (BSC)) should be considered to ensure the ability to continue to manufacture in the event of a system failure.

Ideally, the design of the facility should be informed by the intended manufacturing methodology (automated, semiautomated, manual). This must also include the methods by which excipients such as hetastarch or cryopreservative media are added, and by which in-process and post-process samples are removed. These considerations drive the requirements for quantity and placement of equipment such as biological safety cabinets, centrifuges, freezers, etc. such that they can support the intended manufacturing workflow and, therefore, the space required. Adequate freezer storage for maintaining a growing bank of cord blood units (CBUs) and their associated samples must be considered, with the potential for both growth of storage capacity and rate of unit storage.

Review of the intended personnel, product, ancillary sample, and waste paths through the manufacturing process should be considered with the intention of

reducing potential for product contamination at every step of the process. Where possible, implementation of a unidirectional flow for each of these is recommended to reduce the potential for cross- contamination. Diagrams of each of these paths must be included with the Biologic License Application (BLA) submission in a manner that clearly denotes the path of each and must clearly identify the room(s) involved with each.

Contamination controls for the maintenance of aseptic manufacturing conditions are required for all areas in which the operations for processing of HPC, Cord Blood are performed including, but not limited to, collection, volume reduction, packaging, labeling, cryopreservation, storage, and shipment. These can include in-process controls performed to prevent or identify contamination or cross-contamination, systemic/facility controls such as access control, HVAC systems, water systems, cleaning/sanitization procedures, personnel gowning practices, and an environmental monitoring program.

2.1 General Description of MD Anderson Cord Blood Bank Facility

The MD Anderson Cord Blood Bank (MDACBB) is located in Houston, Texas, in the MD Anderson Cancer Center and administratively within the Department of Stem Cell Transplantation and Cellular Therapy, under the leadership of the Department Chair, Dr. Richard Champlin and under the direction of Drs. Elizabeth J. Shpall and Chitra Hosing. The cord blood bank was originally integrated within the Cell Therapy Laboratory, occupying two bays of a large ten-bay laboratory. After reviewing licensure requirements, it was clear that segregation of products from the remainder of the lab, unidirectional flow of products and personnel, and all of the other requirements of the BLA would require a dedicated facility. After a building/space was identified, MD Anderson Cord Blood Bank leadership embarked on a facility design, consulting with industry experts in cGMP facilities/FDA regulatory requirements.

The current MD Anderson Cord Blood Bank facility was initially commissioned in July of 2013. The International Standards Organization (ISO) classified manufacturing and testing laboratories located within the bank were recommissioned subsequent to renovations undertaken to correct air flow issues in December 2015. The ISO classification of the clean room manufacturing and testing facilities was confirmed by a third-party vendor.

The 10,000 square feet of dedicated space at the MDACBB is divided into the following areas: administration, receiving, preprocessing, manufacturing, testing, cryopreservation, long-term storage, and shipping preparation (Fig. 1). Over 3500 square feet of the ISO-classified clean room facility is designated for the manufacture and testing of HPC, Cord Blood. The manufacturing and testing laboratories meet the requirements for an ISO 7 clean room under ISO standard 14644-1 [10],

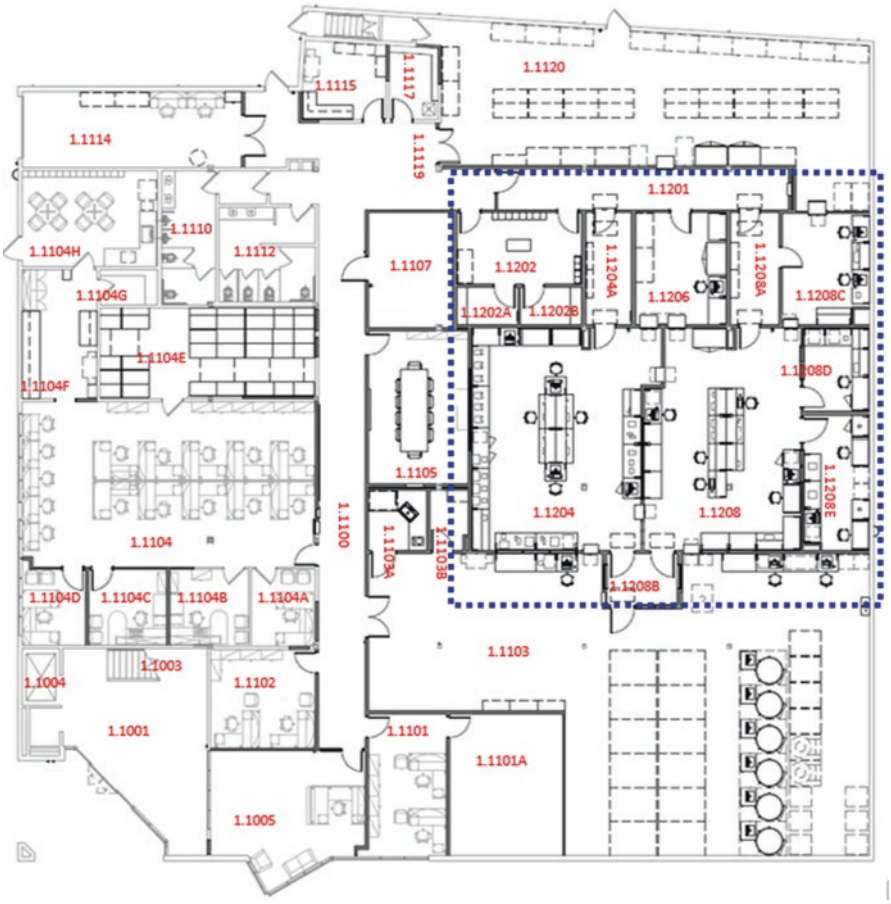

Fig. 1 MDACBB floor plan

while the adjacent areas leading to and from the ISO 7 spaces are classified as ISO 8 (Fig. 2). A cascading pressure differential between the ISO 7 manufacturing area and adjacent areas helps ensure the integrity of the manufacturing area and products produced within is maintained (Fig. 3).

The classified areas of the cord blood bank facility are used specifically for the manufacture, testing, and release of HPC, Cord Blood. There are no other developmental or approved products manufactured or manipulated in the same areas as HPC, Cord Blood. All surfaces of the facility have been designed to reduce particulates and facilitate cleaning and decontamination. The facility consists of a continuous sheet of vinyl flooring that has smooth welded seams. The walls of the facility are painted in an epoxy resin paint to allow for cleaning and reduced particulates. The ceiling of the facility consists of clean room grade ceiling tiles clipped into a gasketed ceiling to prevent movement and allow for cleaning.

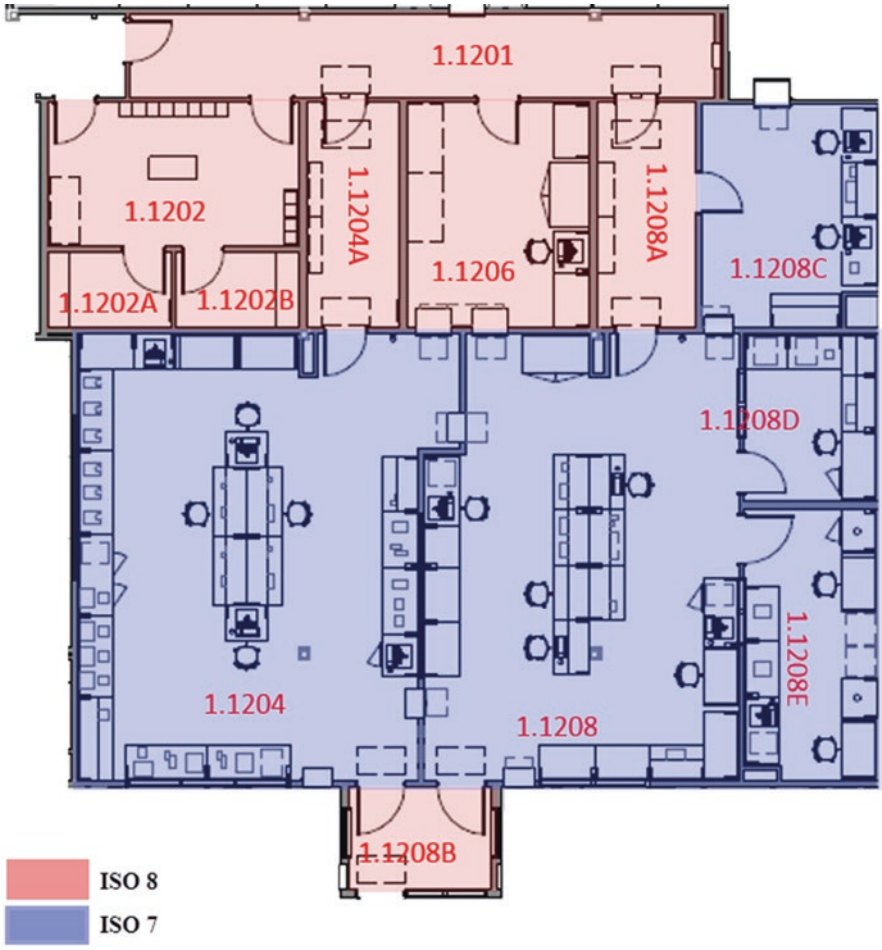

Fig. 2 ISO classifications by room

Approximately 3000 square feet of freezer room space is dedicated to storage of manufactured HPC, Cord Blood and the representative and the retention samples required.

2.2 Access Control

One of the earliest considerations in facility selection/design is how to implement a robust system of access control. The FDA requires a description of the measures implemented to prevent unauthorized access of the manufacturing areas to be

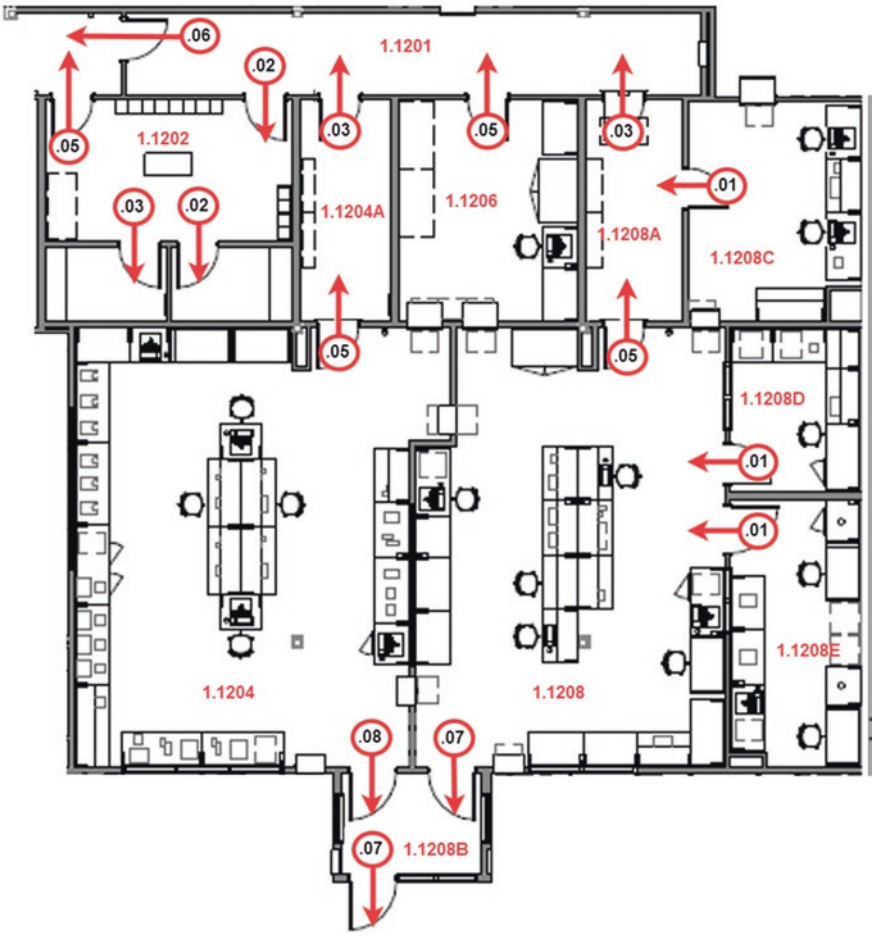

Fig. 3 MDACBB differential pressure cascade

submitted as part of the BLA application. These controls may include limited access entry though keyed access, coded access, card key readers, biometric verification, video surveillance systems, or some combination of controls that is capable of providing robust access control for access to manufacturing facility, access to storage facility, access to quarantined/released supply inventory, and access to manufacturing records both physical and electronic. Facility level access control can also play an important role in the contamination control schema of the facility.

The process of cord blood procurement results in the collection of individually identifiable protected health information (PHI). This information must be kept secured and limited to only those individuals requiring access to perform their job-specific duties as outlined in the HIPAA Privacy and Security Rules.

Access controls for software used in the collection, manufacture, storage, and shipment of products are required to ensure protection of PHI and product. Software access control should be a key consideration in the selection, development, validation, and implementation of any software system implemented for the cord blood bank [21 CFR Part 11] [8].

Ideally the access control schema put into place should be able to limit access to and provide and auditable trail capable of uniquely identifying who entered limited access areas of the manufacturing facility or software application(s), when they were accessed, and in the case of software, any changes that were made.

2.2.1 MDACBB Facility Access Control

All access to the cord blood bank is limited to verified personnel using badge reader access. Entrance to the facility requires a minimum of two levels of access to first enter the lobby of the building and then enter into the cord blood bank. A third level of badge access is required to enter into the freezer room. A fourth level of badge access is required to enter the gowning room of the clean room facility. Video monitoring of the perimeter of the building, lobby, internal corridors, and freezer room is performed 24/7.

2.3 Heating, Ventilation, and Air Conditioning (HVAC)

The HVAC system is another key aspect of the facility design. The cGMP Guidance for industry requires the following of air handling systems:

- Adequate ventilation must be provided [21 CFR 211.46(a)].
- Equipment for adequate control over air pressure, microorganisms, dust, humidity, and temperature must be provided, when appropriate [21 CFR 211.46(b) and 600.11(a)].
- Air filtration systems must be used, when appropriate, on air supplies to manufacturing areas [21 CFR 211.46(c)].

The application for licensure requires the following to be included with the submission:

- A description of the controls used to prevent contamination and cross-contamination, including air handling units, pressure differentials, whether air is once-through or recirculated, and air changes per hour.
- A description of the environmental quality of each room and each aseptic processing area (laminar flow unit)
- A validation summary for the system, including a narrative on the validation utilized, acceptance criteria, and explanation of failures and excursions, including deviation reports and results of investigations.

Key considerations that would drive the HVAC design configuration are the processes performed (e.g., cord blood manufacturing) and the manufacturing methodology (open vs closed system).

2.4 Temperature and Humidity

Of paramount consideration is the ability of the HVAC system to maintain the temperature and humidity ranges specified for your facility/process/equipment despite fluctuations in the external temperature and humidity. Each extreme should be considered for both temperature and humidity independently of one another. The HVAC system must also be able to accommodate the heat output of all equipment that will be maintained in the controlled space. Additionally, the operating temperature and humidity ranges for each piece of equipment utilized must also be taken under consideration. The introduction of additional equipment over the life of the bank, and therefore, cumulative heat load, must be taken into account when determining your HVAC strategy.

2.5 Air Quality

Will the HVAC system be able to meet and/or exceed the air quality specification for the manufacturing environment? The choice to manufacture in a classified or unclassified facility is a decision that will not only have tremendous impact on the design of the HVAC system servicing the manufacturing space but also how that system must be maintained going forward. Consideration must be given as to whether or not a classified space is required, that is to say, if critical manufacturing steps are performed in a Class II/ISO 5 Biological Safety Cabinet or if a functionally closed manufacturing system is employed. Pressure cascades should be designed to limit contaminants from entering the manufacturing space. If an ISO 7/8 space is intended/desired, then decisions must be reached regarding how much of the air will be recirculated, whether or not the air should be terminally high-efficiency particulate air (HEPA) filtered, how many air changes are required per hour to maintain the specifications set forth during facility design, and what amount of the air will be recirculated vs single pass. Maintenance of a classified environment will also result in additional costs for periodic recertification of the system and additional environmental monitoring burden.

2.6 Redundancy

Another important consideration for HVAC design in terms of cord blood licensure is redundancy within the HVAC system. Ideally, redundancy of all components is recommended, including, but not limited to, air handler(s), chiller(s), fan filter units, etc. Loss of the HVAC system, and therefore loss of control of the facility/manufacturing space, may result in the inability to "license" cord blood units manufactured during times when the system is operating out-of-specification (OOS). At a minimum, it would result in the need for the OOS result to be assessed or addressed in terms of the impact on the manufactured product and reported to the FDA. If redundant HVAC systems are in place, it can reduce facility downtime and the impact on the manufacturing process. Depending on the design, redundancy within the system may also allow for better control during periods of extreme weather conditions.

No matter which design choice is selected, it is of chief importance that the HVAC system implemented is able to perform in a manner that allows the facility to consistently meet the defined operational specifications demonstrating consistent control of the air quality in the manufacturing space.

2.6.1 MDACBB Heating, Ventilation, and Air Conditioning

The MD Anderson Cord Blood Bank HVAC system serving the classified laboratory space is designed to provide temperature conditioned air to 48 electric fan-powered HEPA filtration units, which supply the filtered air into the laboratory space. Supply airflow into the clean room plenum is maintained by constant volume valves balanced to maintain controlled air volumes to each space. Two separate air handlers (AHU-1A/1B), each running at 50% capacity, provide the air to the facility. If one air handler fails, the other compensates and is capable of maintaining the required airflow and air temperature to maintain proper function of the classified laboratory space. The air handler units are powered on emergency power circuits. All air supplied to the facility passes through HEPA filters, powered on an uninterrupted power supply, ensuring filtered air is always provided in the clean room. The HEPA filter units provide an air exchange rate of approximately 80 complete air changes per hour in the manufacturing suite and 65 air changes per hour in the testing suites. The air is returned to the plenum via the return air grills located around the perimeter of the room and recirculated through the fan-powered HEPA filter units.

A cascading pressure differential (Fig. 3) is maintained between each room of the facility, the adjacent rooms, and the outside. The manufacturing suite represents the point of highest pressure with all adjacent rooms at a lower pressure. This pressure differential is designed to prevent/limit potential contaminants from entering the manufacturing suite. The air handler units, fan filter units, exhaust fans, room temperature, humidity, and differential pressure cascade are monitored through the Building Automation Services (BAS) by MDACC facilities personnel. The BAS

system automatically notifies the facilities group when a monitored piece of equipment falls out of range. Room temperature, humidity, and differential pressure are also monitored through the annually calibrated, monitoring system maintained by the cord blood bank.

Qualification of the HVAC system, including the test parameters, acceptance criteria, results, and explanation of failures and excursions, was performed by an external vendor. The ISO-classified clean room is independently certified annually. The certification confirms that the facility performs in accordance with ISO standard 14644-1 Class 7 and 8 under operational/dynamic conditions [10]. The certification verifies the face velocity of each HEPA fan filter unit, HEPA filter operation, differential pressure between adjacent areas, room airflow velocity, airflow uniformity, room temperature, room humidity, and airborne particle count. The facility was most recently certified in February of 2021 and found to be operating well within specification (ISO 7 and ISO 8 Certificates). The MDACBB environmental monitoring program monitors the air quality of the clean room on a regular basis.

A separate, completely independent, HVAC system provides air to all nonclassified areas of the cord blood bank including the freezer room, supply storage, corridors, and office space.

2.7 Contamination Control

Prevention of contamination and cross-contamination is at the core of the requirements for cord blood licensure. Cleaning protocols/procedures touch all aspects of the program from facility to final product. A cleaning program must be designed, validated, and implemented in the facility in an effort to limit the potential transmission of organisms. "Selection of cleaning agents and implementing justifiable cleaning process parameters are critical prerequisites for cleaning validation. Consideration should be given to the method of action by which the agent works as well as the required cleaning/contact time necessary to kill the intended range of organisms on the target substrate/surface" [11].

2.7.1 Facility

Consistent and effective cleaning procedures are important in preventing the accumulation of dirt, dust, and other materials that can harbor pathogens and support their growth. A robust, validated program for cleaning of the manufacturing facility and all equipment therein is necessary to effectively minimize the overall bioburden present in the facility. There are a tremendous number of references available in print and online that discuss laboratory and clean room cleaning agents, methods, and equipment and there are a growing number of providers for these products. You must determine the elements that will meet the needs of your facility and build a program accordingly. The FDA will likely review this program in depth during

inspection. The list below is intended to provide a starting point for the development of a facility cleaning program.

- A documented cleaning schedule must be established and maintained.
  - Cleaning agents that cover the range of organisms typically found in the manufacturing environment as well as the range of organisms typically multiple cleaning agents should be identified and validated.
  - It is recommended that cleaning agents be rotated on a defined schedule to limit the potential for the development of resistant organisms isolated during sterility testing of the manufactured product must be identified.
  - The cleaning agents used must be prepared at the dilution/concentration specified by the manufacturer. Proper cleaner concentration and length of time applied are key elements of the agent's effectiveness.
- Dedicated cleaning equipment should be used exclusively for the manufacturing laboratory to limit potential contamination from sources outside of the facility.
- The cleaning equipment selection plays a key role in both the ease and effectiveness of the cleaning and decontamination of the facility.
- Other important considerations include the source of water used, single/multiuse mop heads, dusters, dedicated cleaning equipment (mop handle, buckets), and cleaning methodology such as top-down, single-pass cleaning.
- Standard operation procedures must detail the cleaning methodology including cleaning agents and equipment.
- Staff responsible for performing cleaning must have documented training against the standard operating procedure.
- Documented completion of the scheduled cleaning must include staff performing the cleaning as well as cleaning agents utilized.

2.7.2 Equipment

The FDA expects that "equipment used in the manufacture of HPC, Cord Blood must be of appropriate design, adequate size, and suitably located for its intended use and for its cleaning and maintenance" [21 CFR 211.63]. Furthermore, cord blood banks are expected to have written procedures detailing the processes used for the cleaning and decontamination of all pieces of equipment used in the manufacturing process. Manufacturer guidelines for each piece of equipment should be followed to ensure proper cleaning as well as continued function of the equipment. Cleaning of the equipment before and after every use must be documented, and the products manufactured using the equipment must be traceable to the piece of equipment used.

Per 21 CFR 211.67 (a–c)

- Equipment and/or utensils must be cleaned, maintained, and, as appropriate for the nature of the drug, sanitized, and/or sterilized at appropriate intervals to

prevent malfunctions or contamination that would alter the safety, identity, strength, quality, or purity of the HPC, Cord Blood [21 CFR 211.67(a)].
- SOPs for cleaning and maintenance of equipment, including utensils, must be established and followed [21 CFR 211.67(b).
- Records of equipment maintenance, cleaning, sanitizing, and inspection must be kept [21 CFR 211.67(c)].

Per CB BLA Guidance
- You should provide a brief description of the cleaning procedures and cleaning reagents used (for equipment cleaning procedures and validation). This section should also contain a certification that the cleaning validation for removal of product residues has been successfully completed.
- Procedures for decontamination and cleaning of equipment used to process material in closed containers when there is a breach in the container integrity and leakage of product onto the equipment.
- A written record of major equipment cleaning, maintenance (except routine maintenance such as lubrication and adjustments), and use must be included in individual equipment logs that show the date, time, product, and lot number of each batch processed [21 CFR 211.182]. For example, equipment logs must include the date, time, product, and lot number of each HPC, Cord Blood unit manufactured.

2.7.3 Supplies

Supplies used in the manufacture of HPC, Cord Blood, particularly any supply that comes into direct contact with the product, must be handled and stored in a manner to prevent contamination. When possible, all supplies selected for use should be sterile, single use, and consumables designed for the intended manufacturing purpose. Supplies should be stored off the floor in a manner that allows for cleaning and inspection [21 CFR 211.80 (b) and (c)]. When reasonable, the outer packaging of supplies should be cleaned with an approved cleaner prior to being brought into the manufacturing space. A flow diagram depicting how supplies flow through the manufacturing space is required to be included in the application for licensure. All supplies used in manufacturing must be visually inspected for contamination or damage prior to use.

2.7.4 Personnel

Personnel performing the manufacturing processes represent a significant potential source for product contamination through both direct and indirect interaction with the product. Efforts should be made to limit the potential contaminants that may be inadvertently carried into the manufacturing facility on personnel. This potential risk can be reduced through proper hand hygiene and gowning practices. Laundered

and/or disposable gowning and personal protective equipment, such as scrubs, lab coats, coveralls, disposable shoe, hair, and face coverings all act to protect personnel and limit the outside contaminants that can potentially be carried in on street shoes and clothing. The use of tacky mats and gowning airlocks are additional tools for reducing potential contaminants from being carried into the manufacturing space. A flow diagram depicting how personnel move through the manufacturing space is required to be included in the application for licensure.

21 CFR 211.28, and 600.10(c)

- Personnel should be properly gowned and practice good sanitation practices.
- Only personnel authorized by supervisory personnel should enter limited access areas of your facility.
- Anyone with an apparent illness that could adversely affect the product should report the condition to their supervisor and be excluded from direct product contact.

When implemented properly, a comprehensive proceduralized cleaning and decontamination system for the facility, equipment, supplies, and personnel can significantly reduce the potential for contamination of the manufactured HPC, Cord Blood product.

2.7.5 MDACBB Cleaning/Sanitization Practice

Facility

Cleaning of the MDACBB manufacturing facility is performed by trained staff, on a defined schedule. All cleaning agents are used according to the manufacturer provided instruction/specification(s). Daily cleaning at the completion of all manufacturing and testing activities includes cleaning of doors and handles, windows and ledges, outside of pass through boxes bases and support beams of tables and fume hoods, and chairs. All floors are dry and wet mopped daily using approved cleaners. The facility undergoes a terminal clean at least twice monthly in which all vertical surfaces are cleaned in addition to horizontal surfaces included in daily cleaning. Procedures and cleaning agents used for cleaning of the facility are defined in standard operating procedures. At the start of each production day, staff clean countertops, carts, equipment, and electronics with approved disinfectant, followed by wiping with 70% alcohol before initiating manufacturing processes.

Equipment

All equipment used in the manufacture of HPC, Cord Blood is cleaned before and after use for every product processed using the equipment. Equipment is cleaned using a quaternary ammonium cleaner followed by 70% isopropyl alcohol. Equipment that becomes contaminated due to a breach of container integrity is

removed from use until it can be decontaminated per manufacturer's instructions. All manufacturing operations are performed in a functionally closed system leaving no direct contact between the manufactured cord blood product and equipment. Because of this, validation of removal of cleaning agent residues was not required.

Supplies

All supplies and reagents required for the manufacture of HPC, Cord Blood are inspected upon receipt and are processed through a quarantine/release process. All supplies are cleaned with 70% isopropyl alcohol and enter the facility through pass-through boxes with interlocking doors.

Personnel

All staff working in the clean room are required to gown prior to entering the classified laboratory space. Primary gowning is performed in dedicated gowning room. All staff enter the gowning room by crossing a tacky mat (Dycem™) designed to reduce particulate carried on the soles of shoes. Once in the gowning room, staff wash their hands and then change into laundered scrubs and clean room shoes. Primary shoe covers are placed over clean room shoes. Disposable laboratory coats are worn over the scrubs. Staff don disposable hair bonnets and surgical masks and/or beard covers. Lastly, staff put on disposable gloves and clean the gloves with alcohol foam. Staff cross a second tacky mat prior to entering the clean corridor of the facility. Staff entering the manufacturing or testing suites pass through a secondary gowning room where they are required to don secondary shoe covers. Gloves are once again cleaned using alcohol foam immediately prior to entering the ISO 7 suites. Staff maintain full gowning for the duration of the time they spend in the clean room facility. Staff degown upon leaving the exit air lock and discard all disposable gowning materials.

2.8 Containment Features

Facility design should consider segregation and containment features for areas, operations, personnel, equipment, and waste materials to prevent potential contamination and cross-contamination of manufactured products. Containment features work hand-in-hand with cleaning and decontamination/contamination reduction processes. When designed and implemented properly, these features can act as barriers to potential sources of contamination.

The application for FDA licensure for HPC, Cord Blood manufacturing requires a description of the containment features employed by the manufacturer as part of the establishment description portion of the submission with a specific emphasis on

features of the facility air handling systems and equipment decontamination and cleaning. Potential design considerations for the air handling systems in addition to the previously discussed items may include but are not limited to:

- Segregation of the air handling units (AHU) supplying air to the manufacturing space
- Air supply and return including whether the air is recirculated, single-pass, and/or HEPA-filtered
- Establishment of air pressure differentials between adjacent manufacturing areas including positive and negative pressure manufacturing suites
- Implementation of air locks for employee entry into and exit from the manufacturing suite
- Pass-through boxes for introducing products, supplies, and reagents into the manufacturing space as well as removal of manufactured products and waste from the facility

Procedures detailing equipment decontamination in the event of a breach of container integrity and leakage into/onto laboratory equipment must also be developed and made part of the standard operating procedures to reduce any potential for cross-contamination of future lots of material manufactured on the same device. It is advisable that any procedure recommended or required by the equipment manufacturer be followed when available.

2.8.1 MDACBB Containment Features

The primary contamination control employed by the MDACBB is the use of a functionally closed processing system comprised of sterile, disposable, and single-use materials for the manufacture of HPC, Cord Blood. Function of the closed system has been confirmed though the execution of a media fill protocol as well as through the maintenance of product sterility as verified in a process validation.

As previously discussed, all manufacturing of HPC, Cord Blood is performed in an ISO Class 7 clean room facility. Entry to the facility requires employees to cross a tacky mat upon entry to the gowning suite. Facility access is badge-controlled and limited to staff with documented training of clean room gowning procedures. A magnetic interlock between the doors to enter the gowning room and clean corridor ensure that both doors cannot be opened at the same time. Magnetic interlocks are also employed in secondary gowning areas leading into the manufacturing and testing suites, as well as the facility exit to ensure that the pressure cascade between the manufacturing suite and all adjacent areas is maintained at all times. As detailed in this section under *MDACBB Heating, Ventilation, and Air Conditioning*, air is supplied to 48 electric fan-powered HEPA air filtration units by a pair of redundant air handlers. The filtered air in the facility is recirculated through return air ducting into the plenum of the facility and back to the HEPA filtration units. A differential pressure cascade is established between the ISO 7 and adjacent ISO 8 rooms within the facility. The manufacturing suite is maintained as the highest point of pressure to

prevent potential contaminants from entering the suite from any adjacent room. The air handers, fan filter units, and room differential pressure sensors are all continuously monitored through building automation services.

3 Quality System

As previously stated, the field of public cord blood banking is highly regulated and depending on the organization will have to meet regulations set forth by FDA, FACT, AABB, CAP, CLIA, and other accrediting/inspecting organizations. Central to all of the regulations is the need for a robust quality system (QS) that applies not only to the final manufactured product but to each element of the manufacturing process. The overarching control of the cord blood bank manufacturing facility rests with the QS that has been designed and implemented to ensure continuous quality of the manufacturing facility and processes within. Design of the QS should encompass all facets of the manufacturing facility and process such as materials management, laboratory controls, facilities and equipment, production, and packaging/labeling. Each of these represents a key system element that will be reviewed/inspected in depth during the review of the application for licensure, pre-licensure inspection, and all post-licensure inspections.

In September 2006, the FDA published the guidance "Quality Systems Approach to Pharmaceutical cGMP Regulations" [12], which lays out principles and requirements to ensure the quality of human biological drug products during manufacturing and compliance with cGMP regulations [21 CFR parts 210 and 211] through the implementation of robust quality systems. The guidance outlines six major concepts/requirements for the development of a robust cGMP manufacturing program.

- **Quality:** The established identity, strength, purity, and other quality characteristics designed to ensure the required levels of safety and effectiveness. The quality characteristics being defined extend beyond the manufactured product to the materials used during manufacturing, the manufacturing environment, personnel training.
- **Quality by design and product development:** The concept that the design of the manufactured product and processes used during product development ensure that the product meets a predefined quality at the end of the manufacturing process.
- **Quality risk management:** Risk management is a key component for the mitigation of risk to the quality of the manufactured product through the introduction of process, material, personnel, and environmental changes to the established quality specifications.
- **Corrective action and preventative action (CAPA):** A system designed to investigate, understand the root cause, and correct quality issues with the intent of preventing recurrence of the issue or similar issues.

- **Change control:** A system of managing change to prevent unintended consequences resulting in the inability to meet the quality specifications of the manufactured product. Change control must be employed for all policies, procedures, and systems including facility support and computer systems.
- **Quality unit (QU):** The personnel or group of personnel within an organization tasked with creation, implementation, maintenance, and monitoring of the entire quality system. The QU must operate independently of the manufacturing personnel. The final assessment of each manufactured lot must be reviewed and released or rejected by the QU.

Each of these systems/components is a key element of the QS and will be reviewed by inspectors in detail at all stages of licensure. When implemented effectively, these systems work in unison to ensure the maintenance of product quality and continuous quality improvement. The QU is responsible for the implementation, maintenance, and improvement of the quality management system through training, change control, document management, audits and quality document reviews, risk assessments, product deviation and CAPA reporting, validation of critical supplies, and processes and facility management. The QU must have full oversight of the manufacturing process and is responsible to:

- Ensure that cord blood units (CBU) collected, transported, processed, tested, cryopreserved, stored, listed, and released for administration have the identity, potency, and purity to which they pertain and are safe and effective.
- Maintain education and ongoing training of personnel to facilitate and understand the QS and ensure only trained and qualified personnel perform delegated tasks.
- Ensure CBUs are collected, processed, cryopreserved, listed, released, and distributed in compliance with all required standards and by trained staff.
- Ensure the manufacturing facility and process meets or exceeds regulatory and accreditation agency standards, regulations in compliance with local, state, and federal law and guidance documents.
- Ensure the management of risk assessments through development of product specifications, identification of critical process parameters, and application of the quality plan resulting in controlled change and maintenance of the QS.
- Make the final determination of product acceptability and release for registry listing and/or distribution.

As previously discussed in this chapter, the facility should, whenever possible, be designed around the intended manufacturing process, or when implementing a manufacturing workflow within an existing facility, considerations must be made for the manufacturing workflow that will allow for controlled, repeatable workflow. Furthermore, as the manufacturing process is developed, the intended quality aspects of the product and facility specifications intended to support the quality through the manufacturing process should be at the forefront of the design. Ideally, the manufacturing process will be developed in conjunction with the quality systems that support it. Development with input from the QU informing the process

will help ensure that the final manufacturing process is capable of producing a product that can consistently meet all quality specifications set forth in the guidance for industry documents.

3.1 Establishing Quality Specifications

3.1.1 Product Specifications (HPC, Cord Blood)

One of the first steps that must be undertaken is the establishment of manufacturing specifications for HPC, Cord Blood based on the manufacturing process implemented. The guidance for the CB BLA requires that a list and description of the specific tests which provide information regarding the safety, purity, potency, and identity of the product, and expected results of those tests must be provided with the application for licensure. Table 1 is adapted from the CB BLA guidance and outlines the required and recommended tests and test results required for a product to be licensable. Furthermore, the guidance specifies the minimum required tests to be performed, along with the expected results, for the unit to meet the minimum requirements for licensure.

3.1.2 In-process Specifications

These should also be defined so that each cord blood collection that enters the manufacturing process can be evaluated. These must be established and monitored to ensure that the product meets all specifications prior to release to the next stage of manufacturing. Any unit not meeting the specifications defined in the BLA is unable to be licensed and may need to be rejected/discarded. The specifications below only address the physical and measurable characteristics for the HPC, Cord Blood unit. The list provided is a reflection of common tests/evaluations performed during the manufacture and banking of HPC, Cord Blood and is not meant to be considered as prescriptive nor exhaustive. Each cord blood bank must define the quality parameters that fit the manufacturing processes defined and implemented at the facility. Additional determinations for HPC, Cord Blood acceptability for listing/release and donor eligibility [21 CFR Part 1271] [13] must consider the maternal donor risk and family medical history data collected as part of the cord blood donation process and be made prior to release for registry listing. Consideration should also be given to the requirements of external cord blood banking accreditation regulations such as, but not limited to, FACT and AABB.

Table 1 BLA guidance required and recommended tests and results

Product characteristics[b]	Testing[a]	Sample (type and timing)[a]	Specification
Safety	**Infectious disease testing:** Testing required (21CRF 1271.45 through 1271.90)	Maternal peripheral blood collected within 7 days of cord blood collection – type and timing required (21 CFR 1271.80(a) and (b))	All test negative, except, non-treponemal test for syphilis when specific treponemal confirmatory test is negative. (Cytomegalovirus (CMV) serology results are recorded)
			CMV report
	Sterility Bacterial and fungal cultures – testing required (21 CFR 211.165(b) and 21 CFR 610.12)	HPC, Cord Blood (pre-cryopreservation)[d]	No growth
	Hemoglobin	Cord blood[e] or appropriate donor sample obtained at time of cord blood recovery	No homozygous hemoglobinopathy
Purity and potency[c]	Total nucleated cells (TNC)	HPC, Cord Blood pre-cryopreservation	$\geq 5.0 \times 10^8$ TNC[f]/unit HPC, Cord Blood
	Viable nucleated cells	HPC, Cord Blood pre-cryopreservation	≥85.0% viable nucleated cells
	Viable CD34+ cells (flow cytometry)	HPC, Cord Blood pre-cryopreservation	$\geq 1.25 \times 10^6$ viable CD34+ cells[g]/unit HPC, Cord Blood
Identity	Human leukocyte antigen (HLA)	Cord blood	Report
	Confirmatory HLA typing	Attached segment of HPC, Cord blood	Confirms initial typing
	ABO and RhD	Cord blood	Report

[a]Testing, sample (type and timing), and results are recommended unless specifically noted as required

[b]The PHS Act requires a demonstration that the product is safe, pure, and potent

[c]Other purity and potency assays may be considered under the BLA

[d]Sample may be obtained before or after addition of the cryoprotectant

[e]Cord blood = a sample of unmanipulated cord blood. A red cell sample or other cord blood aliquot obtained after volume reduction may be used for testing with appropriately validated reagents or test systems

[f]Based on 20 kg recipient, a target does of $\geq 2.5 \times 10^7$ nucleated cells/kg and ≥70% post-thaw recovery $+1.7 \times 10^7$ nucleated cells/kg

[g]Based on CD34+ cells ≥0.25% of TNC prior to freezing

Table 2 Cord blood specifications: Release for manufacturing

Assessment	Sample	Analytical method	Specification
Time from collection to receipt at CBB	N/A	N/A	≤44 h post collection
Bag appearance	CBU	Visual inspection	Bag intact (no visible cracks, leaks, or other damage)

3.1.3 MDACBB Cord Blood Specifications

The specifications listed in Tables 2, 3, 4 and 5 have been provided as an example of how a cord blood bank could potentially employ in-process controls at different stages of manufacturing and release in order to ensure that the final product meets the minimum specifications for licensure as defined in Table 1 provided above.

3.1.4 Supplies and Reagent Specifications

Quality specifications must be set for all supplies and reagents used in the manufacturing process. All consumables and reagents must be traceable to the shipment. Whenever possible, sterile, disposable, and single-use supplies and reagents that are approved for human use should be used in the manufacture of HPC, Cord Blood. Supplies and reagents can be divided in two categories: critical and noncritical. A critical reagent or supply comes in direct contact with the cord blood intended for clinical use, whereas a noncritical supply or reagent does not. All supplies and reagent inventory must be assessed to meet predefined quality specification and maintained according to well-defined policies and procedures. The following are suggested methods for the assessment and maintenance of the supply and reagent inventory.

- **Receipt of supplies and reagents:** For all items received, quantity received is verified and receipt condition is noted (no visible damage, received within temperature, etc.) and documented. Damaged supplies or reagents should not be accepted into inventory and should be discarded or returned to vendor.
- **Storage of supplies and reagents:** All received items should be placed in quarantine in an appropriate environment as defined by the manufacturer (critical reagents and supplies) or in a designated area for storage and distribution (noncritical supplies). All supplies and reagents should be stored within temperature ranges specified by the manufacturer in continuously monitored environments. Critical reagents or supplies requiring qualification are quarantined until the qualification procedures have been completed and approved by the quality unit. A Certificate of Analysis should be procured if applicable.
- **Quality assurance review:** Quality assurance reviews all entries for critical and noncritical inventory documented on a product qualification log. This ensures that all qualification requirements are met before the inventory is released for use.

Table 3 Cord blood specification: Release for cryopreservation

Assessment	Sample	Analytical method	Specification
Time from collection to cryopreservation	N/A	N/A	Cryopreserved within 48 h of collection
Final product volume	Cryobag containing buffy coat-enriched CBU	Sepax cell separation system	25.0 ± 1 ml
Bag appearance	Cryobag containing buffy coat-enriched CBU	Visual inspection	Bag intact (no visible cracks, leaks, or other damage
CB appearance	Cryobag containing buffy coat-enriched CBU	Visual inspection	No visible clots > dime size, foreign material or contamination
Total nucleated cell count	Post-process sample of CBU	Cell count/ hematology analyzer	$\geq 9.00 \times 10^8$
Nucleated red blood cells	Post-process sample of CBU	Cell count/ hematology analyzer	<50% of total nucleated cell count

- **Supplies and reagent release:** Once an item fulfills all qualification criteria, it should be accessioned (product name, lot number, manufacturer, expiration date, receipt date, and quantity) into an inventory management system. The system must be able to differentiate between supplies in quarantined and released status and be capable of tracing back to each supply shipment received.
- **Use of supplies and reagents*:*** All supplies should be visually inspected immediately prior to use for appearance (integrity, i.e., breakage of seal or other abnormal appearance). Supplies or reagents utilized during the manufacture of HPC, Cord Blood must be recorded as part of the manufacturing record. The system should allow traceability of every supply and reagent associated with the manufacture of each HPC, Cord Blood unit.

3.1.5 MDACBB Supply Specifications

The MDACBB uses sterile, disposable, and single-use supplies and reagents that are approved for human use in the manufacture of HPC, Cord Blood. Supplies and reagents are divided in two categories: critical and noncritical. A critical reagent or supply comes in direct contact with the cord blood intended for clinical use, whereas a noncritical supply or reagent does not. Each supply has a documented set of specifications that have been predefined by the QU. These specifications include but are not limited to a visual inspection for damage, product sterility/sterilization, manufacturer Certificate of Analysis, and any other specifications determined to critical to the function of the supply within the manufacturing system. Each shipment of supplies received is accessioned into the InvyTrack™ inventory management

Table 4 HPC, Cord Blood specifications: Release for registry listing

Testing	Sample (type and timing)	Specification
Infectious disease testing: HbsAg Anti-Hep B core (HBC) Anti-HCV Anti-HIV-1/2 Anti-HTLV-I/II Anti-*Trypanosoma cruzi* (Chagas) HIV RNA/HCV RNA/ HBV DNA West Nile virus RNA Syphilis Cytomegalovirus (CMV) serology	Maternal peripheral blood collected within 7 days of cord blood collection	All test negative, except CMV and anti-Hep B core (HBC)
		Reactive HBC with nonreactive HBV DNA – listed and designated as ineligible for licensure
Sterility Bacterial and fungal cultures	Cord blood, post-processing red blood cell/red blood cell + plasma fraction	No growth at 14 days
Hemoglobinopathy	Infant donor peripheral blood collected at the time of cord blood collection or post-processing red blood cell fraction	No homozygous hemoglobinopathy
Total CD34+ count	HPC, Cord Blood pre-cryopreservation	$\geq 1.25 \times 10^6$
7-AAD dye exclusion assay – CD45+ cells		≥85.0%
7-AAD dye exclusion assay – CD34+ cells		≥85.0%
Colony-forming units (CFU)		Growth
ABO and RhD	Cord blood unit, pre-processing	Report
Human leukocyte antigen (HLA)	Cord blood unit post-processing red blood cell fraction	Report

Table 5 HPC, Cord Blood specifications: Distribution

Testing	Sample	Specification
7-AAD dye exclusion assay – CD45+ cells	Contiguous sample of cryopreserved HPC, Cord Blood	≥40.0%
7-AAD dye exclusion assay – CD34+ cells		≥70.0%
Colony-forming unit assay		Growth
Confirmatory HLA typing		Confirm initial typing

system and is confirmed to meet all predesignated specifications prior to being released for use at which point in time the supply receives a barcoded identifier that is can trace the supply from the receipt of a unique shipment to within the specific step the supply is utilized within the manufacturing process.

3.2 Vendor Qualification

A risk-based approach for ensuring that vendors (suppliers) of critical supplies and reagents conform to specified requirements, including quality requirements, begins with a documented assessment of potential vendors. A critical vendor is a vendor that supplies a supply, equipment, or services that are categorized as critical within the CBB collection, processing, and testing processes. Once a vendor has been selected, results of the vendor assessment are used to define the type and extent of control to be exercised over the supplier, supply, or service. Records of assessments, approved vendors, type and extent of control exercised over approved suppliers or supplies, and periodic reassessments must be maintained.

3.2.1 MDACBB Vendor Qualification

All requests to add suppliers to the approved list of critical suppliers are initiated through the CBB Change Control Procedure. This facilitates:

- Defining and documenting the requirements of the vendor thoroughly
- Evaluation of the proposed change and the potential risk to product identity, strength, quality, purity, or potency

Vendor assessment may include some or all of these principles, as applicable, and may involve multiple members of the CBB management team and CBB Inventory Team. All assessment activities are documented.

- Does the potential vendor have the ability to supply the service or product quantity required?
- Does the potential vendor meet our budgetary requirements?
- Requirements for vendors supplying donor testing services are at a minimum:
  - FDA tissue establishment registration for testing
  - A list of test kits/methods to ensure each test is approved by the FDA for HCT/P donor testing
  - Manufacturer's inserts for all tests
  - Evidence of periodic inspections and quality audits (current CAP, ASHI, or CLIA accreditation for the scope of testing required by CBB)
- How potential vendors meet the requirements
  - Request samples, if applicable, and assess for acceptability.

  - If it is a service provider, discuss the minimum requirements of the CBB and their ability to deliver the service.

- Identification of the best potential vendor
  - Compare the vendors based on their ability to meet CBB needs at a cost-effective price.
  - Evaluate historical performance if other supplies or services are procured through the vendor.
  - Evaluate top candidates to ensure their documentation provides everything needed to perform a thorough assessment. Examples of documents to request and review, if applicable:
    - Certificates of Analysis
    - Package inserts
    - Third-party certifications
  - Maintain records of vendors not selected during this process.
- Sole source provider: On occasions, a sole source will be identified. Change requests and assessments must be documented in cases were a sole source exists for a critical supply or service.
- Vendor evaluation
  - Assign a risk tier to vendors based on how critical the supply is to the final product.
    - Tier 1 – high risk
    - Tier 2 – moderate risk
    - Tier 3 – low risk
  - For each risk tier, defined controls for both initial approval and post-approval monitoring are described in Table 6 below.
  - For risk tiers requiring the completion of a vendor qualification form by the vendor, signed and dated internal document(s) addressing all questions may be an acceptable alternative. All documents submitted must be reviewed and approved by the quality unit.
- Vendor approval
  - Quality unit must document approval of vendor before vendor is added to list of approved suppliers.
- Post-approval
  - Complete applicable studies/validations for the supply or equipment.
  - Establish a quality agreement if applicable. Agreement must outline the responsibilities and communication requirements for each party for quality-related activities.

Table 6 MDACBB risk-based vendor controls

Vendor type	Tier 1 highest risk	Tier 2 moderate risk	Tier 3 low risk
Manufacturer (materials)	Quality agreement Initial onsite audit Post-Approval Annually monitor quality of shipments, internal quality control results, recalls, and continuation of applicable licensure/registration Biennial re-qualification – completion of vendor qualification Onsite audit in response to noncompliance with specifications and quality requirements	Initial – complete vendor qualification Certificate of Analysis or conformance is retained Post-Approval Annually – monitor quality of shipments, internal quality control results, recalls, and continuation of applicable licensure/registration Re-qualification, if applicable based on findings	Establishment of purchase order or long-standing order Annually monitor quality of shipments, internal quality control results, and recalls Re-qualify, if applicable, based on findings
Service provider/ consultant	Quality agreement Initial onsite audit Post-approval Annually – monitor quality of service and if applicable, continuation of licensure/registration Biennial re-qualification – completion of vendor qualification Onsite audit in response to noncompliance with specifications and quality requirements	Initial – complete vendor qualification Establishment of service agreement or purchase order Post-Approval Annually – monitor quality of service and if applicable, continuation of licensure/registration Re-qualification if applicable based on findings	Establishment of service Agreement or purchase order Annually – monitor quality of service

  - Once a vendor is selected and qualified, if applicable, written agreements should be developed and approved that include measurable performance indicators by which the vendor and supplies can be evaluated.

- Ongoing monitoring/re-qualification of existing vendors
  - Based on the risk tier assigned, ongoing monitoring of approved vendors is performed and may evaluate some or all of the items listed below. Refer to Table 6 for a list of vendor controls post-approval.

- Review of documentation provided by the vendor in response to vendor qualification request
- Renewal of applicable certifications/accreditations
- Review any problems associated with the supply or service provided by the vendor

– Vendors are monitored annually as part of the internal auditing process. The following criteria (as applicable) will be assessed for all established vendors, and re-qualification may be performed based on the findings:

- Quality of shipments
- Quality of service
- Internal quality control results
- Billing and invoicing
- Recalls
- Continuation of licensure/registration
- Compliance with quality requirements

– On-site audits may be required for reasons such as a noted decline in product quality, significant changes to a vendor such as ownership or location, or significant modifications to a special process/product.

3.3 Manufacturing Facility and Equipment Specifications

3.3.1 Specifications

Specifications set for the critical manufacturing space will need to be rigorous for the monitoring and maintenance of the BSC, whereas if the manufacturing will be performed in an ISO-classified clean room laboratory, the specifications for maintaining and monitoring control of the manufacturing space will be increased.

While there is not a definitive set of quality specifications for a manufacturing facility, a risk- based approach should be taken when assessing the parameters measured and monitored. If the manufacturing will be taking place in a classified facility, ISO 14644-1 addresses standards for clean room environments [10]. Below is a list of quality specifications that may be considered for a manufacturing facility:

- Environment
  - Room temperature
  - Room humidity
  - If ISO classified per standard 14644–1
- Airflow
- Air recirculation
- Air changes per hour
- Airflow uniformity

- Airborne particle count (at rest/dynamic)
- Differential pressure gradients between manufacturing rooms and ancillary/adjacent rooms
- Maximum staffing levels for environment
- Specification for cleaning
  - Frequency
  - Cleaning agents including contact time
- Number of concurrent units manufactured
- Gowning requirements
  - Disposable vs laundered gowning
  - Gowning coverage (minimal vs. complete coverage)
- Personnel
  - Showered, shaved, teeth brushed
  - Minimal skin moisturizers; no perfume, after shave, makeup

Regardless of the exact set of specifications determined for your manufacturing facility, it is key that reasonable standards are consistently achievable under normal dynamic use of the space be set. The ability of the facility to meet the specifications established must be measured and monitored. Any excursions from the specifications must be investigated and addressed. If a classified facility is used, periodic recertification of the facility is necessary to ensure continued performance.

A robust environmental monitoring plan for monitoring of critical manufacturing environments must be developed, implemented, maintained, and monitored. Operational (dynamic) and at-rest (static) samples for airborne microbial counts should be collected. The quality unit is responsible for reviewing and tracking results of environmental monitoring and investigating results outside the acceptance criteria. Investigations into out-of-specification results may include, but are not limited to, review of collection technique with collector, correlation of results across sample types, identification of the predominant microorganism isolated, positive sterility rates and microorganism identification, or increased monitoring over a specified period of time. Trending of results and the outcome of investigations should be reviewed periodically.

MDACBB Environmental Monitoring

The MD Anderson Cord Blood Bank has established an environmental monitoring (EM) program to monitor the environmental performance of classified laboratory suites and biological safety cabinets at defined intervals.

In addition to room temperature, humidity, and differential pressure monitoring, the cord blood bank monitors nonviable air particulate counts, active and passive airborne microbial counts, and surface microbial counts throughout the classified areas.

Each day prior to use, ISO 5 classified biological safety cabinets are monitored for nonviable air particulate counts. A 10-minute collection time, equivalent to 28.3 L of air, is utilized to evaluate the number of particles measuring ≥0.5 μm. Acceptance criteria are specified in the procedure for nonviable air particle counts area; excursions are reported to QU and investigated. Weekly, the passive airborne microbial count of each biological safety cabinet is assessed according to specified acceptance criteria for viable microbial counts.

Designated ISO 7 and ISO 8 areas within the gowning, testing, and manufacturing areas are monitored weekly for nonviable particle counts and viable airborne microbial counts. Operational (dynamic) and at-rest (static) samples for airborne microbial counts are collected. Viable airborne microbial counts are collected by aspirating air at a fixed speed and for a fixed time across plated culture media and are reported as colony forming units/volume of air monitored. Viable and nonviable sampling for a location are performed concurrently in order to correlate results.

The EM program also includes the semiannual monitoring of additional locations within the gowning, testing, and manufacturing areas. Annually, an ISO certification of the ISO 7 and ISO 8 areas is performed by a third-party provider.

The QU is responsible for reviewing and tracking results of environmental monitoring and investigating results outside the acceptance criteria. Investigations may include, but are not limited to, review of collection technique with collector, correlation of results across sample types, identification of the predominant microorganism isolated, positive sterility rates and microorganism identification, or increased monitoring over a specified period of time. Trending of results and the outcome of investigations are reported to the Cord Blood Bank Director during regular quality assurance meetings.

3.3.2 Equipment Specifications

Equipment used in GMP manufacturing of HPC, Cord Blood must be of appropriate design, adequate size, and suitably located to facilitate operations for its intended use and for its cleaning/maintenance [21 CFR 211.63]. The suitability of the manufacturing system, and therefore the equipment used, is proven through qualification which is highly dependent on the predetermined specifications for the product manufactured. Specifications must be set for the equipment used in the manufacturing process that are specific to the needs of the manufacturing process. Defined specifications for operation provide the ability to qualify and monitor that each piece of equipment is operating properly and therefore assuring the quality of the manufactured product.

3.3.3 General Guidelines for Equipment Selection

- The system should be suitable for its intended purpose within the manufacturing process.

- The system should be easily cleanable.
- The system should not negatively impact product quality.
- The system should comply with applicable technical rules.

Often equipment is able to perform more than one function. Qualification of the system is achieved through ensuring that the selected equipment can meet predefined endpoints for function. Equipment used must be qualified for the manufacture of HPC, Cord Blood prior to use in the manufacturing system. The qualification process should include:

- **Design qualification (DQ):** A verification that the equipment design meets the needs of the manufacturing system. This may include factors including equipment construction, size, location, ease of cleaning/sterilization, size, operating environment, and more. Whenever possible, devices approved by the governing regulatory agency are recommended (e.g., FDA 510 k).
- **Installation qualification (IQ):** A verification of alignment with design specifications. The IQ should confirm that the instrument has been installed properly. Consideration should be taken to review the manufacturer's installation requirements for the instrument.
- **Operational qualification (OQ):** A verification of alignment with functional specifications. This is used to ensure the instrument is performing as described by the manufacturer. Confirmation that the equipment is functional per the manufacturer including normal and abnormal/alarm states.
- **Performance qualification (PQ):** A verification of the ability of the equipment to meet with user required specifications as part of the manufacturing process.

Performance qualification is the successful proving that the equipment can achieve the user-defined specifications for the use of the equipment within the intended manufacturing system which includes the product, user, equipment, and facility. Whenever possible, equipment should be implemented in duplicate at a minimum to ensure manufacturing may continue should the primary equipment fail. All pieces of equipment need to be validated and/or qualified for use individually. Each piece of equipment must be uniquely identifiable and traceable to the manufacturing batch. A full maintenance history of each piece of equipment that illustrates that the equipment is routinely calibrated, inspected, or checked according to a written program designed to ensure proper performance [21 CFR 211.68(a)] must be maintained. A limited list of equipment specifications is included below to illustrate factors for consideration:

Instrument-Specific Specifications

- Uniquely identifiable
- Operating environment (location/room temp/humidity)
- Power requirements (voltage, emergency power/uninterrupted power supply (UPS))
- Calibration frequency/parameters
- Quality controls for instrument setup (hematology counter/flow cytometry)
- Computer integration/interface

Process-Specific Equipment Specifications

- Fill volume
- Product temperature range
- Length of time to perform specified manufacturing step
- Operating temperature (freezers, refrigerators, incubators)
- Quality controls for instrument performance (assay-specific controls)

A system or systems for continuous monitoring of critical equipment should be implemented that includes an audited history of each item monitored and has the ability to alert the appropriate personnel 24 h a day, 7 days a week. When possible, process-specific equipment specifications should be set for both alert and alarm level functionality for equipment such as incubators, refrigerators, and freezers to ensure that trained staff can be notified in time to recover the equipment such that continuous quality control of the process is possible and the manufactured product quality is not compromised.

MDACBB Equipment Specifications

All equipment housed in the cord blood bank is maintained and used exclusively for the preparation and manufacturing of HPC, Cord Blood. Equipment used in the manufacture of HPC, Cord Blood has been qualified to be of appropriate design, adequate size, and suitably located for its intended use and for its cleaning and maintenance. IQ/OQ/PQ specifications based on the manufacturer's requirements and use in manufacturing are predefined by the QU. Specifications include but are not limited to power requirements, size, space, temperature/humidity, and software version requirements for function within the defined manufacturing process. All equipment is qualified, calibrated, and maintained with preventative maintenance performed as per the manufacturer's specifications and tolerance required for validation, qualification, and operation prior to use in the manufacturing process. Any change of equipment used in the manufacturing process that was not submitted as part of the application for licensure requires that the FDA be notified prior to implementation of the change.

4 Validations

Validation is defined as a process intended to establish documented evidence, which provides a high degree of assurance that a specific process will consistently produce the expected outcome meeting its predetermined set of specifications and quality attributes and be fit for intended purpose. Validation is an important element of quality systems and cGMP regulations. All critical procedures, equipment, testing methods, and processes that have an impact on the integrity, viability, safety, potency, or identity of a CBU must be validated. This applies to any area of CBU

manufacturing: collection, transportation, testing, processing, cryopreservation, storage, thawing, and release for distribution. Any backup process or equipment to be used for downtime/redundancy must also be fully validated in order for the product to qualify as licensed.

Any major change to a process, procedure, equipment/supply, reagent, or environment requires revalidation. Any change to any critical supply, equipment, container system component, or process defined in the application for which licensure was granted will necessitate a change to the CMC and an appropriate notification or request be submitted to the FDA for review and approval.

The application for FDA licensure requires submission of an overall process validation and batch records for a minimum of three consecutive, HPC, Cord Blood units to demonstrate consistent manufacturing of products meeting all defined manufacturing specifications. It is highly recommended that you consider submitting a validation plan for review by FDA prior to submitting your application for licensure.

4.1 Process Validation

A detailed summary of the validations performed for the cord blood manufacturing process must be submitted with the application for licensure and must include at a minimum, validations for cord blood collection, processing, storage, shipment, thawing, and cryoprotectant removal. As outlined in the 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 (CB BLA), the summary must include data from the manufacture of a minimum of three consecutive, separate, HPC, Cord Blood units that meet the product characteristics as well as all additional specifications defined for the manufactured product as part of the quality system.

4.2 Methods Validation/Verification

Methods validation is the process of demonstrating that an analytical procedure is suitable for its intended purpose. An analytical procedure is developed to test a defined characteristic of the drug substance or drug product against established specifications for that characteristic.

Parameters to be evaluated (not all parameters may apply to an analytical procedure):

- **Specificity:** Specificity is the ability to assess unequivocally the target analyte in the presence of components which may be expected to be present.

- **Linearity:** The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.
- **Accuracy:** The closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value, and the value found.
- **Precision:** The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision, and reproducibility.
- **Repeatability:** Repeatability expresses the precision under the same operating conditions over a short interval of time.
- **Intermediate precision:** Intermediate precision expresses within laboratories variations: different days, different analysts, different equipment, etc.
- **Reproducibility:** Reproducibility expresses the precision between laboratories.
- **Range:** The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity.
- **Quantitation limit:** The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.
- **Detection limit:** The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.
- **Robustness:** The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

The Guidance for Industry: Analytical Procedures and Methods Validation for Drugs and Biologics [14] serves as a reference in the determination of which parameter applies for a given test methodology. The CB BLA guidance requires that the validation of all test methods utilized to confirm the specifications for identity, purity, potency, as well as methods used to determine lot-to-lot consistency of the manufactured product be submitted with the application for licensure. It is further recommended that FDA-approved, licensed, or cleared test kits and reagents should be used whenever available. Despite prior approval, FDA expects that test methods performed using these kits are validated in house. Comprehensive experiments must evaluate and document the quantitative performance of an assay, including sensitivity, specificity, accuracy, precision, detection limit, range, and limits of quantitation. Full-assay validation will include inter-assay and inter-laboratory assessment of assay repeatability and robustness.

The assays required for HPC, Cord Blood might include the following:

- Sterility (bacterial and fungal cultures)
- Hemoglobin testing
- Nucleated cell counts (total nucleated cells/nucleated red blood cells)
- Nucleated cell viability assay(s)
- CD34+ cell viability (flow cytometry)
- HLA typing
- ABO blood group and Rh type
- Colony-forming unit assay (CFU)
- Other tests used to confirm identity, purity, and potency of the manufactured HPC, Cord Blood unit

4.2.1 MDACBB Validation Experience

Validation for each of the processes listed above was performed. Some validations were performed as part of the overall process validation while others such as sterility and flow cytometric assays were performed independently and then included within the overall process validation. During inspection, each validation was reviewed in detail as part of the application. Working closely with the FDA during the pre-licensure process to determine the specific parameters and replicates required for approval can greatly reduce the number of iterations that must be performed to provide the validation data expected.

5 Stability Program

Stability is defined by the FDA as the capacity of a drug substance or drug product to remain within specifications established to ensure its identity, strength, quality, and purity throughout the retest period or expiration dating period. A key component of the application for licensure is the definition of the stability program utilized by the cord blood bank. There are two types of stability testing that need to be included to ensure a robust stability program.

5.1 Long-Term Stability (Pre-licensure)

This is used to determine and/or extend the expiration period for HPC, Cord Blood manufactured as described in the application for licensure. This can consist of thawing, washing, and testing the oldest units manufactured per the manufacturing methods described in the application for licensure and evaluating whether or not the units

continue to meet the specifications defined and submitted with the application. This testing may include CBU manufactured prior to licensure.

5.2 Ongoing Stability (Post-licensure)

This is used to determine that units manufactured post-licensure continue to meet the specifications described in the application for licensure. This might consist of thawing, washing, and testing three or more units spanning the previous year of manufacturing, and at least one unit from each year of manufacturing post-licensure.

An example is provided below.

Year 1 post-licensure	3 CBU spanning manufacturing year 1
Year 2 post-licensure	3 CBU spanning manufacturing year 2 1 CBU from manufacturing year 1
Year 3 post-licensure	3 CBU spanning manufacturing year 3 1 CBU from manufacturing year 2 1 CBU from manufacturing year 1
Year 4+	Follow the pattern defined above for all subsequent years during which licensed products will be manufactured

Although it should be confirmed with the FDA as part of the licensure process for your bank/facility, the FDA has allowed banks to utilize units that meet all of the manufacturing specifications defined in the application for licensure but are unable to be licensed/released due to other factors such as donor and family health history for use in stability testing due to the precious and unique nature of each unit of HPC, Cord Blood manufactured and banked.

5.2.1 MDACBB Stability Program

The MDACBB stability program was initiated in 2016 at which point in time, three units processed during the first year of CBB operation, three units from fifth year of operation, and three units from 2016 were thawed and assessed. Every subsequent year, three units representing the earliest representative processing point available are selected, thawed, and evaluated as described in Table 5. The selected units must be manufactured and meet all manufacturing specifications as defined by CBB standard operating procedures but are not required to meet all clinical requirements for listing. Post-licensure, ongoing stability is performed by selecting three units spanning the manufacturing year and thawing, washing, and evaluating each unit to ensure that current manufacturing process continues to produce units that meet the specifications set for HPC, Cord Blood. Each subsequent year, one unit from each

Table 7 MDACBB stability program and acceptability criteria

Test	Result acceptance criteria
Bacterial/fungal contamination	No growth at 14 days
Total nucleated cells (TNC) count recovery	≥70% recovery (Post wash TNC/pre-cryopreservation TNC)
CD34+ % viability	≥70% viable
CD45+ % viability	≥40% viable
CFU assay	Growth
Post thaw use of the CBU cryobag/tubing	No leaks noted during the manipulation of the CBU cryobag/tubing throughout the thaw procedure

manufacturing year post-licensure will be evaluated in addition to the three units from the current manufacturing year. The selected units must be manufactured and meet all manufacturing specifications as defined under the license but are not required to be licensed units. Units having unique or rare characteristics may be spared from selection for stability testing.

CBB Directors and Quality Management of the MD Anderson Cord Blood Bank review the assembled stability data and based on the results determine an expiry date for the cryopreserved cord blood units being stored for clinical use (Table 7).

5.2.2 Out-of-Specification (OOS) Results

A root cause investigation is completed. If no assignable cause for the OOS result can be identified as a result of the investigation, all results are included in the determination of an expiry date.

If an assignable cause for the OOS result is identified, a minimum of three additional units with manufacturing dates adjacent to each failed unit are selected, prepared, and evaluated as described above.

6 Conclusions

- Obtaining the BLA was a major effort requiring the expertise of many different groups, facilities, laboratories, collection and processing leaders, engineers, administrators, and our institutional leaders.
- The process of applying for and maintaining FDA licensure is a major commitment financially and administratively, and support from our institution was critical to the success of obtaining licensure for the manufacture of HPC, Cord Blood.
- It is highly recommended that a strong collaborative relationship be developed with the FDA personnel who are working with you during the application process. The FDA is collegial and willing to work with applicants to obtain the BLA. The shared goal of both the manufacturer and the FDA is to produce HPC, Cord Blood products that consistently meet all identity, purity, and potency spec-

ifications while reducing the risk of transmission of communicable disease. Establishing an early partnership when designing your manufacturing facility and process can help guide and ensure that all requirements and concerns have been addressed prior to submission for licensure.

- There are a number of ways to meet the requirements for licensure that do not require a clean room facility. A clean room/classified manufacturing facility does not make the manufacturing process cGMP compliant. The systems implemented that provide a controlled, documented manufacturing process capable of consistently producing products meeting set specifications for identity, purity, and potency are how cGMP compliance and subsequent licensure for the manufacture of HPC, Cord Blood are achieved.
- Establish your validations early and with guidance from FDA to ensure all necessary information and testing is included. This will reduce the need for additional validations or revalidations to be performed prior to licensure and can greatly reduce the length of the licensure process.
- HPC, Cord Blood is increasingly being seen as a starting material for the development of cellular therapeutics and FDA licensure provides a highly qualified, off the shelf, cGMP grade product for use in the development of biologic drugs. It is highly likely that this is the future for the cord blood banking industry.

References

1. Foundation for the Accreditation of Cellular Therapy website. http://www.factwebsite.org/. Last accessed 4 Aug 2020.
2. AAABB Website. http://www.aabb.org/Pages/default.aspx. Last accessed 4 Aug 2020.
3. American Society for Histocompatibility and Immunogenetics website. https://www.ashi-hla.org/. Last accessed 4 Aug 2020.
4. College of American Pathologists website. https://www.cap.org/. Last accessed 4 Aug 2020.
5. Clinical Laboratory Improvement Amendments FDA website. https://www.fda.gov/medical-devices/ivd-regulatory-assistance/clinical-laboratory-improvement-amendments-clia. Last accessed 4 Aug 2020.
6. Food and Drug Administration Center for Biologics Evaluation and Research. (2014, March). *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. Guidance for Industry*. https://www.fda.gov/media/86387/download. Last accessed 4 Aug 2020.
7. US Food and Drug Administration. *Part 210. Title 21 Code of Federal Regulations. Current Good Manufacturing Practice in Manufacturing, Processing, Packing or Holding of Drugs: General*. www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=210. Last accessed 4 Aug 2020.
8. US Food and Drug Administration. *Part 211. Title 21 Code of Federal Regulations. Current Good Manufacturing Practice for Finished Pharmaceuticals*. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=211 Last accessed 4 Aug 2020.
9. National Cord Blood Inventory Contract Summary (NCBI). *Health Resources and Services Administration website*. https://bloodstemcell.hrsa.gov/about/contracts/national-cord-blood-inventory-contract-summary-ncbi. Last accessed 4 Aug 2020.

10. ISO 14644-1:2015. *Cleanrooms and associated controlled environments — Part 1: Classification of air cleanliness by particle concentration*. https://www.iso.org/obp/ui/#iso:std:iso:14644:-1:ed-2:v1:en. Last accessed 4 Aug 2020.
11. Verghese, G. (1998). *STERIS Corporation. Selection of cleaning agents and parameters for cGMP processes*. https://pdfs.semanticscholar.org/a269/f0e16ea861fe74f543f1263596edb0524601.pdf. Last accessed 4 Aug 2020.
12. U.S. Food and Drug Administration. (2006, September). *Center for Drug Evaluation and Research (CDER) Center for Biologics Evaluation and Research (CBER) Quality Systems Approach to Pharmaceutical CGMP Regulations. Guidance for Industry*. https://www.fda.gov/media/71023/download. Last accessed 4 Aug 2020.
13. US Food and Drug Administration. *Donor Eligibility. Subpart C, Part 1271, Title 21 Code of Federal Regulations*. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=1271&showFR=1&subpartNode=21:8.0.1.5.60.3. Last accessed 4 Aug 2020.
14. Food and Drug Administration. (2015, July). *Center for Drug Evaluation and Research (CDER) Center for Biologics Evaluation and Research (CBER) Analytical Procedures and Methods Validation for Drugs and Biologics. Guidance for Industry*. https://www.fda.gov/media/87801/download. Last accessed 4 Aug 2020.

Indiana University Vector Production Facility (IUVPF)

Daniela Bischof and Kenneth Cornetta

1 Background

Our current vector production facility was a labor of love that took almost a decade from concept to occupancy [1]. In 2002, we submitted a NIH Construction Grant (NCRR C06-RR020128-01) in order to incorporate a GMP cleanroom into the design of a new research building being developed for the Indiana University School of Medicine. After a successful grant review, cleanroom design, construction, and commissioning of the building, the cleanroom was released to us in 2009.

A number of considerations led to the ultimate design of the facility. Our institution had decided we would not generate licensed products. Our major focus was, and remains, the generation of Phase I/II products to assist investigators in their initial gene therapy clinical trial. As our experience was in retroviral and lentiviral vectors, our design focused on these vector systems. For example, these vectors are usually frozen with minimal delay at the site of harvest and processing. Therefore, a specific fill-finish was not incorporated into the design.

A significant challenge was the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules, which at the time recommended lentiviral vector manufacture of greater than 10 l be performed at BSL3 containment. This presented a major issue for cleanroom design, since BSL3 facilities are designed to contain pathogens within the facilities, while cleanrooms are designed with the opposite purpose. To meet these conflicting design requirements, each production suite has individual negative pressure entrance and exit anterooms. These rooms act as air sinks, venting air from the production suite and the outer corridors thus minimizing contamination of the product and the building. Biosafety cabinets within the production suites are also vented to the outside to further limit

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contamination. This complex design required careful balancing of air pressures among rooms and was the major design challenge for the architects and engineers. The air handling system is also independent from all other air handling systems in the building.

While complex, the air handling system does facilitate multiple products being generated at one time given that the production suites do not open onto a common hallway. As the facility operates on a one-way flow of personnel, a passageway was incorporated into the design so individuals could pass from the pre-production to the post-production space without having to enter a production suite. This facilitates maintenance and other activities. Retroviral and lentiviral vectors require storage at −70 to −90 °C, so multiple outlets and monitoring ports were incorporated into the design so quarantined products could be maintained under GMP conditions within the facility while release testing was being conducted. A pass-through autoclave was also incorporated into the facility. Consideration was given to putting a liquid nitrogen portal into the facility to enable the refilling of cryogenic storage vessels, but discussion with others at the time raised the possibility of compromise to the facility integrity at the area where liquid nitrogen would pass through the wall. It appears new systems are more reliable, and we would probably have incorporated them had they been available.

2 Facility and Support Areas

The IUVPF consists of approximately 4300 ft^2 (~400 m^2) of cleanroom space and occupies a portion of the top floor of the Joseph Walther Hall research building. It is an ISO class 8 cleanroom with four ISO class 7 production suites (Figs. 1 and 2).

All personnel enter the facility via the *personnel entry anteroom* that is divided into a dirty and clean side. Prior to crossing over to the clean side, full sterile cleanroom-grade gowning is required. The *pre-production area* (Fig. 3a) houses fridges and freezers for storage of reagents utilized for GMP productions, and the *storage room* provides space for portable reusable equipment, single-use supplies, and additional freezers. Reagents that require preparation (such as media and buffers) and supplies that require assembly (e.g., supernatant clarification filter units and final product bag kits) are formulated in the biosafety cabinet (ISO class 5) located in the *media prep room*. The *media prep room* also holds a water purification system that provides sterile water for humidifying incubators and for reconstitution of disinfectants for cleaning. All materials used for productions are transferred into the cleanroom via the *materials entry anteroom*. Each of the four *production suites* (Fig. 3b) has a dedicated entrance and exit anteroom. Two of the suites have two biosafety cabinets each to allow for the extensive processing involved during lentiviral productions. The other two suites have one biosafety cabinet in each and permit adequate processing space for retroviral productions and the generation of master cell banks. Fully exhausted Class II B2 biosafety cabinets (ISO class 5) in the suites provide the primary containment during open manipulations. Each

Fig. 1 Floor plan of the Joseph Walther Hall sixth floor. IUVPF areas are highlighted

production suite also contains two to three large reach-in incubators to accommodate large-scale vector production. The *post-production area* has a sink to dispose of decontaminated liquids, and a large pass-through autoclave for decontamination of solid waste. The loading end is located in the *post-production area*, and decontaminated waste is retrieved via the *autoclave room* that is located exterior to the cleanroom. The autoclave room also houses a dedicated CO_2 manifold system and several CO_2 tanks that service the incubators in the production suites. Vector products manufactured at IUVPF are stored in the *freezer room* that contains ultralow temperature freezers for both quarantined (i.e., awaiting certification per FDA requirements) and released (i.e., fully certified) products. The *dewar room* houses cryogenic storage vessels where certified master cell banks used in the generation of viral vectors are stored in liquid nitrogen vapor phase. Decontaminated reusable

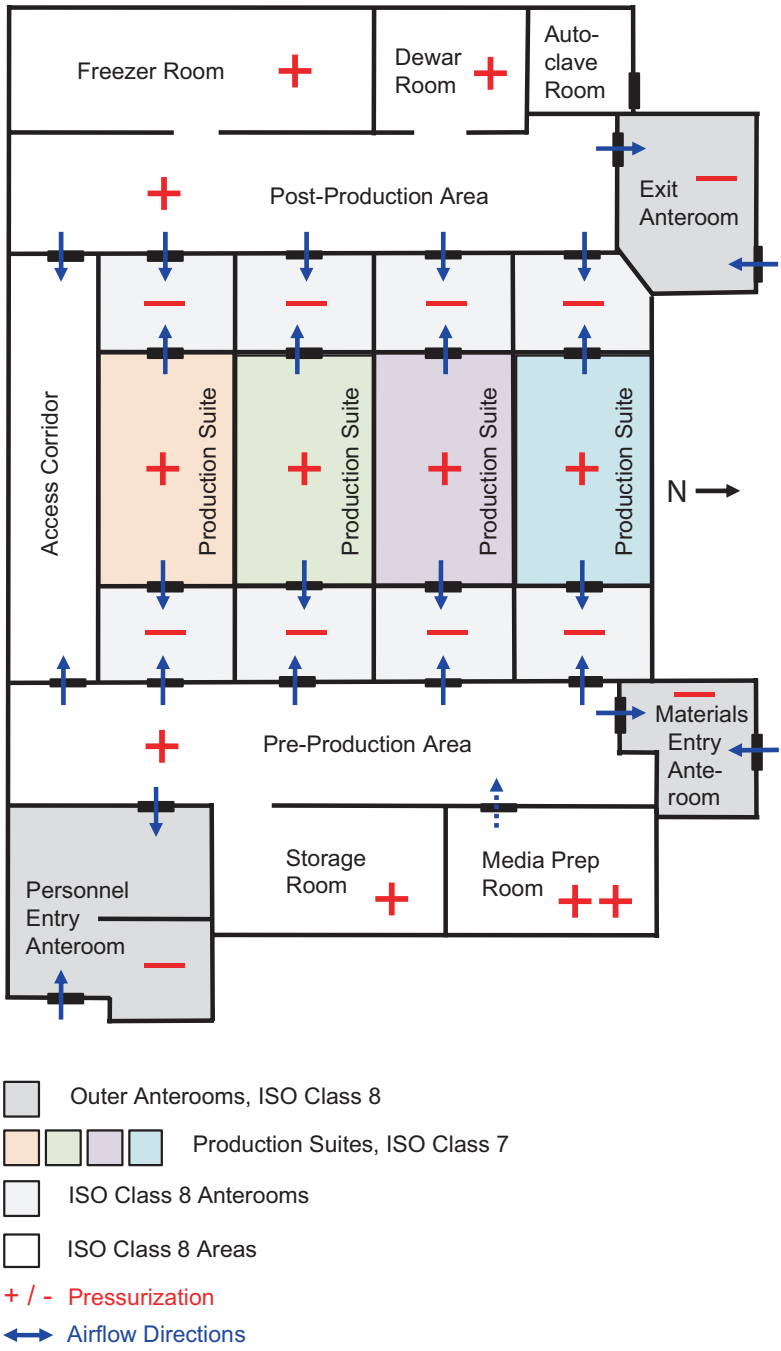


Fig. 2 ISO classification, room pressurization, and airflow patterns in the IUVPF cleanroom

(A)

(B)

Fig. 3 Pre-production area (top) and production suite
(**a**) Pre-production area. The four doors on the left-hand side lead into the production suites. The doorway to the right is the entry to the storage room. The door at the far end leads from the materials entry anteroom into the pre-production area.
(**b**) One of the four production suites containing two biosafety cabinets

equipment is transported via the *access corridor* from the *post-production area* to the door abutting the *pre-production area*. Personnel can then retrieve this equipment from the *pre-production area* without necessitating the removal of this equipment from the cleanroom. Personnel may also pass from the *pre-* to the *post-production* area via the *access corridor*. All personnel and materials exit the facility via the *exit anteroom* leading from the *post-production area*.

Adjoining but isolated from the facility is the ~2500 ft^2 (232 m^2) *mechanical room* that is maintained by campus facility services. It harbors the dedicated air handling system for the cleanroom. This includes a humidification system that feeds into the air handler. The air handling unit has two supply fans and motors associated with it. The fans are sized such that one at full capacity is sufficient to maintain static status for the facility. Both fans run constantly to prevent any type of damage that can occur when one motor sits idle for extended periods. Running both fans and motors allows each to run at a lower speed. Should one of the fans fail, the other automatically ramps up to the required capacity to meet the static set point. Located in the *mechanical room* is also a reverse osmosis water system that supplies the input water for the water purification system located in the *media prep room*. Housed here are also the pressure relief dampers for each of the *production suites*. These allow for the release of surplus air should the pressures in the suites get too high, thus preventing the ceilings and walls in the *production suites* from becoming damaged or blowing out. Physically separating this area from the cleanroom is a design feature that permits repairs even while the facility is operational. Additionally, no equipment is located above the ceilings in the cleanroom – HVAC equipment such as terminal boxes, bubble tight dampers, actuators, and exhaust valves are all located in readily accessible areas outside of the facility.

Adjacent to the *mechanical room* is the *facility systems closet*. This area holds the building management system (BMS) that exclusively services the cleanroom and associated areas. The BMS monitors the facility 24/7. It maintains the room pressurizations, temperatures, and humidities within the established acceptable ranges. It also monitors critical equipment such as in-use incubators, ultralow temperature freezers, and cryogenic storage vessels. Rotating personnel is on call at all times to address any facility or equipment alarms that may occur.

External to the cleanroom are two *storage rooms* totaling approximately 460 ft^2 (43 m^2). These rooms are used to house incoming quarantined materials, and QA released items prior to transferring into the cleanroom for use in GMP campaigns.

Numerous projects require smaller-scale productions to complete studies (such as animal toxicity studies) prior to initiating a gene therapy clinical trial. Either research grade or GMP-comparable product is suitable for these purposes, and such runs do not require a cleanroom environment. IUVPF has research space (~150 ft^2/14 m^2) within a BSL3 laboratory to manufacture small-scale preclinical vector products.

Office space for management and QA, production personnel workspace, and support areas encompass approximately 468 ft^2 (44 m^2).

A controlled access system is in place that limits access to the cleanroom, autoclave room, facility systems closet, storage rooms, the BSL3 lab, and the office and

support areas. The system allows for tracking and access reporting thus complying with GMP requirements. A video monitoring system was also installed in the outer entrance and exit anterooms to further enhance traceability.

3 Heating, Ventilation, and Air Conditioning (HVAC) Considerations

The temperature in the entire cleanroom is regulated to be ≤25 °C. When necessary the air is humidified to maintain a range of 20–60% humidity. The steam-to-steam humidification system utilized reduces the introduction of chemical contaminants from the boiler steam into the cleanroom.

The cleanroom air is 100% exhausted, i.e., the supply is 100% fresh outdoor air, which means there is no return from the facility back into the supply. All supply air is HEPA filtered. Production suites are exhausted through the biosafety cabinets and include supplementary low exhausts to maintain downward airflow. Exhaust air is HEPA filtered prior to discharge. To ensure appropriate laminar flow, the supply air grills are located in the ceilings, whereas exhaust grills are located as low as possible in the cleanroom walls. HEPA filters are challenged at least annually to ensure integrity of the filters.

Airflow changes are maintained as >10 changes per hour for the outer anterooms leading to and from the facility from the "dirty" exterior hallways. All other ISO class 8 areas are kept at >20 changes per hour. Although the requirement for ISO class 7 is 30–60 air changes per hour, IUVPF maintains a more stringent 90–100 air changes per hour.

To meet the requirement for ISO class 7 standards, the *production suites* are positively pressurized in relation to their entrance and exit anterooms. Similarly, the *pre-* and *post-production areas* are positively pressurized compared with the inner (to and from *production suites*) and outer (entry into and exit from cleanroom) anterooms. Since materials for GMP productions are prepared in the *media prep room*, it is maintained at double positive pressure (Fig. 2).

Minimal differential air pressures within all areas of the cleanroom must be maintained at ≥0.04" water. However, IUVPF aims to sustain differential pressures in the 0.08–0.12 inches of water range. Differential pressure monitors are located at all the entrance and exit points to and from the facility, as well as at all doors within the cleanroom. These visual checks allow personnel to confirm that differential pressures are within acceptable limits.

Nonviable particle counts are maintained as per ISO standards, i.e., counts cannot exceed 100,000 particles/ft^3 in ISO class 8, and 10,000 particles/ft^3 in ISO class 7 environments (0.5 μm size). IUVPF has established particle count alert values that are significantly below the ISO criteria.

4 Maintaining Integrity of Vectors Manufactured by IUVPF

To maintain the integrity of vector products generated by IUVPF, which translates to minimizing the potential for product contamination, a personnel and material flow regimen was implemented for the cleanroom. Unidirectional flow of personnel provides the highest degree of stringency – it is accompanied by additional gowning requirements as cleanliness escalates. This includes donning a second pair of sterile gloves when entering an ISO class 7 from 8 environment and then replacing the second pair of gloves and adding sterile sleeves prior to performing open manipulations in an ISO class 5 biosafety cabinet. Acceptable flow patterns for personnel, equipment, and materials are illustrated in Fig. 4a, b.

The cleanroom was designed to minimize surfaces that could potentially harbor microorganisms. The walls and ceilings are all solid and seamless. Walls of the *production suites* are encased in vinyl that is resistant to the disinfectants used for cleaning. The facility has seamless epoxy floors with raised edges. These surfaces minimize areas where microbes can reside and allow for more effective cleaning of the facility.

Robust daily, weekly, monthly, and semiannual facility cleaning procedures are in place to minimize the introduction of microbial contaminants into the cleanroom. Verification of the efficacy of the cleaning program is achieved by performing monthly environmental monitoring (EM), including settling and contact plates, and microbial air sampling. Stringent acceptance criteria have been established for all IUVPF EM.

In addition to standard cleanroom EM, IUVPF performs EM on the inside of refrigerators used for the storage of materials utilized for GMP productions; this includes refrigerators outside of the facility. Each month contact plates are touched to randomly selected materials taken into the cleanroom. Both procedures assist in tracking potential microorganisms transferred into the cleanroom.

5 Lessons Learned

Cabinets composed of a particle board core and coated with high-pressure decorative laminate were incorporated into the original design throughout the facility, including two of the *production suites*. Such surfaces are no longer considered appropriate for GMP cleanrooms and have been replaced with stainless steel counters, tables, and shelving.

Liquid nitrogen tanks from the vendor cannot be disinfected adequately to enable transport into the cleanroom; thus, tanks are cleaned and retained in the *exit anteroom*. Cryogenic storage vessels located in the *Dewar room* must be wheeled into the exit anteroom for filling. As noted in the background section above, a piping system leading into the *Dewar room* for the dispensing of liquid nitrogen would have been optimal.

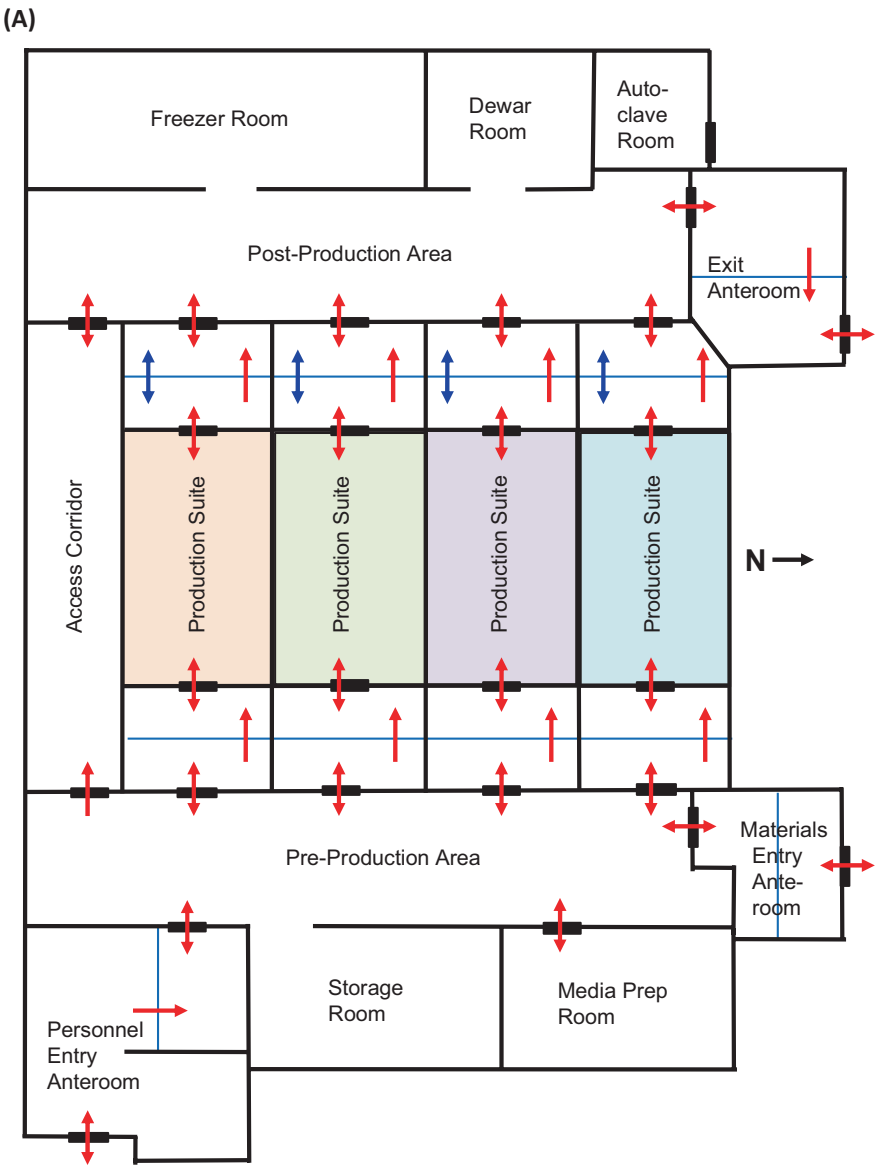
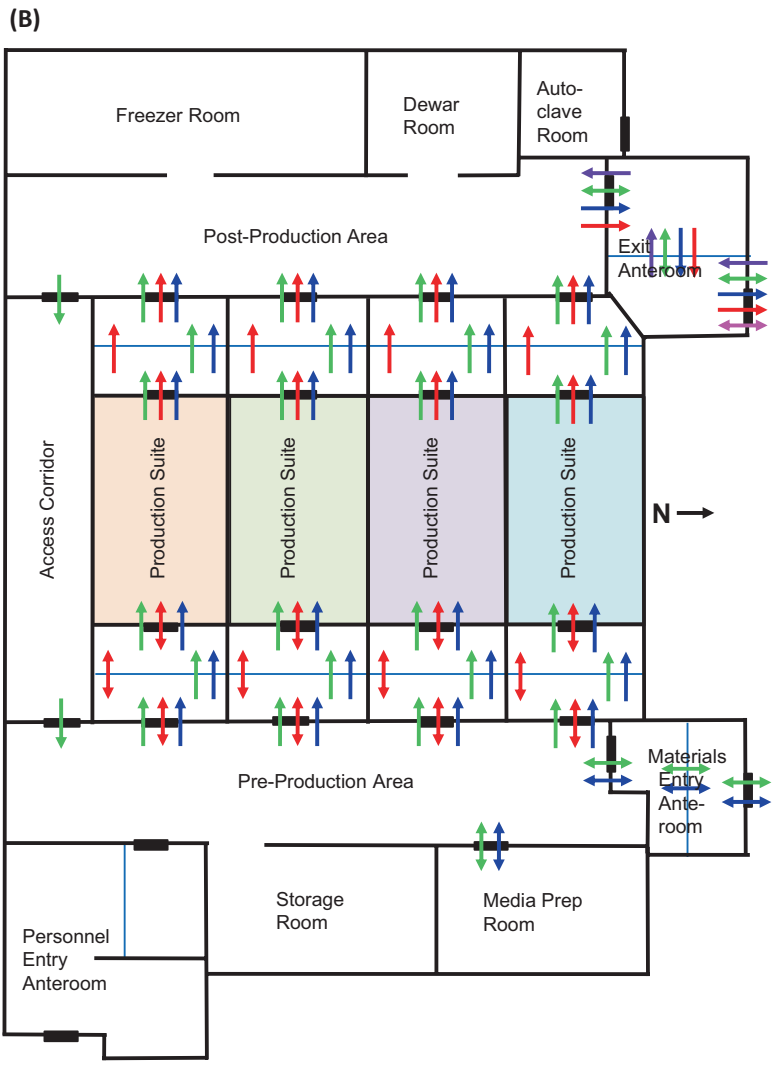


Fig. 4 Personnel and materials flow while cleanroom is operational
(**a**) Personnel flow design
(**b**) Materials flow patterns



Materials flow while cleanroom is operational:
(1) Equipment
(2) Reagents and supplies
(3) Reagents dedicated to a study and vector supernatant
(4) Cells (for storage in dewars)
(5) LN_2 tank
The **blue lines** demarcate between 'clean' and 'dirty' sides of spaces. Once materials have been taken across a blue line into a Production Suite, they are not permitted to be backtracked over the blue line. The only exception is (3) above where these reagents are stored in dedicated fridges located in the Pre-Production Area for the duration of the project.

Fig. 4 (continued)

Ensuring adequate space to accommodate growing staff, processes, document filing, and raw materials can present as a challenge. It is recommended to consider the following points in the designing phase such that reasonable space requirements can be met:

- The full production capacity of the cleanroom, i.e., what is the maximum number of products that can be manufactured in the space?
- What materials (equipment and disposable) are required to achieve the work? How much storage space will this require?
- The number of staff members required to complete projects at maximum capacity. Is there adequate work, office, and other support space?

Maintenance of a cleanroom space is expensive, and these costs must be incorporated into the fees for manufactured products. It is challenging to set pricing considering the large quantities of raw materials required for productions, in addition to personnel and facility costs, both in regard to generating vector products and maintaining the cleanroom. Since some of the products are paid for through the National Institutes of Health grants or contracts, careful cost accounting is required to show the vectors are being produced at cost. We found it helpful to partner with faculty and students in the Indiana University Kelley School of Business who were instrumental in developing a useful tool to estimate cost as a function of output [2]. The tool is open source and can be downloaded on the National Gene Vector Biorepository site (www.NGVBCC.org).

6 Conclusions

IUVPF serves as an example of a facility that has strived to grow with the gene therapy field over the past 25 years. In the process, it has become recognized for generating retroviruses and lentiviruses for clinical use. Adapting to the changing field has been key to the success of our facility. Our primary mission to facilitate academic scientists and physicians in initiating Phase I/II gene therapy clinical trials remains unchanged.

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References

1. Hill, J. B., Cross, S., & Cornetta, K. (2007). Solutions for a gene vector production lab. In *The lab design handbook*.
2. Boeke, A., Doumas, P., Reeves, L., McClurg, K., et al. (2013). Vector production in an academic environment: A tool to assess production costs. *Human Gene Therapy Methods, 24*, 49–57.

Qualification and Commissioning of a New GMP Facility

Adrian P. Gee

1 Qualification Plan

For the purposes of this article, we will assume (i) that the facility plan and construction have been submitted to the specific regulatory agency and received their approval and (ii) that construction of the facility has been completed and the relevant utilities are operational. As soon as possible after the plans have been approved, time should be spent to develop a comprehensive commissioning and qualification master plan (Fig. 1) [1]. This contains all of the elements to be qualified, e.g., equipment lists, heating, ventilation, and air conditioning (HVAC) operations, cleaning procedures, a timeline for implementation; and a list of the staff responsible for each phase. Consideration should be given to including additional information on facility construction, finishes, floor plans, etc. This must be supported by documentation obtained from the contractor(s) and architects. This would include information on the materials used for construction of walls, floors, and ceilings, paints or finishes used, cabinetry, and plans and operational details of HVAC, electrical, and plumbing systems. This provides an essential record of initial information on the facility, added to which should be documentation on the planning process and regulatory interactions. Included with documentation should be details of the handover of the facility from the construction company to the institution and the plan and details for resolution of problems that occur during the start-up phase.

Crucial to the successful operation of a cGMP facility is the development of a comprehensive documentation system. Space precludes a full description of what is required; however, it must record all significant aspects of facility operations from facility management and materials receipt to release and distribution of final products. Central to this system is the use of a formal document control procedure to

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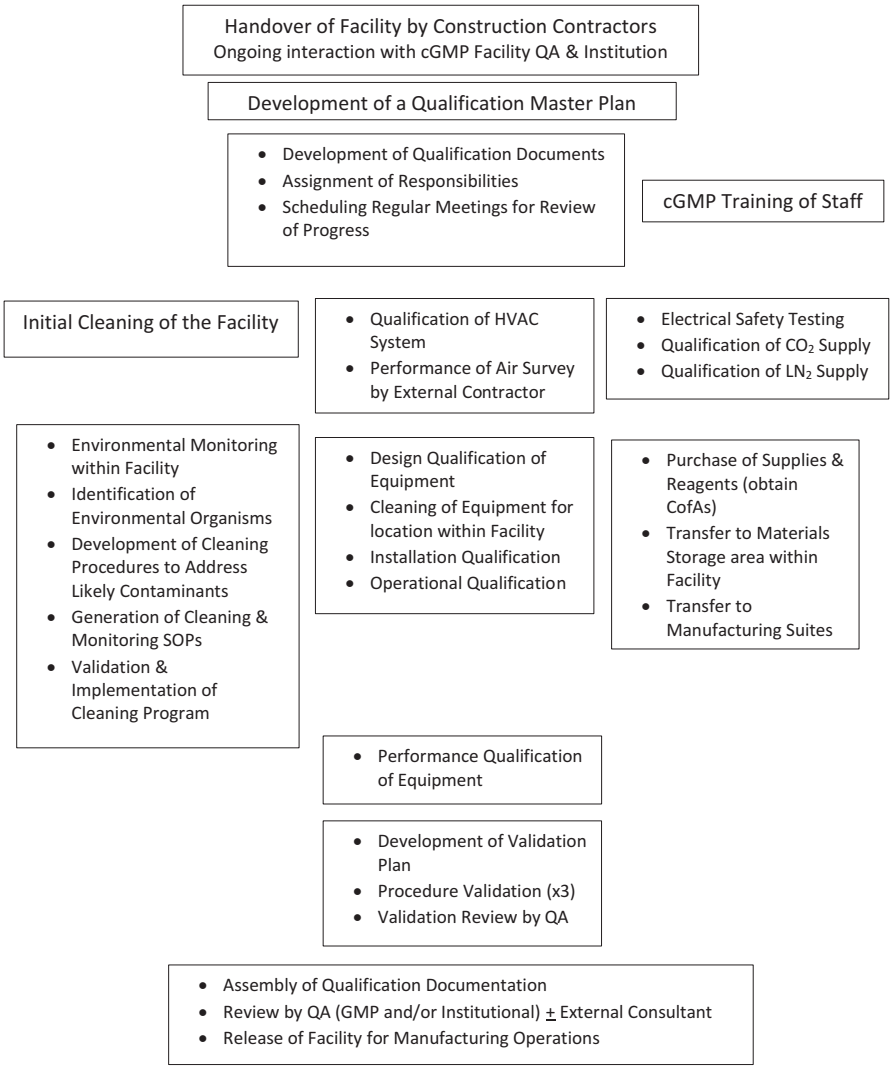

Fig. 1 Flow path for the qualification of a new facility

manage the development, release, revision, use, and recall of paperwork. This is usually under the control of the quality assurance (QA) group. The approved documents must be readily available to all appropriate staff members. In parallel, QA will develop a quality program to monitor and improve facility operations and to detect, document, and remediate any deviations from standard operating procedures (SOPs).

Consideration should be given to the development of specific documentation for the qualification and commissioning (Q&C) activities. In some cases, pre-existing

forms and SOPs may be used, but, given the specific activities that will be performed, it is preferable to supplement these with new documents that more clearly record the Q&C process.

Regular meetings of all concerned must be held to monitor activities and resolve any issues and problems that may arise. It is recommended that specific point persons should be identified to deal with individual tasks, e.g., cleaning and environmental monitoring, equipment installation, and procedure validation. Normally the intended director of the facility will lead the Q&C efforts, although in some cases this may be delegated to a representative of an external organization.

2 Initial Activities

2.1 Alarm Systems

One of the first activities in a new facility includes installation of an alarm/monitoring system. This will monitor equipment performance and environmental conditions when manufacturing activities start. The selection of an appropriate system can be complex since there are many options available. Some academic facilities use the in-built monitoring system; however, this may not provide the level of documentation that is required for cGMP operations. Consideration must be given as to whether a hardwired or wireless system is to be selected. The former may be more reliable but is less flexible if equipment is moved over time. Another consideration needs to be involvement of the information technology department in the institution in selection of the system, since they will frequently be involved in its operation and maintenance. It is, in some cases, possible for the company providing the system to provide off-site services and monitoring capabilities. Whatever system is selected it must be validated before implementation and the data collected must be compliant with FDA electronic data regulations [FDA 21 CFR Part 11] [2].

2.2 Cleaning and Environmental Monitoring

Although the construction company may have performed an initial clean of the new facility, this will not be sufficient for quality control (QC). It will be necessary to perform several cleanings of the area, initially using basic procedures, rather than those developed to support cGMP activities [3]. It is then recommended that environmental monitoring be implemented [4–6]. It is assumed that the HVAC system has been tested and documentation is available to support that it is meeting predetermined specification for the facility. It is recommended that an external contractor should be used for this purpose rather than using the installation company. They should be able to resolve any issues in collaboration with the installers.

At this stage the QC group should have developed an environment monitoring program to include air quality measurements and monitoring of surfaces. This is then implemented on an ongoing basis and the results reviewed. The results should include speciation of all organisms detected and should be reviewed with the infection control department in the healthcare institution with which the cGMP facility is affiliated. This will assist in the development of an effective cleaning program by selecting disinfectants that will combat the detected organisms effectively. These should be supplemented with a second effective disinfectant that will be rotated with the first. Ideally one should be phenolic and one non-phenolic. The disinfectants should be validated by showing their repeated efficacy against isolates of the organisms detected in the facility. The cleaning program should address the movement of cleaning staff, both when cleaning the facility and when removing waste. This should include an evaluation of the potential for product contamination if waste crosses paths with manufacturing supplies and/or in-process or final therapeutic products. This can be addressed by ensuring that there is a suitable time interval between manufacturing and cleaning activities.

The cleaning program that has been developed must be implemented for a period of time before items start to be moved into the facility. During this period electrical safety checks may be performed on all of the outlets. This is usually done by the institution. If there are city or purified water systems present in the facility, these can be tested for contamination. Maintenance of water systems presents a continuing commitment that can be onerous, particularly if organisms are occasionally detected. Consideration should be given to using commercially available sterile water for preparation of media and supplements and perhaps even for cleaning purposes.

2.3 Gas Supply

Checks must also be performed on the gas system supplying carbon dioxide to facility incubators. The gas should be of pharmaceutical grade, and there should be a switchover system to ensure continuous operation of the system when one bank of gas cylinders is emptied. This must be tested as should the operational pressure and the security of attachment of the incubators to the gas supply. Similarly checks must be performed on the supply of liquid nitrogen to both the cell storage vessels and to the controlled rate freezers. It is suggested that the supplies to each be separate, as the freezers can put stress the supply, especially if more than one is in operation. Again the switchover device must be tested as must the ability of the system to supply the anticipated number of qualified storage vessels.

2.4 Equipment

Once the cleaning program is routinely producing satisfactory results, consideration can be given to the movement of equipment into the manufacturing space. Prior to location within the facility, each piece of equipment should be cleaned and re-cleaned once it is in its final location. It is important that all equipment undergo a documented qualification procedure [6, 7]. This consists of four phases, design qualification (DQ), initial qualification (IQ), operational qualification (OQ), and performance qualification (PQ). DQ occurs at the time of selection of a piece of equipment and is intended to ensure that it meets user specifications in terms of capabilities, size, power requirements, intended location, etc. For QC purposes the next phases are of more direct impact. IQ is intended to document the receipt and installation of the equipment in the facility. It will record the date that the equipment is received, document that all parts and components were received, transportation to the location of intended installation, and the installation process including location, connection to utilities, power-up, etc. Included in this procedure will be cleaning of the equipment before it is taken into the cGMP area. It is normal to include all documentation related to a piece of equipment in an individual binder that can then be used to record subsequent calibration, cleaning, and maintenance. OQ is intended to demonstrate that the equipment operates to the manufacturer's specifications. This will include calibration (usually performed by the manufacturer's representative or a calibration company) and operation of the equipment according to the user's manual to demonstrate that it performs as expected. PQ is performed when the location is operating to cGMP specifications and documents that the equipment performs as expected when used according to a specific SOP, i.e., that it produces the results expected as documented in that SOP. Normally, PQ is performed several times to demonstrate consistency of performance, and the equipment is released for cGMP manufacturing purposes after approval of the qualification package by the facility quality assurance group. An additional qualification step is re-qualification. This applies to equipment that has been moved or potentially altered in some way, e.g., after repair or is to be used in a different manufacturing application. It essentially repeats different aspects of the previous qualification steps and may include repetition of IQ, OQ, and PQ. It would apply to equipment moved from an existing location into a new cGMP facility.

2.5 Calibration

As stated above, calibration of equipment is performed on all applicable equipment prior to cGMP use. It is defined as a comparison of the measurements made by a device against a reference instrument or standard check of its precision, accuracy, and limits. The requirements for calibration are defined under Title 21 of the FDA Code of Federal Regulations (CFR), Sect. 820.72 [8]. This indicates that the

standards used to inspect, measure, and test the equipment should be traceable to national or international standards. The guidelines indicate that the calibration is to be performed routinely according to written directions and that this calibration is documented. The acceptable limits for accuracy and precision must be specified and the personnel performing the procedure must be trained. The standards that are used must be traceable to the National Institute of Standards and Technology (NIST) or to in-house standards. There should also be a provision for evaluating adverse effects caused by malfunctioning equipment and procedures for remedial action. The procedures for performing the calibration must be easily accessible to the personnel performing the procedure and must include specific acceptable limits for accuracy and precision. The procedures may be developed in-house or supplied by the company performing the calibration service and should be on file in the cGMP facility. It is normal practice to place a sticker providing calibration information on the piece of equipment after it has been calibrated. This should indicate when the next calibration procedure is due.

2.6 HVAC

HVAC air surveys should conform to the recommendations provided in the FDA Guidance for Industry Sterile Drug Products Produced by Aseptic Processing — Current Good Manufacturing Practice [4] and in the International Standards Organization 14,644 Cleanroom Standards [5]. Due to their complexity, these calibrations are usually carried out by specialized contractors and are normally performed semiannually. Since Class 100 (ISO 5) biological safety cabinets (BSC) provide the primary manufacturing environment for cellular therapy products and vectors, their regular calibration is important, and, again, this is normally performed semiannually by a specialized company. In both cases there are predetermined specifications that must be met, and complete documentation of calibration should be provided, reviewed by quality assurance, and maintained on file.

2.7 Training

Once cleaning procedures and equipment are in place, the staff must be trained on cGMP if they are new to the facility. This should include reading the specific regulations that will be followed by the facility. These may be current Good Tissue Practices (cGTP) [9] and/or cGMP [10]. It is important that they thoroughly understand the difference between the two. cGTP apply to certain tissue types and to products that are minimally manipulated (not cultured ex vivo, activated, genetically modified, etc.), whereas cGMP cover more-than-minimally manipulated human cells, tissues, and cellular and tissue-based products (HCT/Ps). The regulations are provided in the CFR under Title 21 Parts 1–99, 200–299, 300–499,

600–799, and 800–1299 for cGMP and Title 21 Part 1271 for cGTP [10]. Training requires a job description and documentation of training on each facet of facility operations, not only manufacturing activities. It normally includes reading the relevant SOP, asking questions, and observing the particular procedure performed by a trained operator multiple times, followed by performance of the procedure multiple times satisfactorily under supervision by a trained staff member. In subsequent years the competency of a trained staff member must be assessed, and he/she should participate in relevant proficiency tests if available.

2.8 Gowning

One of the first procedures to be followed will be gowning to enter the facility. The levels of gowning are not mandated by the FDA or other regulatory agencies. When working in clean rooms, it is usual to wear overalls, hair covers, shoe covers, gloves, and a face mask. In academic cGMP facilities, these patterns may vary in both the grade of gowning used (sterile versus non-sterile) and the level of gowning followed, e.g., some facilities do not use face masks. This should be determined based on a risk assessment, or gowning may be modified based on initial manufacturing experience. The application of makeup in the facility is not allowed, and some facilities mandate the removal of all makeup and jewelry before gowning. Insertion of contact lenses is also not permitted while inside the facility. In newer academic facilities, two-level gowning is used, in which basic gowning is performed before entering the facility. Once inside, secondary gowning is employed before entering the manufacturing suite. This may include donning sterile gowning materials. This requires an additional gowning area in proximity to each manufacturing suite and an area in which the supplementary gowning is discarded when leaving the manufacturing room. Efficacy of gowning should be monitored as part of the environmental assessment program and should include touch plates of gloves and gowns.

There should be procedures that describe the occupation of clean areas. These would specify the maximum number of staff that may be present in that area, restriction of travel between the manufacturing suites and adjoining areas, and changeover actions to be taken when manufacturing more than one therapeutic product in the same area. Usually the manufacture of multiple products in a single suite must be discussed with and approved by the regulatory agencies. They will expect to see written precautions in place to prevent cross-contamination between products.

2.9 Materials and Reagents

The next stage is to stock the facility with materials and reagents that will be used in manufacturing. For cGMP manufacturing, these will predominantly have been pre-approved by the FDA as part of the IND review procedure. They will require

that the certificate of analysis for each lot be obtained from the manufacturer and maintained on file, together with the results from any supplementary testing required by the regulatory agency. In some facilities an institutional bar code sticker is applied to the item to indicate that it has been released for use; alternatively some other form of sticker may be applied to indicate its release. The item may then be transferred from the receiving area to the materials storage room within the facility. This is normally accomplished by wiping down the exterior packaging of the item using sterile alcohol or an approved disinfectant and transferring to the clean area by use of a pass-through box. The storage area must be organized on a first-in-first-out basis so that the oldest released items are used first. The items required for manufacturing may then be transferred from storage to the manufacturing suites. This may be done either by transferring sufficient supplies for the day's activities or stocking the suite with all of the supplies required for manufacturing the finished product. In the latter case it is important to ensure that all items will be used before their expiration date. Routine checks of expiration dates should be performed by both manufacturing staff and quality assurance audits. Items should be stored in closed cabinets and the external packaging wiped down with alcohol or disinfectant before their transfer to the inside of the BSC. There must be a process in place to record all of the reagents, materials, and equipment used during a manufacturing activity. This may be accomplished by scanning bar codes on the items or by use of a printed checklist of items within the room.

2.10 Validation

Validation of manufacturing practices is one of the next steps [11]. Validation procedures are discussed in detail in another chapter in this volume. It is advisable to initiate manufacturing activities in a phased manner, such that they start with a single product prepared in a single room. The validation plan must be pre-approved by QA and include the expected results, usually the product release criteria. The manufacturing validation should also include the relevant environmental monitoring procedures. The rule of thumb is that three validation procedures are usually performed before the results are reviewed by QA. Given the scarcity of raw material for validation studies, it may be acceptable to prepare products for eventual clinical use provided that they meet all release criteria. If this is the case, it is important that the informed consent makes this clear to the patient/donor so that they understand that this product may not become available for administration should there be a manufacturing problem. The QA review for initial validations should be comprehensive and include all aspects of both manufacturing and facility operations to ensure that everything is operating within specifications. This process can be continued until all manufacturing suites are operational and all manufacturing procedures have been successfully validated.

2.11 Documentation

All Q&C activities should be documented formally in a manual. It is the responsibility of the QA group to monitor qualification activities and to collect all of the associated documentation. This is assembled into a manual that undergoes formal review by the facility QA and, in some cases, by the institutional QA department and/or an external consultant. A successful review is followed by formal opening of the facility for manufacturing activities.

3 Conclusions

Qualification of a new facility is a major step in establishing its suitability to start manufacturing. It provides a degree of assurance that the major facility components meet expectations and that controlled, auditable, and reproducible procedures are in place to initiate activities. Qualification of operations and equipment will continue once the facility has opened. It is up to the senior quality representative to determine when sufficient qualification has been performed and documented to allow operations to start.

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References

1. Browne P (2020) *Commissioning, qualification and validation*. A GMP approach. Coppell, Texas. ISBN-13: 976–1547091256.
2. U.S. Food and Drug Administration (2003) *Part 11 - Electronic Records; Electronic Signatures - Scope and Application*. Guidance for Industry. August 2003.
3. Brown R, Nguyen T (2015) *Facilities & equipment: CGMP requirements. Presentation at Regulatory Education for Industry (REdI): Focus on CGMPs & FDA Inspections*. Silver Spring, Maryland. 15–16 July 2015. https://www.fda.gov/media/92841/download. Last accessed 9 July 2020.
4. U.S. Department of Health and Human Services Food and Drug Administration (2004) *Sterile drug products produced by aseptic processing — current good manufacturing practice*. Guidance for Industry. September 2004. https://www.fda.gov/media/71026/download. Last accessed 9 July 2020.
5. ISO Standards (2015) IS0 14644–2:2015, Clean rooms and associated controlled environments – Part 2: Monitoring to provide evidence of cleanroom performance related to air cleanliness by particle concentration.
6. ISO Standards (2015) ISO 14644-1:2015, Clean rooms and associated controlled environments – Part 1: Classification of air cleanliness by particle concentration.

7. McElroy J (2018) *Writing Compliant IQ/OQ/PQ Protocols — Meeting FDA Expectations*. Pharmaceutical Online website. https://www.pharmaceuticalonline.com/doc/writing-compliant-iq-oq-pq-protocols-meeting-fda-expectations-0001. Last accessed 9 July 2020.
8. Code of Federal Regulations. *Part 820. Title 21. Quality System Regulation*. Subpart 820.72. Inspection, measuring and test equipment. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=820 Last accessed 9 July 2020.
9. Code of Federal Regulations. *Parts 210 & 211, Title 21. cGMP manufacturing, processing, packing, or holding of drugs and finished pharmaceuticals*. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=210 https://ecfr.io/Title-31/Part-210 and https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=211 Last accessed 9 July 2020.
10. Code of Federal Regulations. *Part 1271, Title 21. Human cells, tissues, and cellular and tissue based products*. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=1271. Last accessed 9 July 2020.
11. Department of Health and Human Services Food and Drug Administration (2011) *Process validation: General principles and practices*. Guidance for Industry. January 2011. https://www.fda.gov/files/drugs/published/Process-Validation%2D%2DGeneral-Principles-and-Practices.pdf. Last accessed 9 July 2020.

Part IV
Facility Infrastructure

Environmental Monitoring

Adrian P. Gee

1 Gowning for Monitoring

The FDA does not have specific gowning regulations, but the Guidance [3] recommends that the gown should provide a barrier between the body and exposed sterilized materials and prevent contamination by particles generated by, and microorganisms shed from, the body. It states that there should be an aseptic gowning qualification program to assess the ability of a cleanroom operator to maintain the quality of the gown after performing gowning procedures.

The Good Manufacturing Practice (GMP Guidelines on GMP specific to Advanced Therapy Medicinal Products) published by the European Commission in 2017 provide additional gowning information [4]. For working in a Grade A/B (ISO 5)/Class 100 and background environment, sterile headgear must be worn that totally encloses the hair (and if applicable the beard and moustache). It should be tucked into the neck of the suit. A sterile face mask and sterile eye coverings should be worn together with appropriate sterilized rubber or plastic gloves and sterilized or disinfected footwear. The legs of the suit should be tucked inside the footwear and the garment sleeves into the gloves.

In a Grade C (ISO 6)/Class 1000 environment, the hair and, where relevant, beard and moustache should be covered. A single or two-piece trouser suit gathered at the wrists and with high neck and appropriate shoes or overshoes should be worn.

In a Grade D (ISO 7)/Class 10,000 environment the hair and, where relevant, beard and moustache should be covered. A general protective suit and appropriate shoes or overshoes should be worn. Appropriate measures should be taken to avoid any contamination coming from outside the clean area.

Wristwatches, makeup, and jewelry should not be worn in clean areas.

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2 Monitoring Parameters

The maximum particle counts in a variety of controlled environments are shown in Table 1. There are different recommendations for the maximum number of colony-forming units (cfu) for viable microorganisms in different cleanroom classifications. These are shown in Table 2 from data published by the European Commission Health and Food Safety Directorate [4], the United States Pharmacopeia (USP) [5], and the US Food and Drug Administration [3]. As can be seen, there is some variability [6]. For this reason, the USP has recommended monitoring of the contamination recovery rate (CRR). This is based upon the observation that if human operators are present, microbial contamination at some level is inevitable, and the following findings [5]:

- Real-time monitoring of the particle count does not provide direct information on the microbiological content of the environment.
- The cfu counts are subject to great diversity.
- The microbial monitoring covers only those captured during a narrow length of time.
- Failure of cfu to grow in a sample means only that growth was not discovered, not that the environment is free from contamination.

As a result, the USP <1116> [5] emphasizes that "rather than isolated events, analysis of data upon time would detect changes in the contamination recovery rate (CRR) that may be indicative of changes in the state of control within the environment." The maximum acceptable incidence rates are shown in Table 3. When the incidence rate is used, it is suggested that these be based on actual monitoring results and that these should be re-tabulated monthly. USP recommends that any single excursion of >15 cfu should prompt an investigation, even if the CRR is <1%

Table 1 Cleanroom classifications, activities, and 0.5 μm maximum particle levels

Grade	ISO classification	Class classification	Activity	Maximum particles/m^3 at rest	Maximum particles/m^3 in use
A	ISO 5	Class 100	Aseptic preparation and filling of sterile products	3,520	3,520
B	ISO 5	Class 100	Background environment for grade A zone operations, when needed for transfers and other less-critical tasks	3,520	352,000
C	ISO 7	Class 10,000	Preparation of solutions that need to be sterile filtered	352,000	3,520,000
D	ISO 8	Class 100,000	Handling of components after washing	3,520,000	Not defined

Adapted from *https://high-techconversions.com/gmp-eu-cleanroom-classifications-a-b-c-d/*

Table 2 Limits for cleanroom contamination for different cleanroom classifications

Viable contamination limits					
ISO classification	Class classification	Active air sample CFU/ m^3	Settle plate (9 cm) 4 hr. exposure	Contact plate	Gloves or gown
ISO 5 (grade A)	100	<1* <1~ <3[a]	<1* 1~ -	<1* 3~ (including floor) -	<1* - 3+ Gloves 10+Gown
ISO 5 background (grade B)	100	<10* - <20[a]	5* - -	5* - -	5* - -
ISO 6 (grade C)	10,000	<100* <7~ <100[a]	50* 3~ -	25* 5[a] -	
ISO 7 (grade D)	10,000	<200* <10~ -	100* 5~ -	50* - -	- - 10+Gloves20Gown
ISO 8	100,000	- 100~ -	- 50~ -	Not stated	Not stated

~ FDA Guidance Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice, FDA, September 2004. https://www.fda.gov/media/71026/download
[a]USP <116> Microbiological Evaluation of Clean Rooms and Other Controlled Environments http://ftp.uspbpep.com/v29240/usp29nf24s0_c1116.html/
bEU Guidance 2017. http://academy.gmp-compliance.org/guidemgr/files/annex%2001%5b2008%5d.pdf and Guidance on Good Manufacturing Practices specific to Advanced Therapy Medicinal Products

Table 3 USP recommendations for the contamination recovery rate (CRR) in different cleanroom classifications

Classification		Incident contamination rate			
Maximum contamination recovery rate (CRR)					
ISO classification	Class classification	Active air sample	Settle plate (9 cm) / 4 hr. exposure	Contact plate	Gown or glove
ISO 5 BSC	100	<0.1%	<0.1%	<0.1%	<0.1%
ISO 5 background	100	<1.0%	<1.0%	<1.0%	<1.0%
ISO 6	1000	<3.0%	<3.0%	<3.0%	<3.0%
ISO 7	10,000	<5.0%	<5.0%	<5.0%	<5.0%
ISO 8	100,000	<10.0%	<10.0%	<10.0%	<10.0%

Adapted from http://ftp.uspbpep.com/v29240/usp29nf24s0_c1116.html/

[7]. If the incidence rate is exceeded, corrective actions should be implemented. These may include revision of the sanitization program, increased surveillance of staff practices, review of sampling methods, and/or additional training.

The suggested frequencies of monitoring are shown in Table 4.

Table 4 Suggested monitoring frequency for different cleanroom classifications

ISO classification	Suggested monitoring frequency
ISO 5	Each operating shift
ISO 6	Each operating shift
ISO 7	Daily*/each operating shift[a]
ISO 8	Twice per week

Adapted from: **http://ddkscientific.com/Environmemtal%20monitoring%20of%20clean%20rooms%20.pdf*

[a]USP <116> Microbiological Evaluation of Clean Rooms and Other Controlled Environments http://ftp.uspbpep.com/v29240/usp29nf24s0_c1116.html/

3 Selection of Sampling Locations

The selection of monitoring locations depends on the phase of cleanroom development. During cleanroom certification, the ISO 14644-1 cleanroom standard [8] should be followed. This defines the number of monitoring locations required to certify the cleanroom. For qualification of the cleanroom, a grid system may be used [9], and particle counts are measured during both operational and at rest states. For routine monitoring the locations should be selected based on the degree of control required to prove management over risk to the finished product. The number and locations of sample sites should be based on a risk assessment [10, 11] and the qualification and certification results.

The risk assessment should be based on the following:

- Planned personnel flow.
- Planned material flow.
- Sampling sites should be based on the risk of the activity to be performed.
- Likelihood of contamination from the procedure being performed, e.g., closed versus open system.
- Likelihood of cross-contamination.

4 Center for Cell and Gene Therapy Risk Assessment (CAGT)

The risk assessment performed for our cell processing facility (CPF) concluded that the risk level was relatively low. This was based on the fact that all products were manufactured in a Class 100/ISO 5 environment, all products terminally are tested for sterility, the biological safety cabinets (BSC) are tested regularly, and the risk posed by particle counts in the room was low, since open products were never handled in the room. For this reason, it was decided that it was not necessary to perform

production manufacturing for each product manufactured within a biological safety cabinet but to perform regular monitoring at predefined intervals.

In contrast, the risk assessment for the vector production facility (VPF) showed a potentially higher degree of risk. This was based on the fact that vectors are produced in large volumes and terminal sterility testing is performed on relatively small volumes. Additionally, in contrast to many cell therapy products, vectors may be used in vivo and/or in vitro to treat large numbers of recipients. This risk is reduced by the unidirectional design of the vector facility and the fact that production monitoring is performed on all activities performed in the biological safety cabinet during manufacturing.

Since the CAGT at Baylor College of Medicine has 23 cleanrooms for phase 1 product manufacturing, it is not feasible to monitor every production in every room on a daily basis. For the CPF the particle monitoring locations selected, in addition to the BSC, were one location toward the entrance to the suite and a second location at the other end of the suite. Viable counts are also performed at the two locations, one of which must be between the biological safety cabinet and the centrifuges. These locations are numbered and marked on the floor plan for the facility. The rooms are monitored at least weekly for both particles and organisms. The schedule is established weekly by quality control (QC) and is partly based upon results of prior monitoring procedures. In unused rooms, dynamic monitoring is performed at least monthly by simulating manufacturing activities.

5 Monitoring Devices

5.1 Particle Counters

ISO 146441-1 [8] defines a particle counter as a light scattering device having the means of displaying or recording the count and size of discrete particles in air with a size discrimination capability to detect the total particle concentration in the appropriate particle size ranges for the class under consideration and a suitable sampling system. Most academic GMP facilities use standalone particle counters which are handheld (Fig. 1 Left Panel) and/or portable (Fig. 1 Right Panel).

The handheld systems generally have a lower airflow rate than portable samplers. The chosen counter should be ISO 146441-1 compliant with a counting efficiency that meets ISO 21501-4 [12] and should classify the required number of particle sizes. Handheld counters frequently store the data onboard the counter with the capability to download it via an Ethernet or USB connection. The portable counters have flow rates up to 100 liters/minute and frequently have extension tubes allowing the sampling head to be placed in a restricted space, such as a BSC, thereby avoiding perturbation of the airflow within the cabinet. It is also possible to have permanent particle counters wired into a BSC. There are units that are designed to

Fig. 1 Air particle counters
Left Panel – Handheld; Right Panel – Portable

monitor an entire facility using remotely placed batch and continuous particle samplers.

5.2 Viable Sampling Systems

There are a variety of designs for viable counters [5]. A common choice is the centrifugal sampler. This uses a turbine or a propeller to pull a known volume of air onto a tangentially placed strip (Fig. 2). Atrium devices consist of an autoclavable system containing a media plate onto which is drawn air through holes in the lid via a pump. The slit-to-agar sampler draws air through a slit onto a rotating media plate. The normal sampling capacity is 80 liters per minute which requires a 15-minute exposure if 1 m^3 is to be sampled. The slit-to-agar samplers have been shown to produce lower recoveries than the centrifugal samplers which show selectivity toward larger particles which may result in higher airborne counts [13].

Settle plates (also used for detection of fingerprint contaminants) usually contain either Tryptic Soy Agar (for detecting total aerobic microbes, yeast, and molds) or Sabouraud Dextrose Agar (for total yeasts and molds). They are available with a variety of inhibitors to neutralize the inhibitory effects of disinfectants and antibiotics. The normal exposure time for a 90 mm settle plate is 4 h.

Replicate Organism Detection and Counting (RODAC) plates are used to sample surfaces, e.g., working surfaces, gowning materials, etc. The most widely used plate medium is TSA (Tryptone Soya Agar) containing Tween and lecithin for the

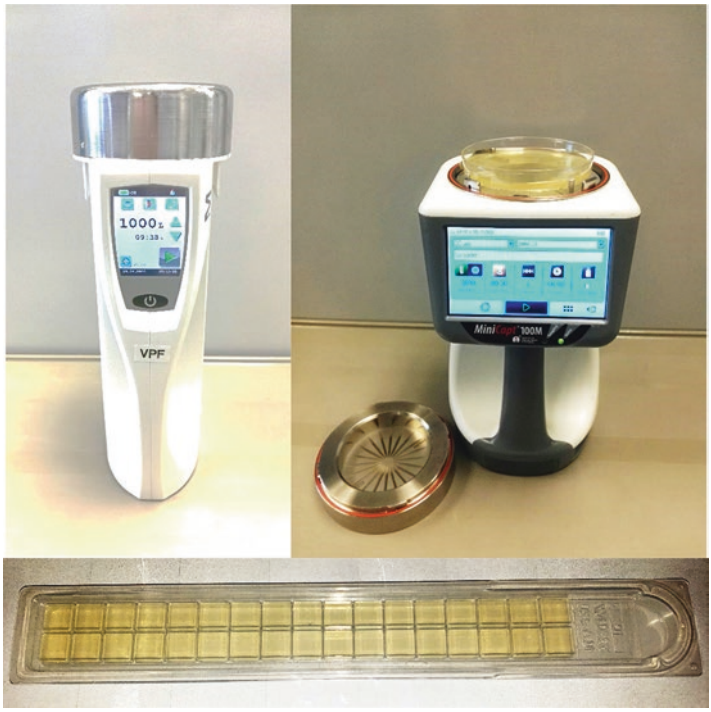

Fig. 2 Viable counters
Left Panel: Viable particle counter – RCS High Flow Touch from MilliporeSigma
Right Panel: Slit-to-agar viable counter
Bottom Panel: Test strip used in RCS counter

inactivation of inhibitory substances. The typical incubation schedule is 2–4 days at 20–25 °C after the initial 3–5 days at 30–35 °C.

6 CAGT Monitoring

Particle counters are placed at the predetermined sampling locations and at least 1 cubic foot (0.028 m^3) is sampled. For viable counts 1000 liters (35.3 cubic feet) of air is sampled. The results are checked against the data in Table 1 and for viable organisms against the active air sampling column in Table 2.

6.1 Biological Safety Cabinet

BSC monitoring in the CPF is performed as follows:

6.1.1 Fallout Plates

Two plates are placed into the BSC and opened. The plates are changed every 4 h. One plate is incubated at ambient temperature for 14 days and the second is incubated at 32.5 °C ± for 14 days. The plates are checked for growth at 2–4, 7, and 14 days. All CFU on all types of plates used are sent for speciation.

6.1.2 Replicate Organism Detection and Counting (RODAC) Plates

The horizontal working surface is sampled using four RODAC plates for simulations of manufacturing. For actual production monitoring, four plates are touched to the surface immediately after completing the production, and an additional four are touched after the work surface has been cleaned. Two plates are placed at the left of the cabinet and two to the right. One of the plates from each location is incubated at ambient temperature for 14 days and the second is incubated at 32.5 °C ± for 14 days. The plates are checked for growth at 2–4, 7, and 14 days. RODAC plates are also used for monitoring the shelves in each incubator on a biweekly basis.

6.2 Touch Plates

Touch plates are used to monitor gowns and gloves during production manufacturing. The plates are briefly touched to the gown or glove fingers and incubated as described for RODAC plates.

The alert and alarm limits for monitoring of BSC are shown in Table 5.

The major issue with viable particle monitoring is the delay between sampling and receiving the results. For this reason, it is useful to check the sterility of the product after the positive monitoring result was obtained. This can be performed by a visual check accompanied by sending out a Gram stain and 14-day cultures. Even if the results are negative, complete cleaning of the BSC is recommended.

Out-of-specification results are dealt with as deviations that document the short- and long-term corrective actions and follow-up activities.

7 Data Management

Environmental monitoring creates a huge amount of data that must be reviewed, filed, and trended. The easiest way to do this is in the form of a spreadsheet or database. This should also include details of the cleaning records for the area that was monitored, the cleaning agents used, and the identity of the cleaning staff, so that these can all be related. To determine the incidence rates for viable counts, the data should be trended on a monthly basis. The chapter on cleaning provides information

Table 5 CAGT alert and alarm levels for monitoring ISO 5/Class 100 (upper panel)and ISO 7/ Class 10,000 environments

Monitoring performed for ISO 5 – Class 100 classification	Alert level Actions to be taken in order of problem severity	Alarm level Actions to be taken in order of problem severity
Particle count (<3,000 / m^3 No action)	Count >3000–3520 / m^3 • Alert QA for action to be taken. • Re-monitor. • Remedial action may require recleaning.	Count >3,520 /m^3 • Close down cabinet. • Re-monitor. • Complete clean. • Test last product prepared. • Decontaminate if necessary. • Filter replacement if necessary. • Requalify cabinet. • Re-monitor.
Viable count (0 CFU / m^3 No action)	N/A	≥1 CFU/strip (0.142 CFU/cu ft) • Alert QA and QC. • ID organism. • Review any subsequent monitoring results. • Examine and/or test last product prepared. • Complete clean. • Shut down cabinet. • Decontaminate if necessary. • Filter replacement. • Requalify cabinet/re-monitor.
RODAC Fallout plate (0 CFU No action)	N/A	≥1 CFU or spreading bacteria • Alert QA and QC. • ID organism. • Review any subsequent monitoring results. • Examine and/or test last product prepared. • Complete clean. • Shut down cabinet. • Decontaminate if necessary. • Filter replacement if necessary. • Requalify cabinet/re-monitor.
Particles (<300,000/ m^3 No action)	≥300,000–352,000/ m^3 • Alert QA and QC. • Re-monitor. • Terminate use of room. • Seek probable cause. • Replace HEPA filter. • Recalibrate.	>352,000 / m^3 • Alert QA and QC. • Re-monitor. • Monitor adjacent suites. • Terminate use of room. • Complete clean. • Seek probable cause/ decontaminate room. • Replace HEPA filter. • Requalify room.

(continued)

Table 5 (continued)

Monitoring performed for ISO 5 – Class 100 classification	Alert level Actions to be taken in order of problem severity	Alarm level Actions to be taken in order of problem severity
Viable counts (<7 CFU / m^3 No action)	≤7 - 10 CFU / m^3 • Alert QA and QC. • Re-monitor. • Complete clean. • Remonitor. • Review other monitoring records for suite. • Reopen if no previous events found.	>10 CFU / m^3 • Alert QA and QC. • Close suite. • Re-monitor. • Complete clean. • Re-monitor. • Review cleaning records for last complete clean. • Decontaminate room. • Replace HEPA filter if necessary. • Requalify room.
Settle plates (0- < 4 CFU No action)	4 CFU • Alert QA and QC. • Re-monitor. • Complete clean. • Re-monitor. • Review other monitoring records for suite\reopen if no previous events found.	≥5 CFU or spreading bacteria • ID organism. • Complete clean. • Re-monitor. • Decontaminate. • Re-monitor.

on decontamination procedures to be used in the case that resistant organisms are detected. There should be at least quarterly reports of environmental monitoring activities to the quality program and an annual report to the director of the GMP facility.

8 Conclusions

A robust environmental monitoring program provides the essential foundation for GMP/GTP manufacturing. It is vital to have an understanding of what potential contaminants are present during manufacturing, their location(s), and whether they are actually present during a procedure. In combination with an effective cleaning and disinfection program, assurance of product safety from a microbiological standpoint can be assured.

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References

1. U.S. Food and Drug Administration. *Code of Federal Regulations. Drug Good Manufacturing Practices (GMP)*. Title 21, Parts 210 and 211. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=211 Last accessed 20 July 2020.
2. U.S. Food and Drug Administration. *Code of Federal Regulations*. Human Cells, Tissues, and Cellular and Tissue-based Products, Part 1271. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=127 Last accessed 20 July 2020.
3. U.S. Food and Drug Administration (2004) Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice. Guidance for Industry. September 2004. https://www.fda.gov/media/71026/download. Last accessed 20 July 2020.
4. European Commission (2017) *Good Manufacturing Practice Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products*. https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-4/2017_11_22_guidelines_gmp_for_atmps.pdf. Last accessed 20 July 2020.
5. United States Pharmacopoeia <1116> Microbiological Evaluation of Clean Rooms and Other Controlled Environments. http://ftp.uspbpep.com/v29240/usp29nf24s0_c1116.html/ . Last accessed 20 July 2020.
6. Duarte R (2009) *Environmental Monitoring of Clean Rooms*. http://ddkscientific.com/Environmemtal%20monitoring%20of%20clean%20rooms%20.pdf. Last accessed 20 July 2020.
7. Agalloco J. and Associates. *USP Guidances on Environmental Control including related USP, FDA, EMEA and PDA Activities*. https://www.pda.org/docs/default-source/website-document-library/chapters/presentations/new-england/usp-guidances-on-environmental-control-including-related-usp-fda-emea-pda-activities.pdf?sfvrsn=6. Last accessed 20 July 2020.
8. International Standards Organization. Cleanrooms and associated Controlled Environments. International Standards ISO 14644-1 https://zoser.com.co/wp-content/uploads/2015/10/ISO%2014644-1%20Version%202015.pdf. Last accessed 20 July 2020.
9. Hartvig, N. V., Farquharson, G. J., Mielke, R., Varney, M., et al. (2011). Sampling plan for cleanroom classification with respect to airborne particles. *Journal of the IEST, 54*(1), 1–15.
10. Stabler Hardiman M. (2014) *Risk-based environmental monitoring*. https://pqri.org/wp-content/uploads/2015/11/Hardiman.pdf. Last accessed 20 July 2020.
11. Sandle, T. (2013). New guidance for environmental monitoring in cleanrooms. *GMP Review, 11*(4), 9–11.
12. ISO Standards. *Determination of particle size distribution – single particle light interaction methods – Part 4: Light Scattering Airborne Particle Counter for Clean Spaces*. ISO 21501-4. https://www.sis.se/api/document/preview/80004178/. Last accessed 20 July 2020.
13. Placencia, A. M., Peeler, J. T., Oxborrow, G. S., & Danielson, J. W. (1982). Comparison of bacterial recovery by reuter centrifugal air sampler and slit-to-agar sampler. *Applied and Environmental Microbiology, 44*, 512–513.

GMP Facility Cleaning and Maintenance

Deborah Lyon and Adrian P. Gee

1 Cleaning

1.1 Requirements and Definitions

The Food and Drug Administration (FDA) requires under Good Manufacturing Practices (cGMP) [1] that any buildings used in the manufacture, processing, packing, or holding of a drug product be of suitable size, construction, and location to facilitate cleaning, maintenance, and proper operations. It also specifies that trash and other refuse be disposed of in a safe and sanitary manner. The facility must be maintained in a safe and sanitary condition and shall be free of infestation by vermin. There must be written procedures assigning responsibility for sanitation and describing the cleaning schedules, methods, equipment, and materials and that these procedures must be followed. There must also be written procedures for the use of suitable rodenticides, insecticides, fungicides, fumigating agents, and cleaning and sanitizing agents. You will find that these procedures may differ based on the location of your facility. For example, a GMP located in an academic building or hospital will also need to comply with guidelines appropriate to those institutions. In these cases, the treatments for rodents and insects may be handled by the building management. Copies of institutional treatment protocols and records should be maintained by the GMP facility. It would be advisable to maintain copies of their treatment protocols and records.

Cleaning may be defined as the removal of visible and microscopic contamination by dirt, extraneous matter, or product residues by mechanical or physical means. It usually requires the use of agents such as detergents or solvents which are used under specific conditions of pH, temperature, time, and solvent concentration.

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These should also not have a deleterious effect on the items being cleaned [2]. In contrast, sanitation may be defined as the reduction of microbiological contamination, usually by the use of chemicals, although heating and vigorous mechanical action, e.g., scrubbing, may also be effective. It is important to use the required concentration of the sanitizing agent and, in most cases, to remove agent residues.

There are differences in the definitions of sanitizing, disinfecting, and sterilizing. A sanitizer should result in bacteria reduction of >99.9% – 3-Log reduction – and should be used on a pre-cleaned surfaces. Use of a disinfectant should result in 100% kill of vegetative bacteria, target viruses, and target fungi. It may require pre-cleaning of the surface on which it is to be used. Use of a sterilant results in 100% kill of all microorganisms, including bacterial endospores (*B. subtilis*, *C. sporogenes*), and always requires pre-cleaning [2]. Determination of the efficacy of sanitizers depends on the number and types of microbes, the nature of the material containing the microbes, and the conditions under which the sanitizing agent is used, including the time of exposure. Disinfectants used in Grade A /ISO 5 /Class 100 zones should be sterile [3].

The limits of contamination by viable microorganisms for various clean room classifications are shown in Table 1. These contaminants include bacteria, fungi, viruses, and spores.

1.2 Environmental Survey

When selecting cleaning and disinfecting agents, it is important to conduct an environmental survey of the facility to determine which organisms are present. From the facility design, a number of sampling sites are selected. These include a variety of locations within the cleanroom environment. For formal cleanroom qualification, the following formula is used to determine the number of locations [4]:

$$N_L = vA$$

where N_L is the minimum number of sampling locations (rounded to a whole number), v is the air sample volume and A is the area of the room or zone in square meters. For routine qualifications a smaller number of strategically located sites may be used. These should be selected based upon risk of contamination to the product, e.g., areas of high activity, sites of open system manipulations, etc. Sampling sites should include biological safety cabinets, work surfaces, and equipment. The organisms that are detected should be sent for identification and subcultured to test for sensitivity to disinfecting agents.

Development of a standard operating procedure (SOP) for the cleaning process should occur after the disinfectant reagents have been selected. It should cover the frequency of cleaning of the various areas, the agents to be used, and the procedure to be followed. The method for cleaning, e.g., mops, sprays, and wipes, must be

Table 1 Limits of contamination by microorganisms for various cleanroom classifications

Viable contamination limits					
ISO classification	Class classification	Active air sample CFU/ m^3	Settle plate (9 cm) 4 hr. exposure	Contact plate	Gown or glove
ISO 5 (grade A)	100	<1* <1~ <3[a]	<1* 1~ -	<1* 3~ (including floor) -	<1* - 3+ Gloves 10+Gown
ISO 5 background (grade B)	100	<10* - <20[a]	5* - -	5* - -	5* - -
ISO 6 (grade C)	10,000	<100* <7~ <100[a]	50* 3~ -	25* 5[a] -	
ISO 7 (grade D)	10,000	<200* <10~ -	100* 5~ -	50* - -	- - 10+Gloves20Gown
ISO 8	100,000	- 100~ -	- 50~ -		

~ FDA Guidance Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice, FDA, September 2004

[a]USP <116> Microbiological Evaluation of Clean Rooms and Other Controlled Environments *http://ftp.uspbpep.com/v29240/usp29nf24s0_c1116.html/*

bEU Annex 1 *http://academy.gmp-compliance.org/guidemgr/files/annex%2001%5b2008%5d.pdf* and Draft Guidance on Good Manufacturing Practices specific to Advanced Therapy Medicinal Products

specified together with the surfaces to be cleaned on each occasion, e.g., ceilings, floors, and walls. There should be a system in place to make changes to the cleaning schedule based on the results of environmental monitoring. The procedure must be documented with details of the areas cleaned, the agents used, and the identity of the cleaning staff. The lot numbers, certificates of analysis, and MSDS sheets should be available for each batch of cleaner or disinfectant used.

1.3 Training of Cleaners

All staff involved in cleaning must receive proper training in the SOPS. Suggested elements of the training program are shown in Table 2.

Table 2 Suggested components of a cleaning training program

Training component	Areas for discussion
Basic microbiology	Types of viable organisms in the cleanroom Endotoxin Methods for their destruction Detection of microorganisms (including CFU) Effects of microorganism on the products
Contamination sources	People Air Water Equipment Manufacturing/testing procedures Cleaning agents
Facility design	HVAC system Airflow as a contamination source Gowning procedures
Cleanroom behavior/personal hygiene	Hygiene importance and components Proper hand washing and sanitization Movement within the cleanroom Handling of materials and equipment in cleanroom
Environmental monitoring	EM samples and how they are obtained How EM data are used Limitations of EM Evaluating EM data to determine proper manufacturing
Cleaning program	Types of dirt present in facility General approaches to cleaning Preparation of cleaning agents Use of cleaning equipment Safety aspects of cleaning agents Cleaning of specific cleanroom components
Disinfection program	Which reagents to use How disinfectants work How disinfectants are chosen Removal of residues Preparation of disinfectants Where to use, how to apply, contact times Safety aspects of disinfectants

Adapted from *https://kupdf.net/queue/tr-70-cleaning-and-isinfection_58d6d1cadc0d60520fc34702_pdf?queue_I*

1.4 Disinfectants

There is a wide variety of disinfectants available, but they vary in their efficacy against different microorganisms as is shown in Table 3 [5, 6].

The efficacy of the disinfectant can be tested by plating the test organism onto a solid surface which is covered and expanded by incubation at room temperature and at 37 °C. The surface can then be cleaned using the recommended procedure to be used for cleaning with the test disinfectant, plus or minus residue removal as appropriate. The surface can then be sampled using suitably screened RODAC plates,

Table 3 Properties of different disinfectants

Disinfectant	Activity				Irritation	Residue	Speed
	Bacteria	Fungi	Viruses	Spores			
Alcohol	Good	Good	Good	None	Medium	No	Yes
Aldehydes	Good	Good	Good	Good	Very high	Yes	No
Amphoteric surfactant	Good	Fair	Fair	None	Low	Yes	Yes
Biguanide	Good	Fair	Good	None	Low	Yes	Yes
Chlorine dioxide/quat blend	Good	Good	Good	Good	Low	Yes	Yes
Hypochlorites	Good	Good	Good	Fair	High	Yes	Yes
Hydrogen peroxide/peracetic blend	Good	Good	Good	Good	High	No	Yes
Phenolic compounds	Good	Good	Fair	None	High	Yes	No
Quaternary ammonium compounds	Good	Good	Good	None	Low	Yes	Yes

Adapted from https://www.copybook.com/companies/shield-medicare/articles/step-by-step-selection-of-cleanroom-disinfectants

which are then incubated and scored. Negative and positive control plates should be tested.

While it is generally recommended that there should be a rotation schedule between different disinfectants, e.g., phenolic and non-phenol-based agents, to prevent the development of resistant organisms, the need to rotate disinfectants has been questioned by Martinez [7] who states that there is no scientific basis for the practice.

When establishing a rotation schedule, it is important to trend the number of colony-forming units recovered, especially following the change between disinfectants to demonstrate that the second disinfectant shows at least equivalent activity to the first. It is also critical to use disinfectants at the recommended or validated concentrations. Too low a concentration will be ineffective and too high a concentration may cause damage to the disinfected surfaces.

Both the European Union (EU) [8] and the FDA have produced validation guidelines based on validation and expectations for cleaning procedures. The FDA document [9] which was valid as of 2014 states that the FDA does not intend to set acceptance specifications or methods for determining whether a cleaning process is validated. It does stress the importance of the development of specialized clean in place methods for specific equipment, use of written procedures, the use of specific and sensitive analytical methods for detecting residues and residual contaminants, and additional sampling of rinse solutions. This FDA Guidance is relatively outdated, and based upon warning letters issued by the Agency, the following areas are now emphasized: (i) use of a plan showing how the cleaning processes, practices, and validation study results are evaluated for each piece of multipurpose manufacturing equipment, (ii) scientific rationale for the cleaning validation strategy that shows that the cleaning processes are effective, and (iii) a summary about updating the cleaning validation protocol with – at least – the worst case scenarios.

Table 4 Phase 1 GMP requirements and methods for compliance

Phase 1 GMP requirement	Compliance
Use of aseptic technique	Use of biological safety cabinet meeting class 100/ISO 5
Use of process simulation using growth medium	Fill validation
Performance of environmental monitoring	Use of settle plates and air sampling
Disinfection	Of workstation, gloves, non-sterile items
Following written SOPs	For aseptic technique, manufacturing, and testing
Proper training	In aseptic technique and sterilization procedures
Equipment performance verification	Sterilizing apparatus, calibration of temperature probes in equipment, maintenance of equipment logs, use of biological indicators
Appropriate final release of products	Review by quality assurance unit

The European Union (EU) validation document [10] is published in Annex 15 Volume 4 of EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use. This is a detailed publication with an October 2015 deadline for implementation. The EU has an expectation that the disinfection procedure will result in a 3–5 logarithm reduction of the contaminating organisms.

In the United States, the FDA has established the level of cGMP compliance it expects from institution performing phase 1 clinical trials in a Guidance document [11]. This indicates that the provisions in Sterile Products/Aseptically Processed Products for phase 1 investigational drugs prepared using aseptic processing should be followed but specifically mentions the requirements shown in Table 4.

There is no such guidance from the EU. However, in 2010, the European Commission published a review on the impact of regulations on the development of advanced therapy medicinal products by academic facilities [12]. It concluded that the regulations had produced some uncertainty and little harmonization at the level of delivery across member states and that this had resulted in stifling the development and commercialization of promising new therapies and that this had been of most impact on early phase trials conducted by academic facilities. It stated that academia has to be recognized as a major contributor and partner in development of these products and that small facilities be endorsed by a more risk-based approach as fostered by the FDA and offered by Annex 2 of the European GMP guide.

1.5 Disinfectant Preparation

There should be a designated area for the preparation of disinfectant solutions, which should be made according to the manufacturer's directions using the recommended diluent. In some cases, facilities have their own systems for producing

Table 5 Contact times and log organism reductions for different organisms

Organism	Disinfecting agent	Contact time	Suggested minimum log reduction
Non-spore forming	Sanitizer	Maximum 90 sec.	>1 log
Non-spore forming	Disinfectant/ sporicide	1–5 min.	>1 log
Mycoplasma	Disinfectant/ sporicide	1–5 min.	>1 log
Mold spores	Sporicide	1–5 min.	>1 log
Bacterial spores	Sporicide	1–5 min.	>1 log

Adapted from Parenteral Drug Association. Fundamentals of Cleaning and Disinfection Programs for Aseptic Manufacturing Facilities. Technical Report 70. 2015 *https://kupdf.net/queue/tr-70--cleaning-and-disinfection_58d6d1cadc0d60520fc34702_pdf?queue_*

water with defined specifications. These should not be used for cleaning unless the water undergoes regular testing for microorganisms and chemistries. Demineralized, deionized, or distilled water may be used for dilution depending on the cleaning/disinfecting agent. These can be purchased from commercial suppliers. For use in Class 100/ISO 5 environments, the disinfectant used must be sterile [3]. This can usually be purchased from the supplier or depending on the agent filtered through a 0.2um filter into a sterile container or by irradiation or autoclaving. The stability of the agents must also be determined based upon the manufacturer's recommendations or by validation, so that it is used before its expiration date. It is possible to purchase automatic dispensers that pre-dilute the disinfectant or cleaning solution prior to use. Cleaning agents should be used prior to disinfectants. In most cases, however, hospital grade disinfectants are formulated with surfactants, dispersants, and chelants to provide a moderate level of cleaning and microbial kill in cleanrooms [2]. Recommended contact times and organism reductions are shown in Table 5.

1.6 Equipment Used for Cleaning

A variety of supplies will be used for facility cleaning. These include mops with disposable cleaning heads, buckets, squeegees, spray bottles, pre-packaged disinfectant wipes, etc. These should all be sterile or at very least disinfected before use. These items are available from a number of specialized suppliers, e.g., MicroNova, MicronSwep from Vileda, and CE Duo.

Table 6 Recommended frequency of cleaning for controlled environments (2)

Classification	Class 100/ ISO 5	Class 10,000/ ISO 7	Class 100,000/ ISO 8	Controlled areas
Floors	Daily	Daily	Daily	Daily/weekly
Ceilings	Daily	Monthly/yearly	Yearly	Yearly
Walls	Daily	Weekly	Monthly	Monthly
Equipment/ fixtures	Daily	Daily	Monthly	Monthly

1.7 Cleaning Frequency

The recommended frequency for cleaning in areas with different classifications is shown in Table 6.

1.8 Cleaning Methods

1.8.1 Two-Bucket Method for Floors, Walls, and Ceilings

The two-bucket method is most commonly used. In this method the disinfectant is in one bucket and either nothing or some disinfectant is placed in the "waste" bucket [13]. The mop head is dipped into the first bucket and the excess liquid allowed to drain off. The mop head is then applied to the surface. When it starts to drag on the cleaned surface, the mop head is dipped into the waste bucket and rung out. It is then transferred to the first bucket and the process repeated. There are three bucket systems in which a rinse bucket is included [2, 13].

During cleaning the mop head is applied to the surface and in a first stroke and it is pulled towards you. It is then lifted and reapplied to the start of the first stroke, allowing an overlap of about 20%, and it is again pulled towards you. The process is repeated for a third stroke. The agent should be left in contact with the surface for the amount of time recommended by the manufacture to achieve effective disinfection. It is recommended that the disinfectant solution be changed after 600 square feet (56 square meters) have been cleaned in ISO 5–6 areas and after 1000 square feet (93 square meters) in ISO 7–8 areas [2].

All surfaces cleaned in a Class 100/ISO 5 environment should be rinsed after cleaning using water for injection or 70% isopropyl alcohol. In Class 10,000/ISO 7 environments, rinsing should be performed as needed to remove residues.

In unidirectional cleanrooms, the cleaner starts working at the entrance door and works towards the exit. They will then leave the facility, re-gown, prepare new disinfectant solutions, and use new mops to clean the next room in the sequence. In multidirectional facilities, the cleaner will start work at the part of the room furthest from the entrance and work towards the exit.

The cleaning burden in cleanrooms may be reduced by the placement of tacky mats at the entrances to the facility and to the manufacturing suites. These are available in washable, e.g., from Cleanroom World, and disposable formats. These should be validated for performance before and during use.

1.8.2 Biological and Laminar Air Flow Cabinets

These should be cleaned before and after each use. Initially the surfaces should be wiped down with 70% sterile isopropyl alcohol starting at the back top of the cabinet and working towards the bottom front area. Care must be taken not to wet the HEPA filter or its covering grate. This is followed by use of a disinfectant most of these contain a cleaning agent. This is applied using a spray bottle containing the sterile agent and sterile wiping cloths, e.g., Texwipe. Application of the disinfectant is helped by the use of a tool such as the Isolator Tool from Micronova. It is recommended that a sporicide be included in the cleaning procedure. After allowing the appropriate contact time, the surfaces should be rinsed with sterile water for injection or 70% isopropyl alcohol. It is recommended that in addition to the daily cleaning, the cabinet undergoes a complete clean, involving removal of the working surface and cleaning beneath it, at regular intervals. A sporicide, applied using a sterile wipe, should be used during a complete clean followed by a sterile 70% isopropyl rinse. A record must be kept of the cleaning procedure and the components prepared in the cabinet before and after the cleaning procedure.

1.8.3 Worksurfaces and Cabinetry

Worksurfaces and cabinetry can be cleaned using disinfectant in a spray bottle and either a mop or device such as the Isolator Tool or wipes. The exterior of cabinets should be cleaned regularly, whereas the interior, if used for wrapped supply storage, may be cleaned on a less regular schedule. When cleaning cabinets, it is important to work down from the top towards the bottom. Phones should also be included in the cleaning schedule as should benchtops, doors (including the tops), and windows if present. If a disinfectant is used that leaves a residue, the surfaces should then be wiped down with 70% isopropyl alcohol. Carts should be cleaned with special attention being paid to the wheels.

1.8.4 Equipment and Tools

The first consideration is whether the piece of equipment or tool can withstand the disinfecting agent. If not, it should be cleaned with a regular cleaning agent and then wiped down with 70% isopropyl alcohol. An appropriate disinfectant should be selected, for example, bleach should not be used on stainless steel or metallic surfaces without careful and extensive rinsing as it will pit the surface and produce

rusting. The normal procedure is to spray the equipment with disinfectant (avoiding any sensitive areas such as sensors) and then wipe with sterile wipers and rinse with WFI or 70% isopropyl alcohol. Sterile wipes may be moistened with the appropriate disinfectant to clean around sensors. Each piece of equipment used during manufacturing must be cleaned before and after use and the cleaning documented. If equipment is not used for an extended period of time, a minimal cleaning schedule should be established.

1.9 Waste Removal

The pathway used for the removal of waste prior to cleaning must be considered. In unidirectional flow facilities, it should be removed via the exit from the manufacturing suite and through the dirty corridor to either the exit or to a pass-through to an external storage area. In multidirectional flow facilities, it is important that waste should not cross the path of the product, the manufacturing staff, or reagents and materials. This can be accomplished by performing the removal after manufacturing or other transfers have been performed.

2 Sources of Contaminants

The most common sources of contaminating microorganisms are shown in Table 7.

2.1 Resistant Organisms

If resistant microorganisms appear within the facility, the first option is to try the alternative disinfectant in the rotation schedule and/or a sporicide for spore-forming organisms. If these fail to achieve decontamination, fogging or gassing of the piece of equipment or room should be considered [14]. The most widely used agent for this purpose is hydrogen peroxide vapor. This can have limited efficacy against

Table 7 Types and commonest sources of facility contaminants

Organism	Commonest source
Gram-negative rods	Water or liquids
Gram-positive rods and fungi	External environment, e.g., soil and air
Gram-positive cocci Non-spore-forming gram-positive rods	Staff

Adapted from Parenteral Drug Association. Fundamentals of Cleaning and Disinfection Programs for Aseptic Manufacturing Facilities. Technical Report 70. 2015. *https://kupdf.net/queue/tr-70--cleaning-and-disinfection_58d6d1cadc0d60520fc34702_pdf?queue_*

Table 8 Relative advantages and disadvantages of decontamination agents

Decontaminant	Advantages	Disadvantages
Hydrogen peroxide vapor	Rapid, broad-spectrum antimicrobial effects	Affected by presence of organic/ inorganic materials, e.g., lipids
	Breaks down into nontoxic substances	Some catalase-producing bacteria show resistance
	Can assess efficacy using chemical and biological indicators	Requires specialized equipment
Formaldehyde	Long and successful history of use	Poor penetration and slow acting
	Easy to handle and inexpensive	Requires effective removal at end of process
	Simple to use & easy to detect	Toxic and carcinogenic
	Broad spectrum activity	Strictly regulated in some areas
	Ffective against M. tuberculosis	Paraformaldehyde deposition

Adapted from *https://www.ivtnetwork.com/article/biodecontamination-cleanrooms-and--laboratories-using-gassing-systems*

bacterial spores and *Mycobacterium species,* but it has less deleterious effects than agents such as formaldehyde on a range of surfaces. Most commonly hydrogen peroxide decontamination uses a non-condensing vapor in four steps. First the area to be decontaminated is dehumidified, and then 35% hydrogen peroxide is vaporized under controlled conditions of temperature, humidity, and pressure to prevent condensations. The supralethal concentration is maintained for a prolonged time period, and finally the area is purged with air to reduce the hydrogen peroxide levels.

Formaldehyde vapor can also be used, but it is toxic and an irritant that can also be explosive at concentrations above 7.7%. It can be used for equipment decontamination, but when used for an entire room, there must be adequate extraction of the vapor. The relative advantages and disadvantages of both methods are shown in Table 8.

It is recommended that gassing and vapor decontamination be performed by specialized companies.

3 Environmental Monitoring

Central to determining the efficacy of a cleaning program is an accompanying environmental monitoring program. This must include air samples for viable and nonviable particles, touch plates for monitoring personnel, RODAC plates for sampling surfaces, and settle plates for determination of fallout organisms. When results indicate excursions from the specifications set for the facility, there must be a deviation report which includes short- and long-term corrective actions. Environmental monitoring is addressed in a separate chapter in this volume.

Table 9 Common cleaning deficiencies

Cleaning deficiencies commonly cited
Failure to use water of adequate quality
Failure to use sterile water for cleaning class 100/ISO 5 areas
Failure to include a sporicide in the disinfectants
Inadequate contact time with disinfectants
Improper dilution of disinfectants
Use of expired cleaning agents
Use of inadequate tools for cleaning
Failure to follow cleaning procedures
Damage to equipment by cleaning procedures
Inadequate disinfectant for cleanroom bioburden
Failure to validate the cleaning procedures

4 Common Cleaning Citations

Regulatory agencies frequently issue warnings related to inadequate cleaning procedures. Some of the findings are shown in Table 9.

5 Maintenance

A brief mention is made of maintenance activities in the following section. This includes maintenance of the facility and of the equipment.

5.1 Facility Maintenance

It is important that facilities be maintained in good working order, and this requires the prompt attention to and resolution of problems. This should, in most cases, be delegated to a facility manager. There should be plans in place to perform scheduled testing and maintenance of the HVAC systems, at least annually, and with changes of the HEPA filters every 2 years. There should be written procedures on file that document what is to be performed. If a water system is in use, the output needs to be tested regularly for contaminants and chemistries. The flooring must be checked regularly for cracks or splits and repaired as necessary. Flooring in contact with liquid nitrogen apparatus requires particular attention as it can be easily damaged by

exposure to the liquid. Walls must be examined for damage and wearing down of the epoxy paint covering. The same is true for ceilings, which should be examined for the tight fit of items which penetrate or attach to the surface, e.g., fire indicators and light fittings. Light fittings should be replaced when necessary. Cabinetry should be regularly examined for damage or corrosion and remedial actions taken. Results of pest control actions must be documented and the program for eradication maintained.

The staff performing repairs must be trained in gowning procedures and preferably should be supervised during the repair. Cleaning may be required after the repair has been completed.

5.2 Equipment Maintenance

There must be an extensive program in place for the cleaning, maintenance, and calibration of all facility equipment. Routine cleaning, following written standard operating procedures, is best left to users in the facility and must be documented by them. Equipment calibration may be performed by the Quality Control Unit or by a contract company or the supplier. This should be performed on a written schedule and all documentation of the procedure maintained. It is normal to place a calibration sticker on the equipment showing the date of calibration and when the next calibration is to be performed. Similarly repairs to equipment must be documented and a formal re-qualification performed if the repair is likely to affect the performance of the equipment. Alarm systems that keep track of facility and equipment operating parameters require particular attention. Some require a daily checkpoint of all sensors and this is supplemented by an annual calibration of the system.

6 Conclusions

A thorough facility maintenance and cleaning program is essential for cGMP compliance and to ensure the safety of products. This must be closely allied to comprehensive environmental monitoring to determine its efficacy. It is important to know the identity of the types of organisms detected in the facility and their sensitivities to various disinfectants and to use this information to design the cleaning and disinfection program. Further protection is provided by the use of closed or functionally closed manufacturing systems.

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References

1. The Code of Federal Regulations. Title 21 – Food and Drugs. Parts 210 and 211; cGMP in Manufacturing, Processing, Packing, or Holding of Drugs and Finished Pharmaceuticals. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=210 and https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=211. Last accessed 17 July 2020.
2. Steris Laboratories (2014) *A risk based cleaning and disinfection program*. https://www.pda.org/docs/default-source/website-document-library/chapters/presentations/pacific-northwest/a-risk-based-cleaning-and-disinfection-program.pdf?sfvrsn=cb7e9e8e_4. Last accessed 17 July 2020.
3. Parenteral Drug Association (2015) Fundamentals of cleaning and disinfections programs for aseptic manufacturing facilities. Technical Report 70. https://kupdf.net/queue/tr-70-cleaning-and-disinfection_58d6d1cadc0d60520fc34702_pdf?queue_. Last accessed 17 Jul 2020.
4. ISO Standards. Cleanrooms and associated Controlled Environments. ISO 14644-1. https://zoser.com.co/wp-content/uploads/2015/10/ISO%2014644-1%20Version%202015.pdf . Last accessed 17 Jul 2020.
5. Shield Medicare. Step By Step Selection of Cleanroom Disinfectants. https://www.copybook.com/companies/shield-medicare/articles/step-by-step-selection-of-cleanroom-disinfectants. Last accessed 17 Jul 2020.
6. United States Pharmacopeia 35 <1072> Disinfectants and Antiseptics https://www.drugfuture.com/pharmacopoeia/usp35/PDF/0619-0622%20%5B1072%5D%20DISINFECTANTS%20AND%20ANTISEPTICS.pdf. Last accessed 17 Jul 2020.
7. Martinez, J. (2008). The rotation of disinfectants principle: True of false. *Pharmaceutical Technology, 33*(2).
8. European Chemicals Agency (2016) *Transitional guidance on efficacy assessment for product types 1-5, disinfectants*. https://echa.europa.eu/documents/10162/23492134/tg_efficacy_pt1-5_superseded_en.pdf/afac1df2-7cdc-acc6-ba98-e9a19ac126c3. Last accessed 17 Jul 2020.
9. U.S. Food and Drug Administration (2004) *Sterile drug products produced by aseptic processing - Current good manufacturing practice*. Guidance for Industry. September 2004. https://www.fda.gov/media/71026/download. Last accessed 17 Jul 2020.
10. European Commission Directorate General for Health and Food Safety (2015) *EU guidelines for good manufacturing practice for medicinal products for human and veterinary use annex 15: Qualification and validation*. https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-4/2015-10_annex15.pdf. Last accessed 17 July 2020.
11. U.S. Food and Drug Administration (2008) *Current good manufacturing practice for phase 1 investigational drugs*. Guidance for Industry. July 2008. https://www.fda.gov/media/70975/download. Last accessed 17 July 2020.
12. European Commission (2010) *Final report summary – Academic GMP (The Impact of Regulations (EC) No 1394/2007 on the Development of Advanced Therapy Medicinal Products (ATMPs): An Academic Perspective)*. https://cordis.europa.eu/project/id/260773/reporting. Last accessed 17 July 2020.
13. Angstrom Supply (2020) *How to clean a cleanroom: Using multiple bucket systems*. https://www.angstromcleanroomsupply.com/multiple_bucket/. Last accessed 17 July 2020.
14. Sandle T (2017) *Biodecontamination of cleanrooms and laboratories using gassing systems*. Institute of Validation Technology. https://www.ivtnetwork.com/article/biodecontamination-cleanrooms-and-laboratories-using-gassing-systems. Last accessed 17 July 2020.

GMP Documentation

C. G. Lindgren

1 GMP Documentation Management Introduction

One of the hardest decisions leaders of a GMP cell manufacturing facility have to make is how to manage their documentation. Until a decade or so ago, GMP documentation management was primarily paper-based. While source documents were paper-based, other electronic homegrown programs or commercial spreadsheet applications were often used as a backup and as a tool to trend and track documentation and data more easily. In more recent years, a variety of software options have become available to provide digital source documentation compliant with 21CFR part 11. Some software platforms have been designed to handle specific aspects of GMP documentation, such as document control, training modules, materials inventory, quality assurance, etc., and may or may not be capable to integrate or communicate with each other. Larger and more complex software which can automate processes and provide data management and reporting from a single software program, such as laboratory information systems (LIMS), are now an option as well. Historically, LIMS were seldom found outside of commercial pharmaceutical or licensed blood banking establishments. However, with the rapid expansion of commercial biotechnology, LIMS vendors have responded with systems now more suitable for cell-based manufactured products.

There are pros and cons to any documentation management choice. Most academic GMP facilities begin with and continue to use paper-based source document management which is suitable and works very well for small facilities. However, the paperwork can become overwhelming if facility activity and personnel expand greatly and/or includes more than a single manufacturing site. It should be noted that electronic software options can be expensive to set up, require initial and ongoing point-of-use validation, and still require a contingency plan if the network that

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supports the documentation software crashes. A carefully researched and thought-out strategy with consideration of risks, advantages, disadvantages, cost, etc. should be undertaken to determine the best choice for documentation management for each manufacturing facility. Regardless of whether a facility chooses electronic options for none, some, most, or all source documentation, all policies and procedures must first be developed.

This chapter will focus on development and documentation management of standard operating procedures (SOPs), but it should be noted that there are various types of documents which fall under the GMP documentation umbrella which share many of the same document management requirements. These include, but are not limited to, specifications, bills of materials, certificates of analysis, forms, labels, reports, usage logs, and qualification and/or validation documentation.

2 Standard Operating Procedures

The use of SOPs ensures that all manufacturing policies and processes are controlled and codified, thereby increasing the likelihood of consistent finished products that will be acceptable for clinical use. In addition, it prevents the expansion of "tribal" knowledge, where operators may perform erroneous procedures based on unreliable information passed down orally from person to person.

The use of SOPs and a document control system are required by Food and Drug Administration (FDA) for both Good Manufacturing Practices (GMPs) and Good Tissue Practices (GTPs) as described in Title 21: Code of Federal Regulations (21CFR 211.100 and 1271.180, respectively). Additionally, accrediting agencies, such as FACT and AABB, also require the use of SOPs (Table 1). While each agency describes baseline requirements and expectations of an SOP system, they do not provide instructions on how to write SOPs or set up a document control program. For the novice, the task of developing and implementing a full SOP program can be overwhelming. However, armed with a little knowledge, the establishment of such a system is relatively simple. The goal of this chapter is to provide useful information and helpful hints to make the process of developing an SOP infrastructure for cell-based therapy a little less daunting.

Table 1 Useful regulatory and professional standard references for documentation practices

Code of Federal Regulations, Title 21, Part 211.188 Batch Production and Control Records. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=211.188
Foundation for the Accreditation of Cellular Therapy (FACT) Standards for Immune Effector Cells, Version 1.1, March 2018, Omaha, NE
FACT-JACIE International Standards for Hematopoietic Cellular Therapy Product Collection, Processing, and Administration, Seventh Edition, March 2018, Omaha, NE
FACT Common Standards for Cellular Therapies, Second Edition, March 2019, Omaha, NE
AABB Standards for Cellular Therapy Product Services – Ninth Edition. July 2019, Bethesda, MD

2.1 Types of SOPs

An SOP should be written for each and every policy and/or procedure that could have an impact on the quality of the product, operator safety, and the safety of the facility and environment. When planning an SOP system, it is often very helpful to review an SOP Table of Contents from another institution or facility that performs similar procedures to your own. Many of the SOPs used by one facility will be applicable to another, although there will almost certainly be some degree of difference, based on the structural design of the facility and the nature of products being manufactured.

Another extremely helpful method for deciding what procedures should be governed by an SOP is to begin with a walk-through of all of the events that take place during manufacturing of a cell product. An example is shown in Fig. 1.

When using such a flow sheet, each square can be further divided into additional SOPs. For example, subdivisions of the training square shown in Fig. 1 include the employee training program, gowning procedures, aseptic technique, employee laboratory exclusion policies, annual competency testing, etc. This is a helpful exercise to undertake to determine if your SOP program is comprehensive, as well as to prevent redundancy or overlapping of procedures.

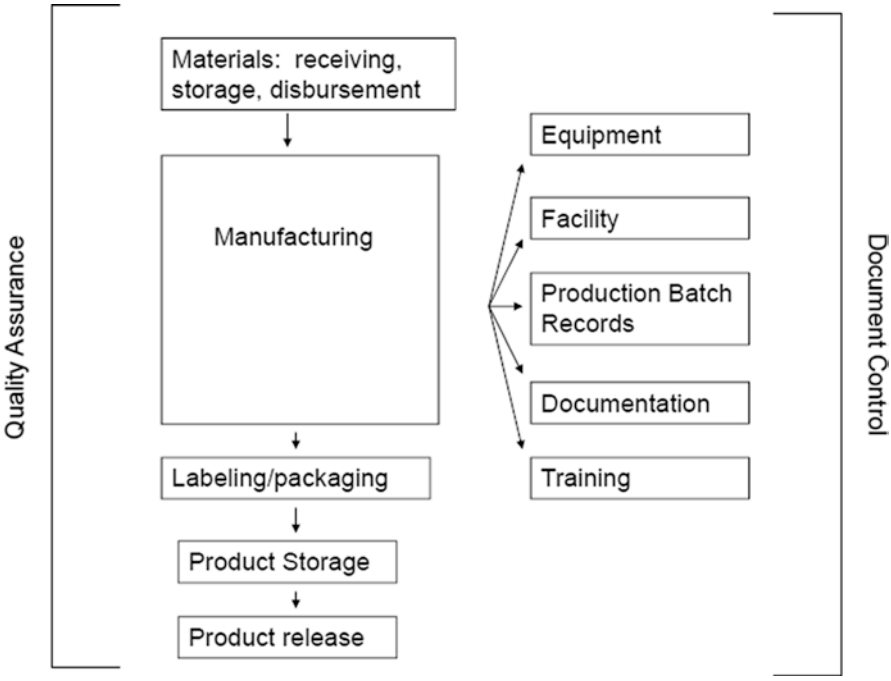

Fig. 1 Flow chart for activities performed in a cellular therapy manufacturing facility

SOPs describe procedures, policies, and processes that support manufacturing of multiple lots of a given product, such as an environmental monitoring plan, facility cleaning, record retention policy, aseptic technique, etc. An SOP which describes and records procedures for a single lot of processed or manufactured cell product is called a Production Batch Record (PBR). PBRs include space for manufacturing personnel to document all steps in the manufacturing process, including lot numbers and expiration dates of manufacturing supplies and reagents, equipment used, calculations performed during manufacturing, signatures of operators and verifiers, cell counts, etc. PBRs will be described more fully in the Product Manufacturing chapter.

2.2 Document Control

Document control is the multicomponent system that provides a process for document approval, issuance, reconciliation, revision, and archiving, as well as protecting documents from accidental or unauthorized use and/or modification. Document control provides the infrastructure for management of large numbers of SOPs. The responsibility for document control can lie with groups as diverse as a small department within a large facility, quality assurance, quality control, the laboratory manager, or another assigned person within a smaller facility. For the purpose of this chapter, the party responsible for performing document control duties will be referred to as DC. The required elements for document control consist of the following:

- Standardized numbering system
- Standardized format and content for SOPs
- Procedure for approval of document submissions and/or revisions
- Distribution (and subsequent return) of SOPs to staff
- Training documentation
- Archiving of retired or obsolete documents
- Periodic review of policies, processes, and procedures

2.2.1 Numbering Systems for SOPs

Development of a system for assigning unique identification numbers to SOPs is essential for efficient and effective SOP use. A numbering system allows for easy organization, tracking, and document control of an SOP system. Numbering can be accomplished in many different ways. Seattle Children's Therapeutic Cell Production Core facility has employed a five-character alpha-numeric system in which the first two characters are letters denoting the document category and the remaining characters within each document category are a sequentially assigned

three-digit number beginning with 001. A description of those document categories and their corresponding two letter characters is provided below:

DC Document control policies and procedures
EQ Equipment-related policies and procedures (operation, maintenance, calibration, etc.)
FA Facility/utility system-related policies and procedures (operation, maintenance, calibration, etc.)
GN General/administrative policies and procedures (SOPs applicable to more than one department)
PR Production/process-related policies and procedures (lot numbering systems, aseptic techniques, waste disposal, labeling controls)
QA Quality assurance/quality control (product release, retest policies, failure investigations, etc.)
SP Specifications for materials, components, and/or product
TM Test methods/analytical procedures
PH Apheresis program procedures
BR Production batch records

2.3 Formatting and Content of SOPs

The appearance of SOPs can vary greatly from manufacturing facility to manufacturing facility, depending on personal preferences; however, a standardized format should be developed and implemented within a facility. One of the first SOPs written should be "SOP Formatting" and should include specifics regarding:

- Font style and size
- Use of bolding and italics
- Contents of headers and/or footers
- Description for numbering sections using Roman numerals, alphanumeric, or numeric outlining

Each SOP must include approval signatures from personnel with oversight over that particular procedure. Generally, these signatures include quality assurance, subject matter expert, facility management, and/or research study investigator as appropriate. Signatures are located either at the beginning or the end of the document. Additionally, the following information must appear on each page of an SOP:

- Title and number
- Revision level
- Effective date
- Page numbers (using a format of X of Y)

The following sections make up the body of an SOP.

2.3.1 Purpose

Provides a brief description of the intended function of the SOP.

2.3.2 Scope

Provides a statement that describes the applicability of the SOP, including identification of those area(s), operation(s), and/or facilities affected by the SOP.

2.3.3 Definitions

Defines any acronyms, abbreviations, and scientific terminology required to understand the SOP.

2.3.4 References

Lists applicable references such as regulations, manuals, investigator protocols, and other SOPs that relate to the SOP. If other SOPs are referenced, include the document number only and not the revision level.

2.3.5 Health and Safety

Describes any health and safety concerns or precautions associated with the SOP.

2.3.6 Equipment and Materials

Lists materials and/or equipment required for use in the SOP.

2.3.7 Procedure

Provides a step-by-step set of instructions for the activities required to perform a specified task or function.

2.3.8 Expected Endpoints

Includes any result reporting, test results, acceptable endpoints, or objectives.

2.3.9 Attachments

List attachments, forms, and data report sheets included as part of the SOP.

2.4 Writing SOPs

Once formatting and numbering issues have been decided, the work of writing the SOPs begins. SOPs should describe in concise and easily understood language the details of all the steps involved in a process or procedure in a chronological manner. They should describe checks and controls that allow for assessment that the procedure meets the desired endpoint requirements, be as simple and short as possible, and they should not conflict with other SOPs. Basically, say what you are going to do and then do what you say.

When adding calculation tables or figures whereby a large unused space remains on the page, notate the empty space as "space left intentionally blank" to confirm the page content is accurate and not an error.

The language used in an SOP should be unambiguous, avoiding words like appropriate and adequate, unless definitions of those words are also included. While it is vitally important not to be ambiguous, on the other hand, it is equally important not to "write yourself into a corner" by setting extremely stringent requirements for procedures in which some flexibility could be allowed. When possible and appropriate, it is best to include a window within which an operation can be performed, such as "every 7–10 days," rather than the more limited "every 7 days."

Language which would seem extremely clear in day-to-day use can be ambiguous in the context of an SOP. As an example, envision a procedure which is described as "performed monthly" in an SOP. Does monthly mean once every 4 weeks? Does monthly mean once every 30 days? What about once every calendar month, in which case the 60-day window between June 1 and July 31 would be within the range and, therefore, compliant? It can be helpful to define what terms like "monthly" mean in your facility.

It is also important to keep in mind that when writing an SOP, sometimes less specific language is wiser than very specific language. An example of this might be a procedure in which 5mls of a liquid are to be pipetted into a 15 ml test tube. You might be inclined to state in your SOP, "Using a sterile 5 ml pipet, transfer 5mls of liquid A into a 15 ml test tube." However, when operators perform this task, they might instead elect to use a 10 ml pipet to transfer the 5mls of liquid. While the gradations on a 10 ml pipet are perfectly adequate to do so accurately, and the procedure has clearly been performed appropriately, you are now in deviation of the SOP because the 5 ml pipet was not used. Therefore, if it is not critical to the product; in this case it might be more prudent to say, "Using a sterile pipet, transfer 5mls of liquid A into a 15 ml test tube."

Whenever possible, have personnel who are to perform the procedures draft the SOP. Document control should allow for easy accessibility of an SOP template and staff should be encouraged to draft SOPs for procedures that fall within their job description. At Seattle Children's Therapeutic Cell Production Core facility, drafts

of SOPs are largely written by the laboratory staff and circulated among themselves for editing, before reaching leadership review and quality assurance for approval.

New employees are excellent reviewers of SOPs. The best way to find out if an SOP is clear and comprehensive is to have someone new try to follow it. They will find the parts that are unclear and difficult to understand and can provide valuable feedback into the clarification of such sections.

Lastly, it can be of enormous value to obtain SOPs from another institution or facility. Use the shared SOP as a starting point, because, as mentioned before, no two facilities are exactly alike, and modifications are likely to be required. For example, the size of your production suites, whether you have a classified cleanroom facility, and the number and type of products being manufactured within your establishment will drive how often you need to clean your facility.

3 Validation Plans

Quite often the procedures described in an SOP are based on data generated within the same facility. The process of approving a procedure based on controlled experimental data is called validation. A validation protocol is written prior to initiation of the testing process and provides a detailed description of how the process will proceed and what the acceptance criteria will be. The validation protocol requires approval signatures prior to initiation and again following execution for acceptance of the validation test results. Validation studies are an integral part of the SOP draft process.

4 Document Approval

4.1 New SOPs

SOPs need to be approved before their first use. However, SOPs in draft form can be used during practice and qualification runs, and in fact it is usually helpful to "test drive" an SOP in draft form to work out any problems and unclear sections prior to final approval. Document control (DC) typically receives the SOP drafts along with a document change request form. An example of the document change request form used at Seattle Children's Therapeutic Cell Production Core facility is shown in Fig. 2.

DC will normally assign the document number to each new draft document received, enter the assigned document number into the header or footer of the document, and verify the correct formatting of the draft document prior to initiating the routing for review cycle.

DC sends copies of the draft to all parties that need to review the document. This can be done either in hard copy or electronically. Reviewers examine the document for

SEATTLE CHILDREN'S RESEARCH INSTITUTE THERAPEUTIC CELL PRODUCTION CORE	SOP no.	DC-004
	Revision no.	10
	Effective date:	10/26/17
STANDARD OPERATING PROCEDURE	Page	11 of 12
Title: Document Control Policies for Standard Operating Procedures and Production Batch Records		

Attachment 1: Document Change Request Form

Section I: To be completed by Originator

Complete for New Documents Only:	
Document Title:	

Complete for Existing Documents Only: ☐ Revise document ☐ Retire document			
Document Title:			
Current Doc. Number:		Current Revision:	
Describe changes and justification (attach additional pages if needed)			

Originator Signature:_____ Date:_____

Section II: To be completed by Manager (N/A if Retiring a Document)

Check one:	☐ New document / revision request is approved ☐ New document / revision request is not approved
Peer Reviewer:	
Comments:	

Manager Signature:_____ Date:_____

Section III: To be completed by Document Control

Check one:	☐ Final draft or retirement request not acceptable; no document implemented or retired. ☐ Final draft acceptable to originator and all signatories; document implemented as described below. ☐ Retirement request acceptable; document retired.		
Check one:	☐ New document / revision impacts other controlled documents (describe impact) ☐ New document / revision does not impact other controlled documents		
Final Document Title:			
Final Document Number:		Effective Date:	
Final Revision:		Technical Authority:	

Document Control Signature:_____ Date:_____

Fig. 2 Document change request form

accuracy and completeness, including verification of any formulae or calculations contained in the document. Reviewers may annotate the document with any comments or modifications that the originator must address prior to final document approval. Once all changes have been collected, reviewed, and completed by the originator, a final version is drafted. A hard copy is generated by DC and routed for approval signatures. DC will assign a date on which the SOP will go into effect. Typically, the effective date will be 5 days or more from the date of obtaining the last approval signature on the document. This delayed effective date is used to allow adequate time for document distribution and staff notification and training for the new SOP.

4.2 Revised SOPs

Individuals who possess a working familiarity with the subject matter usually initiate SOP revision requests. The person initiating the revision will submit a document change request form and request a copy of the effective, current SOP from DC. DC will provide either an electronic or hard copy to the requester, who will either "red line" the changes to be made on the hard copy or use the "track changes" feature of a word processing application when working with an electronic copy. Again, it is helpful to have other staff familiar with the subject matter look at the revision request and provide input before it is returned to DC for routing for review. Any supporting data or documentation justifying the requested changes should also be provided. Documentation of the reason for requesting document revision and providing the supporting data is extremely important as revision of a document may impact regulatory commitments, other SOPs and documents, process, facility, analytical method validation status, etc. If a proposed change impacts other SOPs, they must also be reviewed as appropriate. Once again, DC will assign the revision number and provide the final hard copy for approval signatures.

4.3 Retired SOPs

On occasion, a facility may decide that a particular SOP is no longer needed. The same procedure used for new and revised SOPs of request, review, and approval is carried out, culminating with the retirement of the SOP from active use. It is important to note that any other SOPs that reference the SOP being retired will also need to be revised to remove such references.

5 Document Distribution and Availability

As previously mentioned, one of the key roles of DC is to prevent documents from accidental and/or unauthorized use. Therefore, it is imperative that originals of all the SOPs are kept in a secure location and manufacturing staff only have access to

the most recent revision of an SOP. This is accomplished by a DC distribution system. DC keeps a master list of all SOPs which include the SOP number and title, revision number, effective date, and the location of all copies of the SOP. The master list can be generated in a simple Excel spreadsheet or as part of validated document management software.

Once final approval of a new or revised SOP is completed, it is the responsibility of DC to distribute copies to the appropriate persons and/or departments. For hard copies, copies are typically made and stamped COPY on each page. Your manufacturing facility may also want to have some or all of your SOPs available for viewing electronically by staff. DC can write protect the SOP, watermark it for read-only, or use another indicator that will allow the document to be viewed but not altered.

DC is responsible for collecting and destroying all of the old copies of SOPs in circulation. That can be done by physically collecting the old copies for destruction or by documentation of the destruction of the old copies by persons within or outside of DC. DC will always keep the original revised copy for archive.

6 Production Batch Records (PBR)

The use of PBR SOPs represents another key area for document distribution. Although it is a regulatory requirement that distribution of PBRs is controlled, the methodology used to achieve that goal is not prescribed by the regulatory agencies. Some facilities have a very formal process whereby only the designated DC person/s can issue a PBR for use. Other smaller facilities may elect to have a laboratory manager sign out PBRs or even utilize a policy in which the production staff sign out documents for their own use as needed. When choosing which method to employ, it is important that PBRs are available when needed for use by staff. For example, a too-stringent document sign-out policy could lead to a situation in which the laboratory staff need a PBR for processing a product in the evening and are unable to get it as the authorized distribution person is gone for the day. Whatever system your facility sets up needs to control and document the distribution of the PBRs but be flexible enough to allow the manufacturing personnel to perform their tasks in a timely fashion and deal with any unexpected situations that might arise.

7 Training Documentation

Often the first area that a regulatory agency will inspect during an audit is the employee training records. They want to be sure that personnel performing the work are adequately trained to perform all procedures they are tasked to do. Therefore, a system needs to be in place that documents that personnel training has been completed on new and revised SOPs. A documentation system for training may be easy in theory to design and to initially implement, but it can be one of the most difficult systems to maintain on an ongoing basis.

Some facilities file training documentation records by employee. Every time personnel train on a new or revised SOP, documentation is placed in the employees' file. Another method used by some facilities is to have the training records tied with the SOP. Essentially, all personnel are trained on a new or revised SOP, at which point the personnel sign and date a document that tracks with the master records for that SOP.

These records may be kept through electronic documentation or in hard copy.

8 Archival of SOPs

When an SOP is retired or undergoes revision and is superseded by a new version, the old versions are destroyed within the manufacturing facility to prevent their continued use. However, DC must retain and archive the original version of the revised or retired SOP indefinitely. These documents should be labeled as retired on each page to prevent them from being issued by mistake in the future. Retention of revised or retired documents is important, because during an audit some procedures may have been performed using the previous, now revised SOP and, therefore, may be requested by an auditor. Additionally, retention of old revisions of SOPs can provide a good window from which to view how a process or procedure has evolved over time.

9 Annual Review

It is a GMP/GTP requirement that all policies, processes, and procedures must be periodically reviewed, typically every 1–2 years. Periodic document review is performed to assess the suitability of existing policies and procedures that support clinical product manufacture and is used as an aid in determining the need for changes to or further evaluation of manufacturing processes or SOPs. Typically, quality assurance is responsible for the compilation, evaluation, and documentation of annual procedural review with input and assistance from manufacturing personnel. In addition to reviewing SOPs, review of trends in deviations, rejections of materials, repetitive environmental action levels, and other supporting data are also used to ascertain whether changes in manufacturing procedures and SOPs are needed.

An alternative to using a single proscribed date of review of SOPs used by some facilities is a rolling review process. The total number of SOPs is divided into sections and reviewed at different time intervals with all SOPs being reviewed within the prescribed time period. Whichever method is employed, documentation of the review process is required. This can be achieved using a spreadsheet such as Excel, a simple word processing document, or even showing the review process on the SOP itself. An example of the form that Seattle Children's Therapeutic Cell Production personnel use is shown in Fig. 3.

SEATTLE CHILDREN'S RESEARCH INSTITUTE THERAPEUTIC CELL PRODUCTION CORE STANDARD OPERATING PROCEDURE	SOP number: QA-012 Revision: 06 Effective date: 12/27/18 Page 5 of 5
Title: Annual Procedural Review	

Attachment 1: SOP/PBR Review Report Form

Document Title:			
Document Number:		Revision:	
Last Review Date:			

Reviewed By (QA may assign additional reviewers):

Printed Name	Signature	Date	Needs revision?
			☐ Yes (Describe in comments section) ☐ NO
			☐ Yes (Describe in comments section) ☐ NO
			☐ Yes (Describe in comments section) ☐ NO
			☐ Yes (Describe in comments section) ☐ NO

Comments:

Completed form returned and reviewed by QA: _____ Date: _____

Next required review year: _____

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Fig. 3 Example of an SOP sign-off page

With the development of an efficient and effective document management system and use of SOPs, a quality infrastructure is formed which provides a critical base on which assurance of quality, safety, and reproducibility of products is built.

10 Conclusions

As one can see, timely and accurate documentation is truly the foundation for every activity within a GMP cell manufacturing facility, and there are a variety of approaches to attaining GMP documentation compliance. Careful consideration and planning when designing a document management system which will meet current and future needs for your facility, and is scalable and robust, will ensure successful implementation and continuance of GMP documentation compliance and moreover help ensure the quality, safety, and efficacy of products manufactured within your facility.

Process Validation

Carolyn A. Keever-Taylor

1 Introduction

The Good Manufacturing Practices (GMP) as codified in the Codes of Federal Regulations (CFR) were established primarily for the regulation of human pharmaceuticals and focus on ensuring the identity, strength, quality, and purity of drugs. The Food and Drug Administration (FDA) has responsibility for implementing GMPs. Over time the mandate of the FDA has broadened to include veterinary medicines, biological products, medical devices, cosmetics, and products that emit radiation. Cellular therapy products, in particular, have features that do not permit direct adoption of GMP regulations, such as the inability to terminally sterilize the final product, the small lot size, a broader range of activity, and the variable nature of living cells. Instead of developing a completely different set of regulations for tissues and tissue-based products, the FDA has issued a series of guidance documents for FDA reviewers and industry (including academic facilities) to define practical requirements for meeting specific GMP regulations. These guidance documents are meant to convey current thinking on compliance and may be updated based on time and experience [1, 2]. Process validation has emerged as a central tenet to ensure the purity and safety of cellular therapy products. The emergence of global regulatory paradigms for biological products has further reinforced the importance of validation as a means to achieve a safe, pure, and sterile product [3].

The FDA has defined two major categories of cellular therapy products in Title 21 of the CFR, those that are extensively manipulated and thus require a higher level of regulatory control following GMP and those that are commonly used for the restoration of hematopoiesis and require minimal manipulation. Regulations in section 351 of the Public Health Service Act (PHS Act) apply to cellular therapy products that are extensively manipulated, while regulations in section 361 were

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specifically issued for minimally manipulated tissues and their donor sources. These minimally manipulated "361" products require a lesser degree of adherence to GMP, but nevertheless the concept of process validation does apply to laboratories processing only 361 products. This chapter will focus on current thinking on how a safe, pure, and potent product can be manufactured by control of the manner by which it is produced.

2 Definition of Terms

Multiple terms are used to describe the steps required in process validation (definitions are from the seventh edition of Standards published by the Foundation for the Accreditation of Cellular Therapy (FACT) and the Joint Accreditation Committee of the International Society for Cell and Gene Therapy and the European Society for Blood and Marrow Transplantation (JACIE).

2.1 Process Validation

Establishing, by objective evidence, that the process consistently produces a cellular therapy product meeting its predetermined specifications. In its most recent guidance, FDA defines process validation as the collection and evaluation of data, from the process design stage through commercial production, which establishes scientific evidence that a process is capable of consistently delivering quality product. This is a change from the original 1987 definition which was "establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its pre-determined specifications and quality characteristics."

2.2 Process

A set of interrelated tasks and activities to accomplish a work goal.

2.3 Procedure Validation

Confirmation by examination and provision of objective evidence that particular requirements can consistently be fulfilled.

2.4 Qualification

The establishment of confidence that equipment, supplies, and reagents function consistently within established limits.

2.5 Verification

The confirmation of the accuracy of something or that specified requirements have been fulfilled.

3 Process Validation

The need for process validation is specified as being required in the CFR Quality System Regulations in 21 CFR 820.75 wherever the results of a process cannot be fully verified by subsequent inspection and testing. Where appropriate, major equipment used for the process must also be validated (or qualified). Once validated, procedures for monitoring and control of process parameters must be established to ensure specified requirements are met. Validation activities must be performed by qualified individuals. When changes or deviations occur, the process shall be reviewed and evaluated for the need for revalidation. All validation activities must be documented.

Effective process validation contributes significantly to assuring quality cellular therapy products. According to the FDA [1] the basic principle of quality assurance is that a product that is produced is fit for its intended use. The following conditions exist:

- Quality, safety, and efficacy are designed or built into the product.
- Quality cannot be adequately assured merely by in-process and finished product inspection or testing.
- Each step of a manufacturing process is controlled to assure that the finished product meets all quality attributes including specifications. A validated process ensures that product performance is consistent from batch to batch and unit to unit.

Within a given processing facility, the extent of the validation study is a function of what has been validated previously. For example, if major equipment has undergone qualification for its basic function, then you only need to verify that it functions the same way for the process to be validated, likewise an existing quality program will likely have already been validated. What is needed is to document that the new process works together with previously validated components. All of the

individual procedures could meet the definition of being validated, but the manner in which the procedures interact may result in failure to fulfill specific requirements.

Process validation has three main stages:

3.1 Process Design

Process validation begins with process design. The intent of this stage is to define the commercial process based on knowledge gained through development and scale-up activities. Well-documented product development activities provide key data to the process design stage, including expected cell yields, the percentage of cells with the desired activity, and identification of key steps affecting processing. During process design it is important to identify variations that occur during processing and their sources and to determine the degree of variation and, of most importance, to learn to control variation in proportion to the risk it represents to the process and product. There should be a focus on scientific arguments as part of the process design.

Process validation at the design stage should take into account the inherent variability of the ancillary materials and components that are used and the potential effect on quality if these materials are changed or modified by the manufacturer during scale-up of the process. These data are used to establish benchmarks for quality and production control. For the types of products produced in a cellular therapy laboratory, process design will most often use healthy donor cells. Therefore, the variability that might be expected due to the primary disease or disease treatment of the patient may need to be assessed during the process qualification stage. During process design it is important to:

- Establish a control strategy.
- Break down the process into segments for each part of the procedure and the overall process.
- Identify the limits of critical process parameters and the operational limits of the process.
- Consider regulatory constraints. The control strategy can be established by understanding the variability of the system and carried over to the final stage of process qualification.

Data from small-scale studies can contribute to the overall validation provided such studies can be demonstrated to be an appropriate representation of full-scale production.

3.2 Process Qualification

The process qualification stage is where the process design is confirmed as being capable of providing reproducible commercial manufacturing. Process qualification has two stages, facility design and qualification of equipment and process performance. Products at this stage can be used clinically (with the appropriate IND).

Qualification of equipment generally includes the following activities:

- Selecting equipment construction materials, operating principles, and performance characteristics based on whether they are appropriate for their specific uses.
- Verifying that the equipment is built and installed in compliance with the design specifications (e.g., built as designed with proper materials, capacity, and functions and properly connected and calibrated).
- Verifying that equipment operates in accordance with the process requirements in all anticipated operating ranges. This should include challenging the equipment or system functions while under load comparable to that expected during routine production. It should also include the performance of interventions, stoppage, and start-up as is expected during routine production. Operating ranges should be shown capable of being held as long as would be necessary during routine production.

Qualification of equipment may be done as part of the overall validation or under individual plans. How equipment is used in the specific validation study may require additional testing to ensure that the proper studies or tests can be used, the criteria are appropriate to assess outcomes, the timing of qualification activities, the responsibilities of relevant departments and the quality unit, and procedures for documenting and approving the qualification are in place.

The process performance qualification combines the actual facility, equipment, and trained personnel with the commercial manufacturing process, control procedures, and components to produce commercial batches. The number of batches needed here is based on the variability as established in process design. The greater the variability the more studies that are needed during process qualification. The studies should be done by personnel expected to routinely perform each step. This is a departure from the 1987 Guidance document, where most manufacturers expected no more than three procedures would be required [4].

The level of monitoring and testing should be sufficient to confirm uniform product quality throughout the batch.

In special situations, the FDA will approve commercial distribution of a product before the process qualification is complete. This applies to infrequent processes, such as orphan products, products with a short half-life, or those that are medically necessary.

3.3 Continued Process Verification

This occurs at the stage where products are being commercially manufactured. Ongoing assurance is gained during routine production that the processing remains in a state of control. The goal is continual assurance that the process remains in a state of control during commercial-scale manufacturing. This requires collection and monitoring of data during commercialization. It is critical to have systems for detecting unplanned departures for the process. Evaluating the performance of the process identifies problems and determines whether action is needed to correct, anticipate, and prevent problems from occurring. Data collection should include relevant processing trends, the quality of incoming materials or components, in-process material, and finished products. Appropriate statistical analysis may also be required.

4 The 2011 Guidance Document Vs the 1987 Guidance Document

When the original "Guidance for Industry-Process Validation: General Principles and Practices" was published in 1987, process validation was an unfamiliar concept [4]. The new version from 2011 uses the experience gained during the intervening years to update the approach and to introduce some new concepts [1]. Major differences in the 2011 version include:

- Focus on scientific arguments as part of the process design stage and throughout production instead of mere documentation.
- Requirement for collection of product data throughout the product life cycle and its scientific evaluation to support product quality.
- Introduction of the three-stage approach to process validation.
- The requirement that the number of products at the process validation stage is a function of how variable the process is. Three is not always enough.
- Use of a life cycle approach, such that ongoing assurance is gained during routine production to ensure processing remains controlled. Validation is ongoing as more knowledge is gained. Incorporate risk management.
- A change away from the worst-case concept of 1987 during process qualification. Attempts to encompass upper and lower limits and circumstances of SOPs are not required. The 2011 Guidance indicates that the process qualification lots should be within the expected typical ranges.
- A revision of the concept of revalidation to continued process verification. The 2011 Guidance now requires that changes be assessed against variability limits established during the first two stages of process validation. Changes may or may not require repeat performance qualification.

- Use of a matrix approach where multiple similar products, presentations, or equipment are grouped in one validation exercise is now acceptable, if appropriate.
- Concurrent validation, that was not mentioned in the 1987 guidance, may be allowed under defined circumstances and with appropriate justification.

5 Performing a Validation Study

Process validation is the big picture and is required when a new cellular therapy product is introduced to the laboratory. For a typical cellular processing facility preparing primarily "361" products, smaller validation studies are more typically required. Studies may be performed:

5.1 Prospectively

Before a procedure or process is implemented. This is the expected approach and should occur for any new procedure or whenever a process is implemented in a new facility.

5.2 Concurrently

Usually some studies done prior to implementation with completion during implementation, for example, verification of a device for cell enrichment or depletion.

5.3 Retrospectively

For procedures that have been in place but were not formally validated. This should rarely be needed.

5.4 Revalidation

Secondary to major changes in the procedure or process that might affect product quality. Repeat validation may also be required if sequential product lots fail to meet specifications.

What to Validate

- Processes, policies, and procedures
  - Processing (including cryopreservation)
  - Storage
  - Distribution and transport
  - Product assays and testing
- In-house prepared reagents
- Labels
  - Creation, accuracy of identity, and content
  - Suitability under conditions of use
- Computer systems provided they:
  - Are required to adhere to core GTP functions
  - Perform user-defined calculations
  - Constitute an inventory control system

6 The Validation Plan

The overall approach is to write a validation plan as defined by SOP. The plans should have a consistent format (Validation Study Template). There needs to be a process for data acquisition and analysis. It should include conclusions as to outcome and an implementation plan including staff notification and training. The validation plan should be written and reviewed by the QM program before the study is begun and after the study is completed.

Validation Study Design

- What will be measured?
- How will the measurements be done?
- How many measurements will there be?
- What are the key elements and critical control points that must be controlled?
- What are the expected results?
- What is an acceptable outcome?

Validation Study Results

- Include all raw results or reference their location
- Prepare a summary of results, use tables or figures if appropriate
- Use statistical analysis suitable for the data
- Explain unexpected failing results or repeated testing
- Come to an overall conclusion as to the validation of the procedure or process based on the study results

Key Aspects to Validate

- Analytical systems qualification

  - Equipment must be qualified to validate a procedure

- Method validation
  - Precision – Ruggedness, repeatability, and reproducibility at different levels. Expressed using standard deviation and/or coefficient of variation of replicates.
  - Accuracy – Closeness to an acceptable value. Can be precise but not accurate!
  - Limits – Highest and lowest values that can be handled.
  - Specificity, linearity, and range – Usually apply to assay validation
  - Robustness – Effect of deliberate variations in method parameter (e.g., reagent concentration, temperature)
- System Suitability – Refers to the overall process.
  - Determines potential for effects on other parts of the system.
  - Measures effects on other parts of the system

7 Conclusion

Process validation is required for all cellular therapy products. This process should start from the very beginning when the process is first designed and should continue throughout the product life cycle. Studies should be performed using a written plan

VALIDATION/VERIFICATION PLAN	
Title	
Added to the Validation/Verification Plan Table of Contents as "In Progress" by:	Date:

PURPOSE		
☐ Validation Study		☐ Verification Study
☐ Prospective	☐ Retrospective	☐ Re-validation/Re-verification
The process parameters and outcomes to be assessed in this study include:		

ACCEPTABLE RESULTS
For this study the following minimal measurement parameters are required Precision (repeatability, document stdev or confidence interval): Accuracy (mean compared to true values): Specificity (measure analyte of interest in presence of other substances): Sensitivity (lower limits of detection): Linearity and Range:

PROCEDURE
Study Procedure:
Name of needed SOPs: Name and location of needed manuals:
Study workforms- (indicate location or refer to relevant SOP):

SYSTEM DESCRIPTION
Expected results or function:
Critical Control Points (where things can go wrong):
Key elements (steps to manage to prevent errors):

TARGET REPLICATES

RESPONSIBILITIES	
Equipment installation qualification	
Performance qualification	
Study plan author	
Study plan approval	Laboratory Director

Fig. 1 Validation study template

Data review	Laboratory Director.
Final approval and implementation	Quality Manager

RESULTS	
Inclusive Dates of Study: From:	To:
Workforms:	
Raw data:	
Statistical analysis:	
Graphs:	
Data summary:	

SUMMARY EVALUATION			
Overall description of study results:			
This process is considered:	☐	Validated/Verified	☐ Not Validated/Verified
If Not Validated or Verified give reason:			
Required additional studies or monitoring after implementation.			
Further instruction if disapproved.			

IMPLEMENTATION PLAN		
SOP implementation or modification	SOP Name:	Plan:
Workform creation or modification	Workform Name:	Plan:
Personnel Notification:	Venue:	Plan:
Personnel Training:	Method:	Plan:
Effective Dates	Implementation of New Method:	Cessation of existing Method:

AUTHORIZATION SIGNATURES	
Study plan author	
Director Study Plan Approval	
Director Data Review	
Quality Manager Approval	

Fig. 1 (continued)

RESULTS	
Inclusive Dates of Study: From:	To:
Worksheets:	
Raw data:	
Statistical analysis:	
Graphs:	
Data summary:	

SUMMARY EVALUATION				
Overall description of study results:				
This process is considered:	☐	Validated/Verified	☐	Not Validated/Verified
If Not Validated or Verified give reason:				
Required additional studies or monitoring after implementation.				
Further instruction if disapproved.				

Fig. 1 (continued)

IMPLEMENTATION PLAN		
SOP implementation or modification	SOP Name:	Plan:
Workform creation or modification	Workform Name:	Plan:
Personnel Notification:	Venue:	Plan:
Personnel Training:	Method:	Plan:
Effective Dates	Implementation of New Method:	Cessation of existing Method:

AUTHORIZATION SIGNATURES	
Study plan author	
Director Study Plan Approval	
Quality Manager approval & implementation	

Fig. 1 (continued)

with the parameters defined according to the nature of the cellular therapy product itself, the equipment used, and the exact requirements for manufacturing. The end result should demonstrate that each step of the manufacturing process is controlled such that the finished product meets all quality attributes including specifications. A validated process ensures that product performance is consistent from batch to batch and unit to unit (Fig. 1).

References

1. U.S. Food and Drug Administration. (2011). *Guidance for industry. Process validation: General principles and practices*. January 2011. https://www.fda.gov/media/71021/download. Last accessed 17 Nov 2020.
2. U.S. Food and Drug Administration. (2020). *Guidance for industry. Chemistry, Manufacturing, and Control (CMC) information for human gene therapy Investigational New Drug Applications (INDs)*. January 2020. https://www.fda.gov/media/113760/download. Last accessed 17 Nov 2020.
3. European Medicines Agency, Committee for Medicinal Product for Human Use (CHMP). (2016). *Guideline on process validation for finished products-information and data to be provided in regulatory submissions*. November 2016. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-process-validation-finished-products-information-data-be-provided-regulatory-submissions_en.pdf. Last accessed 17 Nov 2020.
4. Federal Register. (1987). *Guideline on general principles of process validation*. May 1987. 52 FR 17638.

Equipment Qualification

Sara Richman

1 Introduction

Equipment qualification is the final series of inspection and tests to ensure that critical requirements necessary for related product quality are satisfied and that documents and procedures necessary to properly operate and maintain the system are in place. The US Food and Drug Administration does not define qualification, but indicates in Title 21 Code of Federal Regulation Part 211.63 that "Equipment used in the manufacture, processing, packing, or holding of a drug product shall be of appropriate design, adequate size, and suitably located to facilitate operations for its intended use and its cleaning and maintenance" [1].

Qualification is considered a subset of validation. Equipment qualification (EQ) will provide documented evidence that the specific piece of equipment has been installed per specifications (manufacturer's recommendations) and will attain and maintain critical process parameters repeatedly and reliably. EQ will often be used as evidence of regulatory compliance during regulatory audits.

2 Qualification Procedures [2]

There are generally four stages to EQ [3] (Fig. 1):

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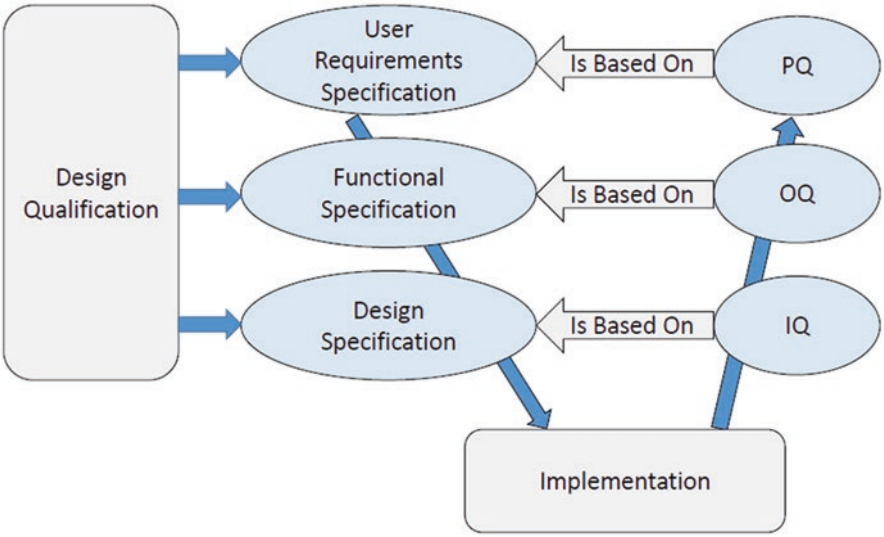

Fig. 1 Process diagram

2.1 Design Qualification (DQ)

DQ defines the functional and operational specifications of the equipment and details the decision-making process in selection of the supplier, i.e., why you have chosen this particular piece of equipment.

2.2 Installation Qualification (IQ)

IQ establishes that the instrument is received as designed and specified, that it is properly installed in the selected environment, and that the environment is suitable for proper operation, i.e., the equipment was received safely and intact and has been properly installed in the correct environment.

2.3 Operational Qualification (OQ)

OQ demonstrates that the instrument will function according to the operational specification in the selected environment, i.e., the equipment functions according to the manufacturer's specifications – in other words does what it is made to do.

2.4 Performance Qualification (PQ)

PQ demonstrates that an instrument performs according to a specification appropriate for routine use, i.e., the equipment works properly for a specific application in which you will be using it, e.g., if you use a centrifuge for general purposes, you may not need to do a PQ, and if, however, you want to be sure that it can be used to separate cells of a specific type, you would perform a PQ.

Once the specific piece of equipment is received and ready for EQ, it should be placed in the location where it will be used. It should be clearly labeled with a sign stating that "Qualification is Progress – Do Not Use." This will ensure that the equipment cannot be used for clinical processing.

3 Design Qualification

List the requirements that the piece of equipment must meet to justify your choice of the specific make and model.

- Provide a description of the equipment (e.g., centrifuge) and the serial number.
- Provide the electrical specification, e.g., voltage, amperage, etc.
- Document any specifications that must be met, e.g., must be capable of centrifugation at 1000 x G.
- Key performance characteristics, e.g., maximum speed, operational temperature, etc.
- Operational ranges, e.g., temperature control range, e.g., 4–24 °C.
- Ease of use, cleaning, and maintenance, e.g., must be able to autoclave parts.
- Sample volume, throughput, e.g., for a pump 1–10 l/h.
- Health and environmental safety concerns, e.g., must be shielded from direct light
- Uniqueness of available instrument – why this is the only acceptable model of the equipment requested.
- Indicate reasons for selecting the chosen supplier and append any supporting documentation – e.g., new equipment must be connected to existing equipment made by same supplier.

This documentation may also be useful to the institutional purchasing department to justify the particular model of equipment selected and the appropriate vendor.

4 Installation Qualification

This procedure is designed to document the installation of the equipment upon receipt. The following procedure should be followed:

- Document the date of receipt and verify that all parts ordered were received, including any ancillary equipment, such as computers and software.
- Document any damaged or missing parts and contact the vendor immediately.
- Check that operating manuals are received, scanned, and filed electronically on a shared drive (electronic manuals are often directly available from the equipment vendor) or keep hard copy with the equipment.
- Confirm that any safety and environmental information pertaining to operation and use of the instrument has been received.
- Document that the intended location meets any specifications provided by the manufacturer, e.g., correct temperature, space around equipment, power source, need for uninterrupted power supply, etc.
- Document the name of the installer, the date of installation, and the location selected.
- Document whether the equipment worked when switched on and whether it performed and passed any self-check routine.
- Indicate whether servicing, maintenance, and calibration arrangements have been made and described as appropriate.
- Document any unexpected events and actions taken.
- Confirm that installation has been performed successfully.

5 Operational Qualification

The purpose of OQ is to determine that the equipment is functioning as per the manufacturer's specifications. If it is to be used for a specialized procedure, a PQ may also be required. It may be possible to combine the PQ with the OQ. You should consult with quality assurance (QA) to determine if a PQ is necessary (Table 1).

Table 1 Additional components of operational qualification [4]

Parameter	Test
Operational conditions (specified by manufacturer, e.g., temperature, pressure, flow)	Verify operation
Testing to be performed	Use documented testing protocol
Controllers, indicators, recorders, alarms, interlocks	Verify proper operation
Sequencing through each operational step	Verify proper sequencing
Key functions and parameters	Check and document
Undesirable operations	Confirm proper operations
Appropriate response under fault or failure conditions	Check and document
Test different operating conditions	Check and document

- The OQ must be performed and approved by QA before the instrument is put into routine service.
- For OQ on equipment, such as water baths, the OQ is designed to demonstrate that the equipment maintains (in this example) temperature with the accuracy and precision described by the manufacturer. For more complex equipment, where the basic specifications must be met, but the equipment will be used in a particular manner during routine use, a PQ should also be performed. For example, an OQ would demonstrate that a centrifuge is capable of maintaining accurate speeds and temperatures, but for certain applications with cells, it may be important to show that the centrifuge is also capable of producing a specific cell product with a particular composition and viability. Under these circumstances a PQ would also be needed
- Document the specifications that will be tested, e.g., for a water bath the temperatures that will be checked.
- Document whether the equipment has been calibrated and checked. Include copies of these with the qualification package.
- The user must develop, and have approved by QA, a standard operating procedure and worksheets, for instrument operation, cleaning, maintenance, and calibration before an instrument is put into routine use

6 Performance Qualification

PQ is performed on equipment that is used in critical procedures, e.g., a magnetic cell separator may meet electrical specifications and pass self-checks as part of OQ, but this does not indicate whether it will separate cells satisfactorily. For this purpose, a PQ is required.

- The specifications for a PQ are established before the PQ takes place, e.g., the PQ must result in cells with > 90% viability and > 70% purity (Table 2).
- Document what cellular material you will use for the PQ. The PQ and the choice of the cellular material must be pre-approved by QA before starting the qualification.
- Attach PQ results with the qualification packet
- Include statistical analysis where appropriate.

The number of PQs to be performed should be determined based upon the criticality of the performance.

Table 2 Additional component of performance qualification [3]

Parameter	Test
Performance qualification plan	Predetermines acceptance criteria to be met
Documentation	Operational SOP, sampling plan
Testing to be performed	Test protocol(s)
Acceptance criteria	Specifications to be met by test samples
Final report	Results, deviations, non-conformances

7 Requalification

Qualified equipment may require requalification if it is:

- Moved
- Repaired
- Damaged
- Fails during use
- Modified (e.g., upgraded)
- The use is changed

Requalification may involve IQ, OQ, and/or PQ depending on what has happened to the equipment since its last qualification.

8 Approval of Qualification

Once the qualification has been performed, all of the appropriate documentation must be submitted for review by QA who will determine whether the qualification aims have been achieved. During this review the equipment must not be used for manufacturing. QA will formally release the equipment for use. The "Qualification in Progress – Do not Use" sign will be removed and archived by QA.

A notification should be sent to the users that the equipment has formally been released for clinical use. All equipment qualification paperwork must be filed along with any calibration documentation and available for review by external auditors. Users of the equipment must undergo formal training before it can be used for manufacturing purposes.

9 Conclusions

Properly qualified, calibrated, maintained, and cleaned equipment is essential for the safe and reproducible manufacturing of cellular therapy products and viral vectors. There must be SOPs in place for performing these procedures and documented evidence that they have been completed.

References

1. Code of Federal Regulations. Part 211. *Current good manufacturing practice for finished pharmaceuticals*. Title 21. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=211.63. Last accessed 7 Aug 2020.

2. International Society for Pharmaceutical Engineering. (2011). *ISPE Good practice guide: Applied risk management for commissioning and qualification.*
3. Gross, J. (2019). *the four stages of qualification*. Fisher Clinical Services Website. https://www.fisherclinicalservices.com/en/learning-center/insights-blog-overview/the-four-stages-of-equipment-qualification.html. Last accessed 7 Aug 2020.
4. Brown, R., & Nguyen, T. (2015). *Facilities & equipment: CGMP requirements*. Presented at Regulatory Education for Industry (REdI): Focus on CGMPs & FDA Inspections. Silver Spring, Maryland 15–16 July 2015. https://www.fda.gov/media/92841/download. Last accessed 7 Aug 2020.

Vendor Qualification and Supply Management

Robert Ott

1 Regulatory Requirements and External Standards

Firms engaged in the manufacture of Type 361 products as defined by the Public Health Service Act for Human Cell Tissue Products (HCT/Ps) are required to follow the Good Tissue Practices regulations defined in 21CFR Part 1271 [1]. Firms engaged in the manufacture of 351 products are required to follow the Good Manufacturing Practices regulations defined in 21CFR Parts 210 [2] and 211 [3]. Both the cGTP and cGMP regulations are closely aligned in many regards. Interestingly, neither set of regulations addresses the topic of "vendor qualification." The cGTP regulations use the term "vendor," but the context is always focused on the material supplied rather than the firm that provided it. There is no mention of the term "qualification," and the term "audit" is used but only in the context of establishing a quality management program. The cGTP regulations do not address the need to evaluate those entities that supply goods and services to the firm. The cGMP regulations do not use the term "vendor"; however, the term "supplier" is used frequently. The term "qualification" is used sparingly and only in the context of personnel qualifications or qualifications of consultants. The term "audit" does not appear in the cGMP regulations, and there is no formal discussion relating to the need to evaluate vendors before sourcing goods and services from them. The Foundation for the Accreditation of Cellular Therapy (FACT) [4] is far more concerned with the vendor qualification than the cGTP and cGMP regulations combined. The "Common Standards for Cellular Therapies," published by FACT, specifically requires that FACT-accredited organizations have within their **"**Quality Management Plan a summary of the policies and Standard Operating Procedures the firm uses for qualification of critical manufacturers, vendors, equipment, supplies, reagents, facilities, and services." The basis of this requirement is to ensure

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that there is control over critical manufacturers and service providers so that goods and services obtained from these firms are reliable and of consistent quality. Vendors and suppliers need to be assessed for compliance with applicable government regulations. The FACT standards go further to state that qualification plans for suppliers need to be established and approved by the quality unit.

In contrast to vendor qualification, the concept of supply management is much more readily addressed in both the cGTP and cGMP regulations. In this regard, the regulations are closely aligned with some subtle differences. Both the cGTP and cGMP regulations specify that materials are to be withheld from use until they are verified to be acceptable [1–3]. Traceability is a paramount concern; both cGTP and cGMP regulations specify the need to maintain records of items used in manufacturing. The FACT standards are in close alignment with cGTP and cGMP regulations in this regard.

1.1 Establishing Your Program for Vendor Management and Supply Management

In most processing facilities, the responsibility for vendor qualification and supply management resides with the Quality Assurance department. The quality unit is typically tasked with building these programs and ensuring that policies and procedures are written and enforced. The procedures must align with the site's quality policy and with applicable external regulations, i.e., 21CFR Part 1271, 21CFR Parts 210 and 211, and FACT requirements [1–4]. Management is responsible for ensuring that the program has sufficient support and is adequately staffed. The personnel assigned to these activities are typically organized into a separate unit/department that may reside under quality or operations. In our facility, the department responsible for receiving, releasing, and issuing materials is referred to as Materials Management and resides under the quality umbrella. Vendor qualification activities are jointly managed by the Materials Management team and Quality Assurance department.

In terms of building your program, it is first necessary to decide what activities the materials group will be responsible for managing. This information should be listed in the Quality Manual along with a *brief* description of the *key* procedures that relate to each process. The intent is just to reference the procedures by name so that the Quality Manual captures all the critical documents that relate to the process. The firm's Quality Manual is typically the most important document that it will produce. It provides the framework upon which the quality system is based and is often the first document requested in an audit.

In terms of processes and procedures to be defined, the following activities relate to management of supplies. A separate list follows for processes and procedures related to vendor qualification.

Supply management procedures:

- Procedure for creating item codes
- Procedure for ordering, receiving, evaluating, releasing, and distributing materials
- Procedure for material transfers
- Procedure for assignment of expiration dates when not assigned by manufacturer
- Procedure for inventory control
- Procedure for domestic and international shipments
- Procedure for disposal of materials
- Procedure for writing and approving release specification

Supplier qualification procedures:

- Procedure for vendor qualification
- Procedure for external audits
- Supplier questionnaire
- Supplier self-assessment
- Procedure for supplier corrective action requests

After establishing these processes and procedures, a gap assessment should be undertaken to verify that the firm is meeting all applicable regulatory standards. Quality Assurance may perform an internal audit, or an external consultant can be contracted to review the quality system. Firms registered with the FDA as processing facilities are subject to ongoing surveillance audits to assess compliance with applicable sections of the Code of Federal Regulations (CFR). It is widely recognized that there are multiple approaches for achieving compliance. There are both low-tech and high-tech solutions for every requirement with matching variability in the level of startup investment required. In my personal opinion facilities should be encouraged to work within their means and build modest systems that meet all requirements and are sustainable within the capacity of their existing resources. When designing the program, firms need to focus first on what is manageable and sustainable. Complexity can be added later when the program has matured and there is a firm foundation upon which to build.

The reporting structure for personnel should be clearly defined in an organizational (ORG) chart. Typically, the materials group will report to either quality or operations. If the materials group reports through operations, it is important that the responsibility for releasing supplies does not reside within the manufacturing group, which creates a potential conflict of interest. As the manufacturing group needs the supplies to perform their job function, they are not necessarily positioned to make unbiased decisions as to their suitability. The determination as to the suitability of supplies and raw materials is best left to an independent group where there are no perceptions of bias. The author recognizes that for smaller organizations, individuals may wear multiple hats and the role of quality and production could be handled by the same individual out of necessity. In cases where the independent oversight of a quality group cannot be obtained, care needs to be leveraged when this

responsibility is transferred to production. While this situation is not ideal, it is a reality for small organizations.

1.2 Qualification of Vendors and Service Providers

Vendors providing a service to your firm are no less critical than those vendors providing supplies used for processing. Common service providers used by cell processing labs may include commercial couriers, pest control providers, laboratories for testing and characterization, janitorial/housekeeping services, calibration/metrology service providers, and those providing preventive maintenance activities for equipment and building systems. Initial selection of the vendor should include evaluation of relevant certifications, such as International Standards Organization (ISO) [5] and FACT. Firms need to establish a process for qualification of vendors and service providers. This process should be defined in a procedure and referenced in the firm's Quality Manual. The firm should maintain a list of all approved suppliers, and this list should be made available to the purchasing department. Purchases should only be made from approved providers. Vendor management is a critical but time-consuming activity. Firms should be encouraged to follow a risk-based approach whereby the level of qualification is tied to the criticality of the product or service supplied by the firm. In the most common risk-based approaches, supplies are grouped into tiers based on criticality, and activities required for qualification are defined specifically for each tier. The principle reason for performing vendor qualification is to ensure that the vendor will consistently be able to supply their goods and services with the requisite quality attributes necessary for manufacturing according to cGMP, cGTP, and FACT standards [1–4]. The scaled or tiered approach to vendor qualification could be represented as:

- Level I, no qualification required.
- Level II, vendor self-assessment required; qualification completion indicates no objectionable findings.
- Level III, vendor self-assessment and on-site audit required; vendor will be considered qualified only after successful resolution of any audit findings.

Many firms have procedures for vendor qualification requiring completion of self-assessments; however few of these firms define how the completed self-assessment worksheets are reviewed and evaluated. The procedure for vendor qualification should describe how vendor self-evaluations are reviewed and processed. It is simply not enough to collect the data. It must be analyzed and processed. The presence of a defined procedure provides assurance that vendors will be assessed fairly. The procedure should describe how the vendor self-evaluations are analyzed and the threshold required for approval. In the event objectionable information is discovered, the procedure should describe how the vendor is to be tracked and monitored, should it be necessary to retain their services. The procedure for vendor qualification should be linked to the vendor self-assessment worksheet. This

procedure should define how the self-assessment is to be evaluated, along with the expectations for vendor responses to audit reports. It is important that the process is impartial and unbiased. The conditions under which vendor qualification can be rescinded, due to objectionable findings or poor performance, should also be defined in this procedure. Supplier Corrective Action Reports (SCAR) are useful as formal documentation of instances where suppliers have failed to meet expectations for the provision of goods and services. As with audits, it is imperative that this process be handled consistently and fairly.

Supplier scorecards are an interesting approach to monitoring the performance of a firm's suppliers. Annual assessments based on these scorecards can provide a means to improve supplier performance. Most reputable vendors will welcome performance feedback. In order to have information to share, a tracking mechanism needs to be established. Potential metrics may include price, on-time delivery, order accuracy, quality of good/service provided, and adherence to delivery specifications. Supplier scorecards are useful for providing an objective measure of vendor performance over a specified time frame. They could also prove invaluable when addressing supplier notifications of price increases.

Vendor qualification is a necessary, but time-consuming activity. Many firms simply do not have the resources required to support on-going site audits. One consideration, as an alternative to onsite audits, is to review vendor qualification information provided by Rx-360, the International Pharmaceutical Supply Chain Consortium [6]. Desk audits are another alternative to on-site audits. A desk audit is another term for a supplier audit that occurs remotely. Documents are typically shared electronically, and the auditor views the material from their home office. However, many firms are reluctant to share documents electronically, even with appropriate confidentiality agreements in place. Desk audits do not allow for direct observation of the manufacturing process, such as that which would occur during a facility tour/inspection.

1.3 Supply Agreements, Quality Agreements/Contracts, and Change Notification

A supply agreement is an agreement between a supplier and a buyer for supply and purchase of products. The agreement specifies the terms upon which the parties agree to supply and purchase products from each other. Typical items included in a supply agreement are:

- Purchase price
- Order forecast
- Order and delivery process
- Change notification
- Sampling and inspection
- Product defects

The order forecast serves to identify the amount of product to be purchased and the anticipated schedule for product delivery. Order and delivery process describe vendor requirements for how orders are to be placed and the customer's requirements for how delivery shall occur. Change notification describes the notification process that the supplier must follow *before* making a change to an item included in the supply agreement. Suppliers are obligated to disclose any change that impacts the form, fit, or function of the material. Besides physical specifications, this information includes packaging, labeling, testing, characterization, and regulatory status. Sampling and inspection define whether the customer requires a sample for testing prior to taking acceptance of the lot/batch. Product defect describes the actions that are to occur when defects are detected in the delivered material. Supply agreements are useful for critical custom items. When qualifying vendors, it is critical to determine if they have a change control program. Deficiencies in change control and the change notification process could lead to unexpected variability that could ultimately impact product quality.

Periodic surveillance audits that include a detailed review of batch production histories are useful for detecting changes in manufacturing processes. Auditors should review past change control notifications from the manufacturer as preparation for the audit.

1.4 Monitoring and Tracking Supplier Performance

The term supplier refers to those entities that provide goods and services to the cell processing facility. The terms vendor and supplier are used synonymously throughout this chapter. It is important to remember that service providers warrant the same consideration as those firms providing goods. Many of the services solicited from external providers are deemed essential as the firm does not have a mechanism to perform these activities for itself. The same can be stated for the supplies and raw materials used in processing. Most goods used in a cell processing lab are sourced from external suppliers. Typically, very few of the supplies and raw materials are manufactured in house. The exception is usually custom media products used for proprietary processes. At a minimum, suppliers should be qualified before being allowed to supply goods or services to the firm. The expectation is that goods and services are procured from approved suppliers and the firm maintains records of which suppliers are approved and those that are *not* approved. The firm should maintain an approved supplier list and this list should be shared with the purchasing department. Quality Assurance personnel should immediately communicate any changes to supplier status to the purchasing department.

In this section, we will discuss the importance of tracking the performance of your suppliers, post-qualification and approval. To begin, supplier management needs to be viewed as a long-term activity. It is not a "one and done" process;

periodic follow-up is required. The process cannot be allowed to run on autopilot. Vendor management involves establishing an ongoing relationship with your suppliers, such that you become familiar with their quality system. Firms need to reassess their suppliers on a periodic basis and to ensure ongoing suitability. Firms have an obligation to ensure that their suppliers are continuing to provide goods and services that meet all specified quality standards and contractual obligations. The important thing to remember is that this is a long-term commitment and the firm's relationship with its suppliers may last for many years. No organization is ever static, and it is not prudent to continue to use suppliers without some formal, documented reassessment process. Firms that achieve initial approval may be unable to meet the same performance standards in a subsequent evaluation. Changes in the supplier's financial health may necessitate changes in staffing and the way the vendor seeks to fulfil their business model. The vendor's commitment to quality may be challenged by resource constraints. Periodic reassessments are valuable for all the reasons highlighted here. One approach that firms may wish to consider is the use of "supplier scorecards." This process involves tracking vendor performance in real time and using the metrics as a "scorecard" to provide performance feedback to the vendor. Examples of the type of data collected may include on-time delivery, material/service quality, price (including consistency), and customer support. The practice of collecting this type of data requires a commitment that cannot be overlooked. However, this valuable data provides objective evidence. Suppliers need to know when they are doing well and when they are not doing well. Silence equates to acceptance and provides no incentive for the supplier to change course. Supplier quality issues need to be detected and addressed expeditiously so that they do not translate into product quality issues with the cellular products. Suppliers that do not respond to product quality issues need to be managed. Big Pharma uses Supplier Corrective Action Reports (SCAR) to document and track product quality issues formally. SCAR should have a corrective action component to address the customer's observations. It is also good practice to maintain qualified backup vendors who can be called upon if the primary vendor is unable to deliver as planned. It is critical to ensure that backup vendors are truly independent and capable of providing the specified goods or services. Frequently reagents are bought, relabeled, and sold by intermediate parties that pass the goods off as their own. This practice has become increasingly common with monoclonal antibodies used for research applications.

2 Pandemics, Supply Chains, and Disaster Mitigation Strategies

The global pandemic caused by COVID-19 has impacted health care systems on every single continent. Processing facilities have not been immune as the demand for personal protective equipment has strangled the supply chain that furnishes this

equipment for medical professionals. Disaster response planning is not a new phenomenon and is a requirement for FACT-accredited institutions. FACT requires a disaster plan which includes actions from the processing facility; refer to FACT Standard D5.1.21 (Cellular therapy emergency and disaster plan, including the Processing Facility response). Disaster preparation is not integrated into the regulations but is a business practice. In my opinion, I have seen many disaster plans and they all suffer from the same set of flaws. The most common finding is that the plans are too superficial to provide actionable guidance in the event of true disaster. In most cases, the plans list the supervisory personnel to notify when a disaster occurs. While this information is certainly a required element of an emergency response plan, it is only the beginning. Disaster plans are arguably very difficult to write as no one can predict with any certainty how the disaster will unfold. However, this difficulty should not be a justifiable excuse for limiting the scope of the plan. There is no way to write a disaster plan that will provide a step-by-step remedy for every potential eventuality. There is, however, value in discussing potential case studies and providing recommended institutional responses. Good disaster response plans are detailed, descriptive, identify the institutional resources available to personnel, and delineate institutional priorities for business continuity. I have had the opportunity to review a few disaster response plans that are truly exceptional. Disaster response plans must be exercised and tested in controlled drills to evaluate their efficacy. Unfortunately, few institutions take the time to run drills and evaluate their outcome. This drill approach helps determine shortcomings in the plan that need to be mitigated. Most importantly, as your customer, I want to know how you are going to continue operations. The global pandemic caused by COVID-19 has stress-tested many organizations. We have all had to adapt, overcome, and find new ways to meet business needs while conforming to the regulations and standards that govern cellular therapy. Undoubtedly, each firm is learning about their vulnerabilities. Some of these may be shared across the industry while others may be institutionally specific. Whatever the case, firms should take the opportunity to update their risk mitigation strategies. Be sure to keep a print copy accessible in a location clearly designated for emergency operations.

Consider these questions when developing your plan. How long would you be able to keep running if your supply chain stalled tomorrow? Know your burn rate and know your targets. What happens if 50% of your staff are unable to report to work? Are staff sufficiently cross-trained and competent to establish continuity? Cross-train now for future incidents. What about access to controlled documentation? Do you have a backup plan in the event your document management system becomes inoperable? Is there a backup mechanism in place to obtain a controlled version of the document? Bottom line include your electronic systems in your disaster plan. How will you replace their function if they are no longer accessible? Final point, take the opportunity that COVID-19 presents and update your disaster response accordingly. I believe strongly in the adage, “those that do not learn history are doomed to repeat it.” The threat to your business, from global pandemics caused by infectious viruses and microorganisms, is real.

3 Selecting Supplies and Establishing Release Specifications

Supplies must be selected and used in a manner that is consistent with the intentions of the manufacturers that produced them. Cellular products should be manufactured using supplies that are approved for *human* use. The materials used in their construction must be commensurate with this purpose. Supplies should be selected and approved by the processing facility director, medical director, and quality director. Supply selection decisions need to be made by an individual with the requisite authority of knowledge of manufacturing cellular products. Supplies should be selected by an individual with the appropriate education, training, and background needed to approve their use. Typically, supplies are selected and approved by the process facility director. Supplies which are approved by a relevant health authority reduce the qualification burden. Once supplies are approved, release specification records provide a mechanism to ensure that the items are sourced from the appropriate vendor/distributor and their essential quality attributes have been checked. A release specification/release record is a document that formally defines the specifications that a material must possess in order to be deemed suitable for release. Without release specifications, there is no *consistent* way to differentiate approved supplies from non-conforming supplies. It is all about consistency when it comes to materials used to process cellular therapy products.

Release specifications/release records should include the following verifiable information:

- Identification of the approved manufacturer (manufacturer's catalog number)
- Identification of the approved distributor (distributor catalog number)
- Identification of the version number of the manufacturer's release record, specification sheet, or certificate of analysis
- Expiration date
- Identification of storage conditions
- Special storage parameters (e.g., protect from light and humidity)
- Sterility (if applicable)
- Endotoxin (if applicable)
- Results of infectious disease testing (if applicable)
- Product-specific parameters
- Space to record test results
- Space to record signatures
- Space to record the results of comparability testing

For critical reagents, components, and supplies, where no suitable clinical or pharmaceutical grade is available, it is necessary to perform comparability testing any time a new batch is received. This activity is required per FACT regulations (D6.2.4.2 Where there are no suitable clinical or pharmaceutical grade reagents available, reagents shall undergo lot-to-lot functional verification). Some firms take the approach of creating a centralized list of all approved supplies. Such a list is useful when designing new production processes. The approved parts list serves a

centralized repository for all raw materials, components, and supplies that have been approved by the firm. Having all items in a centralized list or database makes it easier to build the master production records and pick lists that are required for cGMP manufacturing. Some firms go through an additional step and include information about the supplier's qualification status.

4 Evaluation, Testing, and Release

Products should be reviewed and released by Quality Assurance personnel (or someone functioning in a capacity that is independent from manufacturing). The name of the individuals making the release decision must be recorded. It is a conflict of interest for the same personnel to release products that are to be used for production. There needs to be an independent voice. Supplies that are non-conforming must not be used for production without acceptable justification. Use of non-conforming supplies shall be documented in a variance or deviation report. The risks associated with using the non-conforming supply must be identified in the deviation report. All incoming supplies shall be held in quarantine until it has been determined that they meet release specifications. Firms must have a way of securing unreleased items to prevent their inadvertent use. Unreleased storage areas are necessary to accommodate all applicable temperature ranges. Supplies, that are accepted purely based on the manufacturer's certificate of analysis (COA), should be checked to ensure that specifications are within the acceptable range. Performance testing is strongly recommended for all critical components. Lot-to-lot comparability testing is required when a new lot of a critical reagent is received. It is recommended that firms establish a risk-based approach for determining what items require performance-based testing. Items that are not the appropriate grade require some level of performance testing to justify their suitability.

Release evaluation must be conducted systematically and the outcome independently reviewed by a second person trained in the release process. All items in the shipment must be inspected. A visual inspection coupled with review of the manufacturer's COA is the minimum set of checks to be performed. Visual verification must confirm the integrity of the outer packaging and ensure no overt signs of contamination. Products received with compromised packaging must not be used for manufacturing cellular products. Firms may elect to assign a unique number (receival number/batch number) to differentiate materials from a secondary shipment that may carry the same manufacturer's batch number. Such a process provides a unique and convenient mechanism for archiving release documentation and the manufacturer's specification sheets and certificates. This documentation must be retained per the cGTP regulations [1]. Workstations in the receiving department need to include sufficient space to permit orderly segregation of incoming materials and to prevent mixing of approved and unapproved inventory. Careful consideration needs to be made regarding operator needs. Stations need to be set up in an orderly manner with the operator in mind. Risk of mix-ups is higher when workstations do

not facilitate orderly segregation. Workstations segregated by job function make it easier to continue processes in the event work is interrupted.

5 Issuance and Batch Traceability

Batch traceability is a cGTP requirement. Processing facilities must establish and maintain records of all components and supplies used in the manufacturing of cellular therapy products. Traceability to all components and supplies used in processing must be maintained. Traceability can be achieved through a variety of mechanisms, including inventory control logs and batch production records. Both the cGTP and cGMP regulations speak to batch traceability. Items used for processing should be issued to the batch and recorded in the batch history record [2, 3]. Electronic inventory management systems, with barcode tracking, are capable of creating reports to show items consumed during batch construction. Such systems require that materials are identified as they are scanned into the batch. Expired items are automatically excluded. Batch traceability is also a cGMP requirement. In the event of a product recall, traceability of the supply must be evident so that all cellular products, constructed with the offensive material, can be identified. The inventory management system should permit both forward and backward tracing. "Backward" tracing is where you start with a final cellular product and identify all the materials used in its construction, for example, reviewing batch production records to check materials used in its construction. "Forward" tracing is where you start with a specific supply and determine where the supply was used, for example, reviewing inventory logs to determine which batches contain the supply. Tracing is a laborious and time-consuming activity, especially when performed manually. Fully electronic systems make tracing more efficient. The bottom line is that no matter how tracing is performed, your system must provide an unambiguous answer to where materials were used.

6 Storage

Adequate storage facilities must be available for all supplies, components, and raw materials used in processing. Supplies must be kept under conditions that are consistent with manufacturer's recommendation and adequate for preserving the quality, purity, potency, and efficacy of the material. Systems must be in place to monitor environmental conditions in all storage locations. Excursions from approved storage conditions must be investigated and the results of the investigation shall be documented and risk-assessed. Materials that are deemed objectionable must be culled and removed from controlled inventory. Temperature excursions are typically documented in deviation reports.

Continuous laboratory monitoring systems are recommended and offer 24/7 protection. These programs monitor storage locations and contact personnel following a prescribed call or email list when a critical limit is reached. Typically, there is a delay built into the programming to avoid issuance of nuisance alarms. For example, a firm may elect to set the program to initiate the dial-out process after being out of range for at least 15 minutes. For temperature-controlled equipment, this typically provides a suitable window to allow for instrument recovery following routine use (door opened/door closed). It is critical that personnel update management anytime their contact information changes. Some monitoring programs are also able to send email alerts, but this is typically of limited utility when problems arise in the middle of the night. Monitoring programs should be validated and alarm functionality tested on a regular basis. In conjunction with the periodic testing, it is advisable to use the opportunity to evaluate process personnel for alarm response and system acknowledgment. Simply put, alarm response is one of the most critical activities that staff might perform, as the freezers monitored by these programs may hold thousands of irreplaceable patient samples. Given this criticality, it is advisable to run drills with new staff on how to respond to equipment alarms.

Backup procedures need to be put in place for contingency during system downtime. The procedure should establish a reporting interval during which manual checks of equipment should be made. A record of the manual data checks should be kept with general monitoring records. Provision for backup instrumentation must be available. Refrigerators and freezers will fail on occasion, and it is critical that the facility maintain enough reserve storage capacity at each temperature. Inventory logs must be updated to reflect the temporary storage location for traceability. FACT standards require that accredited organizations have a backup plan in the event of equipment failures. The backup units should be clearly designated as such to facilitate product transfers in the event of an emergency. Firms with a predominantly aging fleet of freezers should give serious thought to increasing the proportion of backup storage capacity.

Storage location shall be monitored for humidity as well as temperature. It is recognized that while most consumables are packaged in way to make them impervious to the effects of humidity, it is important that limits are set and room conditions monitored. Part of the incoming evaluation process for new materials should include an assessment of the manufacturer's recommended storage conditions and the conditions where the item would be stored at the firm. Storage conditions should be defined on the release record. Provisions need to be in place to protect items which are sensitive to light. Opaque bags or dark colored secondary packaging can be employed when it is not possible to create these conditions within the room itself. Be certain to follow local, state, and federal guidelines for storage and disposal of hazardous materials. Remember to follow the guidelines and requirements established by your local institution as well. Most academic facilities are required to follow institutional environmental health and safety regulations. Items should be organized in such a manner as to permit "first-in, first-out" dispensing by materials management personnel. Storage bins are useful for separating materials; and ziplock storage bags can be used for further segregating items within the bins

according to the lot number. Supplies should not be kept in contact with the floor. Further, it is also important to be mindful of fire code regulations regarding room occupancy and storage. For fire safety, it is critical that firms do not stack items too close to the ceiling; fire suppression sprinkler heads should never be blocked.

Most hospitals and academic institutions have a department responsible for environmental health and safety, who should be consulted prior to making changes to a storage location. Firms need to be mindful of accessibility when setting up their storage areas. This detail is important for at least three reasons:

- Items should be able to be stowed and retrieved quickly without creating a harmful situation. Bins used for storage should be clearly marked as to their contents. Operator safety and ergonomics are important considerations.
- Storage locations should be able to be easily cleaned. Periodic cleaning is required by both the cGTP and cGMP regulations. Cleaning of storage locations is typically facilitated when there is reserve capacity allowing for items to be moved without stacking. Mobile storage shelves enhance accessibility and allow for cleaning difficult locations. The procedure for cleaning should describe the cleaning schedule (frequency), the cleaning method and documentation, the cleaning agents and tools required, and the personnel responsible for performing cleaning. Procedures that are unduly cumbersome, and use tools that are selected without operator input, will typically not yield a good outcome.
- Inventory assessments (cycle counts) need to be able to be performed quickly and yield accurate data. This task is delayed and potentially compromised, if the storage locations are not organized optimally.

When designing storage locations, firms need to carefully consider the potential for future growth. Is the current system amenable to growth? Firms, that build their inventory locations to satisfy only today's needs, will likely be sorely disappointed when they reach saturation capacity tomorrow. Storage capacity will ebb and flow with the demands of production. The materials needed today might not always be the materials of choice for tomorrow.

7 Recalls and Product Advisories

Firms. engaged in the processing, testing, packaging, holding, and distributing of cellular therapy products must have procedures and processes for responding to recalls. Upon notification of a recall, firms should first verify that the material included in the recall advisory was received. If received, the firm must determine if any of the impacted inventory remains in-house and ascertain if it was utilized for manufacturing.

Any remaining unused inventory should be secured and placed in a quarantine location immediately. Depending upon the nature of the recall advisory, Quality Assurance personnel may be able to release the item from quarantine following inspection or implementation of other checks as described in the recall advisory.

Any decision to release recalled, quarantined items shall be documented and the justification recorded. Recalled items that were issued to production must be traced. In the event a recalled supply was used to manufacture a cellular product, a determination must be made as to whether the cellular product has been compromised. If the product has already been administered, the patient's physician should be notified. Quality Assurance personnel should be prepared to provide a detailed narrative on the reason for the recall and the role of the supply in the manufacturing process. A manufacturing process flowchart, depicting critical steps, including points of entry for critical supplies, components, and raw materials, should be created. In the event the product has yet to be infused, a determination needs to be made as to whether it remains suitable for patient use. The patient's physician/medical director will need this information to determine if medical intervention is required. Suppliers are required to notify customers when they are aware of product performance concerns that could alter the quality or expected performance of the supplied material. Supply agreements and/or quality agreements should contain language outlining the supplier's responsibility for customer notification during the recall process. It is critical that customers communicate with suppliers as to how they should be notified in the event of a recall. Hospitals and academic research centers are large enterprises with many distinct departments and personnel; frequently, there are multiple buyers and purchasing agents. Critical recall information must be disseminated quickly, efficiently, and effectively transferred to the consumer or end user of the supply. For this reason, it is important that all personnel associated with any part of the institutional supply chain must be trained on how to respond to and communicate recall information. A flowchart or decision tree is a useful tool to help personnel respond appropriately to recalls.

8 Inventory Management

It is essential that firms have procedures for managing inventory. There are many elements that go into this task and they are described in the sections that follow. One item that bears immediate discussion is the role of production planning in the inventory management process. Production activities should be driven by a schedule, and this schedule shall determine how the firm's personnel, facilities, and equipment are to be deployed and prioritized for processing products. This planning process shall also describe how components, supplies, and raw materials are to be allocated in support of this work. It is imperative that there be a process for communicating the production schedule to the personnel responsible for ordering materials or otherwise managing inventory. Production planning is important, but to be meaningful and effective, the decision and outcome must be articulated to all stakeholders. While it is beyond the scope of this chapter, careful consideration needs to be given to assess the firm's overall capacity for processing cellular products. This assessment should translate to all departments that contribute in any capacity to production of cellular products. While personnel resources are paramount to this discussion,

capacity issues can also be triggered by limitations in infrastructure and equipment availability.

Supplies must be available for production processes when they occur and must be within expiry at the point of use. The inability to process patient cells due to lack of processing supplies is unacceptable. The human cells used as progenitors for cellular therapy products are perishable and fresh products must be used within a narrow window. It is critical that processing facilities maintain adequate inventory of all supplies and raw materials used for processing. Firms are recommended to establish regular, recurring production planning meetings, where a production schedule is shared and discussed. This practice is absolutely essential. The schedule should be shared with all staff that are involved with the cellular therapy program and posted in a prominent location. An inventory assessment should be performed after each production planning meeting to determine if adequate supplies are available to process all patients identified on the schedule. Changes to production schedules must be communicated promptly, especially when additional patients are added beyond the original forecast.

Due to the expense of critical supplies and components, and the requirement to use these items within expiry, maintaining a vast surplus is not a realistic option. Most electronic inventory management systems can set targets and reorder points for supplies and raw materials. This functionality allows for placement of orders without human intervention, and they can track inventory and place orders at predetermined minimum values. Electronic systems are only as good as the information they have been provided. Failure to record items debited from the inventory will cause the electronic system to perceive that there is more product available to issue than there really is in inventory. If materials are electronically entered into the inventory system, it is equally important that they are debited out of the system electronically when issued for batch production. Orders will be filled up to an established maximum. These systems work well with minimum intervention; however, the bottom line is that they are only reliable if provided with accurate and timely data.

Most electronic inventory management systems can run on-demand reports to determine inventory levels. These reports are especially valuable to assess site readiness following a production planning meeting. Inventory holdings should be physically verified in an audit to verify accuracy relative to the holdings reflected in the electronic system. Some firms elect to perform cycle counts where a subset of inventory is counted (physically verified) to ensure that numbers reflected in the electronic system are accurate. This practice has merit regardless of whether an electronic system or paper-based system is utilized.

9 Expired Materials

Expired supplies, materials, and components must not be used for processing. Expired items may be suitable for research and development applications with suitable annotation within the study record. Firms are recommended to set targets for

stock based on production history. While it is recognized that materials are expensive, this reality should not create undue pressure to use these items past their expiry. Expiration dates are provided by the manufacturers for a reason, and therefore, firms should be cautious about arbitrarily extending these dates without solid scientific justification. Real-time stability assessments, collected under an approved stability protocol, provide the most objective approach for reevaluating expiry dates. Multiple lots should be included in the stability evaluation to determine if there is any lot-to-lot variability. It may also be prudent to evaluate the material in manufacturing processes for different product types to verify that the material remains suitable for all applications tested.

A systematic process for removal of expired inventory must be implemented. Electronic inventory management systems can generate lists of items that are expired or are about to expire. Manual inventory systems require more maintenance; personnel will need to inspect materials (or inventory log sheets) on a periodic basis to cull expired items. Firms shall write, approve, and follow procedures defining how expiration dates are to be set in the absence of such information from the supplier. Critical items for which expiry dates are not provided by the manufacturer should be performance tested in a real-time stability assessment. The use of expired supplies for processing should only be undertaken in an emergency. Use of expired supplies should be handled as a deviation and the event evaluated using a formal risk assessment. The inventory system must be kept current regardless of whether it is electronic, paper-based, or a hybrid system with both electronic and non-electronic elements. Processing staff need to have confidence that materials will be readily available when needed.

10 Personnel

No discussion on this topic would be complete without consideration for staffing and personnel. Materials management personnel should be trained in applicable sections of the cGTP regulations, cGMP regulations, and FACT standards. Training matrices are recommended and should include the aforementioned items as well as institutional policies and procedures. Most firms have an annual program for safety training. Competency and proficiency assessments should be established for materials management personnel. If shipping of cellular products is performed at your facility, it is recommended that you have personnel who are specifically trained to manage this process. There are many considerations associated with shipping cellular products, and this is best managed by personnel trained specifically for this role. The skills needed for materials management personnel are varied and diverse. A list of skills commonly required, in both academia and industry, is:

- Receiving, stocking
- Handling cryogenic materials
- Programming temperature monitoring devices

- Ordering
- Shipping dangerous goods/loading/unloading refrigerators and freezers (−20 °C, −80 °C, and LN_2)
- Navigating electronic systems, including, but not limited to, inventory management systems, electronic document management systems, Laboratory Information Management System (LIMS) for metrology, deviation/Corrective and Preventive Action (CAPA) management programs, change control programs, and equipment monitoring programs.

A biological background is advantageous for materials management personnel, including limited laboratory experience. This background is useful, especially so that personnel recognize proper precautions for handling biological materials. The basis of this comment is to protect the worker, but also to ensure the material is handled properly and protected from conditions that could lead to its deterioration. If personnel have used these items in a research setting, they are more likely to understand the precautions necessary to safeguard the material. Experience with shipping biologics is also useful and includes knowledge of the permits and documentation that must accompany these shipments.

Personnel should be cross-trained to the highest degree possible. This practice affords managers the greatest flexibility and is the best buffer against the unexpected. In addition to cross-training, it is equally important that all personnel have access to the software needed to perform all job functions that they could be expected to complete. Software that is only locally installed is a risk. Software should be networked, whenever possible, to increase accessibility. Access and permissions can be assigned by the network administrator. The important thing is that the software is available when it is needed. A training matrix should be created for all employees within the processing facility. This matrix should be updated periodically to ensure new and revised procedures are added to the matrix as deemed appropriate and archived procedures are removed. The training coordinator should audit training records periodically to ensure that there are no gaps. Deficiencies in completion of training assignments should be communicated immediately to the quality manager and processing facility director. It is recommended that these training matrix updates are tied to normally recurring events (such as performance evaluations) as a reminder. These training matrices are useful discussion tools for goal planning associated with upcoming performance evaluation periods.

11 Conclusions

Vendor selection, qualification, and management are critical activities that have direct impact on the quality of products manufactured by cell processing facilities. Vendors should be classified according to the criticality of the goods and services they supply, and qualification activities should be commensurate with their risk classification. Vendor self-assessments are a useful tool for gathering data on the

vendor's organization and its quality system. In most cases, these surveys or self-assessments represent the minimum level of qualification necessary. Site audits coupled with supply agreements/quality agreements may be appropriate for those vendors supplying goods and services deemed critical. Management of vendors is an ongoing activity, and it is very important to recognize that vendors should be reassessed periodically. No business is ever static; changes occurring within a vendor's organization may impact their quality system and result in changes to the quality of goods and services supplied. Vendors have an obligation to report substantive changes to their customers, and for that reason their change control program and change notification process should be assessed as part of the initial qualification. The manufacturer of the cellular product is ultimately responsible for the final product quality, and thus they bare ultimate responsibility for the quality of the goods and services used in its production. Given the ongoing demands associated with vendor management, it should be recognized that this program needs to be resourced and staffed appropriately. Clearly, it is an activity that is not without cost, and smaller organizations may need to be creative to build a meaningful vendor management program within the constraints of a limited operating budget. In this chapter, significant attention was applied to the process for managing supplies upon receipt at the cell processing facility. The level of care applied to the management of the supplies should be on par with the effort applied to qualify the firm that supplied them. The release of supplies and management of storage locations and storage equipment are essential activities. The overall program for supply management must be holistic, such that quality is assured from the point the supplies are sourced to the point they are incorporated into manufacture of the final product.

References

1. U.S. Food and Drug Administration. Code of federal regulations. Human cells. Tissues and cellular and tissue-based products. Part 1271. Title 21. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=1271. Last accessed 6 Aug 2020.
2. U.S. Food and Drug Administration. Code of Federal Regulations. Current good manufacturing practice in manufacturing, processing, packing, or holding of drugs; General. Part 210. Title 21. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=210 Last accessed 6 Aug 2020.
3. U.S. Food and Drug Administration. Code of Federal Regulations. Current Good manufacturing Practice for Finished Pharmaceuticals. Part 211. Title 21. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=211 Last accessed 6 Aug 2020.
4. Foundation for the Accreditation of Cellular Therapy website. http://www.factwebsite.org/. Last accessed 6 Aug 2020.
5. International Standards Organization website. http://www.factwebsite.org/. Last accessed 6 Aug 2020.
6. Rx-360, the International Pharmaceutical Supply Chain Consortium website. https://rx-360.org/about_rx360/. Last accessed 6 Aug 2020.

Staffing, Training, and Competency

Diane M. Kadidlo

1 Introduction

Cell and tissue processing laboratories perform a wide variety of complex processes and quality control testing, requiring staff to have an in-depth knowledge of cell biology, analytical methods, and aseptic manufacturing. While many cell therapy staff maybe certified as medical laboratory scientists, in general, they receive little formal training and/or relevant Good Manufacturing Practice (GMP)/Good Tissue Practice (GTP) manufacturing work experience prior to working in a cell therapy laboratory. The onus, therefore, falls on the laboratory to develop an extensive training program to ensure staff are educated, well-trained, and competent. Such training programs must be effective and all-encompassing, focusing not only on developing technical skills but also scientific knowledge, experience in quality system essentials, and understanding biologic regulations, in order to develop independence and critical thinking skills.

Selection of the right employee can be just as challenging as creating a meaningful training program. As the baby-boomer generation rapidly approaches retirement age, there is increased competition for qualified workers, forcing companies to put more of their resources into attracting and retaining the right people for their job needs. According to the Baldrige National Quality Program core values developed by the National Institute of Standards and Technology (NIST), companies that center their attention on the employee through personal learning see improved employee retention, personal satisfaction, and versatility [3]. Investing in the employee's personal career development and well-being through job optimization, training, mentoring, and career enrichment is just as important as the effort that the company puts into the products and services it provides. Integrating the needs of the company, the employee, and the applicable regulatory requirements into the design of a training

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program is key to development of a meaningful training program. These programs are most effective when there is an evolution in the progression of employee learning from knowledge acquisition, through skill mastery, to application, critical thinking, and dynamic problem-solving.

One approach to developing an effective training program is to use the instructional system development (ISD) method [4]. This is a stepwise approach for creating a standardized training program. It consists of five phases: analysis, design, development, implementation, and evaluation. Through each step of the ISD model, informational outputs and controlled process decisions are generated and incorporated into the next phase. The model begins with an assessment of training needs through the evaluation of the job task, regulatory requirements, the organization's needs, and the level of education and experience of the employee. Based upon the training needs assessment instructional objectives, training tools and lesson plans are constructed. Once the preparative work has been completed, the actual training can be performed. The final phase of the ISD model is an evaluation of the trainee and the training process via feedback mechanisms, in order to identify what went well and where process improvements can be made.

2 Analysis

The analysis phase of the ISD model consists of an evaluation of the training needs, whether training is truly needed, analysis of job duties and learning requirements, and development of performance objectives [4]. The first question to ask is whether training is truly needed? If an employee does not know how to perform a task expected of their job, then it is obvious that training is warranted. However, if an employee does not know the job expectations, or lacks resources to do his or her job, then training alone may not be the solution. In the analysis phase information regarding training needs is gathered from employees, subject experts, and the management and quality assurance unit, through interviews, observations, and questionnaires. The subject matter expert can assist in identifying essential job tasks, the key processes to be mastered, and competency measures. Management can provide input on the organization's mandatory training requirements, such as safety, disaster plans, and American Health Insurance Portability and Accountability Act (HIPAA) training. Quality assurance unit can assist in defining the types of training needed to comply with regulatory requirements.

3 Job Description

Included in the analysis phase is an evaluation of the job duties and responsibilities specific for each job class. Every employee should have a written job description that defines the job functions and outlines the physical, technical, educational and

training requirements to perform that role. Job descriptions should include a description of the job responsibilities and a detailed listing of the assigned functions needed to fulfill the expectations of the job (Table 1). Job descriptions should be used to define the training specifications.

Table 1 Job description: Medical laboratory scientist – Cell therapy laboratory

Clinical manufacturing scientist	
1.1.1.1 Core competencies	Knowledge, skills, and abilities needed
Performs a wide variety of complex biologic processing and quality control testing – Functions independently in performing a wide variety of complex biologic manufacturing and testing – Performs biologic manufacturing procedures including positive and negative cell selection, mononuclear cell separation, cell depletion or purification, cryopreservation, cell culture, vaccine preparation, cell activation, expansion, and retroviral transduction – Operates laboratory instrumentation and information systems.	Knowledge of cell biology
	Demonstrates aseptic techniques
	Problem-solving skills
	Working knowledge of instrumentation and ability to take corrective action
Recognizes problems and takes appropriate measures to resolve them – Acts as a resource for problem-solving, corrective action, and troubleshooting for procedures and unexpected events – Initiates proper safety and emergency responses – Consults with management if unable to solve issues – Exercises critical thinking to maintain and improve department productivity and efficiencies	Knowledge of safety protocols, ergonomics, and body mechanics
	Knowledge of infection control principles and practices
	Knowledge of emergency and other relevant policies and procedures
Evaluates testing results and processes for accuracy and appropriate intervention – Determines if test results or process fall within normal parameters and reporting protocols – Correlates data based on clinical knowledge, technical expertise, and other conditions affecting test results or process outcome – Takes appropriate action to recheck abnormal, discrepant, or unexpected results – Directly communicates abnormal or critical results to appropriate parties	Critical thinking
	Knowledge of laboratory testing and significance in human physiology
	Knowledge of relevant factors which can influence testing results
Demonstrates understanding of and commitment to quality assurance, performance improvement, and compliance programs – Documents deviations and action taken – Recognizes and communicates values and trends that exceed the QC decision levels. Takes action to resolve and consult with supervisor as needed – Documents compliance with regulations of governmental or voluntary regulatory services – Collaborates with the customers to promote customer satisfaction	Knowledge of policies and procedures that are based on FDA, AABB, FACT, and CAP standards, as appropriate to the work setting
	Knowledge of quality assurance principles and practices

4 Training Regulations

Ensuring that your training program complies with all applicable regulatory requirements is essential for biologic manufacturing. The Food and Drug Administration (FDA) requires training of all personnel in GMP and GTP regulations [1,2]. *Each persons engaged in the manufacture, processing, packing, or holding of a drug product shall have education, training, and experience, or any combination there of, to enable that person to perform the assigned functions. Training shall be in a particular operations that the employee performs and in current good manufacturing practice (including cGMP regulations in this chapter and written procedure required by these regulations) as they relate to the employee's functions* [1]. The FDA goes on to state that ongoing GMP training must be conducted by a "qualified individual" [1]. The specifications for that qualified individual are up to each institution and should be clearly defined in writing. Professional accrediting organizations such as AABB and the Foundation for the Accreditation of Cellular Therapy (FACT) have also defined requirements for initial and ongoing training, competency, continuing education, and trainer qualifications for all laboratory, medical, and collection staff [5,6].

Based upon the technical, organizational, and regulatory training needs assessment, the next step is to compile a master list of training tasks incorporated. Tables 2 and 3 are examples of general and detailed GMP/GTP master training lists.

From this master list instructional objectives are created that describe what the trainee should to be able to perform, under what conditions they will be able to perform the task, and the criteria for evaluation of the trainee. These objectives support the goals of the training program and aid in development of instructional tools and the establishment of standard performance measures. The following is an example of performance objective for aseptic technique:

- **Performance:** To be able to demonstrate aseptic technique by transferring medium from the primary container to twenty 2mL vials according to current standard operating procedure in a manner that maintains sterility.
- **Condition:** The trainee will be able to perform this procedure independently.
- **Criteria:** The trainee will be evaluated for adherence to the SOP and acceptable microbiological test results.

The final step of the analysis phase is to prepare a document that summarizes the findings and defines the training program. The analysis document should include the training needs, goals and objectives that have been identified, the target audience to be trained, what performance measurements will be used, the financial impact that training will have on the company, and potential obstacles that could impact success. Training takes resources, both financial and human. The development of training tools and documents and performing the actual training takes time, incurs costs, and impacts productivity. Training budgets should be constructed to cover all initial and ongoing staff training costs, and this information should be shared with management, so that sufficient resources are allocated. The analysis documents

Table 2 Master training list

GMP/GTP manufacturing training needs
Orientation
HIPAA
Infection control
Safety
Chemical hygiene
Fire
Disaster plane
Hazardous wastes
Technical processes
GMP/GTP
Aseptic processing
Facility design
Equipment management
Environmental monitoring
Supplies and containers
Quality assurance unit
Process controls
Labeling
Product packaging
Document control
Product testing and release
Storage
Deviations
Recordkeeping
Complaints
Adverse events
Distribution

should also identify potential obstacles that could impede the development of a successful training program, such as insufficient staffing to perform training or lack of funding to purchase training tools. A thorough needs assessment is the foundation of a training program and will be the guiding document for the next phases of ISD model.

5 Design of Training Program

The decisions and outputs from the analysis phase should lead to the creation of a training policy or plan. A training plan is the policy document that defines the organization's expectations, processes, and responsibilities for employee training. Similar to a quality plan, it serves as the governing document that details the

Table 3 Standard operating procedures for a typical cell therapy facility

SOP Title	
Organization and responsibilities	Sterile Tubing Welder
Official signatures and initials	Use and operation of the Sorvall centrifuges
Temperature monitoring system	Use and operation of freezers and refrigerators
Deviation from written procedures	Use and operation of liquid nitrogen storage tanks
Document change control	Liquid nitrogen transfer system
Ordering materials	Use and operation of the ThermoForma Cryomed controlled rate freezer
Visitor access	Use and operation of the ThermoForma Cryomed controlled rate freezer
Gowning for controlled environment areas	Product transport temperature monitoring devices
Facility access cards and keys	Cleaning of reusable equipment
Decontamination of material and equipment	Sterilization of materials and supplies
Material specifications	Transport and storage of hematopoietic progenitor cell products
Batch record review	Shipper integrity testing
Standard operating procedures	Positive aerobic/anaerobic/fungal cultures
Documentation system	Product number assignment
Process validation	Product receipt and inspection
Laboratory notebooks	Quality control and dose adjustment of ABO identical marrows
Laboratory out of specifications investigation procedure	Plasma removal
Labeling of materials	Red cell removal with hydroxyethyl starch
Receiving biologics	Marrow filtration and cryopreservation for EB and MT2008–20 protocols
Sanitization of controlled environment areas	Buffy coat concentration of bone marrow manual method
Production changeover in controlled environment areas	Processing of autologous peripheral blood progenitor cell products
Discard of cell therapy products	Allogeneic peripheral blood stem cell collection for primary transplant
Cell therapy agreements	Donor-derived peripheral blood lymphocytes
Management of Cell Therapy Critical Equipment	Unrelated donor product export
Documentation of equipment problems and repair	Receipt and infusion of unrelated cryopreserved cord and placental blood
Biological safety cabinet – Policies and procedures	Automated cord blood wash using the Sepax 2 RM cell processing system
Clay Adams® Sero-Fuge® 2001 centrifuge	Cord blood wash – Manual method
Operation and maintenance of the Sysmex XS-1000i analyzer	Cord blood kit shipment and receipt

(continued)

Table 3 (continued)

SOP Title	
Organization and responsibilities	Sterile Tubing Welder
Sysmex XS-1000i quality control and quality assurance	Processing of related donor cord and placental blood
Operation and maintenance of pipettes and Stripettors	Cell therapy product salvage
Use and operation of the Sartorius and Mettler Toledo balances	Product thaw and dilution for patient infusion
Operation of the thermoplastic tube sealers	BMT policy – Blood and marrow transplanted-related products: Procedure for Administration
Adverse reactions	Establishing reference values
Return/reissue of cell therapy products	Quality assurance review
Investigation of complaints	Engraftment review
Cell therapy information management	

essential elements of the training program and the organization's intent to comply with applicable regulations. The training plan is the foundation from which training procedures, tools and records are devised [4]. For GxP manufacturing environments, a training policy document may include the following elements [7]:

- **Scope:** Includes the personnel and/or departments that are included in the training plan.
- **Types of training:** Describes the type of training covered by the training plan: technical, GMP and GTP regulations, safety, employee orientation, initial, and ongoing training and competency.
- **Responsibilities for training:** Defines who in the organization is responsible for the training program, training design, and auditing.
- **Personnel training:** Identifies the personnel to be trained. Technical, administrative, janitorial, and management should all be trained in GMP regulations.
- **Timeframe for training:** Defines the timeframe for conducting training including initial and ongoing training.
- **Role and responsibility of quality unit:** Defines the role and responsibilities of the Quality assurance (QA) unit. The QA unit should play an integral role in the training process. While QA staff may not be experts on technical procedures, their role should be to review and approve of training procedures, especially GMP training, for relevancy and usefulness. QA should audit training records for completeness and ensure that instructors are qualified.
- **Learning plans and development process:** This details the curriculum used for training and the approach used for developing training materials, such as the ISD method.
- **Qualification of instructors:** Describes the process for qualifying training instructors.

- **Documentation and record retention:** Defines what constitutes training documentation, such as attendance sheets, tests completions, and/or instructional/training forms. Defines how long training records are maintained.
- **Learning assessments:** Defines measures used for assessing the training, participation in ongoing competency assessments, and the corrective plan for when an employee fails training and/or competency assessment.
- **Program evaluation:** Describes how the training process is evaluated, for example, via feedback and or surveys, and how the data are analyzed and distributed.
- **Reports to management:** Describes the method by which management is made aware of the activities of the training program, such as number of training courses, length of time to complete training, and training feedback.

The value of establishing a policy or procedure that describes the overall training cannot be overstated. It demonstrates the organization's intent to incorporate quality elements in a standardized approach to training and serves as the foundation upon which training strategies, performance measures, supporting procedures, and training modules are developed. From the master list of training requirements, the next step is to establish a standardized approach to training. This should include identifying training delivery strategies and the creation of a lesson plan template. Effective delivery strategies focus on the learning needs of the target audience, in this case the adult learner.

6 The Adult Learner

Since the 1920s, much has been written about adult learners and what motivates them to learn [8]. Unlike children, who prefer a teacher-directed style of learning motivated by rewards, adults tend to want to be more involved in the learning process and prefer to be guided in their training [4]. This arises from the adults' fundamental need to be self-sufficient and in control of their learning [4]. Along with this core principle are several basic assumptions about most adult learners:

- Adult learners are intrinsically motivated to learn.
- Adult learners are self-directed.
- Adult learners have a need for self-esteem, broadened responsibilities, and achievements.
- Adults come to the job place with valuable worldly experiences that make them eager to demonstrate their abilities.
- Adults need the value of what they are being taught [4].

These needs fuel the adult learner to know what is expected of them, so that they can be successful in their job. Training programs should not only be designed to fulfill technical training requirements but should also incorporate the needs of the adult learner into their instructional strategies. Involving the adult learner in establishing his or her training plan, formulating and executing objectives, and assessing

his or her own learning goals will lay the foundation for developing a productive and engaged employee.

With adult learner concepts in mind, a standardized instructional format or lesson plan that demonstrates a logical progression to the mastery of learning objectives and skill acquisition can be created [9]. Use of a lesson plan template ensures that all of the critical elements are covered. A typical lesson format has an introduction, a middle in which core concepts are taught, and a conclusion that summarizes, reinforces, and evaluates the information presented.

The introduction is an overview of the lesson and provides the adult learner with the rationale for learning, performance expectations, measures of success, concepts to be covered, review of the past information, and a training timeline. The core section of the lesson plan includes presentation, demonstration, and application of technical information, feedback and skill refinement or improvement. The last portion of the lesson reorients the learner through the reconstruction of main ideas, integration of the lesson with past lessons, review of objectives and benefit of the lesson, and assessment of performance [10]. Included in the design phase is the decision on how the material is to be presented. Various methods are available including instructor lectures, on-the-job skill development, technology enhanced (e-tools, computer simulations, Internet-based or Internet-assisted courses), self-study, cooperative learning, and inquiry-based, in the form of cases, projects, and problems [11]. In the cell therapy manufacturing setting, the most common approach is instructor-based, one-on-one training of technical staff. This approach most commonly includes the trainee initially observing a procedure and subsequently performing the task independently successfully a minimum number of times [12]. The instructor is typically responsible for gathering and prioritizing key lesson information in a logical order and for ensuring equipment and training space are available. The instructor may use discussion, questioning, role-playing, and/or lecturing and other tools for presenting the information [13].

7 E-Learning

An increasingly popular approach to training, learning, or education is Internet-based learning [13]. The convenience and flexibility of using electronic or e-learning allows training and education to be self-paced, occurring at home or work, at any hour, making training schedule easier to manage. Web-based audioconferences are an excellent means of fulfilling continuing education requirements with the most current information. A quick search on the Internet will reveal a huge variety of web-based training modules, such as mandatory training (HIPAA, safety, and GMP training), many of which are free of charge. With e-learning there is consistency and standardization in the material that is presented, and web-based training material can be update readily. The upfront costs of purchasing interactive software, audioconferences, etc. can be offset by a reduction in the time that the employee instructor requires to prepare training materials. E-learning reduces the need to travel to

attend external workshops and conferences and satisfies the needs of the adult learner by providing a self-paced approach [14].

8 Development

Once the lesson plan has been defined, the next step is development of instructional tools and materials. It is during this phase training that information and materials are gathered, organized, and presented to the trainee in a clear, concise, and logical progression to facilitate effective learning and skill mastery. Using the objectives for the lesson as a guide, key information that the trainee needs to know to master the task should be identified. Training information can be assembled from the standard operating procedures, scientific journals, textbooks, and applicable regulations. Many regulatory and federal agencies' websites (FDA, National Heart Lung and Blood Program Production Assistance for Cellular Therapies (PACT) and cell therapy organizations such as AABB, FACT, and International Society for Cellular Therapy (ISCT)) offer a plethora of cell therapy-related information and presentations that can be easily downloaded and used as instructional materials. Instructional tools, such as videos, interactive software, and pictures of procedures, can all be used to facilitate training. Biologic product simulations or "mock products" are an excellent means of providing hands-on training and for demonstrating aseptic technique. These products allow the instructor to correct deficiencies without stress or potential risk to a clinical product.

Information should be organized in a logical sequence, so that each step builds upon the last. With the information and tools compiled and organized, the final step in development is to prepare a training document.

The training document is the evidence of record and should indicate the type of training performed, the name of the trainee and the instructor, dates of training, the critical training elements, and whether the training was successful. For each training element, acceptable measures of performance should be developed, such as observation, reading assigned material, ability to perform the task independently, a passing test score, etc., as shown in Table 4. For complex technical training, checklists are useful tools for ensuring all critical steps are taught.

9 Implementation

The execution of the training lesson is the output from lesson planning and training preparation. During the training process objectives are demonstrated, reinforced, and evaluated. It is no surprise that an important key to successful training is the role played by the instructor. Charged with ensuring that materials are presented accurately, learned, and practiced, the instructor is also responsible for monitoring, correcting, and providing positive reinforcement to the trainee [9]. The instructor also

Table 4 Training

Objective: Understand and complain with laboratory safety practices and policies	Date completed	Performance acceptable Yes/No	Instructor
1. Review of SOP-519, *General Laboratory Policies*, with instructor			
2. Review of SOP-461, *Biological Safety Cabinets*, with instructor			
3. Review of SOP-595, *Laboratory Safety Plan* with instructor			
4. Review of SOP-623, *Laboratory Disaster Plan* with instructor			
5. Review of SOP-630, *Segregation of Products and Prevention of Cross-contamination* with instructor			
6. Review of general safety/waste disposal policies (SP-001)			
7. Describe policies for disposal of biohazardous and chemical waste. Review list of chemicals used and MSDS manual			
8. Locate the following safety equipment: Handwashing sinks (2), fire pull alarms(3), eyewashes (4), fire extinguishers (3), and safety showers			
9. Describe liquid nitrogen safety precautions			
Training objectives met and competency questions completed successfully YES / NO Reviewer _____ Date_____			

facilitates the progression of learning by creating a supportive environment. Acknowledging achievements and efforts and developing mutual trust by actively listening and judging the action, and not the behavior, are teaching styles that should be required of all instructors. Effective trainers use techniques such as asking questions of the trainee and presenting problem-solving scenarios that will reinforce training concepts and foster confidence and analytical thinking skills [11]. Not all employees make effective trainers. It is management's role to define the qualifications for instructors based upon criteria such as experience, demonstration of competency, and/or additional training. Instructor qualifications should be defined in the training SOPs.

10 Evaluation

Evaluation is the final phase of the ISD model. This includes not only evaluation of the trainee and his or her ability to meet established objectives but also an assessment of the overall effectiveness and value of the training program. Whether through exams, written assessments, or observations, there must be documented objective

evidence that the trainee is competent to perform the specific job functions. Periodic review of the effectiveness of the training program identifies strengths and areas for improvement. Feedback can be obtained from the trainee using surveys and interviews and by asking questions about the adequacy of training, the effectiveness of the trainer, the usefulness of the instruction tools, and for suggestions for improvement [15].

11 Competence

Evaluation of competence prior to the completion of independent performance of a job function and ongoing on an annual basis is a requirement of laboratory accrediting agencies, including College of American Pathologists (CAP), AABB, and FACT [5,6,16]. AABB and CAP have instituted additional requirements for repeat competency assessments for new employees within the first 6 months of employment [5,16]. There are many approaches to assessing competence [17,18,19]. These provide the opportunity to spot errors and introduce improvements to preserve the quality of the product and/or service provided. One strategy for the design of a competency program is to model it on the US federal regulations for clinical diagnostic or testing laboratories. In 1988, the government passed an amendment to the Clinical Laboratory Improvement Act (CLIA'88) that detailed requirements for training and ongoing assessment of competency for laboratory personnel [20,21]. While it is arguable whether the requirements of CLIA'88 apply to GxP manufacturing, the requirements are nonetheless useful. Using the CLIA'88 regulations as a model, the essential components of a competency assessment program are as follows [20]:

- Direct observation of performance.
- Monitoring the recording and reporting of results.
- Review of intermediate test results, quality control (QC) records, proficiency testing results, deviations, and preventative maintenance records.
- Assessment of technical performance via clinical or simulated products or test samples, internal blind testing samples, or external proficiency testing samples.
- Assessment of critical knowledge and problem-solving skills.

Direct observation is a means of assessing adherence to SOPs, technical skills, accurate interpretation and notification of test results, and appropriate completion of quality control processes. Table 5 is an example of competency assessment. Review of intermediate test results, QC records, proficiency testing results, product deviations, and preventative maintenance records can also serve as evidence of competency and understanding quality control and quality assurance. Assessment of technical proficiency can be achieved through use of clinical or simulated products or test samples, internal blind testing samples, or external proficiency testing. The College of American Pathologists offers proficiency surveys for human somatic cells and associated quality control testing methods including hematology, flow

Table 5 Competency assessment

Employee:			Title:		
Competency assessor:			Competency ☐ Annual ☐ Initial		
Process assessed: Receipt and processing of an allogeneic peripheral blood stem cell product					
	Competency measurement A = procedure reviewed B = direct observation C = unknown specimen		Level of competency 1. Competent and can perform independently 2. Competent and can perform independently, able to assess the competency of others 3. Failed competency measurement		
Date	Measurement	Assessor initials	Competencies	**SOP #**	**Level of competency**
	B		Product receipt and inspection	P-22	
	B		Donor eligibility determination	P-23	
	B		Operation of equipment	P-18, P-09, P-26	
	C		Performs all QC testing	P-55	
	B		Demonstrates aseptic technique	P-02	
	B		Completes documentation	P-01	
	B		Product release	P-12	
	A		Adverse event reporting	P-33	
☐ The employee has demonstrated competency in performance all applicable processes associated with the above procedure ☐ The employee requires retraining for the following:_____					
Supervisor Review_____ Date_____					

cytometry, and microbiology. STEMCELL Technologies™, a commercial supplier of clonogenic assay kits, offers proficiency testing to its customers as does Charles River Laboratories, supplier of endotoxin testing kits. Critical knowledge and problem-solving skills can be assessed by asking the employee questions (oral or written) on technical or procedural problems and to test their knowledge of GxP and other regulations. Another approach to demonstrate critical thinking abilities is to ask the employee to document real examples of problem-solving that they have encountered within the past year [21].

Competency requirements should be specific to the job description. Emphasis should be placed on assessing areas that are at a high risk or most critical to product safety, problematic, or prone to error [20]. The qualifications of the competency assessor and the role of QA in the review of competency should be described in the appropriate SOP.

12 Remediation

The intent of competency assessment is to evaluate employee performance, to pinpoint potential problems, and to address issues before product quality or patient care is impacted. Correction or remediation of the problem is an important element of the competency process. Unless the employee is deliberately remiss in his or her performance, remediation should not be punitive, but an educational and improvement process that focuses on the performance and not the behavior [21]. Remediation should include the identification the problem, root cause analysis of the failure, and a corrective action plan. If it is determined that an employee has failed a competency assessment, the first step is to review the event to evaluate the adequacy of competency process to ensure that procedures and objectives were clear and concise and not confusing or ambiguous. If the competency process was acceptable, the employee should be evaluated to ascertain the cause of the failure (knowledge issue, technical error, or documentation error). Based upon these findings, a corrective action plan can be formulated. This may include rereading the procedure and discussion with supervisor and retraining, followed by reassessment, either by observation or exam. As a last resort, the employee may need to be reassigned to another area. During the review process, the employee should not be allowed to perform that particular task until remedial action has been completed and they are deemed competent.

13 Recordkeeping

Records of training and competency must be kept on file, together with documentation of the employee's signature, initials, and inclusive dates of employment. FACT standards required that records be maintained in a confidential manner and as required by governmental laws and regulations [6]. Personnel records should include:

- Job descriptions for all job classes
- Resumes, curriculum vitae
- Relevant degrees as required by job description
- Training records: initial, ongoing
- Institution required training (safety, HIPAA, infection control, etc.)
- Continuing education
- Annual competency
- Annual GMP training

14 Conclusion

An effective training and competency assessment program is the foundation for developing skilled and productive employees. It is integral to ensuring the safety of the products and services provided by GMP/GTP facilities, and its value can not be overstated. By remembering that training is as much about personal satisfaction as it is a technical requirement, we can better design training programs that will be mutually satisfying to the institution and the employee.

References

1. U.S. Food and Drug Administration. Current Good Manufacturing Practice for Finished Pharmaceuticals. Federal Register. 43: 45076. Last accessed 17 Nov 2020.
2. U.S. Food and Drug Administration. (2004). Current good tissue practice for human cell, tissue and cellular and tissue based products establishments; inspection and enforcement. *Federal Register, 69*, 68612–68688.
3. Baldrige National Quality Program. http://www.quality.nist.gov/index.html. Last accessed 17 Nov 2020.
4. American Society for Training and Development, Leslie Kelly ed. (1995). *The ASTD technical and skills training handbook*. McGraw-Hill.
5. AABB. (2019). *Standards for cellular therapy product services* (9th ed.). Bethesda.
6. FACT-JACIE. (2018). International standards for cellular therapy product collection, processing and administration – 7th edition, March 2018.
7. Vesper, J. L. (2000). Defining your GMP training program with a training procedure. *BioPharm, 13*(11), 28–32. November 2020.
8. Lindeman, E. C. (1926). *The meaning of adult education*. New Republic.
9. Amato, H. K., Konin, J. G., & Brader, H. (2002). A model for learning over time: The big picture. *Journal of Athletic Training, 37*, S236–S240.
10. Yelon, S., & Sheppard, L. M. (1998). Instant lessons. *Performance Improvement Quarterly, 37*, 15–20.
11. Heinrichs, K. I. (2003). Problem-based learning in entry-level athletic training professional-education programs: A model for developing critical-thinking and decision-making skills. *Journal of Athletic Training, 37*(4 Supplement), S189–S198.
12. Keever-Taylor, C. A., et al. (2015). Training practices of cell processing laboratory staff: Analysis of a survey by the alliance for harmonization of cellular therapy accreditation. *Cytotherapy, 17*, 1831–1844.
13. GMP Institute a division of ISPE (1998) Designing a Training Lesson Plan. June 1998. http://www.gmp1st.com/trtp/trtp0698.htm. Last accessed 17 Nov 2020.
14. Learn, H. (2005). Web-based programs work for the lab manager. *Medical Laboratory Observer, 2005*(August), 38–41.
15. Lee, C. (1999). Personnel training and competency. *Cytotherapy, 1*(6), 493–508.
16. College of American Pathologists. (2006). Laboratory accreditation program. *Transfusion Medicine Checklist*. October 31, 2006. http://www.cap.org/apps/docs/laboratory_accreditation/checklists/transfusion_medicine_october2006.pdf. Last accessed 17 Nov 2020.
17. George, K. A. (1996). The right way: Staff training and competency assessment. *Medical Laboratory Observer, 28*(12), 44–47.
18. Haun, D. E., & Leach, A. P. (2003). Performing poorly but testing perfectly. *Clinical Laboratory Management Review, 17*(2), 85–87.

19. Tiehen, A. (1999). Competency assessment: Establishing a program. *Clinical Laboratory Management Review, 13*, 275–285.
20. Health Care Financing Administration. (1992). Medicare, Medicaid, and CLIA programs. Regulations implementing the Clinical Laboratory Improvement Amendments of 1988 (CLIA). *Federal Register, 57*, 7002–7186.
21. Sharp, S. E., & Elder, B. L. (2004). Competency assessment in clinical microbiology laboratory. *Clinical Microbiology Reviews, 17*(3), 681–694.

Part V
Product Management

Product Accessioning, Tracing, and Tracking

Jeannette Bloom and Adrian P. Gee

1 General Requirements for Accessioning

Professional standards from accreditation agencies require that there be a prescription for the collection and processing of the product [1]. This provides information on the name, medical record number, blood type, etc. of the intended recipient and donor. It details the cell type to be collected, the date of collection, the product to be prepared, the method to be used, and whether it is to be cryopreserved or administered fresh. It is usually signed by the patient's physician. A copy of the prescription is provided to the collection facility, the medical record, and the cGMP facility at a minimum. Receipt of this document will trigger a number of additional activities. These include obtaining informed consent for the collection procedure (and the intended treatment if not already obtained) and, in the United States, initiation of the donor eligibility assessment. This is required by the Food and Drug Administration (FDA) under Title 21 of the Code of Federal Regulations (CFR) Part 1271 Subpart C [2]. It is intended to apply to Type 361 (minimally manipulated – Good Tissue Practices [GTP]) cell products but is also widely used for Type 351 Investigational New Drug (IND) products to be prepared under current Good Manufacturing Practices (GMP) [3]. Donor screening involves testing for a number of communicable diseases using FDA-licensed, −approved, or -cleared screening tests. This must be performed within 7 days of the collection or 30 days for donors of peripheral blood progenitor cells. Testing is not required for autologous donors; however, some centers perform it on all donors to ensure proper labeling of the products during storage. Testing is supplemented by reviewing the donor's medical records for risk factors for communicable diseases (usually performed using a questionnaire) and by a clinical assessment of the donor. Donors must be classified as eligible, ineligible, or pending eligibility assessment. The use of ineligible donors for

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recipient of GTP products is permitted if the cells are for allogeneic use in a first- or second-degree relative and/or if there is documented urgent need for the cells. This requires specific labeling of the product, consent from the intended recipient, and notification of the recipient's physician. The use of ineligible donors of Type 351 products is not generally permitted and exceptions must be obtained from the FDA. Documentation of the eligibility assessment and the results of tests for communicable disease must be documented by the GMP facility and appropriate labeling of the harvest cells used by the collection center.

Most collection centers use the International Society of Blood Transfusion (ISBT) 128 labeling system which is discussed elsewhere in this volume [4]. The label at completion of collection must contain the unique numeric or alphanumeric identifier; the proper name of the product and the product code, as part of the ISBT 128 label; the approximate volume; the name and quantity of anticoagulant and other additives; and the recommended temperature range for storage. Attached documents should include the recipient name and/or identifier, the identity and address of the collection facility (or donor registry), the date and time of the end of the collection and time zone if applicable, the donor identifier and name (if applicable), a biohazard label (if applicable, together with the appropriate warning information), and a statement "For Autologous Use Only" if applicable. Accompanying information should include the product attributes from the ISBT 128 labeling system.

Following the collection there must be a chain of custody document for the transfer of the cells to the GMP facility. This contains the donor and product information and is signed by the collection center staff releasing the product and the GMP facility staff picking it up for transfer.

2 Labels

There must be systems in place to prevent misidentification and/or mislabeling of the cell therapy product, samples, and associated records. There must also be a system for restricting the use of obsolete labels. If pre-printed labels are used, these must be held upon receipt from the manufacturer pending review against an approved copy or template and then stored using a method to prevent mix-ups. If a print-on-demand system is used, it must be validated to confirm that it accurately prints the identity, content, and conformity of the labels to approved templates. In both cases there should be system for label version control.

When labels are applied, they must leave sufficient area of the container visible to permit inspection of the contents. Labeling information must be clear, legible, and completed in indelible ink, and the material of the label must be validated for storage under the conditions of use. All fields on the label must be completed or designated as "N/A."

2.1 Labeling During Manufacturing

Once in the GMP facility the cells need to be identified for processing. At Baylor Center for Cell and Gene Therapy, a unique "P" number is assigned to all donors and recipients. Unique "C" numbers are issued to all collected cells. These identifiers are tied to both the donor and to the intended recipient by their hospital numbers and name. The accessioning information is maintained on an electronic database, which requires double entry of all information and has a built-in audit trail. The data is printed as hard copy on a regular basis and checked by the quality assurance group. The identifiers are used throughout the processing procedures, usually in the form of a partial label which appears on all containers apart from the initial collection bag and the final product container. Partial labels must contain, at a minimum, the unique numeric or alphanumeric identifier and the proper name of the product. The same identifiers are used on samples taken from the cells for testing, and this information is supplemented by a test request form containing the same elements but with additional identifiers and information. Since we use print-on-demand labels, we maintain a logbook containing the templates of all labels together with their version number. When a label is printed and completed, a copy is generated for the label documentation form which is part of the processing record. The copy of each label is annotated with the name of the person who generated it and the identity of the person who checked the information it contains.

Labels are applied to all containers, culture devices, bioreactors, etc. throughout the manufacturing process. In cases where culture media or reagents are dedicated for use for a particular product, the partial label will also be applied to these containers.

2.2 Final Labels

Final product containers are labeled with the appropriate ISBT 128-compliant label (see elsewhere in this volume). For new products the International Council for Commonality in Blood Banking Automation (ICCBBA) can be contacted to issue a unique product code and defined description for the label.

The label at the completion of processing should contain the unique numeric or alphanumeric identifier, the proper name of the product and the ISBT 128 product code [4], the approximate volume, the name and quantity of anticoagulant and other additives, and the recommended storage temperature. Attachments should include the recipient name/identifier and the donor identifier and name (if applicable). Attached information should include biohazard warning (if applicable and the appropriate information) and the statement "Do Not Irradiate" and "For Autologous Use Only" if applicable. Accompanying information should include product attributes (according to ISBT 128), the date and time the collection ended and time zone

(if applicable), the identity and address of the collection center or registry, the expiration date and time (if applicable), and the ABO/Rh of the donor (if applicable).

3 Storage

For cryopreserved products there must be an inventory system to record the location of the product within the storage device, the date of placement, and the identity of the person adding the product to inventory. If necessary, the status of the donor eligibility determination should be included in the inventory information to act as a reminder at the time of product removal.

4 Distribution and Administration

Processing records should be reviewed and demonstrate the traceability from the donor to the recipient and the recipient to the donor. Before distribution for administration, the product must meet predetermined release criteria, and specific authorization for distribution should be made by the Processing Facility Director, the Processing Facility Medical Director, or their designees. The products must be visually examined before administration for the integrity of the container and the appropriate labeling. A Circular of Information or a document containing the indications, contraindications, side effects, hazards, dosage, and administration recommendations for the product to be distributed must be available. There should also be instructions for handling the product and warnings related to the prevention of the transmission of communicable diseases.

The label at the time of distribution for administration may be a partial label. It must contain the unique numeric or alphanumeric identifier, the proper name of the product, the product code, the approximate volume, the name and quantity of anticoagulant and any additives, and the recommended storage temperature range. Accompanying information should include the recipient name and/or identifier; the product attributes; the identity and address of the collection facility (or donor registry); the date and time of the end of collection; the donor identifier and, if applicable, name; a biohazard label if applicable with the appropriate supplementary information; the name and identity and address of the processing and distribution facility(ies); the statement “Do Not Irradiate”; the expiration date and time (if applicable); ABO and Rh (if applicable); the statement that a leukoreduction filter shall not be used; the statement “For Autologous Use Only” if applicable; and the date of distribution. There must be documentation on receipt of the integrity of the product container and the appearance of the product for evidence of mishandling or contamination and for appropriate labeling.

In the United States the label intended for use on the final product should be submitted to the FDA as part of the Investigational New Drug/Device application.

This will be reviewed by the Agency and the final version must be used in the clinical trial.

Records must be maintained which record the administration date and time, the unique identifier of the intended recipient, the proper product name and identifier, documentation of donor eligibility, and identification of facilities that requested and distributed the product. A Chain of Custody Form should be used at the time of distribution for administration. This will document the date and time that the product was distributed and received, the identity of the transporting or shipping and receiving facilities, the identity of the person(s) responsible for transportation/shipping and receiving the product, the identity of the courier (if applicable), and details of any delays or problems during transportation for distribution.

If a product has to be returned to inventory, there must be documentation in the processing facility records of the events requiring the return, the temporary storage temperature when at the clinical center, the results of product inspection upon return, and any subsequent actions to protect product safety and viability. The Processing Facility Director or designee should consult with the recipient's physician regarding the reissue or disposal of the returned product.

5 Shipment

In many cases products are shipped to other institutions for administration. Shipping is covered elsewhere in this volume; however, some labeling information is provided in this section. The product should bear the ISBT 128 label [4] used at the completion of processing. The shipment is made using an inner container (containing the cells) and an outer container (used for transportation). The following information must accompany the inner container: the date (and time, if appropriate) of distribution, the statement "Do Not X Ray" or "Do Not Irradiate" if applicable, and the statement "Human Cells for Administration" or the equivalent. The statement "Handle with Care"; instructions for handling the shipper, the name, address, and contact information and phone number for the shipping facility; the name address, contact person, and phone number for the receiving facility; and biohazard and/or warning labels as applicable with appropriate information should also accompany the inner container. The outer container must have affixed the statement "Do Not X Ray" or "Do Not Irradiate," if applicable, and "Human Cells for Administration" or the equivalent, the shipper handling instructions, and the shipping and receiving facility name, address, contact person, and phone number. Accompanying information should include the date and time (if applicable) of distribution. Copies of all of this information should be kept within the processing facility documentation. The FDA requires (21 CFR Part 1271.290) [2] that the recipient facility be informed of the shipping facility's ability to track information on the shipped product. We use the form shown in Fig. 1 for this purpose.

CW05.02B.42 Shipping Agreement & Adverse Reaction Notification

The Center for Cell and Gene Therapy at Baylor College of Medicine is providing you with this cellular therapy product at your request. Together with the product we are providing information on the eligibility of the donor, the characteristics of the product and testing that has been performed. The decision on the clinical use of the product is the responsibility of the requesting physician.

As per 21 Code of Federal Regulations Part 1271.290 we are also informing you that CAGT has a system in place to track this product from the donor to its eventual disposition. As a part of this system we will record in this system the unique identifier of the intended recipient. If required, for investigative purposes in the event of actual or suspected transmission of communicable disease by the product your facility will have access to this tracking system.

Donor Eligibility

Documentation of donor eligibility is provided in the documentation sent with the cellular product. In the case that eligibility determination is pending, the cellular therapy product provided must not be transplanted or infused until completion of the donor eligibility determination, except under condition of Urgent Medical Need

Adverse Reactions to Product Administration

In the event of any adverse reaction to administration of the product contact CAGT immediately using the information provided below:

Adrian Gee
Director, Quality Assurance
GMP Facilities, Center for Cell & Gene Therapy
MC3-3320, Suite 1630
Feigin Center, 1102 Bates Street
Houston, Texas 77030

E-mail: apgee@bcm.tmc.edu
Telephone: 832-824-4214
Fax: 832-825-4668

Please have the following information at hand when you contact CAGT

Product Component number: C # on the label:
Date and Time of Administration:
The Unique Identifier for the Recipient:
Description of handling or manipulation prior to Administration:
Nature of Adverse Reaction:

Your contact Information:

In the event of an adverse reaction we will immediately undertake an investigation to determine whether it may be associated with any defect in product manufacturing and testing and will keep you closely informed of our findings.

Thank you

Fig. 1 Sample shipping agreement with information on product tracking and tracing

6 Disposal

If a product is not administered to a patient, it may undergo disposal. This should be carried out under the directives of a pre-written agreement, normally part of the informed consent process, between the intended recipient, or donor, and the storage facility. This must define the length of storage and the circumstance under which disposal would occur, as determined by the processing facility in collaboration with the clinical program. If the intended recipient is still alive he/she should be given the option to transfer the product to another facility. Before disposal there must be documentation of no further need. Disposal should be approved by the Processing Facility Medical Director or the recipient's physician. The methods used for disposal must follow applicable laws for medical waste and biohazardous materials.

7 Conclusions

The ability to trace and track a cell therapy product from collection to administration or disposal is central to cGMP and cGTP manufacturing and the practice of medicine. The involvement of agencies such as the Foundation for the Accreditation of Cellular Therapy [5], AABB [6], and ICCBBA [4] has provided a number of tools to facilitate this procedure, by developing professional standards and international labeling systems. The regulatory agencies have provided a framework for the collection, processing, storage, shipment, and distribution of cellular therapy products, which promise to further standardize practices. The challenge for all of these organizations will be to keep abreast of the rapid developments in the field.

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References

1. FACT Common standards for cellular therapies. Foundation for the Accreditation of Cellular Therapy Website. http://www.factweb.org/forms/store/ProductFormPublic/second-edition-fact-common-standards-for-cellular-therapies-free-download. Last accessed 9 Jul 2020.
2. Code of Federal Regulations. Part 1271 Human Cells, Tissues, and Cellular and Tissue-Based Products. Title 21. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=1271&showFR=1&subpartNode=21:8.0.1.5.60.3. Last accessed 9 Jul 2020.
3. Code of Federal Regulations. Food and Drugs: Parts 1 to 1499. Title 21 https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm. Last accessed 9 Jul 2020.
4. ISBT 128. ICCBBA Website. https://www.iccbba.org/. Last accessed 9 Jul 2020.
5. Foundation for the Accreditation of Cellular Therapy Website. http://www.factwebsite.org/. Last accessed 9 Jul 2020.
6. AABB Website. http://www.aabb.org/Pages/default.aspx. Last accessed 9 Jul 2020.

ISBT 128 in Labeling of Cellular Therapy Products

Zbigniew M. Szczepiorkowski and Paul Ashford

1 Introduction

International standardization of the identification, terminology, coding, and labeling of medical products of human origin (MPHO) allows organizations to transfer information critical to patient safety in an accurate and secure manner regardless of the source and destination of the products and to ensure effective traceability [1].

Developing such a standard is a complex process requiring input from experts in all disciplines of MPHO production and clinical application together with specialists in informatics and terminology [2]. The International Society of Blood Transfusion (ISBT) 128 Standard was specifically designed to achieve these objectives, and today is recognized worldwide as the global standard for the terminology, identification, coding, and labeling of medical products of human origin (including blood, cell, tissue, milk, and organ products) (Table 1). More importantly for the cellular therapy community, it is now required by voluntary accreditation organizations such as AABB, the Foundation for the Accreditation of Cellular Therapy (FACT), the Joint Accreditation Committee of the International Society for Cell and Gene Therapy (ISCT), and the European Society for Blood and Marrow Transplantation (EBMT) – (JACIE). Be The Match (aka National Marrow Donor Program) was instrumental in bringing the ISBT 128 Standard to all apheresis, collections, and transplant centers in the USA.

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Table 1 Medical products of human origin addressed by ISBT 128 standards and corresponding technical advisory groups

Area of interest	Technical Advisory Group	Focus
Blood	APTAG (Asia Pacific TAG)	Regional blood transfusion
	AMTAG (American TAG)	Regional blood transfusion
	EMATAG (Europe, Middle East, Africa TAG)	Regional blood transfusion
Cellular therapy	CTCLAG – Cellular therapy TAG	International cellular therapy
Fecal microbiota	N/A	International fecal microbiota
In vivo diagnostic MPHO	N/A	International in vivo diagnostic MPHO
Human Milk	MBTAG – Milk Banking TAG	International human milk banking
Ocular products	EBTAG – Eye Banking TAG	International ocular tissue banking
Organ transplant	N/A	International organ transplantation
Plasma derivatives	N/A	International plasma derivatives
Regenerative medicine	RMTAG – Regenerative Medicine TAG	International regenerative medicine
Reproductive products	ARTTAG – Assisted Reproductive Technology TAG	International assisted reproductive technology
Tissues	ETTAG (European Tissue TAG)	Regional tissue banking
	ITTAG (International Tissue TAG)	International tissue banking
	NATTAG (North American Tissue TAG)	Regional tissue banking
Topical products	N/A	N/A

The areas of interest include all MPHO products which are included in ISBT 128 Standard: Standard Terminology for Medical Products of Human Origin [5]. Not all the products require technical advisory groups (TAG). All proposed changes to ISBT 128 Standards are approved by the Standards Committee which has representation from all TAGs. Additional information can be obtained from ICCBBB website

Abbreviations: *MPHO* Medical Products of Human Origin, *N/A* not applicable, *TAG* Technical Advisory Group

ISBT 128 is used in more than 87 countries across six continents and disparate healthcare systems. It is widely endorsed by the professional community [3]. It is estimated that over 40 million MPHO are labeled using ISBT 128 each year.

This chapter explains the concepts behind unique identification, standardized terminology, and coding and shows how these are achieved using ISBT 128.

2 Historical Perspective

The need for international standards for MPHO was dramatically highlighted during the First Gulf War (1990–1991). Blood was sent to the war zone from many countries, and major obstacles were encountered due to a lack of standardized identification including duplication of donation numbers and lack of standardization in

the use of codes and their meaning [4]. In response to these events, the Working Party on Automation and Data Processing of the International Society of Blood Transfusion developed the ISBT 128 Standard which was published in 1994. Initial implementations of this system in many countries around the world showed its suitability by accommodating regional changes without substantial structural changes [5]. Importantly, it quickly became apparent that this system may be used not only for blood components but also cellular therapy products and other medical products of human origin [6]. In the late 1990s, a small group of facilities began using ISBT 128 for cellular therapy products [7]. The widespread use required greater international standardization both in terminology and labeling. A multiorganizational effort was initiated with participation of AABB, American Society for Transplantation and Cellular Therapy (ASTCT), American Society for Apheresis (ASFA), Asia-Pacific Blood and Marrow Transplantation Group (APBMT), EBMT, FACT, the International Council for Commonality in Blood Banking Automation (ICCBBA), ISBT, ISCT, JACIE, the National Marrow Donor Program (NMDP), and World Marrow Donor Association (WMDA) [6]. Representatives from these organizations, as well as additional technical experts and regulatory liaisons, comprise the Cellular Therapy Coding and Labeling Advisory Group (CTCLAG). This truly collaborative effort led to significant progress in the use of ISBT 128 across the world [8, 9] (Figs. 1a and 1b).

The name ISBT 128 was derived from the ISBT (www.isbt.org), reflecting the important role this society played in the development of the standard. The number 128 reflects the 128 characters of the ISO/IEC 646 7-bit character set used in ISBT 128 data structures. However, today we also interpret ISBT as Information Standard for Blood and Transplant, which better captures what the standard has become of over the last 25 years [3]. ICCBBA was created specifically for the purpose of managing the ISBT 128 Standard to ensure that it remained fit for purpose in the rapidly

a

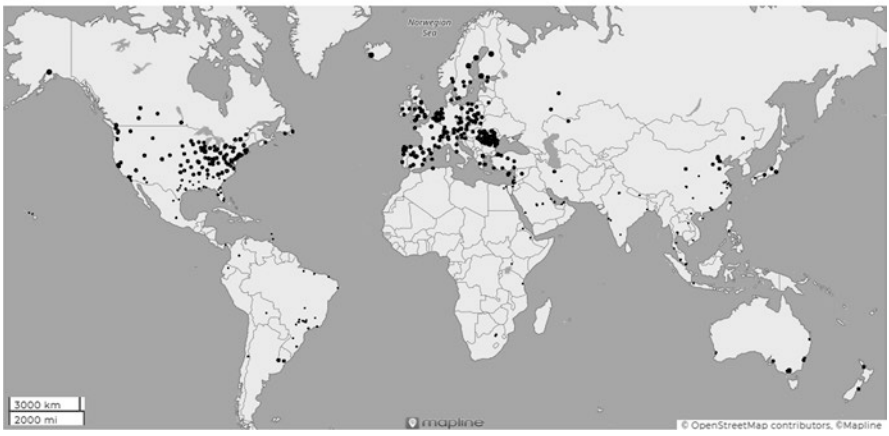

Fig. 1a Worldwide distribution of ISBT 128 in CT facilities as of June 2020

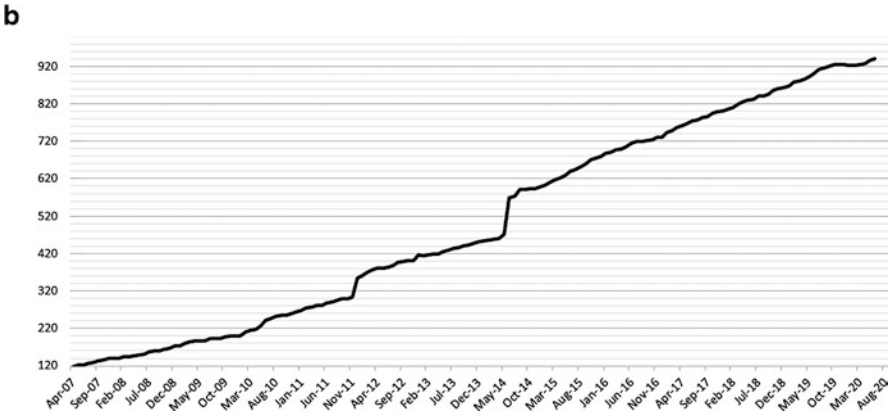

Fig. 1b The growth of ISBT 128 licensed CT facilities

developing fields of MPHO medicine. ICCBBA is a not-for-profit international standard organization responsible for the management and development of the ISBT 128 Standard and became recognized as a non-state actor in official relations with the World Health Organization (WHO) in 2011.

3 Key Elements of ISBT 128

Though many practicing laboratorians may consider ISBT 128 as just a system for cellular therapy product labeling, it is much more. The labeling is but the last step of a complex multilayer construct that assures that standardization can be accomplished. The layers are unique identification, standardized terminology, reference tables, data structures, delivery mechanisms, and labeling.

The ISBT 128 Standard harmonizes information transfer by specifying:

- A donation numbering system that ensures globally unique identification.
- An internationally agreed structured standardized terminology.
- An international reference database of Product Description Codes.
- The information to be transferred, using internationally agreed reference tables.
- The data structures in which this information is placed.
- A bar coding system for transfer of the information on the product label.
- A standard layout for the product label.
- A standard reference for use in electronic messaging.

4 Uniqueness of Donor and Donation

The uniqueness of a product and donation/collection is the foundation of safety and quality of cellular therapy products. The need for unique or distinct identifiers is widely recognized and required by regulation. However, the term unique on its own is ambiguous if not associated with a domain. Thus, a hospital number is unique within a hospital, but may not be so in the wider healthcare community, a social security number is unique within a country but may not be so internationally. Unique identification needs to be established across the domain of use. Regulatory requirements for unique identification of products can be satisfied within the national domain, but cellular therapy products move across national boundaries and so have a global domain. ISBT 128 provides a system for the unique identification of any donation worldwide. It is accomplished by using the Donation Identification Number (DIN) consisting of 13 characters. The DIN comprises of three elements. The first element identifies the facility that assigned the DIN. This could be a collection facility, a processing facility or registry, etc. The second element is the year in which the DIN was assigned. The third element is a sequence number which is controlled and maintained by the facility that assigned the DIN. Figure 2a provides additional explanation of DIN.

ICCBBA assigns facility codes and maintains a database of all registered facilities. There is a look-up program which allows individual facility codes to be matched to a facility name and location. A full listing of all facility codes and corresponding facilities can be downloaded by facilities and vendors licensed by ICCBBA. This information is important when accepting CT products manufactured elsewhere.

Over the last few years another requirement for uniqueness was identified by the transplant community. The number of potential cellular therapy (CT) product donors has been growing rapidly and now exceeds 36 million. Their data are stored in different registries around the world that can be searched for matching donors through the European Marrow Donor Information System (EMDIS). In 2013, lack of a globally standard identifier for these donors led to a serious adverse event where a patient was given a completely mismatched allogeneic unrelated bone marrow transplant because two registries used the same identifier for two different donors [10]. This led to recognition of the critical need to be able to uniquely identify each individual donor irrespective of where his/her registration occurred. The WMDA and ICCBBA worked in partnership, and, after achieving a consensus between multiple stakeholders, the Global Registration Identifier for Donors (GRID) was created [11]. The GRID is a 19-character identifier that incorporates a modulus 32 checksum and is printed in an eye-readable format of five blocks of 4, 4, 4, 4, and 3 characters to reduce the risk of manual transcription errors. See Fig. 2b for more details on GRID.

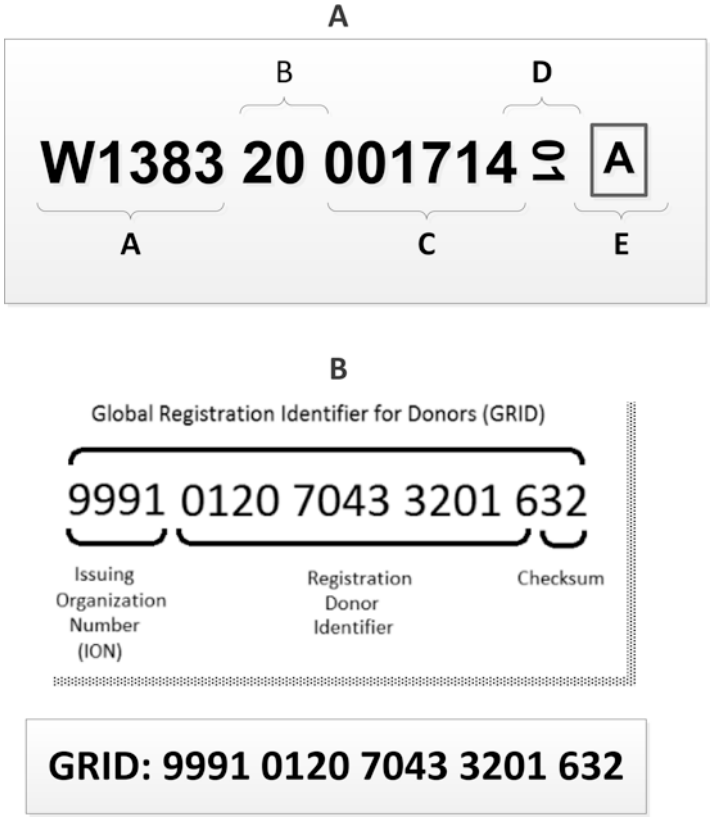

Fig. 2 The description of the Donation Identification Number and the Global Registration Identifier for Donor
(**A/B**) These two numbers contain significant amount of information which is critical to product and donor uniqueness and traceability
(**A**): The Donation Identification Number (DIN). (**a**) identifies the collection facility (in this case Dartmouth-Hitchcock Medical Center, Lebanon, NH, USA); (**b**) identifies the nominal collection year as 2020; (**c**) the sequence number of the collection assigned by the collection facility; (**d**) the two digits printed vertically allow individual bar codes in a number set to be discretely identified, hence providing an option to add process control; (**e**) an additional character is enclosed in a box at the end of the identifier. This is a checksum character used when a number is entered into a computer system through the keyboard to verify the accuracy of the keyboard entry
(**B**) The Global Registration Identifier for Donors. *An example of the full GRID eye-readable format. When printed on a product label (lower figure), the GRID shall be preceded with the upper-case letters GRID and a colon (*i.e.*, GRID:) and placed in the upper right quadrant of the label*

5 Structured Standardized Terminology

The standardized terminology is the basis for common understanding of terms, and it is essential to any standardization [5]. Importantly, there is a need for agreement and consensus among potential users of such terminology. It is achieved by thoughtful analysis and understanding of the products to be defined, especially when the products are used globally where there is a risk that some concepts can be lost in translation. There is a need for appropriate granularity of the definitions. This assures that the transferred information and the quality of the product description are consistent.

A significant effort has been invested in defining basic cellular therapy nomenclature. The initial steps included many stakeholders and led to first definitions included in the Circular of Information for the Use of Cellular Therapy Products [12, 13]. This development also gave an impetus for generation of a more robust and sustainable approach through ICCBBA and its advisory group, i.e., CTCLAG. It was quickly recognized that there is a growing complexity among cellular therapy products and a flexible system needs to be envisioned. This approach went through different permutations leading to our current approach. It consists of classes and attributes. For cellular therapy products, class names are in the format: type of cells, comma, and source of cells (e.g., HPC, apheresis).

The product is further described by core conditions and attributes. The core conditions describe three pieces of information: (1) the anticoagulant, (2) the nominal volume of the original product, and (3) the temperature at which the product should be stored.

The first version of this standardized terminology was published in 2007. CTCLAG meets regularly to review requests for additions to the terminology, and the current version is maintained on the ICCBBA website.

6 Product Database and Product Description Codes

Reference tables are developed once the standardized terminology is in place. They are built to map each item to a suitable code [7]. The reference tables are often complex; however, they need to be constructed to allow for modifications and backward compatibility. This is important especially when the products can be stored for many years prior to their use. It is challenging to construct reference tables which need to accommodate futures changes, some of which are unanticipated, and expansion of terms. Therefore, involvement of clinical experts and information technologists is so important in keeping reference tables relevant and accessible. The reference tables are routinely published for comments and are available to the end users.

An individual cellular therapy product is defined by combining a product class with the appropriate core conditions and attributes. Each combination of class, core

Table 2 Examples of the more commonly used cellular therapy product classes with abbreviations and definitions

Name	Abbreviation*	product definition
Subcategory 1		
CONCURRENT PLASMA, APHERESIS	CP(A)	Plasma collected from the donor as part of an apheresis cell collection procedure
HPC, WHOLE BLOOD	HPC(WB)	A cell product containing hematopoietic progenitor cells obtained from whole blood
HPC, Apheresis	HPC(A)	A cell product containing hematopoietic progenitor cells obtained by apheresis
HPC, Cord blood	HPC(CB)	A cell product containing hematopoietic progenitor cells obtained from cord blood
HPC, Marrow	HPC(M)	A cell product containing hematopoietic progenitor cells obtained from bone marrow
NC, MARROW	NC(M)	A cell product containing nucleated cells obtained from bone marrow
MNC, APHERESIS	MNC(A)	A cell product containing mononuclear cells obtained by apheresis
MNC, UMBILICAL CORD TISSUE	MNC(UCT)	A cell product containing mononuclear cells derived from umbilical cord tissue
NC, ADIPOSE TISSUE	NC(AT)	A cell product containing nucleated cells obtained from adipose tissue
Subcategory 2		
DC, APHERESIS	DC(A)	A cell product containing dendritic cells obtained by apheresis
INVESTIGATIONAL PRODUCT	INV PROD	A product for an investigational study that is accompanied by appropriate identifying study information. This class may be used for a specific product that may be part of a blinded comparison study. Products labeled as investigational product may include different doses or may include an active product or a placebo
iPSC, CORD BLOOD	IPSC(CB)	A cell product containing induced pluripotent stem (iPS) cells obtained from cord blood
MSC, ADIPOSE TISSUE	MSC(AT)	A cell product containing mesenchymal stromal cells derived from adipose tissue
NK CELLS, APHERESIS	NK(A)	A cell product containing natural killer cells obtained by apheresis
T CELLS, APHERESIS	T CELLS(A)	A cell product containing T cells obtained by apheresis
T CELLS, TUMOR	T CELLS(TM)	A cell product containing T cells obtained from a tumor

For a complete list of all products currently defined by ISBT 128, please see publication *Standard Terminology for Medical Products of Human Origin* current version (www.iccbba.com) [5]. *See relationship between subcategory 1 and 2 in* Fig. 3

From the document above: Abbreviations are sometimes needed in documents (published papers, SOPs, etc.). The following abbreviations may be used for this purpose. In some countries, regulations may permit the use of abbreviations on partial labels when space does not permit the use of a full name. Users should consult national regulations for further information. If abbreviations are used on the label, the accompanying documentation must include the full name of the product. No spaces should be present before the parentheses in these abbreviations. This will prevent separation of "HPC" from the parenthetical information when the abbreviation appears at the end of a printed line

Table 3 An example of class, core conditions, and attributes used in practice [7]

	Product description
Component class	HPC, cord blood
Core conditions	NS (anticoagulant not specified)
	XX (variable volume)
	≤ −150 °C (storage condition)
Attributes	10% DMSO
	Other additives: Yes
	Cryopreserved
Product description code	**S1150**

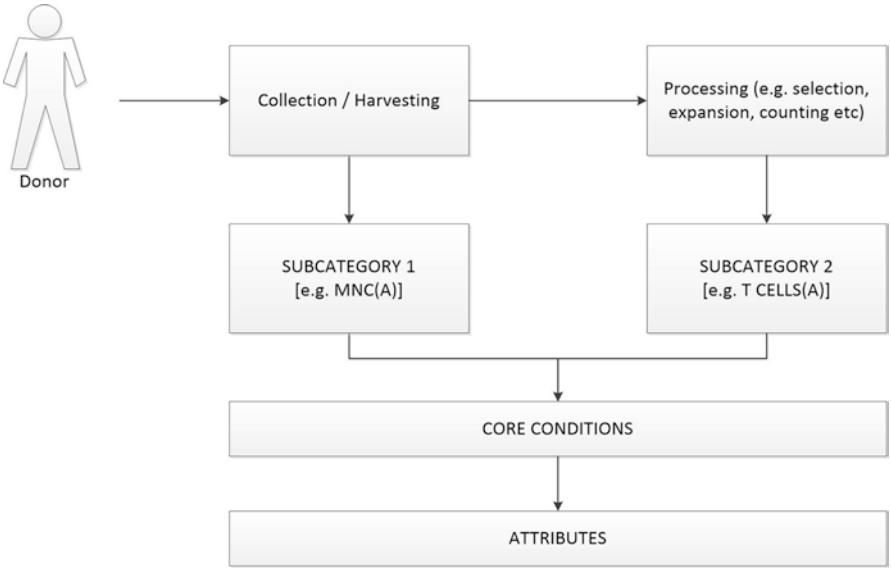

Fig. 3 A simplified diagram explaining relationships between classes, categories, core conditions, and attributes
The cellular therapy product is collected, and, unless further processed or enumerated, it is named using subcategory 1 nomenclature. Core conditions and attributes describe the product in more details. If the product is further processed or enumerated post collection, it carries a subcategory 2 name (see Table 2). Attributes provide additional information as to the type of manipulations used. Often, additional information describing processing steps in greater detail need to be included in accompanying documentation. Information on available core conditions and attributes is provided in Standard Terminology for Medical Products of Human Origin current version (www.iccbba.com) [5]

conditions, and attributes is given a unique Product Description Code (PDC), and these codes are maintained in the Product Description Code Database. The product can be changed by different steps in processing, and such changes are reflected by addition of one or more attributes from the groups and variables. These additional

manipulations are indicated by a different Product Description Code. Table 3 and Fig. 3 illustrate some of the changes, but the reader is encouraged to see more up-to-date information in the newest documents housed on the ICCBBA website [5, 6].

There are many product codes already available in the database, but any new product is defined by stringing together pieces of information from the standardized terminology in a way that unambiguously describes the product. Requests for new PDCs are reviewed by ICCBBA/CTCLAG and, if approved, are assigned a Product Description Code that becomes incorporated into the ISBT 128 Product Description Code Database, ensuring that the product will be accurately identified in any country in the world that is using ISBT 128. If necessary new classes or attribute groups or variables will be incorporated into the terminology by CTCLAG (Table 3 and Fig. 3).

As noted above, the Product Description Code Database is standardized, but the text that appears on the actual label of a product is under national control. This easily accommodates language and regulatory differences between jurisdictions.

The structured nature of the terminology allows PDCs to be grouped according to specific characteristics. This is achieved by use of the "Product Formula" that is available in the Product Description Code Database. This helps to support computerized adverse event analysis and the collection of denominator data and has been used to determine transfusion trends as part of the Food and Drug Administration Biologics Effectiveness and Safety (BEST) Initiative [14, 15].

7 Data Structures

Having defined unique identifiers and standardized product description codes, these need to be presented to computer systems in a machine-readable format that ensures unambiguous interpretation. To achieve this, it is necessary to specify what information is being provided as well as the information itself. Thus, the encoded information must state the context "this is an ISBT 128 Donation Identification Number Data Structure" as well as providing the value of "A99992012345600." All ISBT 128 Data Structures commence with a Data Identifier string which is a set of characters to uniquely specify the context. The opening characters "=" and "&" are allocated to ICCBBA by international agreement [ISO/IEC 15418, ANSI MH10.8.2]. The full Data Identifier references the specific data structure within the ISBT 128 Standard and provides the reading computer system with the syntax rules to allow the information to be extracted and interpreted. One of the primary characteristics of data structures is their clarity and unambiguity, so the risk of transmission of incorrect information is minimized, if not eliminated.

In addition to the Donation Identification Number and the product code, there are additional pieces of information which need to find their way to the final label on the CT product. They may include (1) ABO and RhD blood groups, (2) collection date and time, (3) expiration date and time; (4) collection container catalog and lot

number, (5) Donor Identification Number, (6) patient date of birth, (7) patient identification number, and (8) other relevant dates/times. These elements are incorporated through a wide range of data structures provided by ISBT 128 as illustrated in Fig. 2 and Table 3.

The delivery mechanism is the means of transferring the electronic information between computer systems. The most widely used mechanisms for ISBT 128 data structures are bar codes. Linear bar codes (Code 128) have been in use since the Standard was first developed and are still the most common delivery mechanism. Each linear bar code holds a single ISBT 128 data structure, and therefore multiple codes are usually required to carry all the information associated with a product. Two-dimensional codes (data matrix) are becoming more common, and these allow multiple data structures to be carried in a single code thus reducing scanning times. Work is currently underway to provide a standard approach to transferring ISBT 128 information in electronic messages. This would allow the information encoded on the label to be reduced to the unique identifiers (DIN, Product Description Code, and Division Number) with all other information being available from a linked electronic message. This will be important in the future as the amount of required information continues to increase. All previous information listed above can be delivered by these systems, but their overall capacity and space requirements on the product do differ. Due to the dissociation of information structure from delivery mechanism, the development of new delivery mechanisms should not affect our ability to provide the structured information regarding the product to the end user.

The final element in the coding system is associated labeling. The labeling needs to be able to ensure correct physical assignment of information to the product. The eye-readable printed information and electronically shared information need to be robustly associated with each other, with a high level of confidence. The label on the product serves multiple purposes. One is the safety of the recipient of the CT product. As we still rely on eye-readable content of the label to identify the recipient and the product, it is important that this information is standardized and unambiguous. Critical eye-readable information such as blood groups, product description, and expiration date also appears in fixed positions on the label [6]. This standardization minimizes the risk of error when products from multiple sources are being used as well as allowing patient involvement in the final check when appropriate. The example of the label is provided on Fig. 4.

8 Role of ICCBBA and CTCLAG

It is apparent that the future success of ISBT 128 will depend upon a continuing commitment to design and management. An ongoing dialogue between clinical users, scientists, information specialists, and equipment and software vendors is -critical in supporting rapidly developing clinical practice, especially in cellular therapy.

A

B

QUANDRANT	REQUIRED	OPTIONAL
UPPER LEFT	Donation Identification Number	Collection Date Collection Date and Time Production Date and Time Flexible Date and Time (Encoding the collection or production date and time)
LOWER LEFT	Product Code	
UPPER RIGHT*	Blood Groups [ABO and RhD]	Global Registration Identifier for Donors
LOWER RIGHT**		Expiration Date and Time Flexible Date and Time (encoding the expiration date and time)

Fig. 4 Examples of ISBT 128 labels
More information provided in the following publication *ISBT 128 Standard: Labeling of Cellular Therapy Products* (www.iccbba.com) [6]
(**A**) 100 mm x 100 mm label for cellular therapy product (example). Description: (1) Donation Identification Number (DIN); (2) ABO/RhD; (3) collection date/time; (4) product code; (5) expiration date/time
(**B**) Quadrant bar codes on the 100 mm × 100 mm label. *) required to be printed when known at the time of labeling; **) strongly recommended to be printed when known at the time of labeling
(**C**) Partial ISBT 128 label for cellular therapy product (example from Ref. [7])

C

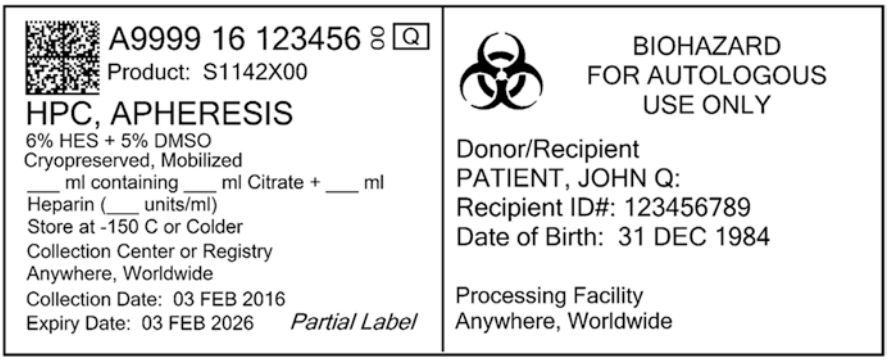

Fig. 4 (continued)

ICCBBA provides the ongoing management support for ISBT 128 and is supported by more than 300 volunteer subject matter experts. The organization is funded from the license fees paid by users of the ISBT 128 Standard.

The Cellular Therapy Coding and Labeling Advisory Group (CTCLAG) is coordinated by ICCBBA and has representation from all the professional societies mentioned earlier in this chapter. Licensed vendors can participate as observers and regulators participate as liaisons. CTCLAG is broadly responsible for reviewing and recommending changes to standards as well creating new classes and attributes for CT products. As the field is rapidly expanding and different new attributes are required, CTCLAG continuously evaluates the appropriateness of our definitions and product descriptions. There is a transparent process for stakeholders to request changes and updates to the standards as well as new product codes. Only some of these requests require CTCLAG review as many of the requests are handled by highly competent ICCBBA technical staff. Further information about ISBT 128, ICCBBA, and CTCLAG is available at the ICCBBA website www.iccbba.org.

9 Practical Considerations for the Implementation of ISBT 128

Implementation of ISBT 128 in cellular therapy laboratory requires significant resources and time. However, the benefits of well-implemented ISBT 128 cannot be underestimated. Many of the laboratories implemented ISBT 128 to comply with local and national regulations, while others needed to comply with voluntary accrediting bodies such as AABB, FACT, JACIE, or NMDP.

Once implemented, ISBT 128 requires maintenance and occasionally updates and qualifications. It has been noted that re-qualifications of already installed

systems are not always performed in a timely fashion. This is a requirement for all accrediting organizations. Such qualification and validation assure the user that the labels are correct and information is properly transmitted from the laboratory information system to ISBT 128.

Another area of concern is related to selection of appropriate codes for manufactured products. It might be a daunting task to identify the correct code, especially if the process is complex or the laboratory personnel has limited experience in this activity.

There are resources available on the ICCBBA website to make this process easier.

10 Conclusions

This chapter briefly summarizes the role of ISBT 128 in supporting international traceability for cellular therapy products. Foundational concepts were introduced, together with their use in practice. This internationally harmonized system has advanced through collaboration and broad stakeholders' involvement. The flexibility of the system, its ability to adjust to the growing complexity of cellular therapy products, and its proactive management all help to ensure its continuing suitability for purpose. Future changes in data management and storage will likely influence how ISBT 128 evolves to serve the cellular therapy field.

References

1. Ashford, P. (2010). Traceability. *Cell and Tissue Banking, 11*(4), 329–333.
2. Ashford, P., & Delgado, M. (2017). ISBT 128 standard for coding medical products of human origin. *Transfusion Medicine and Hemotherapy, 44*(6), 386–390.
3. ICCBBA. (2020). *ISBT 128 – The global information standard for medical products of human origin*. www.iccbba.com. Last accessed 9 Aug 2020.
4. Meeting of Blood Products Advisory Committee. (1997). U.S.F.C.f.B.E.a. Research, Editor. 20 June 1997.
5. ICCBBA. (2020). *ISBT 128 standard: Standard terminology for medical products of human origin* (p. 125). ICCBBA.
6. ICCBBA. (2018). *ISBT 128 standard: Labeling of cellular therapy products* (p. 43). ICCBBA.
7. ICCBBA. (2020). *ISBT 128 for cellular therapy: An introduction* (12th ed., p. 20). ICCBBA.
8. Ashford, P., et al. (2007). Standards for the terminology and labeling of cellular therapy products. *Transfusion, 47*(7), 1319–1327.
9. Ashford, P., et al. (2007). ISBT 128 implementation plan for cellular therapy products. *Transfusion, 47*(7), 1312–1318.
10. Sorensen, B. S., et al. (2016). Inadvertent completely HLA-mismatched allogeneic unrelated bone marrow transplant: Lessons learned. *Bone Marrow Transplantation, 51*(7), 1016–1018.
11. Neller, J. K., et al. (2017). Global registration identifier for donors (GRID) of hematopoietic stem cells: Road to automation and safety. *Transfusion Medicine and Hemotherapy, 44*(6), 407–413.

12. AABB. (2003). *Circular of information for the use of cellular therapy products* (1st ed.).
13. AABB. (2018). *Circular of information for the use of cellular therapy products*. October 2018 ed. Bethesda, Maryland.
14. CBER. (2019). *CBER biologics effectiveness and safety (BEST) system*. Cited 17 August 2020. Available from https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/cber-biologics-effectiveness-and-safety-best-system
15. Obidi, J., et al. (2018). Advancing the use of the ISBT-128 coding system in electronic health records to monitor blood transfusion prevalence in the United States. *Transfusion, 58*, 167A.

Product Processing, Manufacturing, and Administration

Adrian P. Gee

1 Regulatory Issues

The regulations apply to the preparation and use of cellular therapy products in different areas of the world and are addressed in individual chapters in this volume. In the USA products must be manufactured under either current Good Tissue Practices (cGTP) [1] or Good Manufacturing Practices (cGMP). These are detailed in the Code of Federal Regulations (CFR) in Parts 1–99, 200–299, 300–499, 600–799, and 800–1299 for cGMP [2] and Title 21 Part 1271 for cGTP [1]. The latter require the assessment of donor eligibility, but are in many respects, the practice (manufacturing) regulations are similar to those applying to cGMP but are somewhat less stringent. It is important to understand which regulations apply to which types of products.

A primary difference is the degree of ex vivo manipulation that is used to prepare the products. Minimally manipulated cells (procedures that do not alter the relevant biological characteristics of the cells or tissues) are regulated under cGTP [3]. These cells must also be intended for homologous use (the repair, reconstruction, replacement or supplementation of a recipient's cells or tissues with a product that performs the same basic function(s) in the recipient as in the donor) and must not be combined with another article, e.g., a matrix or scaffold. This category includes cells that are not cultured ex vivo, activated, or genetically modified. Procedures covered under cGTP do not require submission of a protocol to the US Food and Drug Administration (FDA – the Agency) but do require reporting of certain deviations and unexpected events to the agency.

In contrast, cGMP regulations are followed when preparing products that are to be used under an Investigational New Drug (IND)/Device (IDE) application [4]. This requires submission of a comprehensive packet of information to the FDA,

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which includes preclinical data, a chemistry, manufacturing and control (CMC) section on product manufacturing, [5] and the proposed clinical protocol. cGMP regulations are very comprehensive and cover all aspects of manufacturing including facility specifications, management of materials, manufacturing, closures, release and distribution, and quality issues. Allowances are made, however, for the phase of study for which the product is to be manufactured. Most academic cGMP facilities are engaged in phase I/early phase II studies, which are intended to demonstrate product safety and preliminary evidence of efficacy. The FDA has published a guidance document on the level of cGMP compliance that is expected when preparing products for these applications [6]. Although there is a trend to exceed these expectations, this guidance should be regarded as the current regulatory stance, although additional requirements may be raised when the associated IND application is reviewed.

In practice, for facilities that manufacture both cGTP and cGMP products, it is easier to follow a hybrid of both sets of regulations, e.g., by following cGMP manufacturing regulations, while including eligibility assessment for all donors.

Details about the manufacturing environment that are provided by the FDA for cGMP manufacturing are rather generic. They indicate that they should be of a suitable size, construction, and location to facilitate cleaning, maintenance, and proper operations. This includes provision of adequate space for equipment and materials to prevent mix-ups; performance of operations within specifically defined areas; adequate lighting; provision of equipment for adequate control over air pressure, microorganisms, dust, humidity, and temperature when appropriate; adequate potable water and drainage; safe and sanitary sewage and waste disposal; adequate washing and toilet facilities; adequate sanitation/pest control with written procedures; and maintenance of the facility in good repair.

2 Facility Design and Review

For cGMP manufacturing, it is probably true to say that the FDA expectation is that this be performed within a cleanroom facility. Academic cleanrooms vary greatly in design, with older facilities being rather basic, while the latest are much more sophisticated. A relatively simple solution is to use modular (ready-made) cleanrooms that are located within the building shell. These are available from a number of companies. Alternatively, pharmaceutical isolators, which are composed of a single unit, consisting basically of a biological safety cabinet (BSC) environment linked to an incubator, may be purchased and placed into a preexisting laboratory. A more expensive choice is to build or renovate existing space to create a cGMP facility. The design of these is discussed in several chapters in this volume. The best design will depend on the type(s) and numbers of product(s) to be prepared. Some facilities in which a single (or very restricted) number of products is to be manufactured opt for large rooms containing multiple BSCs and incubators, while multiproduct facilities generally opt for a larger number of smaller rooms. It is

recommended that facility plans should be submitted to the FDA prior to construction [7]. There are several levels of review available.

The first is a Design Review in which conceptual drawings, proposed layouts, and flow diagrams are submitted. It is expected that the complete final plans and any questions will be made available to the Agency. This is usually the only review requested by academic cGMP facilities. For commercial entities additional review opportunities include pre-construction, construction/equipment installation, and qualification and pre-production reviews. During the Design Review, the FDA will raise concerns about the plans, and the submitter can respond to these either by justifying the existing plan by providing information that addresses the concern(s) or by modifying the plans. Review concerns often focus on the potential for product contamination and cross-contamination, and these can be anticipated by providing product, staff, and waste flow diagrams with the floor plans. Requests for plan reviews can be addressed to the Office of Compliance/Division of Manufacturing and Product Quality at the Center for Drug Evaluation and Research or the Office of Compliance and Biologics Quality/Division of Inspections and Surveillance at the Center for Biologics Evaluation and Research at the FDA.

For manufacture of cGTP products, the use of a cleanroom facility may be avoided. Several academic facilities prepare these products in unclassified space containing several BSCs. If this is proposed, every attempt should be made to use functionally closed systems within the BSC, and there should be written procedures in place to address the prevention of cross-contamination and contamination.

3 Reagents and Materials

Reagents and materials should either have received FDA approval for cGMP procedures or be of pharmaceutical grade for cGTP manufacturing [5, 6]. The FDA may request additional testing where reagents do not meet specifications. If pharmaceutical grade reagents are not available, the highest grade should be substituted and tested for potential toxic or adverse effects on the appropriate cells. Certificates of Analysis (CofAs) for all reagents must be obtained and kept on file together with the results of any supplementary testing performed. Sometimes CofAs are not available for materials. In such cases it is usually possible to obtain from the manufacturer a statement attesting to the fact that the material is produced according to cGMP regulations. It is recommended that sterile materials and reagents always be purchased rather than relying on in-house sterilization. The central sterile supply facilities in most hospitals do not follow FDA regulations for sterilizing drug supplies or their components. Similarly, the use of in-facility autoclaves requires temperature mapping and extensive documentation of autoclave performance. This means that sterilization has to be performed by external contractors to FDA specifications – a process that is both expensive and cumbersome. Membrane filtration (0.2 μm) for sterilization of small volumes of reagents is, however, generally acceptable.

If water is used to prepare reagents or media, it is recommended that this be purchased rather than using water from in-house purification systems. These require routine maintenance and the water must be tested regularly.

4 Manufacturing Techniques

Manufacturing procedures should, wherever possible, use closed or functionally closed systems. These are increasingly becoming available, except for techniques where small numbers of cells are being prepared. There are a variety of cell separation systems that are closed or functionally closed, and these are supplemented by bioreactors and other closed cell culture systems. Functionally closed systems involve aseptic transfer of cells using bags, lines, sterile connect devices, and closeable culture systems. Where these are used, sterility testing should be performed at cell transfer points (critical control points).

A number of automated or semiautomated culture systems are now commercially available, e.g., the Prodigy from Miltenyi [8] (Fig. 1), the Quantum Bioreactor from Terumo [9] (Fig. 2), and the Xuri Cell Expansion System from Cytiva [10]. These vary in their ability to incorporate user-initiated changes, which limits their flexibility for different applications, particularly in academic centers where process development for phase I applications is critical. Another limitation is that they can only be used to manufacture one product at a time and their expense often precludes the purchase of multiple units for use in busy facilities. The availability of such

Fig. 1 The Prodigy cell separation and culture device from Miltenyi Biotech [8] (from Miltenyi Biotech)

Fig. 2 The Quantum Bioreactor from Terumo BCT

systems may decrease the need for cleanrooms, however, since their closed or functionally closed design means that they could be located in unclassified space.

Devices are now available for automating part of the manufacturing process. For example, the Lovo Cell Processing System from Fresenius Kabi is capable of washing and concentrating cell populations by use of a spinning membrane [11]. Cell separation, concentration, and washing systems for use with mesenchymal progenitor cells are also available from Kaneka Medix [12]. Sepax cell separation (Fig. 3) and cell washing devices are available from Cytiva. [13]. The GatheRex device from Wilson Wolf is a cell harvesting system [14] that uses pumps to collect cells from the G-Rex culture devices [15, 16]. There are also devices that use sonic waves to perform cell separations and microfluidic systems that use gentle controlled fluid flow to concentrate viral vector around cells (Draper).

The primary manufacturing location for most cellular therapy products is the Class 100 (ISO5) biological safety cabinet (BSC). In the USA, these are often placed in a Class 10,000 (ISO7) cleanroom environment. In Europe, they are usually located within Class 1000 (ISO6) space. BSC should be calibrated semiannually and, where possible, should have in situ particle counters. There should be an environmental monitoring program for BSC that is followed during manufacturing. This will include particle and viable counts, surface monitoring using RODAC (Replicate Organism Detection and Counting) plates, fallout plates, and touch plates used to monitor the gowning and gloves of technical staff. This should be supplemented with particle and viable counts in the room in which the BSC is located. Staff should be trained both in aseptic technique and in the proper use of the BSC. This must include proper cleaning, not blocking airflow grids, transferring

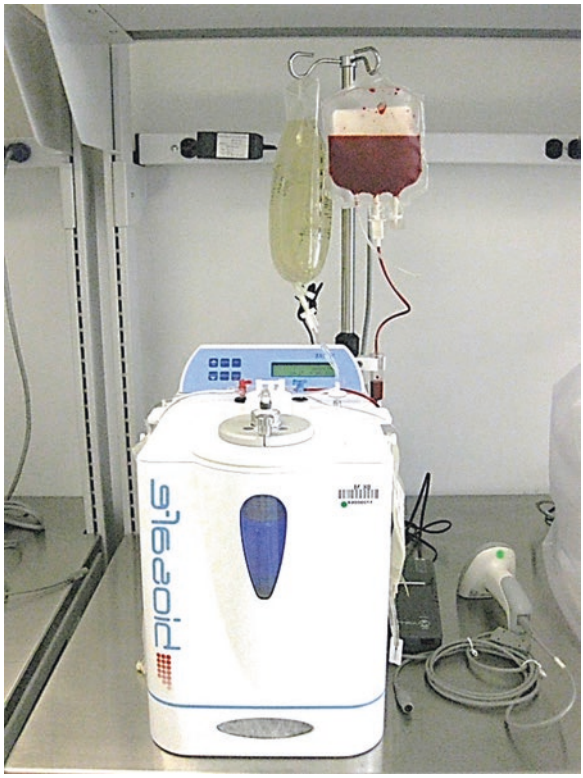

Fig. 3 Sepax cell separation device from Cytiva

items into the BSC, allocation of space within the BSC (supplies and reagents to the left, work area in the center, and waste containers to the right), and proper working stance (arms inserted straight on and well inside the BSC, keeping the nose and mouth away from the window opening, working slowly and deliberately and not shielding materials from the downward air flow). Proper training in the use of automatic and serological pipettes is also essential and should include unwrapping of serological pipettes, avoidance of passing air through liquids, not touching the necks of culture flasks, and proper discard of pipettes.

5 Culture Media

In most cases culture media must be supplemented with serum and other reagents before use. A record should be kept of the preparation of complete media including each ingredient, its manufacturer, and expiration date. Where possible serum-free media should be used. The FDA is increasingly asking for measurement of residual fetal bovine serum or bovine serum albumin levels in products such as vectors. In some cases, it may be possible to determine the approximate concentration by

calculation of the dilution factors during washing steps. An alternative supplement is human AB serum or human platelet lysate from appropriately screened donors.

Although a number of pharmaceutical grade media are under development, many academic facilities use research grade material. In 2001 the Center for Devices and Radiological Research of the FDA issued a Final Guidance on tissue culture media for human ex vivo tissue and cell culture processing [17]. This requires submission of information on the base medium and a list of all additives and supplements and their anticipated ranges. The purity of each component should be stated with justification as to its use. The purpose of each chemical component should be stated and evidence provided to demonstrate the lack of toxicity and performance in maintaining cell function. Stability data should also be provided to support the expiration date. It must be tested for sterility and endotoxin. For widely used media, it is sometimes sufficient to submit the manufacturer's formulation and a Certificate of Analysis.

Expiration dates of the supplemented medium should be based on the shortest expiration date of any supplement and on practical experience on the ability of the complete medium to support cell growth. The medium should be tested for sterility before use. It is recommended that bottles of complete medium should be allocated for use with an individual patient's cells to reduce the chance of cross-contamination.

6 Manufacturing Validation

When manufacturing is performed under cGMP regulations, it is likely that during the IND application process the FDA has approved the use of the relevant reagents and the SOPs for manufacturing and testing the cellular product for release and distribution. In some cases, the application will contain results of the validation of manufacturing, if not, then this must be accomplished before routine preparation of product takes place [18]. One difficulty that arises is the lack of raw material with which to perform validation runs (usually three in number). One possibility is to use cells that are collected with the intent to treat the patient if the validation results meet the release criteria. In such a case, it is important to ensure that the patient/donor gives informed consent that clearly explains that a manufacturing failure may occur, resulting in that product not being available for clinical use. cGTP procedures must also undergo validation before use, and, in both cases, there must be documentation of staff training in the procedure and of continued competence in its performance.

7 Changeover Procedures

When handling multiple cell cultures within a manufacturing suite, changeover procedures must be followed. These are written SOPs that detail the procedure to be followed when a second or subsequent cell culture from a different donor is handled in the same room and BSC. Changeover procedures should include return of the first culture to the incubator, removal of all documentation associated with handling of those cells, documented cleaning of all equipment (e.g., BSC and centrifuge), changing gowning or donning sterile sleeve covers, retrieval of documentation associated with second cell culture, and placement of second cell culture and allocated reagents into the BSC. In parallel a record must be maintained of all reagents, materials, and equipment used during the handling of each cell culture. This must be supplemented by keeping records of each cell product processed using each piece of equipment. It is advisable to submit changeover procedures to the appropriate regulatory agency before handling multiple products within a manufacturing room. The possibility of cross-contamination can be evaluated by HLA typing the donor and final cell products that express Class I or II molecules.

8 In-Process Testing

In-process testing should be performed at critical control points (CCP) during the manufacturing. A CCP is defined as is the point where the failure of an SOP could cause harm to the product. It is a point, step, or procedure at which controls can be applied and a potential hazard can be prevented, eliminated, or reduced to acceptable (critical) levels. Examples of CCP include performance of cell counts to ensure adequate growth or cell recovery, evaluation of sterility after cell manipulation procedures, and expression of gene products after transduction of cell products. With experience on validated procedures, it may be possible to eliminate some of these. However, their inclusion makes it simpler to detect potential manufacturing problems and provide opportunities for process improvement. The FDA recommends that at least three consecutive runs be performed initially followed by semiannual qualifications [19].

9 Product Labeling

Products must be adequately labeled during all phases of manufacturing. A system of unique identifiers for the components and the donor or intended recipient should be used at a minimum. Standard-setting organizations provide guidance as to the information that should be used on labels at various points during production. The labeling should be indelible and easily readable. It may be supplemented with bar

codes. Final cGMP products should be labeled using the label(s) submitted in the IND application. Increasingly the international ISBT 128 labeling system [20] is being used for this purpose. This provides a standardized format that employs both bar coding and eye-readable information. The system is managed by International Council for Commonality in Blood Banking Automation (ICCBBA) [20], which has an official relationship with the World Health Organization. As new products are developed, ICCBBA issues a unique product code which can be used on the label and is readable internationally. Most standard-setting organizations that deal with cell products have now mandated the use of ISBT 128 labeling.

10 Finish and Fill

The finish and fill procedures for products stored in multiple aliquots, e.g., vectors, should be validated. There are now automated devices for the addition of cryoprotectant and filling of the freezer bags, e.g., the Finia Finish and Fill System (Terumo BCT) [21]. Vanrx Pharmasystems Inc. also makes a variety of finish and fill devices. Validation of manual systems is accomplished in the form of media fills [19], which can be also used to simulate complex manufacturing procedures [22]. Media that supports microbial growth, e.g., tryptic soy broth (TSB), is pipetted or automatically dispensed into sterile vials over a period that corresponds to a normal aliquoting run or a complex procedure. This should be performed under normal operating conditions, e.g., with the representative number of staff, rotating staff members, at the normal fill speed, etc. The vials are then usually incubated at 20–35 °C for 14 days. Alternatively, they may be incubated at 20–25 °C for 7 days followed by 7 days at 35 °C. The vials should be examined by quality control (QC) before and after incubation. Turbidity of the medium is interpreted as microbial growth and must be recorded. If <5000 vials are filled, there must be no evidence of contamination. If 5000–10,000 vials are filled, a single contaminated vial must be investigated, and if two or more are contaminated, the validation must be repeated. Environmental monitoring must be performed during the fill procedure. This includes pre-filling and in-process particle counts in the room, viable in-process counts in the room, fallout and RODAC plates in the BSC, and, in some cases, particle counts in the BSC. The vials should also be weighed to determine the fill volume accuracy and a positive microbial control, e.g., *Bacillus subtilis* and a negative control, e.g., TSB, included. It is advisable to test selected vials after incubation using the approved USP sterility test and to perform bacteriostasis/fungistasis testing on the TSB.

11 Cryopreservation

Many cell products are cryopreserved and stored prior to administration. Most centers use 5–10% volume/volume dimethyl sulfoxide (DMSO) as the cryoprotectant and a controlled rate freezer for freezing. Pharmaceutical grade DMSO is now available and should be used. Some standard-setting organizations require that each lot should be qualified before use in cryopreservation procedures. Controlled rate freezers compensate for temperature changes at the eutectic point and generally freeze products to approximately -100 °C before transfer to long-term storage (Fig. 4). Most facilities use a generic freezing program that is independent of the cell type; however, there is increasing evidence that various cells may respond differently to cryopreservation, .e.g., NK cells do not show good functional activity after initial thawing. This has stimulated investigators to examine a range of other cryoprotectants and freezing protocols.

An alternative freezing method is provided by the CoolCell Containers (BioCision) [23]. These are used to freeze cryovials, which are placed into the container which has a solid thermoconductive core and is fitted with a lid. This is then placed into a -80 °C freezer, usually overnight. This provides a cooling rate of 1 °C/minute. The following day the vials can be placed into vapor phase liquid nitrogen storage. These containers are available in a variety of configuration which can accommodate cryovials of different sizes. Similar results can be obtained with an earlier device, the Mr. Frosty (Thermo Scientific) [24], in which the cryovials are placed into a container of isopropyl alcohol which is similarly refrigerated overnight at -80 °C.

12 Long-Term Storage

Long-term storage is usually in the vapor phase of liquid nitrogen to avoid cross-contamination between products, which has been described for cells stored in the liquid phase [25]. Products with known contamination with infectious agents are normally overwrapped (double wrapped) to provide extra protection. Banks in which the liquid nitrogen is contained in absorbent material are now available (MVE Fusion series) [26]. The FDA does not allow the use of donors with positive infectious disease markers for allogeneic cellular therapy product preparation without specific permission. It is normal practice to freeze aliquots of the bulk product to provide additional samples if testing needs to be repeated or for use in stability testing protocols. These should be frozen and stored under identical conditions to the bulk product. Storage vessels should be fitted with multiple alarms (liquid nitrogen level, temperature at the top and bottom of the storage vessel, adequate liquid nitrogen supply, etc.), and opening and closing the lid should be minimized to avoid transient temperature fluctuations.

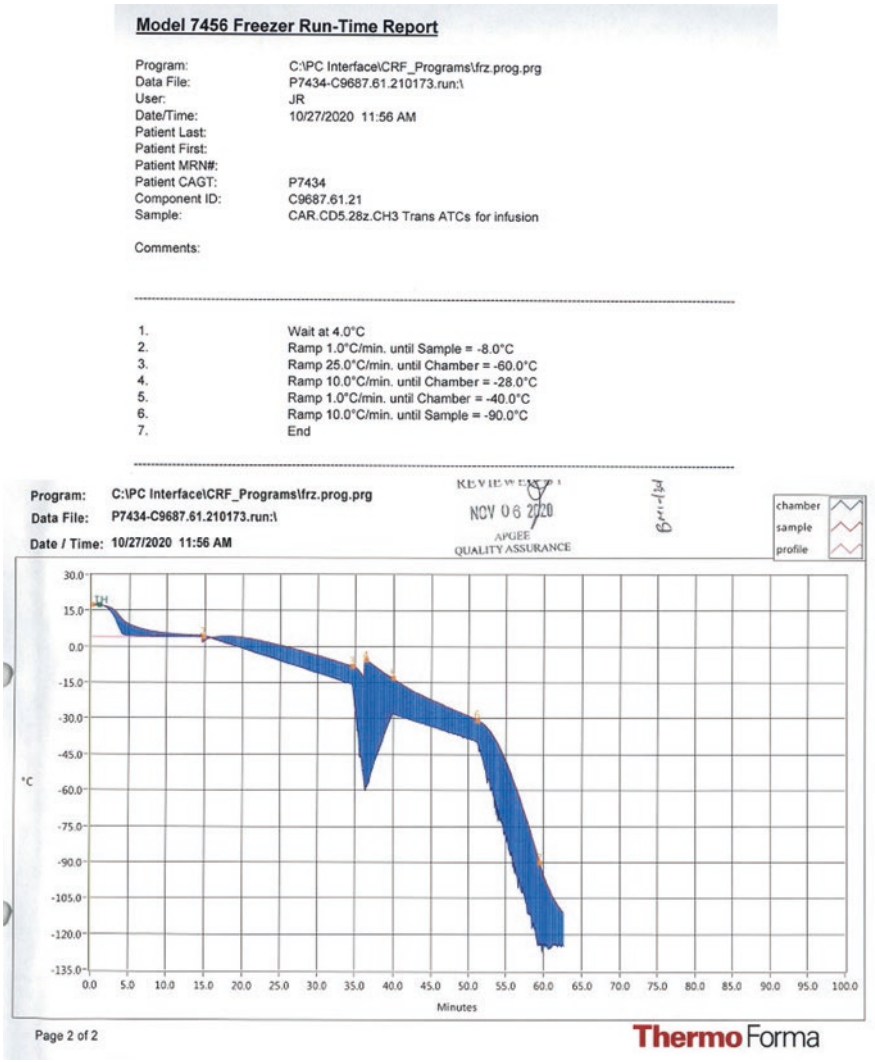

Fig. 4 Typical freezing program and curve

13 Release Testing

Release testing provides evidence that a product is suitable for clinical use. It is required for cGMP products, and the tests to be performed are described in various FDA Guidance documents and may be supplemented during the IND/IDE application review [5]. The results of these tests are presented in a Certificate of Analysis (CofA). This details the tests to be performed, their sensitivity or limit of detection, the identity of the testing facility, the specification for the test results, and

the actual result obtained. If the cGMP product is cryopreserved, this provides sufficient time for release testing to be performed. If, however, the product is to be administered without freezing, an alternative testing strategy must be used. This will include performing mycoplasma, functionality testing, and 4- or 7-day sterility cultures while the cells are still in culture. These results will be supplemented at the time of preparation for administration by endotoxin testing and Gram staining, in addition to submission of 14-day sterility cultures.

The CofA is usually generated by the quality assurance unit following review of the processing records and test results. It is co-signed by a Laboratory Medical Director who attests to the suitability of the product for clinical use. In the case of cGTP products, a CofA is not normally used. Some of these products are administered immediately after preparation and, although 14-day sterility tests are submitted, the results are only available after the product has been administered. If a positive result is obtained from the 14-day test, it is important to identify the contaminating organism and evaluate its antimicrobial sensitivity and to have a procedure in place to notify the recipient's physician immediately. In most cases cryopreserved cGTP products are thawed and administered without further manipulation. Fourteen-day sterility testing may be performed on the thawed samples. It is not normal practice to submit Gram stains on these products. In contrast, when a thawed product is manipulated after thawing, e.g., washed, it is usual to submit a Gram stain and endotoxin test in addition to 14-day sterility tests.

Many release tests cannot be performed in real time, e.g., USP sterility testing; however, a number of rapid tests have been developed. These include endotoxin testing using the FDA-approved and USP/EP-compliant Endosafe device (Charles River) [27]. There are also approved kits for mycoplasma testing by PCR (MycoTOOL (Roche) in the USA and EU (38) and Mycosart (Sartorius) in the EU [29]). Flow cytometric analysis for cell purity and transgene expression is also a rapid method; however, many potency/functionality assays require several days to perform.

14 Product Administration

14.1 Documentation

A prescription for product administration should be provided to the facility in which the product is being stored. This should specify (1) the identity of the intended recipient (with supplementary identifiers, such as medical record number), (2) the location at which the administration is to take place, (3) the time and date of administration, (4) the product to be administered, and (5) the dose to be administered. This should be signed by the intended recipient's physician and the laboratory medical director. Upon receipt by the cell processing facility, it is a good

idea to verify the location of the product if it is cryopreserved to make sure that there are sufficient cells available.

At the site of administration, cross-checking of the product and intended recipient's identity is documented by laboratory and medical staff. The start and stop time for product administration is recorded together with any details of changes in the recipient's condition and any adverse reactions. If an adverse reaction occurs, this must be fully investigated as to its likely cause and the medical actions taken. In the case of IND products, severe adverse reactions must be reported to the regulatory agency rapidly and any other adverse reactions documented in the annual IND report.

14.2 Cryopreserved Product Administration

Products that have been cryopreserved must be thawed for administration. If the product is to be administered without further manipulation, the normal practice is to transport it to the patient's bedside while still frozen. This can be achieved using a small dry shipper or other container suitable for holding liquid nitrogen. The usual method is to transport the product on a cart which contains all of the items required to thaw it and prepare it for administration. We have found it useful to use a checklist to ensure all of the required items are on the cart prior to transportation (Fig. 5). The frozen product is removed from inventory by two staff members who cross-document its location, identity, and removal from storage. The product is placed into the liquid nitrogen container and transported to the intended site for administration. Normally the staff transferring the product will wear portable oxygen monitors to ensure that any escaping nitrogen vapor does not adversely affect the oxygen levels in confined spaces such as elevators. We normally thaw products in baths containing sterile normal saline at 37 °C. This process is speeded up by pre-warming the saline bags in a microwave. Upon arrival, the identity of the product is again cross-checked by the laboratory and medical staff. If the product is in a bag, it may be placed in an outer sterile plastic bag for thawing. Products in vials are normally thawed without additional protection. There are automated thawing devices now available, e.g., the VIA Dry Automated Thawer from Cytiva [30] and the ThawSTAR CB (Medcision) [31] for bags and ThawSTAR (Biocision) [32] and the CellSeal Automated Thawing System (CATS) (Asymptote) [33] for cryovials.

The product is usually administered intravenously by hanging the bag containing the cells or by drawing vialed cells into syringes and injecting them via the catheter. We normally submit samples of thawed products for sterility testing.

Fig. 5 Thaw cart

14.3 Thawed and Fresh Product Administration

If a cryopreserved product is to be manipulated before administration, it is thawed in the cell processing facility under aseptic conditions. The manipulation, e.g., washing to remove cryoprotectant, is performed, and additional release testing, e.g., Gram stain and 14-day sterility testing, may be required.

For fresh products the release testing is more complex. Most regulatory agencies recommend performing non-stat tests several days before the anticipated administration of the product. These would include mycoplasma by PCR, sterility testing, functionality, etc. On the day of preparation for administration, the final product should be tested using stat tests, e.g., Gram stain for sterility, MycoAlert for mycoplasma, and Endosafe for endotoxin. Fourteen-day sterility testing should also be submitted with a plan of action in place as to what to do if the result comes back positive. This usually involves immediately informing the recipient's physician, identification of the contaminating organism, and assessment of antibiotic sensitivity. An investigation of the positive results is also required. In the USA, administration of a hematopoietic cell product that tests positive requires notification of the regulatory agency.

Thawed and fresh products are usually transported to the site of administration under conditions that have been validated to maintain their stability. These studies are usually performed as part of the IND package submitted to the regulatory agency. Similar studies are required to assess the stability of stored frozen products.

15 Conclusions

An understanding of the appropriate regulations that apply to manufacturing cell and gene therapy products for evaluation in clinical trials provides a firm foundation for the selection of the procedure(s) to be used. In general, manufacturing should employ closed or functionally closed systems, rigorous aseptic technique, and pharmaceutical grade reagents. Where these are not available, the highest purity reagent should be selected, and, for cGMP manufacturing, details of its characteristics should be submitted to the appropriate regulatory authority for review. Products must be release tested using appropriate assays.

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References

1. U.S. Food and Drug Administration. Code of Federal Regulations Part 1271, Title 31. Human cells, tissues, and cellular and tissue based products. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=1271. Last accessed 12 Nov 2020.
2. U.S. Food and Drug Administration. Code of Federal Regulations Title 21. Parts 1–1499. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm. Last accessed 12 Nov 2020.
3. U.S. Food and Drug Administration. (2017). Regulatory Considerations for Human Cells, Tissues, and Cellular and Tissue Based Products: Minimal Manipulation and Homologous Use. Guidance for Industry and Food and Drug Administration Staff. November 2017. https://www.fda.gov/media/109176/download. Last accessed 12 Nov 2020.
4. U.S. Food and Drug Administration Investigational New Drug (IND) Application. FDA Website. https://www.fda.gov/drugs/types-applications/investigational-new-drug-ind-application. Last accessed 12 Nov 2020.
5. U.S. Food and Drug Administration. (2008). Content and review of chemistry, Manufacturing, and Control (CMC) Information for Human Somatic Cell Therapy Investigational New Drug Applications (INDs). Guidance for FDA Reviewers and Sponsors. April 2008. https://www.fda.gov/media/73624/download. Last accessed 12 Nov 2020.
6. U.S. Food and Drug Administration. (2008). CGMP for phase 1 investigational drugs. Guidance for Industry. July 2008. https://www.fda.gov/media/70975/download. Last accessed 12 Nov 2020.
7. U.S. Food and Drug Administration Pre-operational Reviews of Manufacturing Facilities. FDA Website. https://www.fda.gov/inspections-compliance-enforcement-and-criminal-investigations/field-management-directives/pre-operational-reviews-manufacturing-facilities. Last accessed 12 Nov 2020.
8. CliniMACS Prodigy Instrument. Automated Processing in a Closed System. Miltenyi Website. https://www.miltenyibiotec.com/US-en/products/clinimacs-prodigy.html#gref. Last accessed 12 Nov 2020.
9. Quantum Cell Expansion System. Terumo Website. https://www.terumobct.com/quantum . Last accessed 12 Nov 2020.

10. Xuri Cell Expansion System W25, Cytiva Website. https://www.cytivalifesciences.com/en/us/shop/cell-therapy/systems/xuri-cell-expansion-system-w25-p-06192h. Last accessed 12 Nov 2020.
11. Lovo Automated Cell Processing System. Fresenius Kabi Website. https://chooselovo.com. Last accessed 12 Nov 2020.
12. Bone Marrow MSC Separation Device. Kaneka Website. https://www.kaneka.co.jp/mdcell/msc/english/index.html. Last accessed 12 Nov 2020.
13. Sepax C-Pro Cell Processing System. Cytiva Website. https://www.cytivalifesciences.com/en/us/solutions/cell-therapy/products-and-technology/cell-processing/sepax-c-pro-cell-processing-system. Last accessed 12 Nov 2020.
14. GatheRex Closed System Cell Harvest Video. Wilson Wolfe Website. http://www.wilsonwolf.com/closed-system-video/. Last accessed 12 Nov 2020.
15. G-Rex: The gold standard for T cell therapy. Wilson Wolfe Website. http://www.wilsonwolf.com/g-rex-t-cell/. Last accessed 12 Nov 2020.
16. The Next Big Advance in CAR T-cell Treatment will be Smarter Cell Therapy Production. Draper Website. https://www.draper.com/news-releases/next-big-advance-car-t-cell-treatment-will-be-smarter-cell-therapy-production. Last accessed 12 Nov 2020.
17. Tissue and cell culture processing applications – Final class II special controls guidance document for industry and FDA reviewers. https://www.fda.gov/medical-devices/guidance-documents-medical-devices-and-radiation-emitting-products/tissue-culture-media-human-ex-vivo-tissue-and-cell-culture-processing-applications-final-class-ii. Last accessed 12 Nov 2020.
18. U.S. Food and Drug Administration. (2011). Process validation: General principles and practices. Guidance for Industry. January 2011. https://www.fda.gov/files/drugs/published/Process-Validation%2D%2DGeneral-Principles-and-Practices.pdf. Last accessed 12 Nov 2020.
19. U.S. Food and Drug Administration. (2004). Sterile drug products produced by aseptic processing—Current good manufacturing practice. Guidance for Industry. September 2004. https://www.fda.gov/med2ia/71026/download. Last accessed 12 Nov 2020.
20. ISBT 128. ICCBBA Website. https://www.iccbba.org/. Last accessed 12 Nov 2020.
21. FINIA Finish and fill System. Terumo Website. https://www.terumobct.com/finia. Last accessed 12 Nov 2020.
22. Flexible Pharmaceutical Vial Filling. Vanrx Website. https://vanrx.com/products/pharmaceutical-vial-filling-machine/. Last accessed 12 Nov 2020.
23. CoolCell® Cell Freezing Containers. Biocision Website. http://www.biocision.com/products/coolcell-cell-freezing-containers/. Last accessed 12 Nov 2020.
24. Mr. Frosty™ Freezing Container. Thermo Scientific Website. https://www.fishersci.com/shop/products/thermo-scientific-mr-frosty-freezing-container-nalgene-mr-frosty-freezing-container-1-2ml-cryogenic-tubes-pc-clear-w-blue-lid-1-cs/1535050. Last accessed 12 Nov 2020.
25. Hawkins, A., Zuckerman, M., Briggs, M., et al. (1996). Hepatitis B nucleotide sequence analysis: Linking an outbreak of acute hepatitis B to contamination of a cryopreservation tank. *Journal of Virological Methods, 60*, 81–88.
26. MVE Fusion Series. Chart Industries Website. https://www.chartindustries.com/Life-Sciences/Stainless-Steel-Cryogenic-Freezers/MVE-Fusion-Series. Last accessed 12 Nov 2020.
27. Endosafe® nexgen-PTS™. Charles River Website. https://www.criver.com/products-services/qc-microbial-solutions/endotoxin-testing/endotoxin-testing-systems/endosafe-nexgen-pts?region=3611. Last accessed 12 Nov 2020.
28. MycoTOOL Mycoplasma Real-Time PCR Kit. Custom Biotech Website. https://custom-biotech.roche.com/home/Product_Details/3_5_3_19_3_2.product-classes%5CTestKits.html. Last accessed 12 Nov 2020.
29. Microsart® AMP Mycoplasma. Sartorius Website. https://www.sartorius.com/shop/ww/en/usd/applications-laboratory-cell-culture/microsart%C2%AE-amp-mycoplasma/p/SMB95-1001#. Last accessed 12 Nov 2020.

30. VIA Thaw Dry Automated Thawers. Cytiva Website. https://www.cytivalifesciences.com/en/us/shop/cell-therapy/systems/via-thaw-dry-automated-thawers-p-09720. Last accessed 12 Nov 2020.
31. Biolife Solutions ThawSTAR Website. https://www.biolifesolutions.com/product/thawstar-cb-cryobag-thawing/ Last accessed 12 Nov 2020.
32. ThawSTAR® CFT1.5 Automated System for Thawing Cells. BIOLIFE Solutions Website. https://www.biolifesolutions.com/product/thawstar-cft1-5-thawing-cells/ Last accessed 12 Nov 2020.
33. CellSeal Automated Thawing System - CATS-100 (CellSeal Automated Thawing System) FCCID Website. https://fccid.io/RR-aSy-CATS-100. Last accessed 12 Nov 2020.

Transport and Shipment of Cellular and Gene Therapy Products

Chy-Anh Tran and Adrian P. Gee

1 General Packaging Requirements

Cell and gene therapy products are either transported fresh or cryopreserved depending on the product's storage requirement to the clinical site for infusion. Cell collected for processing are routinely transported fresh.

The shipping facility must have a standard operating procedure (SOP) for transportation and shipping. Taking time and care to establish the packaging, transport, and shipping requirements will ensure successful delivery of the therapeutic cell collection or therapeutic product.

First, the product should be packaged in a secondary sterile bag to minimize the risk of spilling. This should be sealed before it is placed within the transport cooler. The transport cooler should be a closed, insulated container to maintain a specified temperature appropriate for the particular type of product. An electronic temperature data logger should be included inside the container with the therapeutic product during transport or shipment.

The transport time must be minimized and there must be an alternative means of transport in case of an emergency.

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2 Transportation of Fresh Cells

Fresh cells are routinely transported from the collection facility to the processing facility or from the processing facility to the site of their administration.

Fresh products are standardly stored at 2–8 °C for a short period (<48 h). These can either be shipped at ambient temperature or on cold packs in an insulated cooler. It may be necessary to occasionally add anticoagulant to bone marrow during shipment.

2.1 General Packing Considerations for Fresh Cells (Fig. 1)

General packing consideration for non-cryopreserved cells should include:

- Use of a leak-proof primary container, such as a transfer pack or tube.
- Wrapping the product in sufficient absorbent material to absorb all of the contents (generally, one absorbent pad per one transfer pack bag or one absorbent pad per two tubes).
- Placement of the primary inside a secondary bag.
- Multiple products should be individually wrapped and placed singly within a secondary bag/container to prevent contact or breakage.
- Refrigerated (2–8 °C) gel packs (24 oz or larger) or a frozen gel pack placed outside an inner container holding the product and a refrigerated gel pack.
- Consideration of placing the secondary bag inside a rigid tertiary container, such as a cardboard box or picnic cooler (Fig. 1).
- The shipment must contain a continuous temperature monitor to record the temperature over the period of travel. There are many battery-powered temperature monitors available from which data can be downloaded to a computer and the records printed, e.g., Logger-Trac Datalogging Traceable Thermometer from Traceable Products and TempTale from Sensitech.
- There must be a document inside or fixed to the outer shipping container that contains all of the information required on the outer container as shown in Table 1 [2].
- There must be a secured rigid outer shipping container, such as a cooler or insulated box. This must be made of material adequate to withstand leakage of the contents, pressure changes, and any other conditions incident to ordinary handling during transport or shipping. Suitable insulated shipping boxes are available from Grainger and Sonoco™ ThermoSafe from Fisher Scientific. There is a carrier with an inbuilt temperature monitor (BioT™ Carrier).

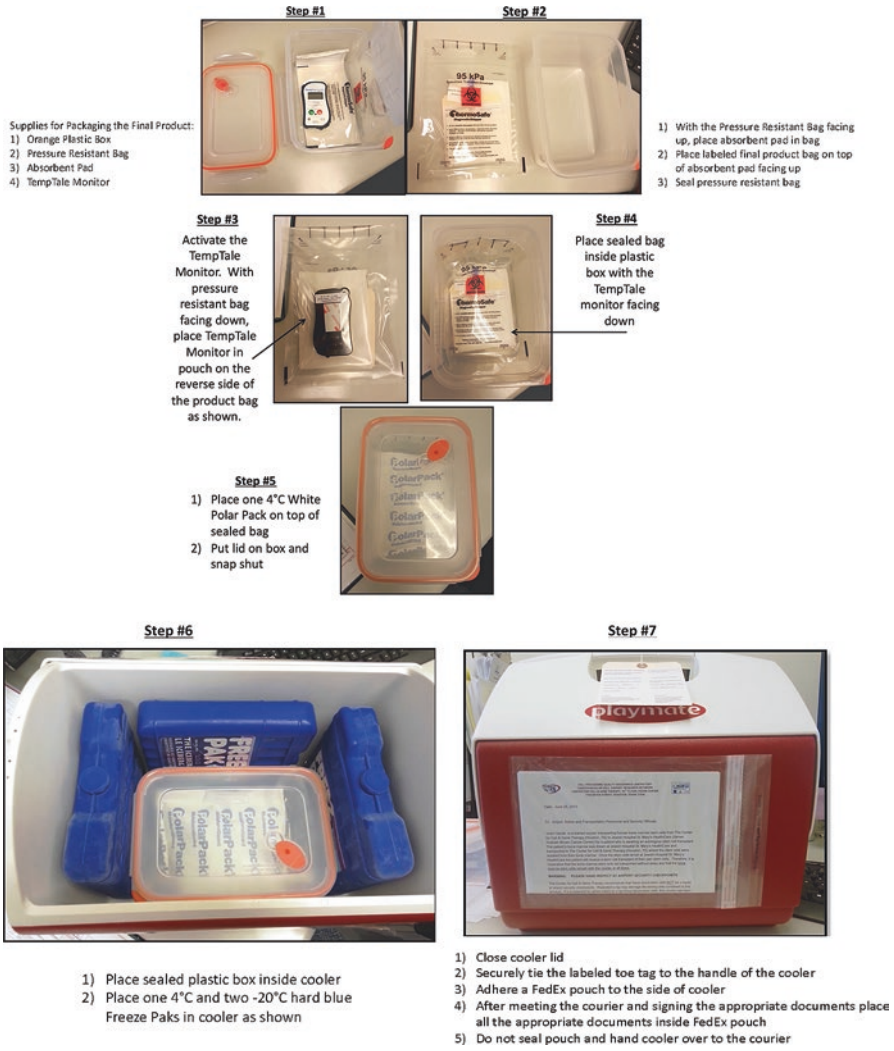


Fig. 1 Sample instructions for shipment of refrigerated fresh cells
This figure shows the steps in preparation of a refrigerated product for shipment. The product was contained in a small transfer pack, which was placed in a pressure-proof bag. This was packed in a rigid plastic container, which was in turn packed into a picnic cooler. The cooler was transported via Federal Express

2.2 General Labeling Considerations

Attention must be paid to appropriate labeling of the outer container. This must include:

- Sender's name and address
- Recipient's name and address

Table 1 Documentation to accompany shipped cells in the USA [2]

Documentation	Allogeneic eligible donor	Allogeneic ineligible donor	Allogeneic incomplete donor eligibility
Name, address, telephone number, e-mail of shipping facility, and contact information for person responsible for shipment	X	X	X
Name, address, telephone number, e-mail of receiving facility, and contact information for person responsible for product receipt	X	X	X
Statement that the donor has been found to be either eligible or ineligible based on donor screening and testing	X	X	
Summary of records used to determine donor eligibility	X	X	
Name and address of facility making eligibility determination	X	X	
Listing and interpretation of the results of all communicable disease tests performed	X	X	X
Statement that testing was performed by laboratory meeting regulatory requirements	X	If applicable	If applicable
Statement that donor eligibility determination is not complete			X
Statement noting reason for determination of ineligibility		X	
Statement that product must not be administered until donor eligibility determination has been completed, except for urgent medical need			X
Listing of tests or screening that has not been completed			X
Results of tests or screening that has been performed			X
Documentation that physician using the product was notified of incomplete eligibility testing or screening			X
Instructions for product use	X	X	X
Instructions for reporting serious adverse reactions or events to the distributing facility	X	X	X

Adapted from FACT Standards 7th Edition

- Exempt human specimen label
- "Do Not X-Ray" label

Full labeling at the time of product distribution is shown in Table 2 [2].

Table 2 Labeling of cell therapy products at time of distribution [2]

Label element	Affixed (AF) Attached (AT) Accompanying (AC)
Unique numeric or alphanumeric identifier	AF
Proper name of product	AF
Product code (ISBT 128)	AF
Recipient name and/or identifier	AT
Identity and address of collection facility or donor registry	AC
Date and time collection ended and (if applicable) time zone	AC
Approximate volume	AF
Name and quantity of anticoagulant and other additives	AF
Recommended storage temperature range	AF
Donor identifier and (if applicable) name	AF
Biohazard and/or warning labels	AT
As applicable:	
"WARNING Advise Patient of Communicable Disease Risk" "NOT EVALUATED FOR INFECTIOUS SUBSTANCES" "WARNING Reactive Test Results for (name of disease agent or disease)"	AT AT AT
Identity and address of processing and distribution facilities	AC
Statement "Do Not Irradiate"	AF
Expiration date (if applicable)	AF
Expiration time (if applicable)	AF
ABO and Rh of donor (if applicable)	AC
RBC compatibility (if applicable)	AC
Statement indicating that leukoreduction filters shall not be used	AF
Statement "FOR AUTOLOGOUS USE ONLY" (if applicable)	AF
Date of distribution	AC

Adapted from FACT Standards 7th Edition

3 Shipment of Cryopreserved Products

Cryopreserved products have been frozen and stored in the vapor phase of liquid nitrogen at ≤−150 °C. These products are frozen using a cryoprotectant, such as DMSO media/solution mixture, or formulated using commercially available cell cryopreservation media, such as CryoStor® (Sigma-Aldrich), HypoThermosol® (BioLife Solutions), BloodStor® (BioLife Solutions), or in defined and serum-free cryopreservation media, such as mFReSR™/FreSR-S™, MesnCult™, or STEMdiff™ (STEMCELL Technologies), etc.

For cryopreserved products, the transport container should be suitable for the storage temperature range, which should be below −120 °C. This can be achieved using a dry shipper (Fig. 2), in which the liquid nitrogen is absorbed into the foam lining of the shipper. The unabsorbed liquid is poured out of the container prior to

Fig. 2 Liquid nitrogen dry shipper and external shipping container **The inner dry shipper is shown at the bottom of the figure. The temperature logger is the red box fixed to the lid. The inner container is placed into the outer plastic shipper shown at the top of the figure**

transport or shipment. Dry shippers are available in a number of sizes that are suitable for transportation of cryovials, e.g., Cryo Diffusion and Taylor Wharton (Fisher Scientific), or bags, e.g., MVE (Chart) or Taylor Wharton (Fisher Scientific). For short-term (~3 h) transport, the CryoPod™ system is available. It can hold a box of vials and some cassette sizes and is particularly suitable for hand-carrying frozen samples.

A temperature recording device should be used during shipment. These are usually battery operated and attached to the lid of the dry shipper. There are a number of models available, e.g., ELPRO from Libero G of Switzerland and ShipsLog3™ from Planer of the UK. These can be programmed to take readings at defined intervals and the data can be downloaded for presentation in graphic or tabular formats. If required, manipulation of the shipper can be documented using externally applied single-use tilt indicators, e.g., SpotSee (International). These detect whether the shipper has been tilted more than 80° during shipment.

All products should be considered potentially infectious and should be handled using universal precautions for handling of tissue and blood products.

3.1 Dry Shipper Qualification

Dry shippers should be validated to maintain their temperature for approximately 48 h beyond the longest anticipated shipment time. Validation is best performed under stress conditions, i.e., placing the charged shipper at high and low ambient temperatures during the recording period. This can be achieved by placing the shipper in a boiler room or cold storage refrigerator. When this is done, it is a good idea to record the external temperature as well as the internal temperature from the data logger. The validation interval varies between institutions. Some revalidate the shipper after return from each shipment, while others perform annual or semiannual validations [3].

The American Society for Testing and Materials (ASTM) [4] and the International Safe Transit Association (ISTA) [5] both provide standardized validation methods using a variety of simulated shipping hazards (Fig. 3). The simulated tests provide qualitative pass-fail criteria; thus, the end user should conduct their own transport and shipping evaluation with real transfer conditions to test the robustness and failure modes of the chosen transport, shipping procedures, sequence of conditions, and environments.

3.2 Packaging of Frozen Shipments

- Precautions must be taken when handling liquid nitrogen. The user must wear a splash-proof lab coat, cryogenic gloves, and a face shield. Closed-toed shoes must also be worn.
- The dry shipper should be charged with liquid nitrogen the night before the anticipated shipment [6]. This is done by weighing the empty shipper and then

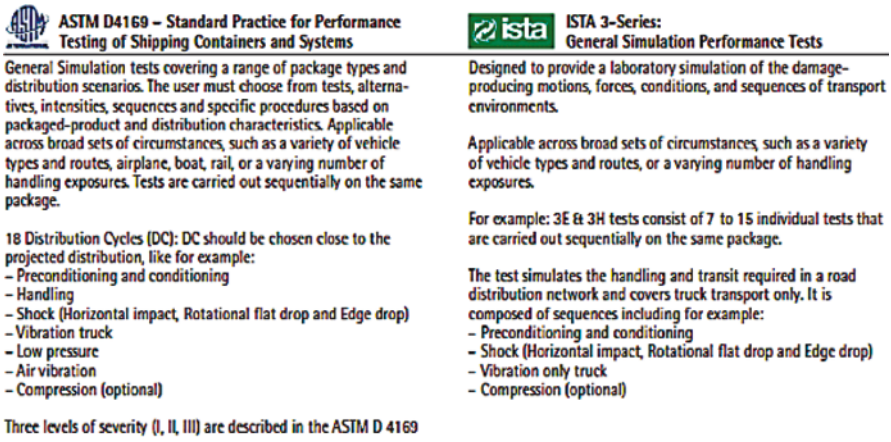


Fig. 3 ASTM and ISTA Standards. (From Association A3P)

filling it with liquid nitrogen to the bottom of the neck opening. This level should be maintained during charging. The shipper is then left to absorb the liquid nitrogen as determined by maintenance of a constant level of liquid nitrogen over time. The shipper can then be reweighed, the remaining liquid nitrogen is then decanted, and the shipper reweighed to check the amount of absorbed liquid nitrogen.

- Pre-cooled shipping boxes and/or canisters are then transferred to the charged shipper. If these are contained within a secondary container, e.g., vials within a secondary container, this should contain enough absorbent material to absorb the product should it thaw out.
- The temperature data logger should then be activated.
- Additional materials needed for both fresh and cryopreserved products are transparent outer pouches or large ziplock bags for transporting documents to be included within the shipment.

3.3 Documentation Requirements for Cryopreserved Shipment

In order to authorize a cell therapy dose for infusion, a cell therapy product should be ordered by a product or physician's order form that is approved by the principle investigator or attending clinician. This form is to be sent to the manufacturing site or product storage site to request the release of the cell therapy product for delivery.

The storage/manufacturing site will prepare the dose for transport or shipment. The quality unit (QU) responsible for the released product will check their inventory for the requested product in the freezer inventory. The QU will verify the product label matching the information on the product order form and arrange for transport or shipment to the designated receiving site.

Accompanying the therapeutic product should be a completed product transfer form which should have at minimum the relevant information shown in Table 1 [2] for different types of donors. If the shipped product is intended for distribution for administration at the receiving center, then the information shown in Table 2 [2] should also be made available to the receiving center. Also provided with the shipment should be:

- Batch or lot numbers
- Description of the cell product
- Container type in which the product is being shipped
- Number of samples in the shipment
- Certificate of analysis (where applicable)
- Sample storage conditions
- Instructions for administration
- A chain-of-custody form which documents each step of the shipping procedure, the personnel involved, and the times and dates on which transfer steps took place

It is also important to confirm with the receiving center that they have the ability to store the shipment on arrival, e.g., by providing the freezing cassette dimensions.

If the receiving center has little experience in handling the product that is being shipped, it is a good idea to offer to send a dummy practice product together with the instructions for preparation for administration.

3.4 Shipping Companies for Cryopreserved Products

If shipping is to be handled by the institution making the product available, the staff may have to undergo institutional training in shipping regulations, such as those of the International Air Transportation Association (IATA) [7–9], Occupational Safety and Health Administration (OSHA) [21 Code of Federal Regulations (CFR) Part 1910.1200] [10], and the US Federal Government, including the Department of Transportation [Title 49 CFR Parts 100–185] [11] and US Post Office. The shipping facility should have a detailed standard operating procedure that describes how products are to be prepared for shipment, labeled, packaged, shipped, and received. Training is an important component of a successful transport and shipment of the cell and gene therapy products. The staff responsible for transporting the material should have training for preparation of appropriate transport container, packing of the material and container, and specific transport or shipping requirements. In cGMP setting, a staff member should verify that the correct products are properly packaged for transportation and that the packaging is performed according to effective standard operating procedures and forms.

There are a number of companies that will transport dry shippers. These include Federal Express and most of the commercial express shipping organizations, some of which have specialized cold chain shipping services [12]. Courier companies, e.g., World Courier, will provide more specialized shipment procedures, e.g., preparation of paperwork, permits, etc., and are particularly useful for international shipments [13]. For institutions lacking dry shippers, a complete shipment system, including qualified shipping containers, is offered by Cryoport (located in the USA, the Netherlands, and Singapore), Fisher Clinical Services, and Cytiva (formally GE Life Sciences). Vendor qualification should be performed if an external shipping company is to be used.

3.5 Action upon Receipt of Shipments

Table 3 [2] shows the actions that should be taken upon the receipt of frozen cellular therapy products for distribution and administration. These include follow-up of the clinical status of the recipient in terms of engraftment, detection of infused cells, adverse reactions, etc. in addition to therapeutic effects.

Table 3 Actions performed on receipt of product

	Action
1	Use of written SOP for receipt of cellular product shipments
2	Confirmation of receipt to shipping facility
3	Verification of integrity of cellular product container
3	Visual examination of cellular product for microbial contamination
4	Confirmation of correct product labeling
5	Verification of correct shipping conditions (e.g., temperature log printout)
6	Review and verification of product specifications
7	Determination if product must be quarantined
8	Confirmation of receipt of all relevant documentation including donor eligibility information
9	Return of empty dry shipper to shipping facility
10	Notification of clinical use, engraftment times, etc. and any adverse reactions

To ensure consistent usage and results of the transport and shipping equipment, be sure a standard operating procedure is drafted for the operation and maintenance of the transport carrier and temperature logger. In addition, training should be provided by read and understanding (RUT) of the procedure, and additional on the job training (OJT) should be performed and documented. To ensure the integrity of the equipment, schedule calibration according to the SOP and file the documentation records with the associated equipment as well as entering it into the equipment logger.

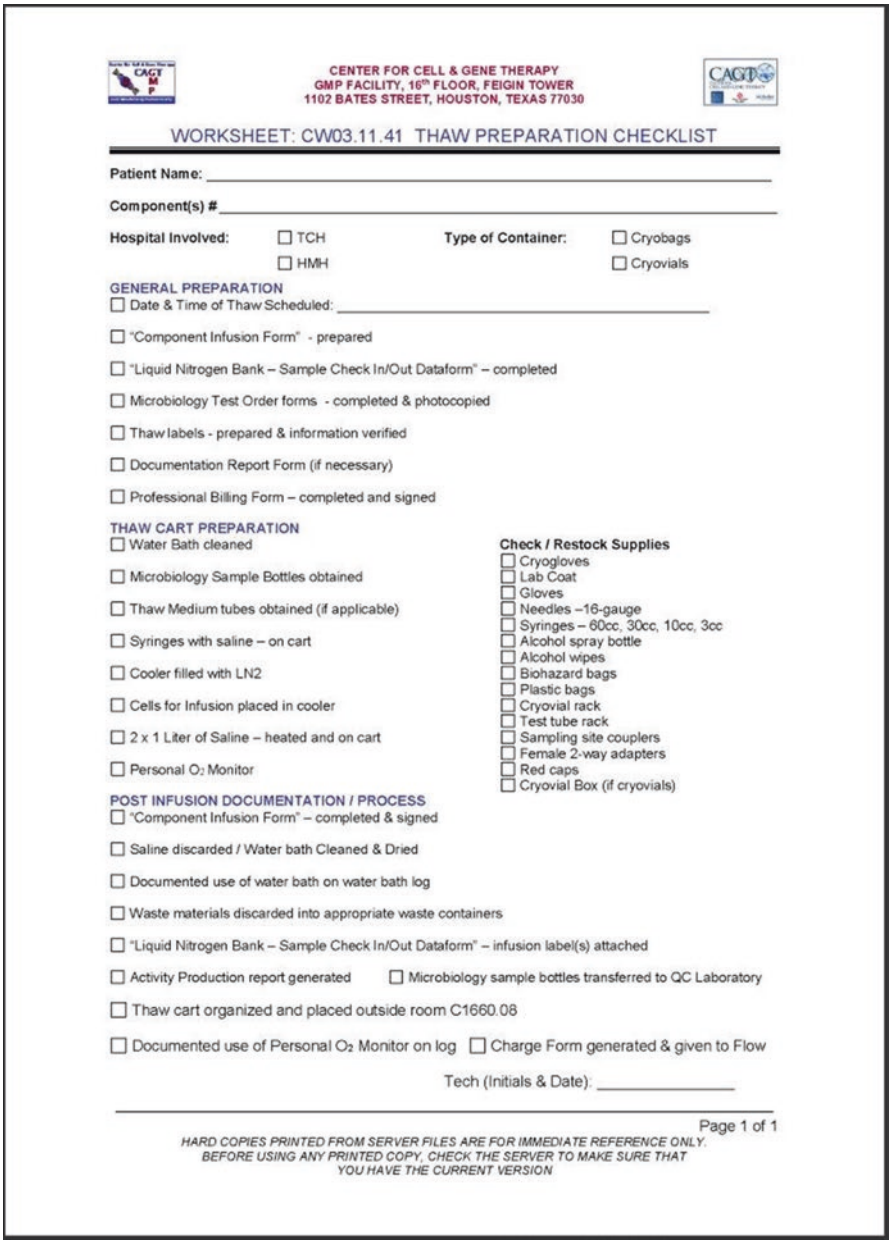
4 Transportation for Patient Administration

4.1 Cellular Therapy and Hematopoietic Products

Most cellular therapy products are transported frozen to the bedside, then thawed, and administered. The transportation can be accomplished using a small dry shipper or insulated container holding liquid nitrogen and fitted with a lid. This should be safely transported, preferably on a cart, by properly attired staff wearing personal oxygen monitors, e.g., BW Clip 2 Year O_2 Single Gas Detector BWC2-X, in case of spillage of liquid nitrogen in a confined space, such as an elevator.

Normally a checklist is used to confirm that all the equipment and supplies needed for administration are available on the thaw cart (Fig. 4). A calibrated water bath is included. This is brought to temperature before transportation and contains either sterile saline or water.

Upon arrival the identity of the product is reconfirmed before the bag containing the product is thawed. It may be placed in a plastic bag during immersion. After examination for bag integrity, the product is usually hung for administration. In the case of products in vials, these may be thawed in a water bath of electrical vial thawing device, e.g., the ThawSTAR® CFT2 from BioLife and the CellSeal® Automated Thawing System from Cook Regentec and Asymptote. Electrical



CENTER FOR CELL & GENE THERAPY
GMP FACILITY, 16th FLOOR, FEIGIN TOWER
1102 BATES STREET, HOUSTON, TEXAS 77030

WORKSHEET: CW03.11.41 THAW PREPARATION CHECKLIST

Patient Name: _____

Component(s) #_____

Hospital Involved: ☐ TCH ☐ HMH Type of Container: ☐ Cryobags ☐ Cryovials

GENERAL PREPARATION
☐ Date & Time of Thaw Scheduled: _____
☐ "Component Infusion Form" - prepared
☐ "Liquid Nitrogen Bank – Sample Check In/Out Dataform" – completed
☐ Microbiology Test Order forms - completed & photocopied
☐ Thaw labels - prepared & information verified
☐ Documentation Report Form (if necessary)
☐ Professional Billing Form – completed and signed

THAW CART PREPARATION
☐ Water Bath cleaned
☐ Microbiology Sample Bottles obtained
☐ Thaw Medium tubes obtained (if applicable)
☐ Syringes with saline – on cart
☐ Cooler filled with LN2
☐ Cells for Infusion placed in cooler
☐ 2 x 1 Liter of Saline – heated and on cart
☐ Personal O_2 Monitor

Check / Restock Supplies
☐ Cryogloves
☐ Lab Coat
☐ Gloves
☐ Needles –16-gauge
☐ Syringes – 60cc, 30cc, 10cc, 3cc
☐ Alcohol spray bottle
☐ Alcohol wipes
☐ Biohazard bags
☐ Plastic bags
☐ Cryovial rack
☐ Test tube rack
☐ Sampling site couplers
☐ Female 2-way adapters
☐ Red caps
☐ Cryovial Box (if cryovials)

POST INFUSION DOCUMENTATION / PROCESS
☐ "Component Infusion Form" – completed & signed
☐ Saline discarded / Water bath Cleaned & Dried
☐ Documented use of water bath on water bath log
☐ Waste materials discarded into appropriate waste containers
☐ "Liquid Nitrogen Bank – Sample Check In/Out Dataform" – infusion label(s) attached
☐ Activity Production report generated ☐ Microbiology sample bottles transferred to QC Laboratory
☐ Thaw cart organized and placed outside room C1660.08
☐ Documented use of Personal O_2 Monitor on log ☐ Charge Form generated & given to Flow

Tech (Initials & Date): _____

Page 1 of 1

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Fig. 4 Checklist for product thaw cart
Checklist for determining that the correct materials and equipment are placed onto the cart used to transport a cryopreserved product to the bedside for thawing and administration

thawing devices are also available for products in bags, e.g., VIA thaw™ dry automated thawing device from Cytiva and the ThawStar CB from BioLife.

Vial contents are then drawn into a labeled syringe or transferred to a small labeled transfer pack for administration.

If the product is to be manipulated before administration, e.g., washed, This is performed in the GMP facility and sterility samples (and potentially other release tests) are performed before administration. Sterility testing is usually performed by STAT Gram stain accompanied by conventional 14-day sterility testing. The results of the long-term test must be conveyed to the recipient's physician when they are available, and there must be an SOP in place to address actions to be taken in case of a positive result. In some cases, the product will be administered to the recipient within a sterile field. This requires preparation of product containers filled aseptically and with a sterile outer surface. After filling, these should be double bagged in sterile plastic bags for transportation for administration.

4.2 Administration of Viral Vectors

Viral vectors usually require thawing and adjustment of viral concentration before administration to patients. This should be done in the GMP facility. Once adjusted using a suitable buffer (preferable one with USP certification), the dilution is transferred to a labeled syringe or small transfer pack. These can be transported, once placed into a plastic bag, on ice in a cooler to the patient's bedside or site of administration. Alternatively, syringes can be packaged in cooling sleeves, e.g., T-Pak Porta-Sample Instant Ice Transporter from Cardinal Health. Vectors should be stability tested after thawing and transportation to determine the maximum time for which they can be held before administration.

5 Shipment of Viral Vectors

Viral vectors are usually stored frozen below −70 °C and must not be exposed to unnecessary temperature fluctuations. They may be contained in tubes or bags. Shipment on dry ice is usually acceptable; however, it has been reported that carbon dioxide gas can affect adenoviral vector stability [14]. It is, therefore, a good idea to pack the vector in a gas impermeable bag, e.g., Flexibles SealPAK 50424 from Ampac™, during transportation. This bag should also contain sufficient absorbent material to soak up the vector if it should thaw out. If smaller volumes of vector are to be shipped, it is advisable to place them into a sealed primary container (Fig. 5 the yellow plastic container holding absorbent material) which is then placed into a secondary box. This is then shipped in the outer shipping container holding the dry ice (Fig. 5 bottom panel). The particular shipping configuration must be validated before use, to ensure that temperature is maintained beyond the anticipated shipment

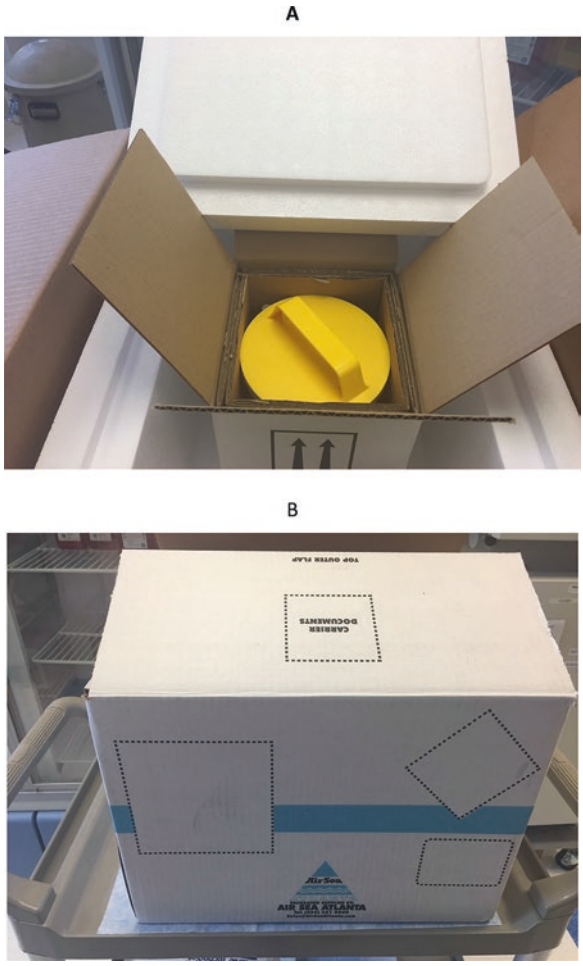

Fig. 5 Containers for viral vector shipment
Panel A shows the inner plastic container and box used to pack small volumes of viral vector. This is placed into the larger shipping container shown in Panel B, which is packed with dry ice. Alternatively, larger volumes of vector can be packed into a plastic bag containing absorbent materials and placed directly onto the dry ice in the shipping container. The outer container is a Thermal Control Unit from Air Sea Atlanta. The box is labeled with UN stickers: (i) UN 1845 indicating that the contents are human or animal materials that are being transported only for the purpose of diagnosis or investigation and (ii) UN1845 indicating that the shipment contains dry ice

time. In some cases, if a courier service is used, they will agree to top off the dry ice during prolonged shipments.

Countries may have regulations regarding the shipment of replication-competent and non-replication-competent viruses. If a shipping service is used, then this information can usually be obtained from the company. If a courier service is used, they will often collaborate on the completion of the relevant documentation.

6 Conclusions

The biological nature of cellular therapy products places them at particular risk from mishandling during transportation. Fortunately, many products can be cryopreserved and shipped relatively easily. Others, such as NK cells, are adversely affected by freezing and are better transported fresh. It is safe to say that whatever shipping conditions are chosen they must be carefully validated to ensure that they do not harm product safety or efficacy.

References

1. Lambert, J. L. (2020) State of the industry: The financial, clinical, and scientific landscape for cell and gene therapies. *Cell & Gene Therapy Insights, 6*(1), 47. https://www.insights.bio/immuno-oncology-insights/journal/article/11/state-of-the-industry-the-financial-clinical-and-scientific-landscape-for-cell-and-gene-therapies. Last accessed 24 Aug 2020.
2. Foundation for the Accreditation of Cellular Therapy. (2018). *FACT-JACIE standards for hematopoietic cellular therapy*. Seventh Edition, March 2018. http://www.factwebsite.org/ctstandards/. Last accessed 24 Aug 2020.
3. Food and Drug Administration. (2011). *Guidance for industry process validation: General principles and practices*. January 2011. R1. https://www.fda.gov/files/drugs/published/Process-Validation%2D%2DGeneral-Principles-and-Practices.pdf. Last accessed 24 Aug 2020.
4. ASTM (American Society for Testing Materials). (2014). *D4169: Standard practice for performance testing of shipping containers and systems*. Website: https://www.astm.org/. Last accessed 24 Aug 2020.
5. ISTA (International Safe Transit Association). *General simulation performances tests*. Website: https://ista.org/. Last accessed 24 Aug 2020
6. Charging a dry shipper training video. You Tube website: https://www.youtube.com/watch?v=6wNbk3gTJHA. Last accessed 24 Aug 2020.
7. International Air Transport Association Infectious Substances Shipping Guidelines (ISSG). IATA website: https://www.iata.org/en/publications/store/infectious-substances-shipping-guidelines/. Last accessed 24 Aug 2020.
8. International Air Transport Association Dangerous Goods. IATA website: https://www.iata.org/en/programs/cargo/dgr/. Last accessed 24 Aug 2020.
9. International Air Transport Association Pharma & Healthcare Products Handling. IATA website: https://www.iata.org/en/programs/cargo/pharma/. Last accessed 24 Aug 2020.
10. OSHA website: 21 CFR Part 1910,1200. https://www.osha.gov/laws-regs/regulations/standardnumber/1910/1910.1200. Last accessed 24 Aug 2020.
11. Title 49 CFR Parts 100-185. https://www.ecfr.gov/cgi-bin/text-idx?sid=585c275ee19254ba07625d8c92fe925f&c=ecfr&tpl=/ecfrbrowse/Title49/49cfrv2_02.tpl. Last accessed 24 Aug 2020.
12. Federal Express Cold-Chain Services. Fedex website: http://www.fedex.com/pt_english/shipping-services/industry-solutions/supplychain/coldchain.html. Last accessed 24 Aug 2020.
13. World Courier Temperature Control Solutions. World Courier website: https://www.worldcourier.com/expertise-temperature-control-solutions. Last accessed 24 Aug 2020.
14. Nyberg-Hoffman, C., & Aguilar-Cordova, E. (1999). Instability of adenoviral vectors during transport and its implications for clinical studies. *Nature Medicine, 5*(8), 955.

Regenerative Medicine: The Newest Cellular Therapy

Bita Nickkholgh, Darren Howard Martin Hickerson, Cynthia Wilkins, Julie Allickson, and John Jackson

1 Introduction

Regenerative medicine has the potential to change the way healthcare is delivered today with the recent approval of several cell therapies and the maturation of tissue-engineered organs making their way to the clinic. The concept of regenerative medicine combined with cell-based therapy and tissue engineering provides a promising approach for the treatment of serious diseases. In fact, many of the regenerated constructs are in the process or have been already approved for clinical trials. Examples of such constructs in increasing complexity include the 3-dimensional (3D) cartilage constructs with chondrocytes [1–4]; several flat structures for regeneration of skin; hollow organs such as tissue-engineered bladder, urethra, vagina, and trachea [5–10]; and solid organs such as bioengineered penile tissue [NCT03463239].

As discussed earlier in this book, a combination product is a therapeutic product that uses more than one component. Accordingly, many products in regenerative medicine field, specifically tissue engineering, are regulated as combination products as they are a combination of cells, biomaterials, and drugs (growth factors, cytokines, chemokines) to provide engineered tissue for restoring the function of compromised tissues [11, 12]. The success in creating functional engineered tissues lies in the integration of cells, biomaterials, and signaling systems, also known as the tissue engineering triad [13]. Final development and manufacturing of these bioengineered products that are intended for clinical trials need to be approved by the FDA.

In November 2017, the FDA announced a comprehensive policy framework for the development and oversight of regenerative medicine products, including novel

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A. P. Gee (ed.), *Cell Therapy*, https://doi.org/10.1007/978-3-030-75537-9_33

cellular therapies [14]. This risk-based framework includes a suite of guidance documents focusing on safety and effectiveness of these therapies and reiterates FDA's commitment to bring new effective therapies to patients as quickly and safely as possible.

In this chapter, we discuss different aspects of regenerative medicine products in relation to FDA regulations.

2 Tissue Engineering in Regenerative Medicine

In regenerative medicine, cells are used as cell derivatives (such as extracellular matrix), intact cells (such as muscle progenitor cells), or as part of engineered tissues. Based on the origin of cells, the settings for delivering final products have been discussed as autologous, allogeneic, or xenogeneic [15].

2.1 Autologous

In autologous regenerative medicine, a patient's own cells are used for development of the cell- based or regenerative engineered product. Biopsies are obtained and specific cell types isolated, expanded, and processed (possibly seeded onto a biomaterial scaffold to form a construct) according to clinical manufacturing specifications and finally delivered back to the patient. The quality, viability, and availability of autologous somatic or stem/progenitor cells greatly depend on the patient's age and health status. The manufacturing process should incorporate a rapid two-directional shipping model for biopsies and final products. The model design should include stability testing of the bio-product accounting for maximum transport time to ensure ideal viability of cells. An ideal model would be a semiautomated, closed system that employs single-use disposable equipment and allows for final product tracking in real time. Commercial production costs would be manageable by utilizing the same infrastructure repeatedly while still maintaining distinct, parallel product flows with near-zero chances of cross-contamination.

Many of the tissue-engineered organs prepared for clinical use can be categorized in this setting.

2.2 Allogeneic

In allogeneic regenerative medicine, a "universal donor" provides the cells used in engineering the regenerative engineered product. Mesenchymal stem cells (MSC) or induced pluripotent stem cells (iPS) of young and healthy donors, for example, have been suggested as a valuable tool for tissue repair and cell therapeutic

applications [16]. The universal donor hypothesis of MSC was based on the immune-privileged theory of these cells. However, it is now well known that MSCs are not immune-privileged, but rather immune evasive which means allogenic MSCs have a lower immunogenic potential compared to other allogeneic cell types. It is critical to consider that although implanted MSCs may not express MHC class II, this will likely always be activated and/or expressed in vivo at sites of inflammation [17]. The immunomodulatory effects of these cells, particularly the effect on promoting innate immunity and suppression of T cells and B cells, support their consideration as universal donor [18]. Careful HLA matching and even some degree of immunosuppression may be suggested to avoid serious side effects such as graft-versus-host disease. Recently, tissue sources, such as amniotic fluid, umbilical cord blood, amniotic membranes, or placenta, have also attracted increased attention as they are readily accessible which is amenable to scaling up in manufacturing. Strategies for scaling up regenerative medicine therapies can borrow extensively from similar strategies used in biological, medical device and cell/tissue banking industries that support the production of biopharmaceutical therapeutics such as vaccines, viruses, antibodies, and other recombinant proteins [16].

The use of mesenchymal stem cells to treat acute respiratory distress syndrome (ARDS) including COVID-19-related ones is a good example of allogeneic regenerative medicine [19–21].

2.3 Xenogeneic

Xenogeneic approaches are similar to allogeneic, but the cells or tissue-engineered products are (at least partially) derived from a nonhuman source. These are cells or animal-based biomaterials that are manufactured, sometimes by genetic engineering, into a clinical cell therapy or tissue-engineered products. If human body fluids, cells, tissues, or organs have ex vivo contact with the living, xenogeneic materials, the final product will be considered as xenogeneic. As the source of the product is nonhuman, additional tests are needed to ensure product safety.

A hypothetical application of xenogeneic cells in clinic is the transplantation of xenogeneic tissue-specific cells into the tissues afflicted with tumors. This may induce cellular and humoral immune response and rejection of xenogeneic cells, which concomitantly revive immune system against tumor cells [22].

Porcine extracellular matrix, for example, has been used in the form of a membrane or as a powder in applications such as repairing membrane defects [23, 24] or accelerating wound healing [25].

Regardless of the source of the cells, target areas for consideration in the tissue engineering process include harvesting the biological material, biological engineering of the final product, stability for packaging and storing the final product, and ultimately a shipping system for transport of the final product to the clinical facility. Maintaining the quality, stability, safety, and efficacy of the regenerated product is critical.

3 Biomaterials

The biomaterials used in tissue or organ regeneration are usually classified as acellular tissue matrix or manufactured scaffolds. A biomaterial alone can sometimes provide cues for regeneration or graft/implant integration. However, incorporation of growth factors into biomaterials could improve the results drastically because growth factors are known to promote healing or regeneration [26]. An ideal biomaterial should provide efficient host tissue integration, with minimal foreign body reaction and robust functional repair of the affected site.

3.1 Acellular Tissue Matrix

Acellular tissue matrix is an allograft/xenograft tissue that is chemically processed to remove all cells while preserving the remaining bioactive matrix. It works by providing a bioactive matrix consisting of collagens, elastin, blood vessel channels, and bioactive proteins that support revascularization, cell repopulation, and tissue remodeling. The rationale behind using native acellular tissue matrix is taking advantage of the extracellular matrix (ECM) proteins that are site-specific and provide protein "footprints" of previous resident cells [27] because ECM proteins are among the most conserved proteins. Decellularization also removes immunogenic cells and molecules while theoretically retaining structure as well as the mechanical properties and material composition of the native extracellular matrix [26]. Decellularized tissues either are recellularized by autologous cells or used without the recellularization step. The latter was used to repair large muscle defects in a human patient [28].

Acellular tissue matrix is referred to as a native ECM scaffold, a biocompatible scaffold that can also be generated by exposing cadaveric tissues to decellularization. Decellularization is the removal of cells without compositional, biological, or mechanical disruption of the ECM via physical (e.g., freeze/thaw cycles), enzymatic (e.g., trypsin), and chemical protocols (e.g., SDS or Triton X) [27]. The removal of xenogeneic or allogeneic cellular contents via decellularization could theoretically produce an essentially minimally immunogenic scaffold with a native intact structure for new tissue regeneration. A wide diversity of decellularized native ECM products (both allogeneic and xenogeneic) are FDA-approved and clinically used [27]. Because it is fully biocompatible, it can be implanted safely, and it induces the formation of host-derived connective tissue while providing mechanical support and tissue augmentation [27]. Decellularized human valves and vascular grafts are good examples of allograft matrix, used for treating congenital heart defects in both pediatric and adult patients in different clinical trials [29, 30]. FDA-approved decellularized bone grafts, used in various orthopedic surgeries, are examples of allogeneic acellular matrix derived from cadaveric tissue [27]; and FDA-approved small intestine submucosa (SIS) scaffold as a graft for different clinical repairs is an example of xenogeneic-derived acellular matrix [31].

The detergents and procedures used to strip cells and other immunogenic components from donor organs and techniques to re-cellularize stripped tissue before implantation are actively being optimized [28]. Because the starting raw materials can be from both human and animal sources, issues related to contamination and disease transmission are critical (the FDA has very strict rules and procedures for reporting the origin and health of the sources). Once living cellular material is completely removed, these naturally derived scaffolds are then processed downstream like any other medical device product. Issues related to quality control, release testing, packaging, shipping, and logistics are fairly well-determined and established [16].

3.2 Manufactured Scaffolds

Manufactured scaffolds may be fabricated from synthetic or natural material. These scaffolds possess at least some aspects of the material properties and structure of target tissue. Naturally derived materials, such as purified extracellular matrix components or algae-derived alginate, or synthetic polymers, such as poly-lactide-coglycolide (PLGA) and polyethylene glycol (PEG), can be used in fabrication of manufactured scaffolds. Hydrogels are composed largely of water and are often used to form scaffolds due to their compositional similarity to tissue. These polymers can be engineered to be biodegradable, enabling gradual replacement of the scaffold by the cells seeded in the graft as well as by host cells [28]. Combining materials with different properties can enhance scaffold performance, as was the case of composite PLGA-coated polyglycolide (PGA) scaffolds that were seeded with cells and served as bladder replacements for human patients [8].

The advantage of synthetic scaffold materials lies in their controlled, well-characterized composition, degradation, and physical properties [27]. Their disadvantage lies in the relative paucity of organ-specific structure and cell type-specific niches [27].

If scaffolds are used in combination with the therapeutic cells, the scaffold's structural architecture and mechanical characteristics will be driven by clinical specifications for the patient's defect and should be addressed specifically for FDA approval.

4 Biomaterials Considerations

Biomaterials that are intended for clinical applications must meet diverse and stringent requirements and should be tested for cytotoxicity, sensitization, hemocompatibility, pyrogenicity, implantation, genotoxicity, carcinogenicity, reproductive and developmental toxicity, and biodegradation [32]. To be approved for a clinical application, biomaterial-based products must also meet the highest requirements for

physical and mechanical characteristics appropriate to their intended use and desired time to biodegradation in vivo. Depending on the application, the main characteristic of a biomaterial-based product can be mechanical strength/elasticity, defined porosity, or surface roughness/chemistry, as required [33].

Many biomaterials can be tested for toxicity; however, the specific design for acute or subchronic toxicity testing should be guided by the regulatory requirements and the proposed end use of the product. Safety testing for biological products, if the biomaterial product is combined with biological molecule and/or cells, is to be conducted as required by FDA regulations [21 CFR parts 600] [34]. The biomaterial-based product will need thorough characterization prior to clinical testing. Physical, chemical, and mechanical characteristics—as well as biological interactions, effects, and product manufacturing—are among the requirements detailed under the FDA Medical Device Regulations [21 CFR 820-Quality System Regulation] [32].

Physical characterization can include examination of the final product (after sterilization) using light microscopy, scanning electron microscopy, atomic force microscopy, and testing for permeation of aqueous fluids. Chemical characterization of the surface of biomaterials can be done using electron spectroscopy for chemical analysis, static secondary ion mass spectrometry, contact angle measurements, infrared surface studies, and infrared spectroscopy and nuclear magnetic resonance for bulk analysis of the biomaterial. Mechanical characterization would include measurements of stress–strain ratios, strain to failure, and flex fatigue testing over time, while thermal properties (including viscoelastic properties) of the final product can be determined using differential scanning calorimetry and thermogravimetric analysis. One of the challenges regarding physical characterization of biomaterial-based products is the match between the mechanical properties of the product (device) and the target tissue in hard tissues such as bone or cartilage, in elastic tissues such as skin, or in soft tissues, such as the brain or liver. The type of the target environment (static or flexing) would also be another consideration. From a tissue interaction perspective, cell–material interactions, biodegradation of the biomaterial (applicable in the case of biodegradable polymers), and controlled release of bioactive molecules would be prominent considerations [35–38]. With respect to biodegradable polymers, attention should be given to the decay of structure and mechanical properties of scaffolds during degradation and the sustaining of strength and toughness of the product through its intended lifecycle in vivo [33].

For medical devices intended for human use, the biocompatibility testing of biomaterials used in single or multicomponent devices will depend on the nature of the end-use application. The medical device development process is multidisciplinary and involves multiple steps and processes, from the choice of a candidate material through characterization of the finished product. Therefore, it is recommended that biocompatibility testing takes place at several logical points in the development process. In general, the evaluation process involves initial screening of raw materials, biocompatibility testing of all device components, safety and efficacy testing of the final product, and testing during product release, followed by post-marketing/audit testing.

Any sterilization of a medical device or biomaterial requires validation of the sterilization method appropriate to the method, materials, and intended duration of implant. For example, ethylene oxide sterilization of an implanted device will require both testing for ethylene oxide residuals to below specified values and challenge testing to ensure the sterilization method is sufficient to remove contaminating organisms. A permanent implant requires lower residuals than a temporarily implanted device. Furthermore, additional testing will be required to ensure the sterilization method has no impact on functionality or integrity of the biomaterial or device. All additional biocompatibility testing must be performed on the fully processed and sterilized final biomaterial.

Safety and efficacy (potency) testing for a biomaterial-based device includes testing physical and biological aspects with reference to the entire device, duration of use, and target tissue interactions [39]. Depending on the nature of the device, its site of use in the body (nature of body contact), and duration of contact, the FDA recommends a number of tests be conducted that measure the short-term and long-term biological effects of biomaterial-based devices, including cytotoxicity, sensitization, irritation or intracutaneous reactivity, systemic toxicity (acute), subchronic toxicity, genotoxicity, implantation, and hemocompatibility. Additionally, supplemental evaluation testing needs to be done when initial evaluation tests are not conclusive. FDA guidance "Use of International Standard ISO-10993, 'Biological Evaluation of Medical Devices – Part 1: Evaluation and Testing" addresses the test selection, general and specific testing considerations for the required tests, the use of animal safety studies instead of some biocompatibility tests, assessment of the known chemical toxicity entities, and finally, content of the biocompatibility report [32].

The workflow for regenerating tissues includes careful selection of the cell source and biomaterials based on the intended goal of treatment and the target organ complexity.

4.1 Level of Complexity in Regenerative Medicine Tissue Engineering

The goal of tissue engineering is to assemble functional constructs that restore, maintain, or improve damaged tissues or whole organs. The level of complexity depends directly on the shape, size, and functional complexity of the organ and the clinical purpose of the regenerated product.

4.2 Extracellular Matrix: External Applications

Extracellular matrix (ECM) is a primary factor in the process of forming a new structural network and tissue. Materials such as tissue base gels, ECM powders, and tissue sheets, which resemble the functional aspects of the ECM (at least partly) or

augment its function, have therefore been targeted for regenerative medicine purposes. Preparation generally requires tissue isolation, decellularization, and lyophilization or cryopreservation. Applications can be as simple as applying coverage (bandage, ointment, or powder) or as complex as a dimensional tissue replacement.

4.3 Flat Organs

Although regeneration of flat organs appears less complicated compared to more dimensional organs, the complexity of manufacturing an organ structure still exists. Generally, more than one cell type is required to build various layers of tissue upon the scaffold structure. Skin, muscle, and articular cartilage are some examples of flat organs generated for the purpose of clinical use [40, 41].

4.4 Hollow Organs

Cylindrical and spherical hollow structures are more complex than flat tissue constructs. Depending upon the design, custom biomaterial scaffolds may be required to provide the appropriate shape and platform to build the tissue-engineered construct. Specialized bioreactors are often necessary in order to provide an adequate culture environment to accommodate construct maturation. The overall process usually involves expansion of two or more cell types, preparation of the scaffold and cells for cell seeding, and construct maturation, which may occur in multiple phases depending on the number and type of cells required. Spherical hollow organs are generally larger than tubular hollow organs, have thicker walls and a greater functional load that requires development of more advanced signal processing within the organ following implantation, and need for deep vascularization. Nutrient exchange and respiration can present a greater challenge for standardized manufacturing at this level. Tissue-engineered bladder is an example of a spherical organ; the urethra, vagina, and trachea are examples of cylindrical hollow organs [5–8].

4.5 Solid Organs

The structural and functional variety and complexity of the solid organs, such as the kidney, liver, and lung, make their regeneration very challenging. Although the functionality, vascularization, and regulatory signaling of these organs are very complicated, foundational methods that apply to manufacturing these organs are being developed today. Bioengineered penile tissue [42], lungs [43], and bio-artificial endocrine pancreas are examples of such sophisticated organs [44]. Figure 1 shows an example of various steps in production of tissue-engineered (solid) organ.

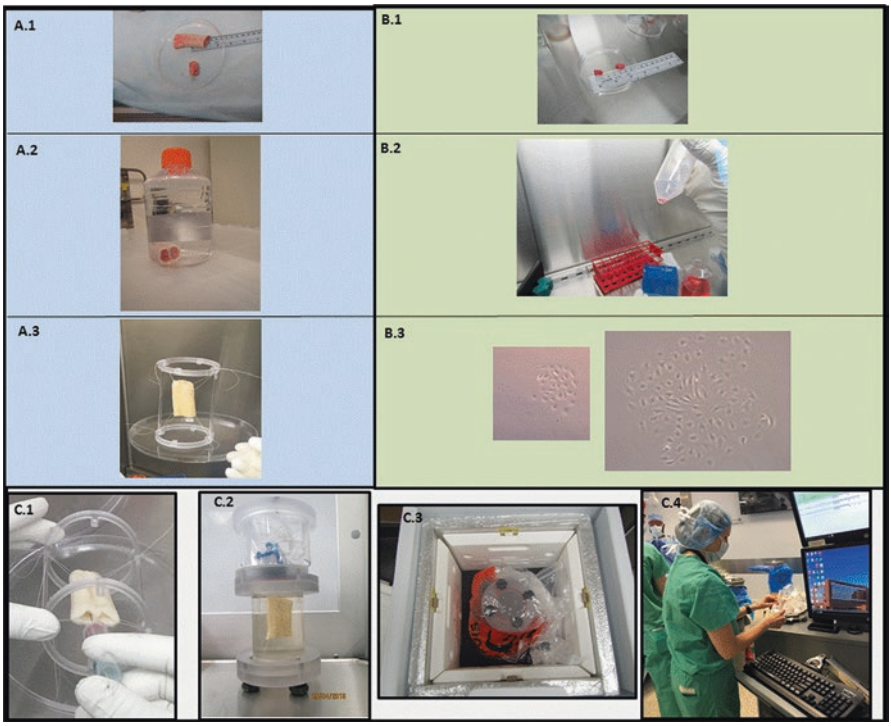

Fig. 1 Steps in the process of generating a tissue-engineered organ in regenerative medicine
The example shown is production of a portion of tissue-engineered corpus cavernosum for penile damage repair
A1–3: Allograft organ production from cadaveric organ: *A.1, allograft biomaterial organ procurement for scaffold production and isolation of tissue; A.2, decellularization of scaffold tissue; A.3, final preparation and lyophilization of scaffold*
B 1–3: Autologous patient cell isolation and expansion: *B.1, patient biopsy procurement and processing; B.2, isolation and selection of autologous cells; B.3, expansion of two cell types*
C1–4: Final construct production for implant: *C.1, seeding scaffold with autologous cells to form construct; C.2, maturation of construct in bioreactor; C.3, temperature-controlled packaging and transport of final product to clinical facility; C.4, delivery to operating room for implant*

5 Key Problems in Regenerative Medicine Therapies

5.1 Vascularity in Tissue-Engineered Structures

In order to contribute functionally and structurally, implanted grafts need to properly integrate into the body. For cell-based implants, integration with the host vasculature is of primary importance for success [26]. Vascularization of engineered tissues requires the body's own angiogenic response, which may be exploited by introducing angiogenic growth factors. A variety of growth factors have been implicated in angiogenesis, including vascular endothelial growth factor (VEGF),

angiopoietin (Ang), platelet-derived growth factor (PDGF), and basic fibroblast growth factor (bFGF) [26]. Application of growth factors may be ineffectual, however, without a proper delivery modality, because of a short half-life in vivo and potential toxicity and systemic effects of bolus delivery. Another approach for promoting graft vascularization at the target site is to prevascularize the graft or target site before implantation. Endothelial cells and their progenitors can self-organize into vascular networks when transplanted on an appropriate scaffold [26]. Combining endothelial cells with tissue-specific cells on a scaffold before transplantation can yield tissues that are both better vascularized and possess tissue-specific function [45]. Taking advantage of decellularized tissues which already have the tracks of advanced tissue vascularization is another promising approach. Despite many attempts to optimize vascularity in tissue-engineered organs or structures and recapitulate the intense architecture of solid organs, vascularization of a regenerated tissue (especially in solid organs) is still considered one of the key hurdles in the field.

Innervation of the engineered tissue by the host will also be required for proper function and integration of many tissues and is particularly important in tissues where motor control, as in muscle tissue, or sensation, as in the epidermis, provides a key function. Innervation of engineered tissues may be induced by growth factors, as has been shown in the induction of nerve growth [26]. Innervation of a regenerated tissue is even less developed compared to vascularization and needs more research to be understood and developed.

5.2 3D Bioprinting

Bioprinting includes the design, prototyping, and fabrication of three-dimensional (3D) anatomical structures (e.g., organs, skin, cartilage, bone) that can be used in therapeutic approaches. While cell placement within manually seeded scaffolds is generally poorly controlled, 3D bioprinting can create structures that combine high-resolution control over material and cell placement within engineered constructs [46]. Two of the most commonly used bioprinting strategies are inkjet and microextrusion [47]. Inkjet bioprinting uses pressure pulses, created by brief electrical heating or acoustic waves, to create droplets of liquid or hydrogel that contain cells (often called bio-ink) in the nozzle [48]. Microextrusion bioprinting dispenses a continuous stream of bio-ink or scaffold material onto a stage [49]. Both are being actively used to fabricate a wide range of tissues. For example, a hybrid inkjet bioprinting and electrospinning system has been used to engineer cartilage by alternating layer-by-layer depositions of electrospun polycaprolactone fibers and chondrocytes suspended in a fibrin–collagen matrix. Cells deposited this way were found to produce collagen II and glycosaminoglycans after implantation [50]. Microextrusion printing has also been used to fabricate aortic valve replacements using cells embedded in an alginate/gelatin hydrogel mixture. Two cell types, smooth muscle cells and interstitial cells, were printed into two separate regions,

comprising the valve root and leaflets, respectively [26]. One of the key hurdles in 3D printing is the idea of using a universal bio-ink for manufacturing bioengineered tissues [40]. Developing a 3D bioprinter that could be housed in a patient facility setting would enable real-time biofabrication of a product that could be brought into the surgical suite [16]. Although 3D bioprinting has not been used for regeneration of tissue used in clinical trials, there are hopes that in the near future, 3D bioprinters will be used for manufacturing tissue and structures for implant, potentially reducing or replacing the need for real-time cadaveric organ donations [40].

5.3 Undeveloped Quality Control Metrics and Lack of Standards

Regenerative medicine is relatively new; consequently, new standards are being developed gradually by the FDA and other organizations. There are challenges for process standardization (including definition of the criteria to measure in-process product characteristics through standardized assays) and scale-up and scale-out cell expansion using bioreactors [16]. A key component of standardization is the ability to measure key product and process quality attributes in a reliable and reproducible manner. The importance of the process metrics is highlighted by the fact that the process can change the product. Identifying measures for clinical effectiveness and safety are needed and would be achieved through assays of several types targeted for the mechanism of action for a particular product [16]. There are many challenges associated with clinical product quality control, and these challenges increase exponentially with the complexity of the clinical product. For reproducibility and scalability of process, assays must be fully understood.

5.3.1 Nondestructive Quality Testing

Quality control in regenerative medicine clinical manufacturing often relies on sacrificial constructs that are processed in parallel with clinical products. These materials are evaluated as a surrogate for the product that is intended for therapeutic application. The use of these sacrificial constructs diverts resources from production of the therapeutic product and may not accurately reflect the quality of the product which they are meant to represent. Advanced strategies for in-process quality assessments are needed. One promising solution currently under development is an array of biosensors that continuously monitor protein biomarkers in the media. Current biosensors are sensitive to the femtomolar range and offer real-time data regarding function and viability of the monitored tissue construct. Many cells secrete specific protein biomarkers indicative of functionality. Additionally, some cell types contain discriminate proteins within the cytoplasm that are released into the media upon cell death. These observations provide an opportunity to monitor cellular behavior

within a multicellular construct by nondestructive methods. Development of standardized and multiplex biosensors for specific cells and tissues would provide a powerful tool in automated biofabrication. Biosensors for microbial pathogens would also provide a means to monitor and ensure product sterility in real time. In vivo imaging represents another avenue for powerful in-process quality control. These technologies could be based on fluorescence, refraction index beam scatter, or any number of additional imaging techniques. Nondestructive gross visual inspection of clinical products during manufacturing could provide critical quality control data. Improvements in quality control in clinical manufacturing would assure product consistency, save money by identifying deficient products early in the production process, and assure product safety in terms of sterility [12].

5.3.2 Potency Testing

Potency testing represents one of the more abstract and difficult challenges of regenerative medicine. In small molecule pharmaceuticals, potency can usually be determined as a chemical function that the drug performs under experimental conditions. This potency can be tested on a large production run of pharmaceuticals that can be used to treat thousands to millions of patients. The potency of a (hypothetical) tissue-engineered kidney is that it makes urine at a sufficient rate to remove toxins from the blood and maintain chemical homeostasis in the patient. Just as a donor organ for transplant, this is a difficult functional potency to measure in a test tube prior to implant, and it needs to be performed on each organ generated, not on a batch that represents hundreds to thousands or more. At present, most regenerative medicine potency measurements are made as a function of the number and characterization of cells present in a cell therapy or the size and subcomponent characterization of a tissue-engineered organ. Sometimes this can be performed by testing a surrogate construct as described above. Where sacrificial construct testing is not possible, the cellular components can be tested before seeding, and thus the potency assay must rely on prior qualification of the method through destructive testing during process development and translation. Future advances and development, as well as additional regulatory guidance, will improve the understanding and approaches to potency testing as the field of regenerative medicine matures.

5.4 Scaling Up

Tissue-engineered products face a particular challenge for scale-up, as currently most of them fall into the category of personalized medicine. High cost and insufficient automation are two of the most important challenges in scaling up a regenerative medicine product. The product can take weeks or months to produce, requires hundreds of work hours, and necessitates the engagement of customized and often costly logistical operations to reach the patient. For scaling up, it is important to identify

processes that can support production for multiple products or patients at once. Subsequently, automation of as many parts of the process as possible would be an advantage for scaling up [40]. Resources and resource classifications, a delivery network to move the product from resource to resource, and a transaction management system that will track products throughout the network (providing real-time information on the products in the supply chain) are necessary for efficient scale-up [16].

6 Future Promises in Regenerative Medicine

Although regenerative medicine from the innovation, achievement, and regulatory aspects is in a promising path, there are five important requirements that need to be overcome for successful results in the field:

- The need for common constituent components that will be part of many regenerative medicine products and reproducible to well-defined quality standards.
- The need to better define product standardization and characterization so that quality assessments can be developed and product quality can be ensured with respect to both the product and the production processes used for the product.
- The need to develop efficient scaled-up or scaled-out manufacturing processes and systems prior to FDA approval.
- The need to define and develop appropriate supply chain and logistics models so that gaps between research and product translation can be realized through well-thought-out product development and well-engineered production systems.
- The need to develop flexible modular manufacturing systems for biologics [16].

One of the common constituents mentioned as a candidate for high-volume production is induced pluripotent stem cells (iPSCs). Creating common resources such as setting up standardized, well-characterized, and well-defined research and clinical-grade iPSC lines that both academia and industry could use with little variability between production batches would be a major step forward. Identifying key "standards" for both products and processes is critical to assuring reliable quality. Defining critical-to-quality (CTQ) attributes and placing acceptable tolerance limits on products is a requirement for any manufactured product. The successful implementation of the road map illustrated in this perspective will enable the field to develop cost-effective manufacturing processes that permit the successful commercialization of these next-generation medical products [16].

7 FDA and Regenerative Medicine

The regulatory pathway for a specific tissue-engineered product may not always be straightforward and based on the nature of the product and its utilization; determination of the final categorization may require early communication with the FDA. Early

interaction with the FDA and other agencies on regulatory pathways for regenerative medicine and the benefits of engaging industry can help to accelerate clinical translation. Recently, the FDA developed guidance documents to expedite the process for regenerative medicine therapies for serious conditions [51]. INTERACT, RMAT, and CATT are examples of these programs, discussed briefly and followed by additional regulations and guidance.

7.1 Initial Targeted Engagement for Regulatory Advice on CBER Products (INTERACT) Program

The FDA Center for Biologics Evaluation and Research (CBER) has implemented the INTERACT meeting program to help accelerate the development and approval of innovative medical products. The program has been designed to foster timely engagement with CBER on issues critical to early product development. It's also aimed at helping innovators meet the FDA's science-based requirements more effectively. It replaced the existing CBER pre-pre-Investigational New Drug (PPIND) meeting process for all products. The benefit of these meetings is to allow sponsors that are not yet ready for a pre-IND meeting to receive feedback from CBER. INTERACT meetings will enable sponsors to engage with the FDA early in the development process and obtain advice on a wide range of development-related topics. INTERACT meetings can be used to clarify CBER's expectations regarding product development programs and to help facilitate more efficient product development [52].

Through an INTERACT meeting, sponsors can obtain initial, nonbinding advice from FDA regarding chemistry, manufacturing and controls, pharmacology/toxicology, preclinical designs, and/or clinical aspects of the development program. This informal meeting can assist sponsors conducting early product characterization and preclinical proof-of-concept studies; initiate discussion for new delivery devices; inform sponsors about overall early-phase clinical trial design elements; and identify critical issues or deficiencies for sponsors to address in the development of innovative product [52].

If there are specific questions on the adequacy of the selected animal models, study design (e.g., endpoints, dose levels, route of administration, dosing regimen), and acceptability of innovative preclinical testing strategies, products, and/or delivery modalities, an INTERACT meeting would be the path to choose early on. The program provides advice on modification of a preclinical program or study design, as applicable, to ensure judicious use of animals.

7.2 Regenerative Medicine Advanced Therapy (RMAT) Program

A regenerative medicine therapy can be designated as a regenerative advanced therapy if it meets certain criteria: the regenerative medicine therapy, which is defined as a cell therapy; therapeutic tissue engineering product; human cell and tissue product; or any combination product using such therapies or products (except for those regulated solely under Section 361 of the Public Health Service Act and part 1271 of Title 21, Code of Federal Regulations) should intend to treat, modify, reverse, or cure a serious or life-threatening disease or condition. It is very important that the preliminary clinical evidence indicates that the therapy has the potential to address unmet medical needs for such disease or condition.

RMAT path has all benefits of the fast track and breakthrough therapy programs, including early interaction with the FDA. Regarding the preliminary clinical evidence to demonstrate the potential of a regenerative medicine therapy to address unmet medical needs, the FDA generally expects that such evidence would be obtained from clinical investigations specifically conducted to assess the effects of the therapy on a serious condition. Such clinical investigations, particularly at the initial stages of product development, may not always be prospective clinical trials with a concurrent control. In some cases, clinical evidence obtained from clinical investigations with appropriately chosen historical controls may provide sufficient preliminary clinical evidence of the potential to address an unmet medical need. In other cases, preliminary clinical evidence could come from well-designed retrospective studies or clinical case series that provide data systematically collected by treating physicians. Such clinical evidence may be from studies conducted outside of the United States. All the details about RMAT path has been documented in Section III C of the recent guidance entitled "Expedited Programs for Regenerative Medicine Therapies for Serious Conditions" [51].

It is important to know that the request for RMAT designation must be made either concurrently with submission of an IND or as an amendment to an existing IND.

7.3 CBER Advanced Technologies Team (CATT) Program

CATT is a new mechanism to address regulatory challenges associated with novel advanced manufacturing technologies. CATT intends to facilitate the development of novel complex products regulated by CBER [53].

As mentioned earlier, regenerative medicine is a relatively young field in introducing highly complex products in clinical scale with many challenges in advanced manufacturing, testing, and quality control platforms. As there are limited

experience in the field, CATT particularly could be beneficial in providing appropriate guidance through discussion and bidirectional education.

This program has provided opportunities for innovators to access early interactions with CBER prior to filing a regulatory submission. When requesting a meeting from CATT, project description should include a brief explanation about the novelty and uniqueness of the submitted technology/product and with a specific description of the impact of the technology/product in terms of improved biological product manufacturing, characterization, quality, safety, or efficacy. The request should be concluded by a summary of the manufacturing/developmental plan and any regulatory or technical questions or challenges for implementation. Inquiry requests submitted to CATT should focus on novel technologies that can have a significant impact on product development, manufacturing process, control strategies, and even regulatory implications [53].

7.4 Other FDA Guidance Documents

In the United States, the Codes of Federal Regulation (CFRs) are the laws to follow for regulatory compliance. To help apply these in tissue engineering and regenerative medicine products, the FDA offers various nonbinding guidance documents. Where compliance requires adherence to standards or specifications set by other organizations such as the International Organization for Standardization (ISO), United States Pharmacopeia (USP), the Public Health Service (PHS), and the National Institute of Standards and Technology (NIST), those documents will generally cite those sources. Specifically, which CFRs and guidance documents apply depends on the categorization and phase of clinical trial or licensure. Below are representative CFRs and guidance documents that may apply in tissue engineering and regenerative medicine. A representation of these regulations is summarized in Table 1.

7.5 (PHS) Act 351

Section 351 of the *Public Health Service (PHS)* Act defines a biological product as a "virus, therapeutic serum, toxin, antitoxin, vaccine, blood, blood component or derivative, allergenic product, or analogous product, etc. applicable to the prevention, treatment, or cure of a disease or condition of human beings." FDA regulations and policies have established that biological products include blood-derived products, vaccines, in vivo diagnostic allergenic products, immunoglobulin products, products containing cells or microorganisms, and most protein products. Biological products subject to the *PHS Act* also meet the definition of drugs under the *Federal Food, Drug, and Cosmetic Act (FDC Act)* [54]. As regenerative medicine products

Table 1 Representative US regulation governing GMPs, GTPs, or related tissue processing requirement

Regulation No.	Description
21 CFR 312	Investigational new drug application
21 CFR 812	Investigational device exemptions
9 CFR 113.53	Requirements for ingredients of animal origin used for production of biologics
21 CFR 4	Current good manufacturing practice (CGMP) requirements applicable to combination products
21 CFR 11	Electronic records; electronic signatures
21 CFR 210	Current good manufacturing practice in manufacturing, processing, packing, or holding of drugs; general
21 CFR 211	Current good manufacturing practice for finished pharmaceuticals
21 CFR parts 600 through 680	Other applicable regulations for biological products
21 CFR 820	Quality system regulation
21 CFR Part 1271	Human cell, tissue, and cellular and tissue-based products (HCT/Ps)
Section 351 of the Public Health Service (PHS) Act (42 U.S.C. 262)	Drugs, devices, and/or biological products requiring clinical trials
Section 361 of the Public Health Service (PHS) Act (42 U.S.C. 264)	Human cells, tissues, and cellular and tissue-based products (HCT/Ps) meeting certain minimally manipulated and homologous use criteria

are combined products that may include or utilize any of the mentioned biological products, they may need to follow the regulations under Act 351 when appropriate. It is important to mention that hormones such as insulin, glucagon, and human growth hormone are regulated as drugs under the *FDC Act*, not biological products under the *PHS Act*. The effect is that regenerative medicine products that fall under the definitions provided in PHS Act 351 will require clinical trials under an IND application. Those regenerative medicine products that can be classified solely as HCT/Ps and meet the minimally manipulated and homologous use criteria defined in PHS Act 361 do not require clinical trials.

7.6 Twenty-First Century Cures Act (Cures Act) of 2016

The twenty-first Century Cures Act (Cures Act), signed into law on December 2016, is designed to help accelerate medical product development and bring new innovations and advances to patients who need them faster and more efficiently. The Regenerative Medicine Advanced Therapy (RMAT) offers a new expedited option for certain eligible biologics products and the Breakthrough Devices designed to speed the review of certain innovative medical devices are both part of twenty-first Century Cures Act of 2016 [55].

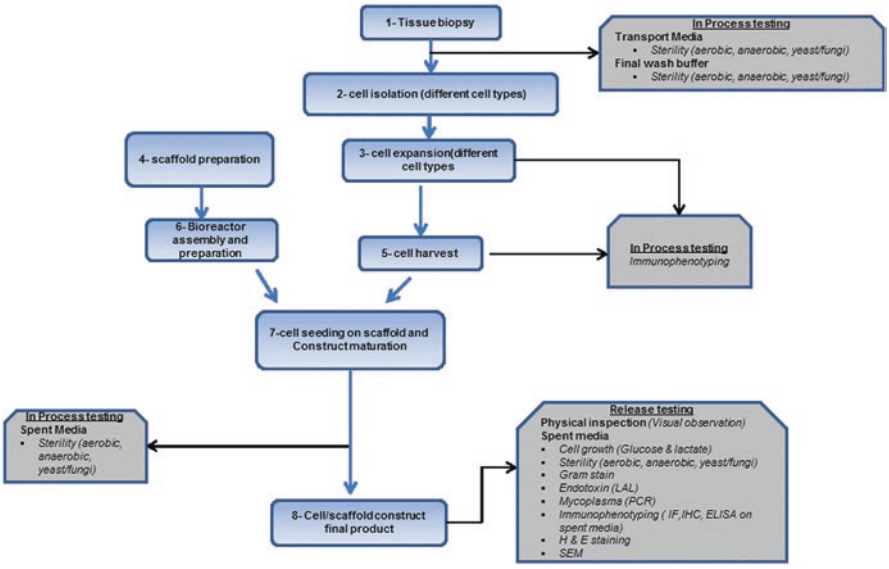

Fig. 2 A generic example of tissue-engineered organ workflow
IHC immunohistochemistry, *IF* immunofluorescence, *H and E* hematoxylin and eosin, *SEM* scan electron microscopy

8 Conclusions

A generic, high-level workflow for the production, testing, and final product preparation of tissue-engineered organs is shown in Fig. 2. This workflow can be used (with modifications) as a template for designing translational processes for regenerated tissue. Such a workflow has been used in many of the translational programs at Wake Forest Institute for Regenerative Medicine Manufacturing Development Center (WFIRM-MDC).

References

1. Adachi, N., Ochi, M., Deie, M., et al. (2014). Implantation of tissue-engineered cartilage-like tissue for the treatment for full-thickness cartilage defects of the knee. *Knee Surgery, Sports Traumatology, Arthroscopy, 22*(6), 1241–1248.
2. Mumme, M., Barbero, A., Miot, S., et al. (2016). Nasal chondrocyte-based engineered autologous cartilage tissue for repair of articular cartilage defects: an observational first-in-human trial. *Lancet, 388*(10055), 1985–1994.
3. Ma, N., Wang, H., Xu, X., et al. (2017). Autologous-cell-derived, tissue-engineered cartilage for repairing articular cartilage lesions in the knee: study protocol for a randomized controlled trial. *Trials, 18*(1), 519.

4. US Food and Drug Administration. (2016). *FDA approves first autologous cellularized scaffold for the repair of cartilage defects of the knee*. https://www.fda.gov/news-events/press-announcements/fda-approves-first-autologous-cellularized-scaffold-repair-cartilage-defects-knee. Last accessed 14 Aug 2020.
5. Law, J. X., Liau, L. L., Aminuddin, B. S., & Ruszymah, B. H. (2016). Tissue-engineered trachea: A review. *International Journal of Pediatric Otorhinolaryngology, 91*, 55–63.
6. Raya-Rivera, A., Esquiliano, D. R., Yoo, J. J., et al. (2011). Tissue-engineered autologous urethras for patients who need reconstruction: an observational study. *Lancet, 377*(9772), 1175–1182.
7. Raya-Rivera, A. M., Esquiliano, D., Fierro-Pastrana, R., et al. (2014). Tissue-engineered autologous vaginal organs in patients: a pilot cohort study. *Lancet, 384*(9940), 329–333.
8. Atala, A., Bauer, S. B., Soker, S., et al. (2006). Tissue-engineered autologous bladders for patients needing cystoplasty. *Lancet, 367*(9518), 1241–1246.
9. Joseph, D. B., Borer, J. G., De Filippo, R. E., et al. (2014). Autologous cell seeded biodegradable scaffold for augmentation cystoplasty: phase II study in children and adolescents with spina bifida. *The Journal of Urology, 191*(5), 1389–1395.
10. Boyce, S. T., & Lalley, A. L. (2018). Tissue engineering of skin and regenerative medicine for wound care. *Burns & Trauma, 6*, 4.
11. US Food and Drug Administration, HHS. (2005). Definition of primary mode of action of a combination product. *Final rule. Federal Register, 70*(164), 49848–49,862.
12. Hunsberger, J. G., Shupe, T., & Atala, A. (2018). An industry-driven roadmap for manufacturing in regenerative medicine. *Stem Cells Translational Medicine, 7*(8), 564–568.
13. Murphy, C. M., O'Brien, F. J., Little, D. G., & Schindeler, A. (2013). Cell-scaffold interactions in the bone tissue engineering triad. *European Cells & Materials, 26*, 120–132.
14. US Food and Drug Administration. (2017). *FDA announces comprehensive regenerative medicine policy framework*. https://www.fdagov/news-events/press-announcements/fda-announces-comprehensive-regenerative-medicine-policy-framework. Last accessed 14 Aug 2020.
15. Hunsberger, J. G., & Hickerson, D. H. M. (2020). Bio-manufacturing for regenerative medicine. In R. L. Robert Lanza & J. P. Vacanti (Eds.), *Principles of tissue engineering* (5th ed., pp. 1469–1480). Academic.
16. Hunsberger, J., Harrysson, O., Shirwaiker, R., et al. (2015). Manufacturing road map for tissue engineering and regenerative medicine technologies. *Stem Cells Translational Medicine, 4*(2), 130–135.
17. Ankrum, J. A., Ong, J. F., & Karp, J. M. (2014). Mesenchymal stem cells: immune evasive, not immune privileged. *Nature Biotechnology, 32*(3), 252–260.
18. Weiss, A. R. R., & Dahlke, M. H. (2019). Immunomodulation by Mesenchymal Stem Cells (MSCs): Mechanisms of action of living, apoptotic, and dead MSCs. *Frontiers in Immunology, 10*, 1191.
19. Matthay, M. A., Calfee, C. S., Zhuo, H., et al. (2019). Treatment with allogeneic mesenchymal stromal cells for moderate to severe acute respiratory distress syndrome (START study): A randomised phase 2a safety trial. *The Lancet Respiratory Medicine, 7*(2), 154–162.
20. Atluri, S., Manchikanti, L., & Hirsch, J. A. (2020). Expanded Umbilical Cord Mesenchymal Stem Cells (UC-MSCs) as a therapeutic strategy in managing critically Ill COVID-19 patients: The case for compassionate use. *Pain Physician, 23*(2), E71–E83.
21. Leng, Z., Zhu, R., Hou, W., et al. (2020). Transplantation of ACE2(−) mesenchymal stem cells improves the outcome of patients with COVID-19 Pneumonia. *Aging and Disease, 11*(2), 216–228.
22. Huang, C. P., Chen, C. C., & Shyr, C. R. (2018). Xenogeneic cell therapy provides a novel potential therapeutic option for cancers by restoring tissue function, repairing cancer wound and reviving anti-tumor immune responses. *Cancer Cell International, 18*, 9.
23. D'Eredita, R. (2015). Porcine small intestinal submucosa (SIS) myringoplasty in children: A randomized controlled study. *International Journal of Pediatric Otorhinolaryngology, 79*(7), 1085–1089.

24. Mosala Nezhad, Z., Poncelet, A., de Kerchove, L., et al. (2016). Small intestinal submucosa extracellular matrix (CorMatrix(R)) in cardiovascular surgery: a systematic review. *Interactive Cardiovascular and Thoracic Surgery, 22*(6), 839–850.
25. Cookbiotech, SIS. https://www.cookbiotech.com/technologies/
26. Mao, A. S., & Mooney, D. J. (2015). Regenerative medicine: Current therapies and future directions. *Proceedings of the National Academy of Sciences of the United States of America, 112*(47), 14452–14,459.
27. Song, J. J., & Ott, H. C. (2011). Organ engineering based on decellularized matrix scaffolds. *Trends in Molecular Medicine, 17*(8), 424–432.
28. Mase, V. J., Jr., Hsu, J. R., Wolf, S. E., et al. (2010). Clinical application of an acellular biologic scaffold for surgical repair of a large, traumatic quadriceps femoris muscle defect. *Orthopedics, 33*(7), 511.
29. Patterson, J. T., Gilliland, T., Maxfield, M. W., et al. (2012). Tissue-engineered vascular grafts for use in the treatment of congenital heart disease: from the bench to the clinic and back again. *Regenerative Medicine, 7*(3), 409–419.
30. Brown, J. W., Elkins, R. C., Clarke, D. R., et al. (2010). Performance of the CryoValve SG human decellularized pulmonary valve in 342 patients relative to the conventional CryoValve at a mean follow-up of four years. *The Journal of Thoracic and Cardiovascular Surgery, 139*(2), 339–348.
31. Cook Biotech. (2020). *Small Intestine submucosa (SIS)*. https://www.cookbiotech.com/products/. Last accessed 14 Aug 2020.
32. US Food and Drug Administration. (2018). *Overview of device regulation*. https://www.fda.gov/industry/regulated-products/medical-device-overview. Last accessed 14 Aug 2020.
33. Allickson, A. A. J. (2014). *Translational regenerative medicine*. Academic. https://doi.org/10.1016/C2012-0-06956-6
34. US Food and Drug Administration. (2018). *CFR – Code of Federal Regulations Title 21*. https://www.fda.gov/medical-devices/medical-device-databases/code-federal-regulations-title-21-food-and-drugs. Last accessed 14 Aug 2020.
35. Williams, D. F. (1992). Mechanisms of biodegradation of implantable polymers. *Clinical Materials, 10*(1–2), 9–12.
36. Lewandowska-Szumiel, M., & Komender, J. (2000). Interaction between tissues and implantable materials. *Frontiers of Medical and Biological Engineering, 10*(2), 79–82.
37. Shi, D. (2009). *Nanoscience in biomedicine*. Tsinghua University Press/Springer.
38. Sanz-Herrera, J. A., & Reina-Romo, E. (2011). Cell-biomaterial mechanical interaction in the framework of tissue engineering: insights, computational modeling and perspectives. *International Journal of Molecular Sciences, 12*(11), 8217–8244.
39. Guthrie, K., Bruce, A., Sangha, N., Rivera, E., & Basu, J. (2013). Potency evaluation of tissue engineered and regenerative medicine products. *Trends in Biotechnology, 31*(9), 505–514.
40. Hickerson, D., Somara, S., Fernandez, A. M., Lo, C., Meinecke, T., Wilkins-Port, C., & Allickson, J. (2020). The next wave: tissue replacement and organ replacement. In A. A. Vertès NJD, D. Smith, & N. Qureshi (Eds.), *Second-generation cell and gene-based therapies: Biological advances, clinical outcomes, and strategies for capitalization* (p. 846). Elsevier Science.
41. Vijayavenkataraman, S., Lu, W. F., & Fuh, J. Y. (2016). 3D bioprinting of skin: a state-of-the-art review on modelling, materials, and processes. *Biofabrication, 8*(3), 032001.
42. Andrew, T. W., Kanapathy, M., Murugesan, L., et al. (2019). Towards clinical application of tissue engineering for erectile penile regeneration. *Nature Reviews. Urology, 16*(12), 734–744.
43. Petersen, T. H., Calle, E. A., Zhao, L., et al. (2010). Tissue-engineered lungs for in vivo implantation. *Science, 329*(5991), 538–541.
44. Llacua, L. A., Faas, M. M., & de Vos, P. (2018). Extracellular matrix molecules and their potential contribution to the function of transplanted pancreatic islets. *Diabetologia, 61*(6), 1261–1272.

45. Lesman, A., Koffler, J., Atlas, R., et al. (2011). Engineering vessel-like networks within multicellular fibrin-based constructs. *Biomaterials, 32*(31), 7856–7869.
46. Ozbolat, I. T. (2015). Bioprinting scale-up tissue and organ constructs for transplantation. *Trends in Biotechnology, 33*(7), 395–400.
47. Murphy, S. V., & Atala, A. (2014). 3D bioprinting of tissues and organs. *Nature Biotechnology, 32*(8), 773–785.
48. Cui, X., Boland, T., D'Lima, D. D., & Lotz, M. K. (2012). Thermal inkjet printing in tissue engineering and regenerative medicine. *Recent Patents on Drug Delivery & Formulation, 6*(2), 149–155.
49. Derby, B. (2012). Printing and prototyping of tissues and scaffolds. *Science, 338*(6109), 921–926.
50. Xu, T., Binder, K. W., Albanna, M. Z., et al. (2013). Hybrid printing of mechanically and biologically improved constructs for cartilage tissue engineering applications. *Biofabrication, 5*(1), 015001.
51. US Food and Drug Administration. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/expedited-programs-regenerative-medicine-therapies-serious-conditions. Last accessed 14 Aug 2020.
52. US Food and Drug Administration. https://www.fda.gov/vaccines-blood-biologics/industry-biologics/interact-meetings. Last accessed 14 Aug 2020.
53. US Food and Drug Administration. https://www.fda.gov/vaccines-blood-biologics/industry-biologics/cber-advanced-technologies-team-catt. Last accessed 14 Aug 2020.
54. US Food and Drug Administration. https://www.fda.gov/drugs/development-approval-process-drugs/new-drugs-fda-cders-new-molecular-entities-and-new-therapeutic-biological-products. Last accessed 14 Aug 2020.
55. US Food and Drug Administration. https://www.fda.gov/regulatory-information/selected-amendments-fdc-act/21st-century-cures-act. Last accessed 14 Aug 2020.

Cellular Therapy Applications for COVID-19

Joshua M. Hare and Aisha Khan

1 Introduction

The novel coronavirus, SARS-CoV2, which first affected humans in Wuhan, China, in December 2019 has created a major healthcare and socioeconomic crisis worldwide [1]. The most common lethal manifestation of SARS-CoV2 is a cytokine storm-driven acute respiratory distress syndrome (ARDS) [1, 2]. The mortality of this syndrome was as high as 90% among the first patient cohorts, but over just a few months, with the introduction of uniform clinical practices and emerging evidence-based interventions, has fallen to approximately 25% [3, 4]. Nonetheless, the rapid and aggressive nature of this viral-driven ARDS has challenged healthcare systems worldwide and prompted an unprecedented rate of clinical trial conduct aimed at developing additional and more targeted evidence-based support for novel treatment strategies. Treatment strategies can be broadly classified into (1) preventative (i.e., vaccines), (1) anti-viral agents, and (2) anti-inflammatory.

2 COVID Cellular Therapy

Cell-based therapy (CBT), which has been in clinical testing for two decades, was very early on hypothesized to have a potential therapeutic role for COVID-19 pneumonia [5]. The rationale for this approach is based upon a long-standing safety profile of intravenous infusions of culture-expanded adult stem cells, coupled with a well-characterized immunomodulatory effect [6]. Importantly, although CBT suppressed inflammatory cytokines, it does not inhibit physiologic immune reactions

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targeting infectious agents; rather CBT augments T helper cell reactions and thus may augment anti-viral responses. In addition, smaller clinical trials had been previously conducted in ARDS patients with mixed results [5, 7]. In 2020, several open-label studies appeared, along with the initiation of phase III trials. In this chapter we will review the biological underpinnings of cell therapy in COVID-19 pneumonia, the early-stage clinical trials, and the design of ongoing phase III clinical trials aimed at generating data to support regulatory approval.

3 Biology of Cell Therapy for COVID-19 Pneumonia

Culture-expanded adult cells that have been in clinical testing for close to 20 years were originally termed mesenchymal stem cells (MSCs), but are alternatively named mesenchymal stromal cells or medicinal signaling cells [8, 9]. These cells, obtained from a diverse range of tissue sources, are characterized by their ability to self-replicate in culture and by the cell surface marker CD105 in the absence of hematopoietic markers such as CD34. Their role as bona fide "stem cells" remains controversial; in this regard, they clearly possess trilineage differentiation potential [10], but they have not been convincingly demonstrated to engraft and differentiate in various tissue lineages in vivo.

MSCs possess therapeutic properties mediated, in part, by the release of secondary factors that include cytokines, growth factors, exosomes, and organelles [8, 11]. Among the effects of MSC infusions is an immunomodulatory effect that can substantially dampen a cytokine storm milieu [8] while at the same time boosting T helper cell responses, possibly B-cell responses, and releasing anti-pathogen factors.

4 Virology of Coronaviruses and Cytokine Release Syndrome in COVID-19

In a very short period of time following the first clinical reports, the SARS-CoV2 virus was isolated and sequenced [1]. It is a betacoronavirus similar in structure to the SARS virus; both viruses bind to the angiotensin-converting enzyme 2 receptor (ACE2), which results in their ability to enter and infect cells. This receptor is found on cardiopulmonary cells, as well as monocytes and macrophages, and possibly dendritic cells. Infection of monocytes and macrophages leads to the production of IL-6 (Fig. 1) [12]. Levels of IL-6 correlate with clinical outcomes in patients, further substantiating the role of the cytokine release syndrome in the pathobiology of COVID-19 pneumonia. IL-6 also drives the production of C-reactive protein, making this clinically available biomarker also useful in monitoring disease progression in COVID-19. Lymphopenia is another clinically predictive hallmark of COVID-19 [13].

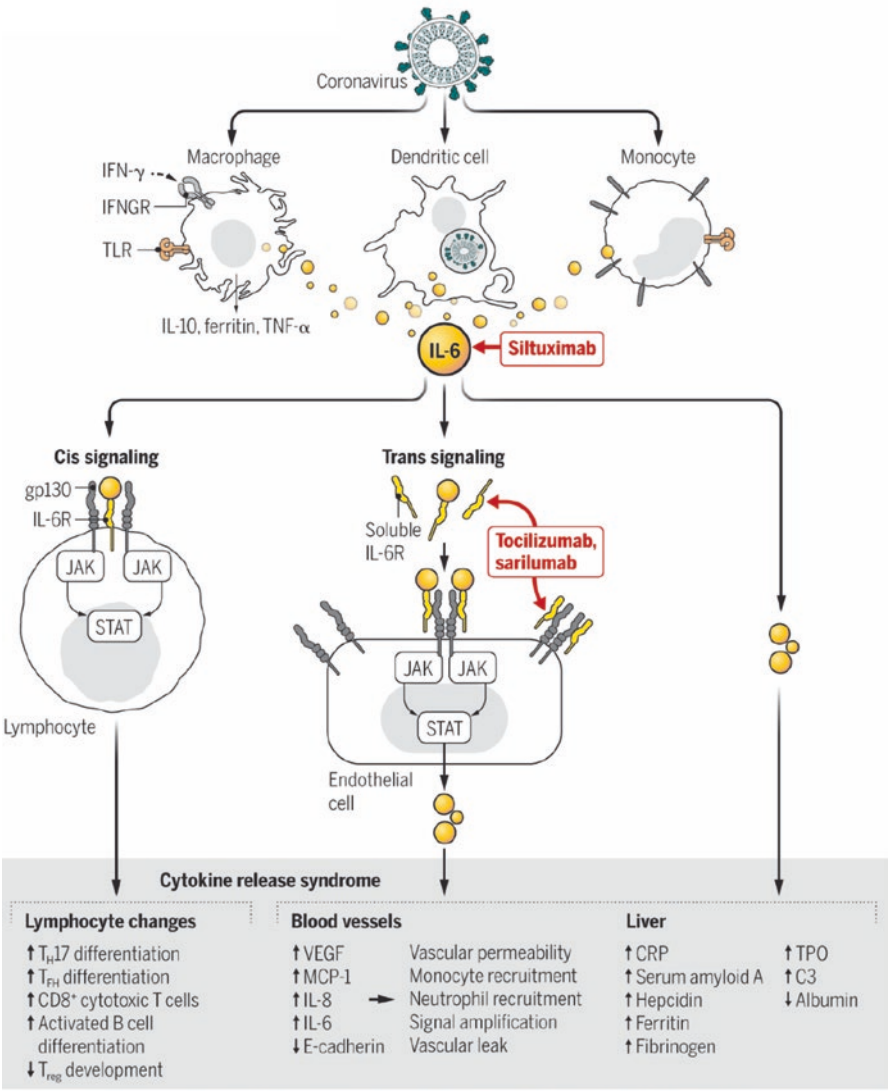

Fig. 1 Cytokine release syndrome in severe COVID-19 [1]. (Adapted from Moore and June. Cytokine Storm. Science, 2020)
Infection with the SARS-CoV2 virus activates monocytes, macrophages, and dendritic cells. This immune activation results in the release of the cytokine interleukin-6, which in turn amplifies the immune response through cis and trans signaling mechanisms. In cis signaling, IL-6 binds to the gp130 receptor on lymphocytes, leading to TH17 and B-cell activation. In trans signaling, IL-6 binds to the soluble IL-6 receptor, creating a complex of activating numerous cell types including endothelial cells. The resulting cytokine release syndrome drives the disease pathophysiology of COVID-19 clinical syndromes. This pathophysiology forms the basis for anti-cytokine strategies, including targeted IL-6 approaches and comprehensive immunomodulatory strategies such as the use of cell-based therapy

IL-6 also drives vascular permeability by stimulating secretion of VEGF and MCP1 in endothelial cells. This vascular leak phenomenon can lead to vascular permeability and leak driving the ARDS process and contributing to impaired respiratory gas exchange. It is increasingly recognized that vascular damage contributes to the pathophysiology of COVID-19 syndrome [14].

5 Effects of MSCs on the Immune System

One of the earliest observations regarding MSCs is that they can be safely administered as an allograft without the requirement for any immunosuppression. Early reports indicated that MSCs could actually suppress a mixed lymphocyte reaction (MLR). MSCs appear to be able to interact with numerous components of the cellular and humoral immune system, essentially converting exuberant immune responses to ones that are more physiologic (Fig. 2). With regard to the CRS, MSCs can modify both macrophage and dendritic cell responses. This immunomodulatory effect forms the basis for the use of CBT to treat graft versus host disease [15].

The immunomodulatory properties of MSCs in terms of modifying cytokine responses also contribute to their potential therapeutic effects in non-CRS disease, such as congestive heart failure [9] and aging frailty [16]. In these conditions, reductions in circulating TNF-α levels may correlate with clinical outcome [17]. Moreover, MSCs also reduce elevated VEGF levels and augment endothelial function in the failing circulation [18]. Thus, these effects may be general markers of therapeutic responsiveness to MSC infusions.

6 Early-Stage Clinical Information

The first report of the therapeutic use of cell-based therapy in COVID-19 originated from Leng et al. in Wuhan in February 2020 [19]. In this report of 7 patients from Beijing, affected patients were infused with 1×10^6 cells per Kg of body weight. The infusions were reported to reverse lymphopenia and reduce both C-reactive protein (CRP) and TNF-α levels. Clinically, pulmonary function improved, and 3 of the 7 patients were discharged from the hospital. Subsequently, Sanchez-Guijo and colleagues reported a case series of 13 COVID-19 patients administered with allogeneic adipose-derived MSCs [21]. In a similar fashion to Leng et al., the infusion dose was ~1×10^6 cell per Kg of body weight [20]. Patients receiving the infusions were mechanically ventilated and received between 2 and 3 infusions. Patients tolerated the infusions well, and there were several indications of potential clinical response, including prompt attenuation of elevated cytokine levels and reversal of lymphocytopenia (Fig. 3).

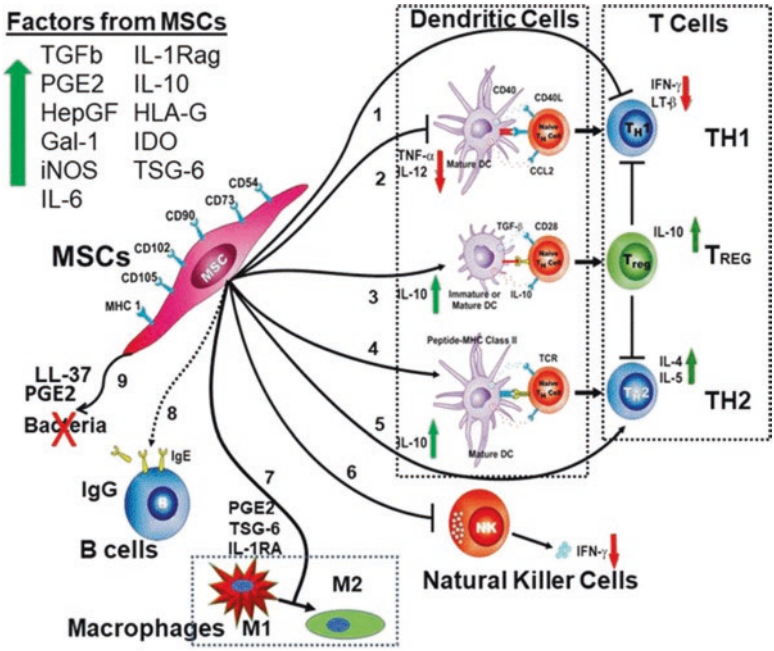

Fig. 2 MSC-immune cell interactions [8]. (Adapted from Pittenger, et al. 2019 – MSCs and the immune system)
MSCs engage in numerous interactions with immune effectors and cells. MSCs release at least 11 factors that have immune activity. Importantly, specific cell interactions have been elucidated. Notably, MSCs dampen immune responses in the cellular pathways that are activated by COVID-19 – monocytes, macrophages (pathway 7), and dendritic cells (pathways 2, 3, and 4). In other specific interactions with cellular effectors, MSCs can dampen exuberant immune responses while preserving anti-pathogen responses. MSCs acting on T cells (pathways 1 and 5) reduce inflammatory T H1 and increase T Regs and T H2 cells altering cytokine profiles including a decrease in IFNγ and increase in IL-10, IL-4, and IL-5. MSCs affect dendritic cells (pathways 2, 3, and 4) by decreasing pro-inflammatory mature DC1 with a decrease in TNF-α and IL-12 and an increase in immature DC and DC2, with increased expression of IL-10. When MSCs interact with natural killer cells (pathway 6), there is a decrease in the expression of IFNγ. When macrophages interact with MSCs (pathway 7), there is a conversion from a pro-inflammatory M1 phenotype to an anti-inflammatory M2 phenotype, associated with increased PGE2, TSG-6, and IL-1RA. The impact of MSCs on antibody secretion from B cells (pathway 8) remains controversial, but there is accumulating support that MSCs release factors that inhibit bacterial growth by a direct or an indirect mechanism (pathway 9)

6.1 Mechanism for Cytokine Suppression

As previously mentioned, MSCs have a profound immunomodulatory effect that manifests as a substantial reduction in inflammatory cytokine levels. Moreover, this occurs without an adverse side effect profile typically seen with corticosteroids. MSCs do not increase the risk of infectious disease, nor do they increase the risk of dysregulation in glucose metabolism or osteopenia [6]. The mechanism of action

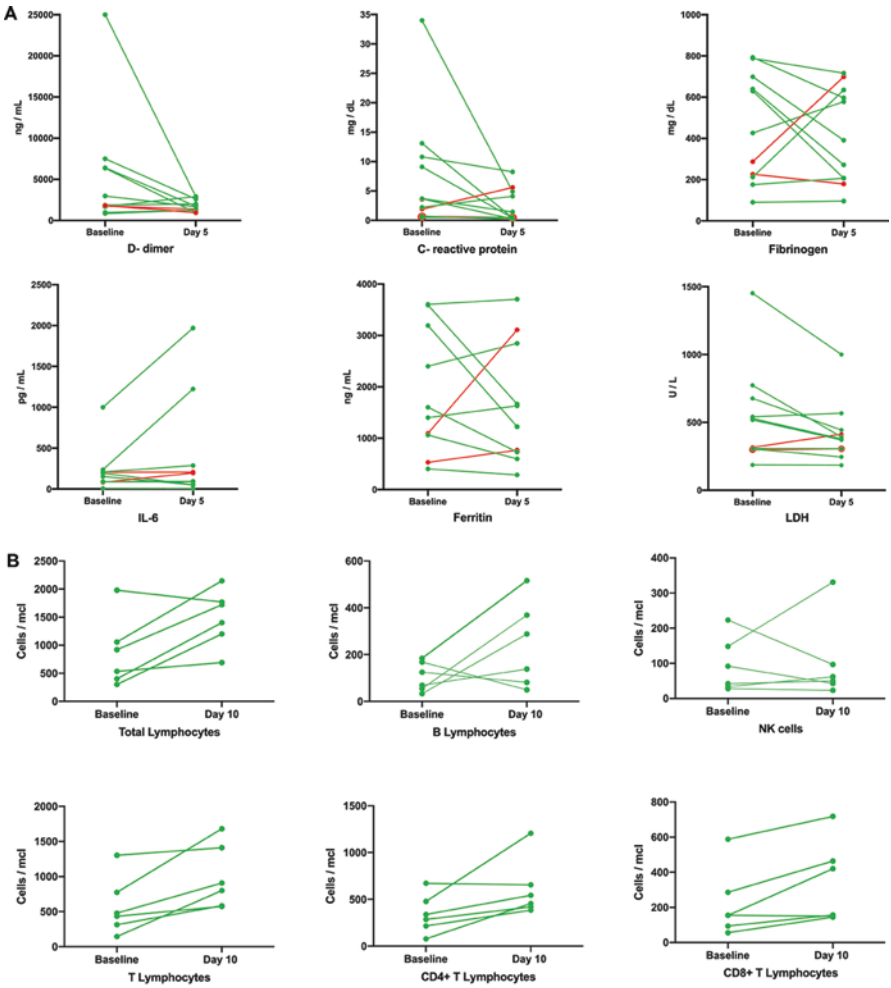

Fig. 3 Impact of MSCs on cytokine levels [21]
Early open-label series documenting treatment of COVID-19 patients with intravenous allogeneic adipose-derived MSCs. (**a**) Cytokine levels. (**b**) Circulating lymphocyte levels. (Reproduced from Sanchez-Guijo et al. – impact of MSCs on cytokine levels) [21]

remains incompletely elucidated, but likely occurs through the release of anti-inflammatory cytokines as well as through potential cell-cell interactions (hetero-cellular coupling) (Fig. 2) [11].

7 Pivotal Clinical Trials

Based upon the biological mechanisms of action, multiple pivotal clinical trials testing CBT for COVID-19 pneumonia have been initiated. ClinicalTrials.gov (on 7/31/2020) reveals that there are at least 50 ongoing trials, worldwide. Importantly, several biotechnology companies have simultaneously initiated pivotal trials that together will provide a large data set of trial information. The majority of these trials target patients with early-onset disease, with varying severity of Berlin criteria ARDS. Other smaller trials are designed to target less severe COVID-19 (i.e., patients who do not require mechanical ventilation), patients with more severe disease (i.e., patients requiring extracorporeal membrane oxygenation), or patients presenting primarily with cardiac involvement (i.e., patients with elevated biomarkers of cardiac injury).

8 Examples of Ongoing Pivotal Multicenter Clinical Trials

Mesoblast plans to enroll 300 participants in 1:1 fashion in a phase III clinical trial of its MSC product Remestemcel-L. The subjects' enrollment criteria include moderate or severe ARDS, using the Berlin criteria, employing a dosing regimen of 2 infusions, 4 days apart, of 2 million cells/Kg body weight. The primary endpoint of this study is all-cause mortality at 30 days (Table 1).

Athersys, a US-based biotechnology company, will enroll 400 participants with new acute-onset moderate to severe ARDS, as defined by the Berlin criteria and clinical diagnosis of COVID-19 of its MSC product, MultiStem. The study's primary endpoints are ventilator-free days and safety and tolerability.

Pluristem, an Israeli biotechnology company that manufactures placental culture-expanded MSCs, is also conducting a phase II trial of 140 participants. In this trial, cells will be administered intramuscularly. Primary outcome is ventilator-free days, and secondary outcomes are all-cause mortality and duration of mechanical ventilation (Table 2).

ACT-NOW is an academic consortium that has initiated a multicenter trial that will enroll patients in a trial comparing bone marrow-derived and umbilical cord-derived culture-expanded MSCs. This phase II program aims to address whether

Table 1 Selected placebo-controlled clinical trials of allogeneic CBT for COVID-19 pneumonia

	Phase	CTG	Tissue source/dosing regimen
Mesoblast	III	NCT04371393	Bone marrow/200 M cells IV, 2 doses 4 days apart
Athersys	II/III	NCT04367077	Bone marrow/900 M cells IV, 1 dose
Pluristem	II	NCT04389450	Placenta/15 injections IM – 2 administrations a week apart
ACT-NOW	II	Pending	Cord blood vs bone marrow/100 M cells IV, 3 doses over 3 days

Table 2 Berlin criteria definitions [2]

Classification	Symptoms
Mild ARDS	PaO2/FIO2 200 to 300 mmHg
Moderate ARDS	PaO2/FiO2 > 100 mmHg and ≤ 200 mmHg, on ventilator settings that include PEEP ≥5 cm H_2O
Severe ARDS	PaO2/FiO2 ≤ 100 mmHg on ventilator settings that include PEEP ≥5 cm H_2O

cell source plays a role is the efficacy of CBT. This trial will enroll Berlin Class I–III patients and will have a primary endpoint of 28-day mortality.

While the above described clinical development efforts employ disparate tissue sources, products, and dosing regimens, there is sufficient harmonization of patient enrollment criteria that a totality of evidence should emerge as to the clinical role of culture-expanded CBT products in the treatment of COVID-19 ARDS.

9 Expanded Access

A growing number of clinical centers have made investigational MSCs available to treating physicians, and several hundred patients are likely to have been treated with CBT in the United States. These experiences offer potentially insightful treatment responses (Fig. 4) and facilitate mechanism of action studies. Widespread clinical use will depend on the successful completion of phase III clinical trials (Table 1).

10 Conclusions

Cell-based therapy is undergoing rigorous testing as a treatment option for COVID-19 syndromes. Culture-expanded CD105+ cells have a long-standing track record of clinical testing and have an outstanding safety profile, including use as an allograft. These cells possess powerful immunomodulatory effects that are well-suited to address the cytokine release syndrome characteristic of COVID-19 pneumonia. Early-stage trials reveal suppression of CRS and potential clinical benefits. These findings are undergoing rigorous testing in pivotal trials, which if successful could lead to FDA approval of CBT for this syndrome.

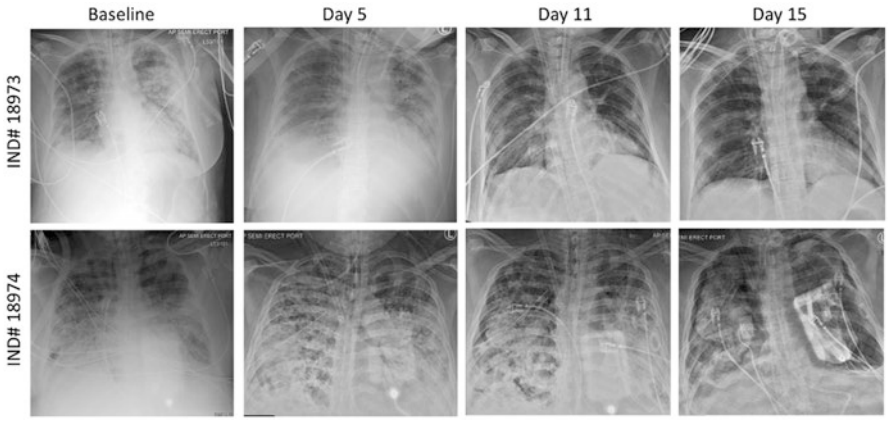

Fig. 4 Chest X-rays of patients with COVID-19 pneumonia treated with IV allogeneic MSCs CXRs revealing marked resolution of pulmonary infiltrates in patients with COVID-19 pneumonia receiving IV allogeneic MSCs

References

1. Zhu, N., Zhang, D., Wang, W., Li, X., et al. (2020). A novel coronavirus from patients with pneumonia in China, 2019. *The New England Journal of Medicine, 382*, 727–733.
2. Moore, J. B., & June, C. H. (2020). Cytokine release syndrome in severe COVID-19. *Science, 368*, 473–474.
3. Ranieri, V. M., Rubenfeld, G. D., Thompson, B. T., Ferguson, N. D., et al. (2012). Acute respiratory distress syndrome: The Berlin definition. *Journal of the American Medical Association, 307*, 2526–2533.
4. Gupta, S., Hayek, S. S., Wang, W., Chan, L., et al. (2020). Factors associated with death in critically ill patients with coronavirus disease 2019 in the US. *JAMA Internal Medicine, 180*(11), 1436–1446.
5. The RECOVERY Collaborative Group. (2020). Dexamethasone in hospitalized patients with Covid-19 — Preliminary report. *The New England Journal of Medicine*. https://www.nejm.org/doi/pdf/10.1056/NEJMoa2021436. Last accessed 18 Sept 2020
6. Qu, W., Wang, Z., Hare, J. M., Bu, G., et al. (2020). Cell-based therapy to reduce mortality from COVID-19: Systematic review and meta-analysis of human studies on acute respiratory distress syndrome. *Stem Cells Translational Medicine*. https://doi.org/10.1016/j.eng.2020.02.006. Last accessed 18 Sept 2020.
7. Thompson, M., Mei, S. H. J., Wolfe, D., Champagne, J., et al. (2020). Cell therapy with intravascular administration of mesenchymal stromal cells continues to appear safe: An updated systematic review and meta-analysis. *EClinicalMedicine, 19*, 100249.
8. Matthay, M. A., Calfee, C. S., Zhuo, H., Thompson, B. T., et al. (2019). Treatment with allogeneic mesenchymal stromal cells for moderate to severe acute respiratory distress syndrome (START study): A randomised phase 2a safety trial. *The Lancet Respiratory Medicine, 7*, 154–162.
9. Pittenger, M. F., Discher, D. E., Peault, B. M., Phinney, D. G., et al. (2019). Mesenchymal stem cell perspective: Cell biology to clinical progress. *NPJ Regenerative Medicine, 4*, 22. https://doi.org/10.1038/s41536-019-0083-6. Last accessed 18 Sept 2020.

10. Banerjee, M. N., Bolli, R., & Hare, J. M. (2018). Clinical studies of cell therapy in cardiovascular medicine: Recent developments and future directions. *Circulation Research, 123*, 266–287.
11. Cao, Y., Gomes, S. A., Rangel, E. B., Paulino, E. C., et al. (2015). S-nitrosoglutathione reductase-dependent PPARγ denitrosylation participates in MSC-derived adipogenesis and osteogenesis. *The Journal of Clinical Investigation, 125*, 1679–1691.
12. Golpanian, S., Wolf, A., Hatzistergos, K. E., & Hare, J. M. (2016). Rebuilding the damaged heart: Mesenchymal stem cells, cell-based therapy, and engineered heart tissue. *Physiological Reviews, 96*, 1127–1168.
13. Scheller, J., Chalaris, A., Schmidt-Arras, D., & Rose-John, S. (2011). The pro- and anti-inflammatory properties of the cytokine interleukin-6. *Biochimica et Biophysica Acta, 1813*, 878–888.
14. Diao, B., Wang, C., Tan, Y., Chen, X., et al. (2020). Reduction and functional exhaustion of T cells in patients with coronavirus disease 2019 (COVID-19). *Frontiers in Immunology, 11*, 827.
15. Ackermann, M., Verleden, S. E., Kuehnel, M., Haverich, A., et al. (2020). Pulmonary vascular Endothelialitis, thrombosis, and angiogenesis in Covid-19. *The New England Journal of Medicine, 383*, 120–128.
16. Kurtzberg, J., Abdel-Azim, H., Carpenter, P., Chaudhury, S., et al. (2020). A phase 3, single-arm, prospective study of Remestemcel-L, Ex vivo culture-expanded adult human mesenchymal stromal cells for the treatment of pediatric patients who failed to respond to steroid treatment for acute graft-versus-host disease transplant. *Biology of Blood and Marrow Transplantation, 26*, 845–854.
17. Golpanian, S., DiFede, D. L., Khan, A., Schulman, I. H., et al. (2017). Allogeneic human mesenchymal stem cell infusions for aging frailty. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences, 72*, 1505–1512.
18. Hare, J. M., DiFede, D. L., Rieger, A. C., Florea, V., et al. (2017). Randomized comparison of allogeneic versus autologous mesenchymal stem cells for nonischemic dilated cardiomyopathy: POSEIDON-DCM trial. *Journal of the American College of Cardiology, 69*, 526–537.
19. Premer, C., Blum, A., Bellio, M. A., Schulman, I. H., et al. (2015). Allogeneic mesenchymal stem cells restore endothelial function in heart failure by stimulating endothelial progenitor cells. *eBioMedicine, 2*, 467–475.
20. Leng, Z., Zhu, R., Hou, W., Feng, Y., et al. (2020). Transplantation of ACE2(−) mesenchymal stem cells improves the outcome of patients with COVID-19 pneumonia. *Aging and Disease, 11*, 216–228.
21. Sánchez-Guijo, F., García-Arranz, M., López-Parra, M., Monedero, P., et al. Adipose-derived mesenchymal stromal cells for the treatment of patients with severe SARS-CoV-2 pneumonia requiring mechanical ventilation. A proof of concept study. *EClinicalMedicine.* https://doi.org/10.1016/j.eclinm.2020.100454. Last accessed 18 Sept 2020.

Part VI
Professional Standards and Support Organizations

Professional Standards for Cellular Therapy: The Foundation for the Accreditation of Cellular Therapy (FACT)

Phyllis I. Warkentin

1 Historical Background

The Foundation for the Accreditation of Cellular Therapy (FACT) is the standards setting and accreditation arm of the two founding societies, each dedicated to quality and progress in cellular therapies, ISCT, and the American Society for Transplantation and Cellular Therapy (ASTCT) [1]. FACT was originally founded as the Foundation for the Accreditation of Hematopoietic Cell Therapy (FAHCT). The name was changed in December 2001 to encompass, in addition to hematopoietic cell (HC) products and therapies, the new and exciting therapies using mesenchymal stem cells, dendritic cells, targeted lymphocytes, genetically modified cells, pancreatic islets, and others. This change followed the lead of the parent organization, the International Society for Hematotherapy and Graft Engineering (ISHAGE), which changed its name in 2001 to the International Society for Cellular Therapy (ISCT) and in 2017 further broadened its scope and name to the International Society for Cell and Gene Therapy. The Regulatory Affairs Committee of ISCT developed the first draft of Standards for Hematopoietic Cell Collection and Processing in 1994. The other parent society of FACT, ASTCT, was formed in 1993 as the American Society of Blood and Marrow Transplantation (ASBMT), a professional society of physicians and investigators involved in the clinical conduct of hematopoietic cell transplantation (HCT). In 2019, ASBMT changed its name to better align with its membership and the expansion of cellular therapy. The ASBMT Clinical Affairs Committee developed the first Clinical Standards for Hematopoietic Cell Transplantation. Believing that quality care can only be achieved if both clinical and laboratory issues are addressed and that professionals in the field are best positioned to set these standards, the ISHAGE laboratory standards and the ASBMT clinical standards were merged into a single document in December 1994. This

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formed the foundation for the first edition of FAHCT S*tandards for Hematopoietic Progenitor Cell Collection, Processing & Transplantation*, published in 1996 [2]. These Standards are unique in breadth and depth, applicable to all phases of the collection, processing, cryopreservation, storage, and administration of hematopoietic progenitor cells (HPCs) regardless of tissue source (bone marrow, umbilical cord blood, peripheral blood, or other tissue source) and to "therapeutic cells," nucleated cells collected for use other than as HPCs. Standards define an infrastructure required for the safe and efficacious collection, processing, storage, and use of HCs; define the minimum education and experience necessary for staff; and require an ongoing assessment of patient outcome, including neutrophil and platelet engraftment and 100-day and 1-year morbidity and mortality.

FACT representatives worked with colleagues from the European Group for Blood and Marrow Transplantation (EBMT) and ISCT-Europe to establish the Joint Accreditation Committee of ISCT-Europe and EBMT (JACIE) [3]. The primary aim of JACIE is to improve the quality of HCT in Europe through its accreditation and education programs and to work toward international harmonization of standards and regulations. JACIE adopted the first edition of the FAHCT Standards in 1999 [4]. The second edition of the Standards was jointly reviewed by FACT and JACIE; subsequent editions have been jointly developed and entitled *FACT-JACIE International Standards for Hematopoietic Cellular Therapy Product Collection, Processing, and Administration* [5]. The first programs were accredited in North America in 1998. FACT and JACIE collaborated in three training workshops in Barcelona, Spain (January 2000, March 2001, and May 2002), to share accreditation tools and experience and to initiate the European accreditation program. Following a pilot project in Spain between 2000 and 2003, during which FACT inspectors performed the first on-site survey, the JACIE accreditation program was fully implemented in January 2004 with support from the European Union under the Public Health Programme (2003–2008). The JACIE accreditation process is similar but not identical to the FACT process described below. Currently, JACIE lists 242 accredited programs in 25 countries [6]. During implementation of this process, JACIE inspectors and staff found that almost all centers were functioning at a high level of excellence, with the majority having only minor deficiencies noted at the on-site inspection. When formally surveyed, these centers reported that implementation of JACIE accreditation required a significant investment of time and resources; however, all believed that the result was demonstrable improvement in the accredited program [7].

FACT Standards also apply to the administration of hematopoietic progenitor cells derived from cord blood; however, additional standards are required for the complexities of cord blood collection and banking. In collaboration with members of the International NetCord Foundation, a network of independent cord blood banks, these additional standards were promulgated. The first edition of *NetCord-FACT International Standards for Cord Blood Processing, Testing, Banking, Selection and Release* was developed by consensus of international experts in the field, initially published in June 2000, and revised in 2002 [8]. These Standards superseded all cord blood standards in FAHCT *Standards for Hematopoietic*

Progenitor Cell Collection, Processing and Transplantation, excepting those clinical standards related to the transplantation of cord blood cells. In 2017, the International NetCord Foundation consolidated its activities within the World Marrow Donor Association (WMDA). The name NetCord is preserved in the title of the NetCord-FACT Standards to acknowledge the contributions of the pioneers in cord blood banking whose expertise was critical to the development of these Standards. The scope of the NetCord-FACT International Standards encompasses all phases of donor recruitment, screening, and testing; cord blood unit collection, transport, testing, processing, storage, and release for clinical use; and assessment of clinical outcomes [9]. All cord blood banks are required to maintain a comprehensive quality management program; to document training of all collection and processing staff; to utilize validated methods, supplies, reagents, and equipment; to maintain product tracking; and to maintain details of clinical outcome. These comprehensive standards apply to both unrelated donor cord blood units and units collected and stored for a specific family or designated recipient. These Standards form the basis for the voluntary accreditation of cord blood banks worldwide. The first cord blood banks achieved FACT-NetCord accreditation in 2004.

2 Recent Advances

In response to rapid advances of cellular therapies into clinical trials and the wide variety in product quality, FACT developed the *Common Standards for Cellular Therapies* in 2015 to cover the collection, processing, and administration of cellular therapy products [10]. Based on the hematopoietic cell and cord blood banking standards, these fundamental standards are applicable to any cell type, cell source, clinical application, phase of product development, or clinical trial. This includes minimally or more than minimally manipulated cells collected from non-hematopoietic cell sources as defined in 21CFR1271.3(f), cells collected from hematopoietic sources and processed and administered under approved research protocols for new indications or non-homologous use, and a variety of cell types, including pancreatic islets, hepatocytes, adipose-derived cells, and others. Included are basic requirements for quality management, process controls for facilities, personnel, equipment, procedures, testing, labeling, and transport. Outcome measures are primarily for safety. These Standards are intended to form the basis for voluntary accreditation in early-phase products or applications and the foundation for additional discipline-specific or product-specific standards in collaboration with relevant experts.

3 FACT Standards for Immune Effector Cells

FACT Standards for Immune Effector Cells represent the first application of this concept of building upon the Common Standards [11]. Novel cell therapies outside of traditional transplantation are increasingly being developed and utilized in clinical trials worldwide, including cytotoxic lymphocytes, natural killer (NK) cells, genetically modified T cells expressing chimeric antigen receptors, or engineered T-cell receptors directed against tumor-associated antigens, tumor-infiltrating lymphocytes, dendritic cells, and others. Four chimeric antigen receptor (CAR)-T cell products directed toward the CD-19 antigen have achieved licensure in the USA, and others are poised to do so. Given the challenges of unique logistic and toxicity profiles, the need for novel supportive care and medications, and the rapid expansion of these therapies, cell therapy experts sought to apply existing FACT Standards to these new therapies and to develop additional specific guidelines to ensure safety. Third-party payers, manufacturers, and regulatory agencies expressed similar interest to promote safe clinical trials and continued assurance of proper handling and use of products in the expectation of good patient outcomes.

FACT published the first edition of *FACT Standards for Immune Effector Cells* in January 2017 [11] with collaborators representing its founding organizations, ASTCT and ISCT, academic cell therapy experts, and representatives of the American Society for Gene and Cell Therapy (ASGCT) and the Society for Immunotherapy of Cancer (SITC) [12]. Simultaneously, the standards unique to immune effector cells were added to *FACT-JACIE International Standards for Hematopoietic Cellular Therapy Product Collection, Processing, and Administration* as Edition 6.1 with the acknowledgment that many transplant programs are active in the clinical trials and therapeutic use of these cells [13].

4 Standards

4.1 Standards Development

All FACT Standards are developed by consensus of experts active in the field and are based on established evidence from the literature whenever possible. Standards are reviewed by legal counsel and internally for technical accuracy, consistency, and regulatory compliance. The FACT Board of Directors appoints a Chairman of the Standards Committee, who works with the FACT Chief Medical Officer and appropriate subcommittees to develop each new edition. For the hematopoietic cellular therapy standards, JACIE appoints a co-chair, and FACT and JACIE each appoint members to subcommittees for clinical, apheresis collection, laboratory processing, and quality management standards. With the eighth edition under development, an additional subcommittee has been added to address immune effector and genetically modified cellular therapies. Additional expertise is sought for specific issues

such as histocompatibility as needed. For cord blood banking standards, working subcommittees include quality management, cord blood collection, processing, selection, and distribution. Representatives of both public and private cord blood banks are included. The Standards development process follows these steps:

- The Standards Steering Committee determines any global changes for each new edition, noting advances in the field and lessons learned from the prior edition, and surveys accredited programs for recommended changes and new standards.
- Subcommittees review the assigned portions of the existing Standards. Each Standard is reviewed, and it is retained, revised, or deleted as appropriate.
- New Standards are proposed and considered for applicability in all sections of the Standards.
- The resulting draft is reviewed by legal counsel and reviewed and approved by the FACT and JACIE (EBMT) Boards of Directors for publication for public comment.
- Each subcommittee reviews each comment received during the 90-day public comment period, and revisions are made as indicated. FACT staff maintain consistency across the sections of each document and among the different sets of Standards.
- The final version is approved by legal counsel and the Boards of Directors and published. Accredited programs are expected to be in compliance with the new edition by its effective date 90 days after publication.
- Each edition of FACT-JACIE Standards is accompanied by an Accreditation Manual that contains guidance material including an explanation of the intent of the standard, evidence that may be requested or provided as documentation of compliance, and examples of processes that would be compliant with the Standard. The Accreditation Manual uses universal language to the extent possible and includes references to Food and Drug Administration (FDA) regulations and guidance documents and to governmental regulations of the European Union, the Australian Therapeutics Goods Administration, and others. FACT-JACIE Standards and the accompanying accreditation guidance manual are available in print and online at the FACT website (www.factwebsite.org).

4.2 Current Standards

4.2.1 FACT-JACIE International Standards for Hematopoietic Cellular Therapy Product Collection, Processing, and Administration

The seventh edition of Hematopoietic Cellular Therapy Standards is designed to provide minimum guidelines for facilities and individuals performing hematopoietic cell transplantation and related cellular therapies. These Standards apply to:

- Hematopoietic progenitor cells (HPCs), defined as self-renewing and/or multipotent stem cells capable of maturation into any of the hematopoietic lineages,

lineage-restricted pluripotent progenitor cells, and committed progenitor cells from hematopoietic sources (bone marrow, umbilical cord blood, peripheral blood, or other tissue source).

- Nucleated cells or mononuclear cells from any hematopoietic tissue source (marrow, peripheral blood, umbilical cord, and placental blood) collected for therapeutic use other than as HPCs. These cells are used for any clinical indication, may be further enumerated or identified by CD designation or other methodology, or may be used in further manufacturing of products for administration.
  - For HPCs or mononuclear cells derived from umbilical cord or placental blood, these Standards apply only to the administration of the cellular therapy product, applying the relevant clinical and processing standards for product preparation and transplantation. Standards for cord blood collection and banking are available in a separate document, *NetCord-FACT International Standards for Cord Blood Collection, Banking, and Release for Administration*, available at www.factwebsite.org [9].
- Immune effector cells derived from these sources, defined broadly as any cells, in vitro modified or not, that are capable of eliciting or modulating an immune response. This broad designation includes cellular therapy products with widely diverse manufacturing methods, constructs, clinical indications, and safety and toxicity profiles. Individual programs and responsible personnel must understand the immune effector cell products in clinical use, the spectrum and timing of potential, and anticipated toxicities associated with each product or type of product, implement relevant evaluation and mitigation strategies, and apply these Standards appropriately to each situation.

Standards are organized by services provided, divided into Clinical, Cell Collection, and Cellular Therapy Product Processing sections. Since the fifth edition of Standards in 2008, collection standards have been subdivided into Section CM (Bone Marrow) and Section C (Apheresis). Where common themes occur in all areas, the standards are aligned to be consistent across the services provided.

Central to the Standards is the requirement that all clinical, collection, and processing facilities develop and maintain a comprehensive quality management plan that includes at least the following components: defined organizational structure; personnel requirements including qualifications, training, competency, and continuing education; process and procedure development; a system for document control; procedures for audits; agreements; outcome analysis and product efficacy; audits; management of errors, accidents, complaints, and adverse events, including management of products with positive microbial culture results; product tracking; and validation and/or qualification of critical procedures, reagents, supplies, equipment, and facilities. The current edition also includes many of the regulatory requirements from the US FDA and the directives of the European Union. Cellular therapy product terminology, coding, and labeling requirements follow *ISBT 128* or, if previously established prior to these Standards, Eurocode [14].

Standards for each participating service or facility in the cellular therapy program require minimal procedure volumes and leadership experience, adequate facilities, specific content and format for standard operating procedures, and specific personnel. Participating services are required to maintain active communication with each other. Histocompatibility testing must be performed according to current Standards and protocols by a laboratory accredited by the American Society for Histocompatibility and Immunogenetics, the European Federation for Immunogenetics, the College of American Pathologists, or equivalent accrediting body. An international FACT Histocompatibility Committee determines equivalency. All other laboratory testing must be performed by laboratories appropriately licensed and/or accredited for the specific assays.

Clinical standards define a blood and marrow transplant program as an integrated medical team housed in defined location with a Clinical Program Director(s) and common staff training programs, protocols, and quality management systems that uses hematopoietic cell collection and processing facilities that meet FACT-JACIE Standards. Clinical standards enumerate required support and consultative staff and list the processes that must be covered by a written Standard Operating Procedure (SOP). Requirements are defined for autologous and allogeneic donor evaluation, selection, eligibility, and consent. Minimum guidelines for recipient care are also defined, including informed consent; safe administration of the preparative regimen or high-dose therapy, radiation therapy, and the cellular therapy product; management of complications; provision of post-transplant and long-term follow-up care; procedures addressing the indications for and safe administration of extracorporeal photopheresis; and similar processes for the safe and efficacious administration, management, and follow-up of immune effector cells and other cellular therapy products. Standards describe the appropriate management of clinical research and Institutional Review Board-approved protocols. Clinical programs must keep complete and accurate records as measured by the Data Audits of the Center for International Blood and Marrow Transplant Research (CIBMTR) and must follow clinical outcomes, both as individual patient results and aggregate data, and compare these outcomes to national or international benchmarks. As described below, corrective action plans are required when these benchmarks for data accuracy and patient outcomes are not met.

Cell collection Standards define elements common to both bone marrow- and apheresis-derived peripheral blood hematopoietic cells and detail those requirements unique to each cell source. Cell collection services must maintain quality management and written SOPs as described above, have policies for personnel training and competency, and ensure the facility is appropriate to the procedures performed. Donor selection, evaluation, and management standards are included and applicable as the collection facility is responsible for these activities. There are also standards for product labeling, storage, and transportation and shipping. These standards apply to allogeneic and autologous donors and to products that will be administered as collected, processed and stored, or collected for further manufacturing. Coding and labeling according to ISBT 128 is required [14].

Comprehensive laboratory standards detail requirements for quality management; personnel; process controls; inventory management; validation and qualification of facilities, supplies, reagents, and equipment; labels and labeling; storage; transport; and records. Facilities are expected to define, monitor, and maintain environmental conditions appropriate to the type of processing performed and to the amount of manipulation (minimal or more than minimal) and the regulatory requirements of any applicable investigational new drug application (IND). The intent is to establish that the laboratory is operated in a responsible and responsive manner; that deviations in processes or products are documented, investigated, reviewed, and reported; and that appropriate corrective and preventive actions are implemented. Non-conformities relevant to the purity, safety, or potency of the product must be documented and communicated to the clinical team and potentially to regulatory bodies. Laboratory personnel are expected to follow clinical outcome as one measure of product safety and efficacy.

FACT Standards have historically been process-oriented and focused on quality processes as surrogate measures believed to lead to desirable outcomes. The goal of benchmarking is continuous quality improvement. Internal monitoring of several metrics of outcome, such as engraftment rate, overall and treatment-related morbidity and mortality, acute and chronic graft-versus-host disease (GVHD), and infection, has always been a requirement of the Standards. Improvement in patient survival by compliance with these Standards was reported in 2011 [15]. The sixth edition of FACT-JACIE Standards first required measurement of patient survival against national or international benchmarks in 2015 [16]. Although patient outcome may appear to be the optimal measure of quality, any specific outcome is often the result of many factors, some outside of the control of the transplant team. The intent of measurement of outcomes against national or international benchmarks is for each transplant team to assess its processes and outcomes thoroughly, identify causes of death, and address those issues that could result in improved survival. Following extensive study and professional consultation, FACT determined that program-specific 1-year survival data, as collected, processed, and published by the CIBMTR based on the Stem Cell Outcomes Database [SCTOD], provided the most reliable and least ambiguous risk-adjusted measure of outcomes [17]. The SCTOD was developed by CIBMTR as part of the C.W. Bill Young Transplantation Program through a contract awarded by the Health and Human Resources and Service Administration of the US Department of Health and Human Services and authorized by the Stem Cell Therapeutic and Research Act of 2005 (Public Law 109-–129) and reauthorized by the Stem Cell Therapeutic and Research Reauthorization Act of 2010 (Public Law 111–264) and the Stem Cell Therapeutic and Research Reauthorization Act of 2015 (Public Law 114–104). The SCTOD includes data on all allogeneic (related and unrelated) hematopoietic cell transplantations performed in the USA or using donor grafts procured in the USA. With these data, risk-adjusted 1-year survival is calculated for each transplant center using a complex methodology that factors in variables known to significantly influence transplantation outcomes, such as disease, HLA matching by donor and graft type, comorbidities, and demographics [18].

FACT Standards recommend that accredited programs achieve expected outcomes for 1-year survival. For programs submitting allogeneic transplant data to CIBMTR, the SCTOD center-specific outcomes report is the benchmark used by FACT. In this analysis, each program has its own confidence intervals for expected outcome. In theory, all programs can succeed. Corrective action plans (CAPs) are required when this benchmark is not achieved. The FACT Clinical Outcomes Committee reviews all CAPs at a minimum annually. The goal is to help programs to critically evaluate processes and outcome data to make appropriate programmatic changes that will improve patient outcomes. Programs that perform only autologous transplantation or that do not report to CIBMTR still must compare their outcomes using comparative data applicable to their programs and patient populations. Comparative data sources are available, such as the C.W. Bill Young Cell Transplantation Program's US Patient Survival Report or the Disease-Specific HCT Indications and Outcomes Data from Be The Match/National Marrow Donor Program. JACIE is pursuing similar efforts in Europe.

In addition to outcome analysis, FACT-JACIE Standards benchmark data management and accuracy. FACT Standards have always required that clinical programs keep complete and accurate records. CIBMTR also has a comprehensive data audit program to ensure the quality and accuracy of the research database and the integrity of the center-specific outcome analysis. In 2017, FACT eliminated on-site data accuracy assessments and adopted the report of the periodic CIBMTR data audit as the evidence of compliance with the data requirements of Standards. The CIBMTR benchmark of $\leq$3% critical field error rate was adopted. Failure to achieve this benchmark requires programs to submit CAPs, identify the root cause of errors, and make indicated improvements as demonstrated by internal and external data audits. CIBMTR assesses CAPs as part of their internal quality management. The FACT-CIBMTR Data Audit Committee also assesses CAPs, works with programs on implementation and internal audit processes, and interacts periodically with the FACT inspectors who assess on-site implementation and data management.

4.2.2 FACT Standards for Immune Effector Cells

The first edition of *FACT Standards for Immune Effector Cells* was developed in the recognition that novel cell therapies have applicability beyond the traditional transplant setting [11]. In this context, immune effector cells are defined as "cells used to modulate, elicit, or mitigate an immune response with therapeutic intent," including T and B cells, natural killer (NK) cells, dendritic cells, and mesenchymal stem cells. These Standards define the infrastructure needed to ensure safe administration of immune effector cell products, whether shared with the transplant program or existing separately.

These Standards include elements of the Hematopoietic Cellular Therapy Standards excepting those specific to transplantation and build specific guidelines based on known products in clinical trials and/or commercially available. There is a core requirement for a comprehensive quality management plan and program as

described above, to include personnel requirements; training; competency; written SOPs; document control; appropriate facility design and maintenance; control and qualification of equipment, supplies, and reagents; validation of processes and detection; investigation; reporting; and corrective and preventive action in response to accidents, errors, adverse events, deviations, and complaints.

Specific guidelines are based on the known products in clinical trials and/or commercially available. Special attention is directed at the known toxicity profiles of currently available chimeric antigen receptor-modified T cells, namely, the potential for tumor lysis; cytokine release syndrome requiring rapid escalation of care to intensive care units; neurologic toxicity including encephalopathies characterized by aphasia, seizures, and cerebral edema; and unusual immunologic manifestations such as hemophagocytic lymphohistiocytosis. Standards do not prescribe a specific management strategy, but require that all physicians, nurses, and other providers be adequately trained in the recognition of the complications and demonstrate competency in responding to them, that pharmacy formularies are adequate to treat anticipated toxicities, and that programs have institutional guidelines for all caregivers. Coordination and education are required across multiple treatment teams involved with the patient or the product. Standards require designation of appropriate consultants in transfusion medicine, pharmacy, neurology, intensive care, and others so that communication is assured. Data management standards are also critical. There must be appropriate staff trained and available to collect and submit data on product safety, efficacy, and clinical outcomes. These data should be reviewed by the program director and presented as part of periodic quality management meetings. Submission of data for long-term follow-up to the CIBMTR Cellular Immunotherapy Data Resource (CIDR) is highly recommended. Standards also require periodic audit of the accuracy of data elements included in the CIDR forms.

The scope of Immune Effector Cell Standards is the clinical unit, the collection facility responsible for the starting cellular therapy product, and the cell processing facilities as relevant, either manufacturing or receiving the final product and preparing it for administration. As for hematopoietic cell standards, these require that clinical programs utilize collection and processing facilities that meet FACT Standards. However, it is acknowledged that manufacturing sites vary. Regardless of where manufacturing occurs, responsibilities for chain of custody, product storage, verification of cellular therapy product identity, and management of adverse events must be clearly defined. If manufacturing occurs at a third-party or commercial site, Standards require written agreements to ensure quality and regulatory oversight of manufacturing and maintenance of the chain of identity and chain of custody throughout all hand-offs and transportation from collection of the starting cellular product to administration of the final product to the intended recipient.

4.2.3 NetCord-FACT International Standards for Cord Blood Collection, Processing, Testing, Banking, Selection, and Release

These Standards, currently in the seventh edition [9], are intended for the field of cord blood banking, in which a cord blood bank is defined as an integrated team responsible for the collection, processing, testing, banking, selection, and release of cord blood units for administration. It is important to note that the Standards begin with maternal donor recruitment, consent, and screening and the cord blood unit collection, rather than only covering those processes occurring in the laboratory. The Standards apply to both the banks responsible for cord blood units collected, stored, and reserved for use by a designated individual or family ("private" banking) as well as to those banks responsible for units collected, stored, and donated for use by unrelated recipients. There are some differences between standards for family units and unrelated donor units; however, in all cases, the goal is to optimize collection and processing so that all units will be viable and efficacious when selected for patient use. While most Standards are similar between the types of donation, the processes to meet these Standards often differ. The nature of the collection sites and the relationships among the bank, the cord blood unit collector, the donor, and the collection facility are among the prominent differences in the Standards for related and unrelated units.

Similar to the Hematopoietic Cellular Therapy Product Standards, these standards require that each cord blood bank establish and maintain a comprehensive quality management program that covers all aspects of the operation and includes at least the following: organizational structure; personnel requirements, qualifications, training, and competency; systems for document creation, review, control, and maintenance; quality assessments and audits; detection, investigation, reporting, corrective and preventive action, and follow-up of errors, accidents, biological product deviations, adverse events, and complaints; validation, qualification, calibration, and maintenance of equipment, supplies, reagents, and materials; inventory control for reagents and products; process controls; systems for product identification, labeling, and tracking; facilities and safety management; donor suitability determination; vendor qualification; and agreements with third parties. There are also standards for unique issues that may face a bank, such as inventory transfer or interruption of operations at established collection or laboratory sites. Comprehensive processing, storage, and labeling standards are consistent with *ISBT 128* terminology and labeling requirements or Eurocode if previously implemented. The bank staff is required to follow clinical outcomes from each unit released for transplant in sufficient detail to ensure that the procedures in use continuously provide a safe and effective product. This includes viability, nucleated cell recovery, and microbial culture results from the thawed unit, adverse events associated with administration, and recipient outcome, engraftment, chimerism, survival, and graft-versus-host disease.

4.2.4 FACT Common Standards for Cellular Therapies

The *FACT Common Standards for Cellular Therapies* are the fundamental standards, based on current FACT-JACIE Hematopoietic Cell Transplant Standards and NetCord-FACT Cord Blood Banking Standards, that are applicable to any cell type, cell source, clinical application, and phase of development or clinical trial [10]. Minimal requirements in these Standards are for quality management, good documentation practices, process controls for facilities, personnel, equipment, procedures, testing, labeling, storage, and transport and for outcome measures, primarily safety endpoints initially, with potency and efficacy endpoints phased in as appropriate. The premise is that quality management can serve as a bridge between basic research and translational medicine by providing tools that will prepare the investigator for the next steps. Quality management begins the orderly creation of documents and records, ensures availability of equivalent and qualified reagents and supplies and adequate vendors, and provides suitable data related to the successful procedures required for regulatory submission and approval. Quality management begins to establish a culture within the research environment that improves quality and increases the likelihood of success.

Specific elements of quality management required by Common Standards include:

- Design controls
- Process and production control
- Facility and equipment control
- Reagent, supply, and material control
- Records, documents, and change control
- Management of occurrences including deviations and corrective and preventive action

Quality management promotes patient safety. Controlled processes for procedure development and staff training enhance cell collection and processing to protect the integrity and safety of the product and promote safe and effective delivery of the cells and evaluation of the patient before and after administration. Monitoring and self-assessments detect non-compliances, and deviation management requires rapid identification of and response to adverse events. Evaluation and analysis of outcome data identifies results of cell therapy for individual patients and ongoing trends in aggregate results to note needed improvements.

Early adoption of quality management also protects the research environment. The regulatory burden should allow for an environment of research and development that permits advancement in the field. Experts active in the field are in the best position to set Standards that are practical and manageable. Peer assessment fosters an environment of transparency and improvement and increases confidence in veracity of the research. Collaboration in data collection and reporting can advance the field.

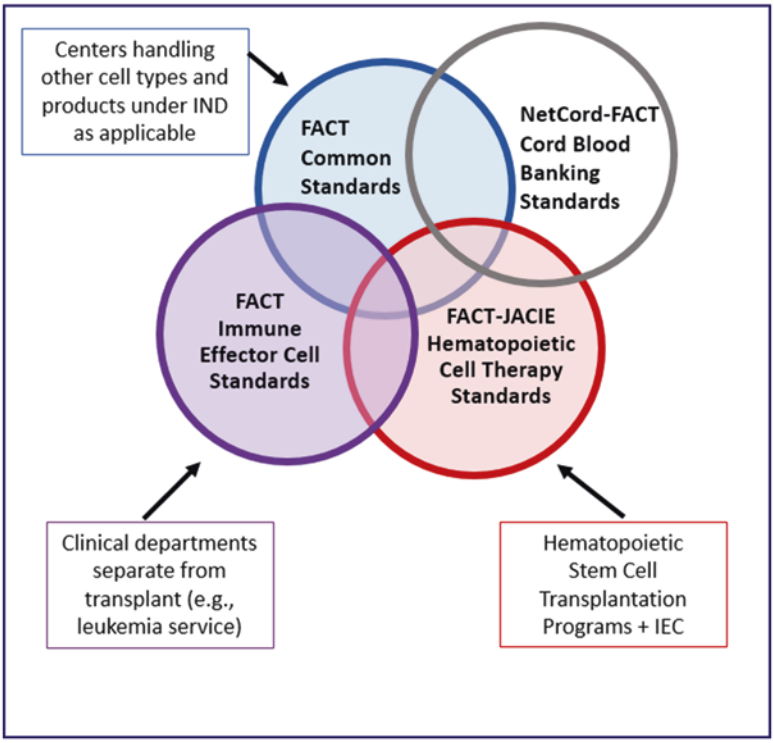

Fig. 1 Relationship among the different FACT Standards and identification of the applicable standards for a specific accreditation applicant

The relationship among these four sets of Standards is illustrated in Fig. 1. All sets of standards and accompanying accreditation manuals are available for download or purchase of a hardcopy at www.factwebsite.org.

5 Accreditation

The goal of the FACT Accreditation Program is to raise the quality of performances for all cellular therapy programs and services in the expectation that such improvements will lead to better patient outcomes [1]. The process is peer-based and intended to be educational rather than punitive to allow capable and committed personnel to achieve accreditation. FACT conducts periodic training programs designed to assist the applicants in preparation for accreditation. FACT-JACIE Standards are singular, but JACIE maintains its own accreditation program. The structure of the processes is similar, but there are some logistical differences between the FACT and JACIE processes. FACT manages the accreditation process for cord blood banks worldwide using a process similar to that for HPC transplant.

FACT accreditation is voluntary and based upon documented compliance with the current edition of Standards through submission of written documents and an on-site inspection. Characteristics of the Accreditation Program that set it apart are the qualifications of the inspectorate, the consistent review by an established Accreditation Committee, and the comprehensive on-site inspection that includes all clinical, collection, laboratory, and storage facilities for hematopoietic cell therapy and immune effector cell programs and for cord blood banks; all administrative, processing, and storage facilities; and all or a percentage of fixed and non-fixed collection sites.

FACT on-site inspectors meet relevant educational requirements for their positions and are active in the field in the area they inspect with a minimum of 1–2 years experience. Inspectors are individual members of the parent professional societies of FACT, ISCT or ASTCT, or members of relevant related societies, WMDA NetCord Working Group, the Cord Blood Association, or the American Society for Apheresis (ASFA). Each inspector has completed required FACT training that includes a minimum of online modules, an in-person training workshop, a trainee inspection, and satisfactory completion of a trainee inspection report and exam over the FACT process and the current edition of Standards. For each on-site inspection, a team is chosen based on the complexity of the applicant program so each area is covered. Inspection teams should be from geographically distant regions and must have no conflicts of interest with the applicant program.

The Accreditation Committee of Hematopoietic Cell Transplantation and Immune Effector Cell Therapies is a standing committee of the FACT Board of Directors, chaired by the FACT Chief Medical Director. Membership is composed of experienced inspectors and the Chairperson of the Standards Committee and members of the Board of Directors, ensuring that all specialties of clinical transplantation, other cell therapies, cell collection, and cell processing are adequately represented. There is a separate Accreditation Committee similarly constituted for cord blood banking accreditation, chaired by the FACT Chief Medical Officer with membership representing the inspectorate, the Board, and international accredited cord blood banks. These committees are responsible to review the reports of the on-site inspection team and determine the outcome of the inspection which defines the next steps in the process for the applicant. Each report is reviewed to ensure the standards are equitably applied and requirements to demonstrate compliance are consistent among applicants.

Eligibility for accreditation is based on criteria specified in the Standards and includes a specified volume of procedures or patients treated and a minimum quantity and quality of staff present and performing relevant procedures for a minimum of 12 months prior to accreditation. Accreditation is for a program, not for individuals or for individual cord blood units in inventory. Facilities apply for the type of accreditation based on services offered.

5.1 Types of Accreditation

5.1.1 Hematopoietic Cell Transplantation

Facilities eligible to apply for accreditation are clinical transplant programs, HPC collection facilities, and/or HPC processing laboratories. If applying separately, a clinical transplant program must utilize both a collection facility and a cell processing laboratory that meet FACT-JACIE Standards and that have a clearly defined contractual or reporting relationship.

- Clinical program accreditation may be for allogeneic or autologous transplantation or both and for adult or pediatric transplantation or combined adult/pediatric. A collection service may provide services for clinical transplant programs that are or are not FACT-accredited but must use a processing laboratory that meets FACT-JACIE Standards. If a clinical program is administering immune effector cells, it is expected to be in compliance with these standards and will be accredited for immune effector cell therapies whether using clinical trial products or commercial products. Cell collection from bone marrow is generally assumed to be a part of a clinical program, although marrow collection accreditation is not required of the program if marrow is not used in that program.
- Collection facilities (apheresis) may be accredited as part of a single transplant program if it provides service to only that program. Apheresis facilities may be accredited independently if providing services for more than one program. Apheresis facilities may provide services to accredited and/or non-accredited programs or may collect cells only for further manufacturing. There is no distinction between autologous and allogeneic collections. Collection from pediatric donors or patients requires compliance with additional standards but is not separately accredited.
- Cell processing facilities may be accredited as part of a single clinical program or independently. These facilities may also be accredited in association with an apheresis provider. Cell processing accreditation is for minimal manipulation, more than minimal manipulation, or both. Definitions used are those of the FDA, wherein more than minimal manipulation is defined as processing that alters the relevant biological characteristics of cells. FACT-accredited laboratories manufacturing immune effector cells for clinical administration are required to be accredited for more than minimal manipulation. If an accredited transplant program administers immune effector cells manufactured by a GMP-compliant laboratory not related to the usual, FACT-accredited facility, such a laboratory must be accredited or in compliance with Immune Effector Cell or Common Standards within this current accreditation cycle. Commercial pharmaceutical companies are eligible for accreditation, but it is not required.

5.1.2 Immune Effector Cell Accreditation

Programs may be accredited for immune effector cell therapies as part of the hematopoietic cell transplant program as described above. A clinical program administering immune effector cells and not performing any hematopoietic cell transplantation may be accredited as a stand-alone immune effector cell program. The applicable standards for each model of care are illustrated in Fig. 1. It is acknowledged that there may be variations in these models of care in the programs administering immune effector cells. For purposes of accreditation, FACT defines the clinical program to be accredited based upon the involvement and responsibilities of the designated attending physicians.

5.1.3 FACT Accreditation for Cord Blood Banks

Cord blood banks that collect units for unrelated donor use, for directed use by the donor or donor's family, or both types of units are eligible for accreditation. The current edition of FACT-NetCord Standards differentiates the requirements for any model. However, to be eligible, the bank must assume responsibility for all aspects of cord blood banking, including advertisements, maternal donor recruitment, collection, transportation, shipping, processing, storage, listing, release for administration, and recipient follow-up. Services can be done internally or may be contracted, but the bank retains responsibility. There is no separate accreditation for laboratory only in cord blood banking. All facilities of the bank will be inspected. A percentage of fixed and non-fixed collection sites will be seen at each visit. Collection sites to be visited are chosen to be representative of each variable in the collection process, including collection method (ex utero vs. in utero); type of collector (midwife, physician, bank employee, hospital employee); distance from the processing facility, bank, and/or intermediate storage facility if applicable; and the mode of transport or shipping (staff delivery, parent delivery, courier, express shipment). The accreditation process allows for various bank structures and processes to be used to meet the Standards. Non-fixed sites that collect fewer than 50 units per year for a specific bank will not be visited in person, although data may be assessed.

5.1.4 Accreditation Under FACT Common Standards

FACT Common Standards apply to any cell type or source. Accreditation under the Common Standards only applies to those cell types and sources not included under the more specific standards. Accreditation is currently only available in North America. The application must be specifically approved by the FACT Board of Directors, which determines the information to be required. Eight facilities are currently accredited under FACT Common Standards.

6 Accreditation Process

The accreditation process is completed in the FACT Accreditation portal and consists of the following steps according to the timeline shown in Fig. 2:

- *Eligibility Application*: A demographic application describing the applicant organization, staff, locations, and services that is submitted after an organization has determined it meets the criteria and has the commitment to pursue accreditation.
- *FACT review*: Accreditation Coordinator reviews the Eligibility Application for completeness and documentation of appropriate volumes of procedures or patients treated. Coordinator creates a customized Compliance Application based on the applicant's activities and services.
- *Compliance Application*: This is a checklist of questions pertaining to each standard and constitutes the document the inspectors will use to assess the applicant at the on-site inspection. Some of the standards require that documents such as credentials, SOPs, licenses, or policies submitted to FACT. Guidance related to each standard is readily available on the application to assist the applicant. New applicants are allowed 12 months to complete this phase since time may be required to create required processes and SOPs. Compliance applications are submitted to FACT when the applicant is confident that all standards have been met.
- *Preparation for on-site inspection*: The FACT Coordinator reviews the submitted documents and application. Additional information is requested via the online portal if necessary, and applicants are informed of any issue that may not be complete. The inspection team is selected and confirmed.

The date of the inspection is selected by the applicant to be a date when all key personnel and program sites will be available to participate. The inspection team is selected from the available inspectorate based upon the size and complexity of the

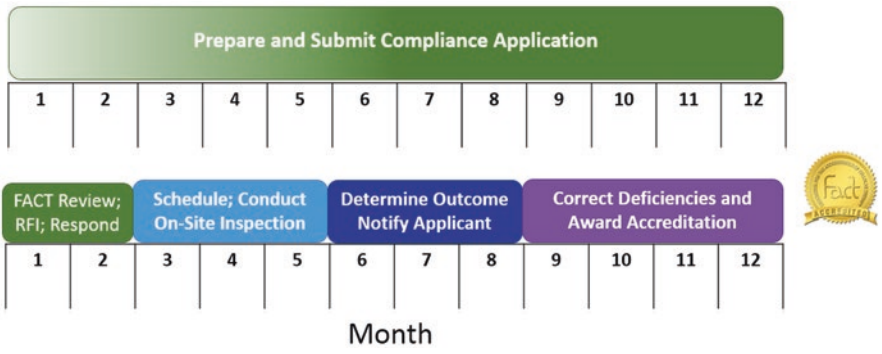

Fig. 2 Timeline for initial FACT accreditation
Applicants may choose to proceed more rapidly

applicant program, assuring that members of the team have the training and experience necessary to assess all HCT activities. Inspectors may be replaced if the inspector or applicant perceives a potential conflict of interest. When the assignment has been confirmed, the inspectors are given access to the applicant's online Compliance Application and all uploaded documents that serve to verify compliance with the Standards. The inspection team has a minimum of 3–4 weeks to review the application and related documents, to request additional information if needed, and meet by teleconference to prepare for the inspection.

- *On-site inspection*: The on-site inspection is completed in 1–2 days, starting with introductions and ending with a summation of major observations, but not a final determination of accreditation status. The on-site inspection is intended to be educational for both inspector and applicant as there are many ways to meet a Standard. Observations made at the on-site inspection are recorded electronically on the Compliance Application. This checklist methodology is effective in focusing the content of the inspection on the Standards and in promoting thoroughness and consistency among inspectors and inspections. The inspector determines whether the applicant program is compliant or noncompliant with each Standard. If noncompliant, there are two options:
  - A deficiency is any observation that does not comply with a mandatory requirement, stated in the Standards as "shall." It is also referred to as a citation.
  - A variance from recommendation is the failure to comply with a standard stated as "should." A variance requires explanation but no change in practice is required.

Each inspector submits a report of the area she or he inspected, and the Team Leader completes the final report. Reports are summarized by the Accreditation Coordinator and expected responses added to each citation in a report for committee review.

Important information is added as applicable from two FACT Committees whose works enhances the depth of the accreditation review and promotes assistance provided to programs. The Clinical Outcomes Committee assesses program that fail to meet expected 1-year post-transplant survival. Accreditation can be renewed if the program is adequately addressing the root causes of decreased survival and implementing the corrective action plans to improve [17]. The Data Audit Committee assesses programs that fail to meet the data accuracy benchmark of ≤3% critical field error rate as determined by the periodic CIBMTR Data Audit. The Committee assists programs in root cause analysis and implementation of corrective action plans and provides this added information to the Accreditation Committee. Continued accreditation is at risk if a program fails two successive CIBMTR audits.

- *Accreditation Committee review*: The committee verifies the consistency of the citations and determines next steps in the process. The potential next steps are:

  - No citations noted. Program is awarded accreditation.
  - Few citations. Program will be accredited following correction of deficiencies and submission of documentation to the FACT Accreditation Office.
  - Larger number of citations and/or more significance severity of deficiencies: The applicant program must submit documentation of correction of all deficiencies and implementation of any new processes as required. The Accreditation Committee will review adequacy of responses before accreditation is awarded.
  - More significant deficiencies in one or more parts of the program: The program must submit documentation of correction of all deficiencies. Verification of corrections and compliance with Standards will occur via a reinspection on-site of all or a portion of the applicant program.

- *Accreditation Awarded*: Accreditation is awarded after deficiencies have been corrected and applicable conditions listed above have been satisfied. Accreditation is valid for 3 years. Program personnel are notified, and all accredited programs are listed with the accredited services on the FACT website at www.factwebsite.org and announced in the newsletter.

  - *Annual report*: Each accredited program and facility reports annually on the number of patients treated and on any significant changes in location, personnel, or services. In addition, programs may be asked to document compliance with specific Standards that are new and problematic or were cited at the prior inspection.
  - *Accreditation renewal*: Programs are expected to have completed the renewal process prior to the expiration date of the prior accreditation. Approximately 14 months before the expiration date, the Program personnel will be notified to begin the renewal process which is essentially identical to the initial accreditation. The Standards applicable is the edition current on the day of the on-site inspection.

7 Current Accreditation Statistics

Currently, there are 291 FACT-accredited facilities worldwide. This includes 234 facilities accredited for cellular therapy in the USA, Canada, Mexico, Brazil, Australia, New Zealand, and Singapore. There are 184 unique clinical programs, of which 89 are also accredited for immune effector cell therapies with additional applications pending. There is also one accredited stand-alone immune effector cell program. In North America, this represents over 90% of eligible HCT programs. Forty-four of the laboratories are accredited for more than minimal manipulation processing. Fifty-four cord blood banks in 27 countries are FACT-accredited. The FACT website is the official listing of currently accredited facilities and the services for which they are accredited.

FACT volunteer inspectors are active in the field of hematopoietic cell transplantation and cellular therapy who meet additional requirements detailed at www.factwebsite.org. Importantly, all inspectors are affiliated with a FACT- or JACIE-accredited program or cord blood bank, making the on-site inspection a peer-to-peer educational experience for both inspector and the applicant. There are over 225 active FACT inspectors.

8 Common Citations

The initial inspection summaries of the 145 programs or facilities inspected under the first edition of the FACT Standards (1996) were reviewed to determine the most frequently cited deficiencies and variances from recommendation. The results from the first 76 programs have been published [19]. Results were recently updated and further for apheresis centers [20, 21]. Similar to the results observed by JACIE [7], the results of on-site FACT inspections demonstrate that most programs are functioning at a high level of quality and have addressed most of the Standards. Deficiencies observed often represent failure to completely address a Standard or to update practice based upon a new requirement in a new edition of Standards.

It is, however, unusual for a program to have no deficiencies observed on-site, even when the program has been continuously accredited for many years [22]. Potential explanations for the continued frequency of these deficiencies include the large number of standards, new requirements in each edition of Standards, and complex processes within each Standard. In addition, inspectors have improved, both in their understanding of Standards requirements and in the quality they expect to see in the applicant program [22].

The most commonly observed deficiencies have remained relatively constant over time and across editions of Standards. An assessment of deficiencies related to the third and fourth editions of FACT-JACIE Standards revealed that the total numbers of deficiencies were decreased between the third and fourth editions, despite the increase in the total number of standards; however, the areas in which deficiencies were documented were similar. The top issues were quality management (QM), policies and procedures, donor selection, evaluation, management, and labeling, cited across all areas of the HCT program. Deficiencies represented a failure to perform specific required activities or failure to describe the ongoing quality activities in the QM plan. Standard operating procedures deficiencies included deficiencies both in format and content.

Quality management deficiencies were also most commonly seen in inspections under the fifth and sixth editions of FACT-JACIE Standards. In 245 reports reviewed by the Accreditation Committee covering the sixth edition (2015), there were 12.5 citations on average per report. The marrow collection service accounted for the smallest number of citations. The remainder were approximately equally distributed among clinical, aphaeresis collection, and laboratory processing. Of these citations, 31–40% were related to the quality management Standards. With introduction of the

immune effector cell standards into the hematopoietic cell therapy standards with Edition 6.1 of the FACT-JACIE International Standards for Hematopoietic Cellular Therapy Product Collection, Processing, and Administration (2017) and the seventh edition (2018), many citations initially were related to full integration of these new requirements into the existing quality management structure of the program, particularly related to outcome analysis, adverse events, and audits specifically related to these therapies.

9 International Expansion of Accreditation

FACT has an international presence, demonstrated in accredited hematopoietic cell transplantation, immune effector cell therapies, and cord blood bank accreditation in 27 countries. In addition to the traditional accreditation pathway described above, there are three initiatives that represent alternatives in specific regions.

9.1 FACT-JACIE International Stepwise Accreditation

Increasing worldwide interest in hematopoietic cell transplantation accreditation prompted FACT and JACIE to collaborate with the Latin American Bone Marrow Transplantation Group (LABMT) to develop and administer the Stepwise Accreditation Program. Initial efforts focused on educational outreach sessions to convey the benefits of accreditation and explain the requirements of the Standards at various transplant society meetings in Argentina, Brazil, Chile, Mexico, and Peru. Similarly, JACIE participated in WBMT workshops in Latin America. This collaboration is designed to reduce confusion inherent in two accrediting bodies utilizing one set of Standards.

The mission of FACT-JACIE International program is to improve the quality and safety of bone marrow transplantation in lower-middle-income economies through compliance with established standards, creating a framework for quality management, education, and training. The stepwise process allows programs additional time and preparation by providing the option to achieve full accreditation in three incremental steps over 6 years. The Standards were divided into three levels, from relatively basic, easier to achieve Standards to the most advanced Standards, with continuous improvement built upon the foundation of quality management. The process is administered in a combination of Spanish and English; the on-site inspections are conducted predominantly in Spanish, the language of the applicants. The process includes an initial application, submission of the compliance checklist for the first level, an on-site inspection, review by the FACT-JACIE International Accreditation Committee, determination of the deficiencies to be corrected, and correction of those deficiencies. The applicant program will be certified for Level One. Each successive level will follow the same process and require up to 2 years,

awarding a certificate at the completion of each level. The sixth edition of Standards (2015) was used for this process, and there are no IEC Standards currently included in the stepwise process. A program accredited in the stepwise process would apply for IEC add-on after achieving full accreditation, i.e., in 6 years if the program took the full time allowed to complete the three steps. Programs can choose to proceed more rapidly. The stepwise process will be updated to new editions of Standards as these are available; however, each program will be allowed to complete a step in the same edition of Standards as it started.

Currently, there are fifteen programs involved at various stages in the stepwise accreditation process.

9.1.1 FACT-SBTMO Accreditation in Brazil

The FACT-JACIE International Stepwise Accreditation is primarily tailored to Spanish-speaking countries in Latin America. The Sociedade Brasileira de Transplante de Medula Óssea (SBTMO) invited FACT to develop a joint FACT-SBTMO accreditation, offering education, training, and accreditation to its Portuguese-speaking colleagues and society members in Brazil. The program was initiated in 2018, with translation of the Standards into Portuguese and FACT participation in the SBTMO annual meeting for workshops and other education. The goals of the collaboration are:

- To enhance efforts to improve quality and patient safety at cellular therapy programs, clinical units, apheresis centers, and processing laboratories
- To increase access for patients to internationally accredited blood and marrow transplant programs in Brazil
- To provide an accreditation program at an affordable cost

Initial efforts have been focused on basic education and quality management training for centers in Brazil. The educational program will offer in-person workshops and online resources to assist programs in implementation of the quality management systems, focusing on establishment and structuring of the quality management program and quality assessment activities.

One program in Brazil was FACT-accredited prior to initiation of this program. A survey has revealed that at least 25 additional programs are interested in pursuing FACT accreditation. Initial applications are being submitted. The program is planned to follow the current FACT process for full accreditation. Following submission of the Eligibility Application, each organization is assigned a FACT and SBTMO coordinator to assist with questions and completion of the Compliance Application. While inspections will be administered and coordinated in English, the on-site inspections will be conducted by a combination of English and Portuguese-speaking inspectors. Results of the on-site inspection will be reviewed by the joint FACT-SBTMO Accreditation Committee. Responses to deficiencies will also be reviewed by the FACT-SBTMO Accreditation Committee and approved by both the FACT and SBTMO Boards of Directors.

9.1.2 FACT-India Working Group

The FACT Global Affairs Committee has also conducted educational activities in association with the Asia-Pacific Blood and Marrow Transplantation Group and the Indian Society of Haematology and Blood Transfusion Haematocon meetings. As a result, the India Working Group of the FACT Global Affairs Committee was established to assist blood and marrow transplant groups in India develop quality systems and achieve FACT accreditation. Representatives of 10 transplant groups have expressed interest and have received copies of the FACT Quality Manual and access to online education. These programs are completing their internal self-assessments of readiness to apply for FACT accreditation.

10 Conclusions: Significance of FACT Accreditation

FACT accreditation helps a cancer program attain its ranking among America's Best Hospitals, published by *U.S. News & World Report* [23]. Since April 2007, FACT accreditation for allogeneic HCT has been awarded one point toward best hospital status. FACT accreditation for autologous HCT only is awarded one-half point. In addition, *U.S. News & World Report* includes FACT accreditation as a factor in the selection of the Top Ten America's Best Children's Hospitals [23].

FACT-JACIE Standards have achieved international acceptance, as best demonstrated by the joint authorship and committee membership in Standards development. In Australia, the Therapeutics Good Administration has accepted the collection and laboratory standards as the regulation for the field. In the USA, cooperative clinical trial groups require institutions entering patients on HCT trials to have FACT accreditation. Most insurance companies require HCT programs to disclose FACT accreditation status as part of the application for Center of Excellence designation. As CAR-T cell products have achieved licensure in the USA, manufacturers have required that these products be administered in a FACT-accredited clinical program.

In 2009, following the recommendation of the Advisory Council on Blood Stem Cell Transplantation (ACBSCT), the US Health Resources and Services Administration (HRSA) recognized FACT as an organization to accredit cord blood banks participating in the National Cord Blood Inventory program. HRSA and FACT work collaboratively to ensure that cord blood banks accredited by FACT and holding NCBI contracts maintain high-quality operations that are compliant with established accreditation standards and NCBI requirements throughout the accreditation period.

Beginning in 2010, cellular therapy programs that have achieved 10 years of continuous accreditation are periodically surveyed about the impact of FACT accreditation on their program and associated challenges. To date, respondents have unanimously agreed that FACT accreditation has positively impacted the quality within their programs. Peer perception, attitude of the affiliated institutions toward

the program, insurance reimbursements, number of transplants performed, and participation in clinical trials were also identified as having been positively impacted. Challenges identified, including time and resources required to maintain accreditation, keeping current with the standards, and reviewing, revising, and organizing documents, provide valuable feedback to FACT in development of future educational opportunities, tips, and tools to assist programs in compliance. Peer-to-peer advising and a consulting service are newly launched initiatives based on the needs identified by applicant and accredited programs.

Further, data from JACIE-accredited centers suggested that clinical outcome is improved when HCT is performed in an accredited program [23, 24]. Additionally, significant improvements in donor care, including consenting and follow-up, were seen after introduction and implementation of FACT-JACIE Standards fourth edition in 2011 [25].

References

1. Warkentin, P. I., & Shpall, E. J. (2016). Hematopoietic cell procurement, processing, and transplantation: Standards, accreditation, and regulation. Chapter 36. In S. J. Forman, R. S. Negrin, J. H. Antin, & F. R. Appelbaum (Eds.), *Thomas' hematopoietic cell transplantation* (5th ed., pp. 414–424). Wiley Blackwell & Sons Ltd.
2. Foundation for the Accreditation of Hematopoietic Cell Therapy. (1996). *Standards for hematopoietic progenitor cell collection, processing & transplantation* (1st ed.). Foundation for the Accreditation of Hematopoietic Cell Therapy.
3. Kvalheim, G., Urbano-Ispizua, A., & Gratwohl, A. (2000). FAHCT–JACIE Workshop on Accreditation for blood and marrow progenitor cell processing, collection and transplantation. Barcelona, Spain. *Cytotherapy, 2*, 223–224.
4. Joint Accreditation Committee of ISHAGE-Europe and EBMT. (2000). Standards for blood and marrow progenitor cell processing, collection and transplantation. *Cytotherapy, 2*, 225–246.
5. Foundation for the Accreditation of Cellular Therapy and Joint Accreditation Committee of EBMT and Euro-ISCT. (2018). *FACT-JACIE international standards for cellular therapy product collection, processing, and administration* (7th ed.). FACT and JACIE.
6. Samson, D., Slaper-Cortenbach, I., Pamphilon, D., et al. (2007). Current status of JACIE accreditation in Europe: a special report from the Joint Accreditation Committee of the ISCT and the EBMT (JACIE). *Bone Marrow Transplantation, 39*, 133–141.
7. NetCord, Foundation for the Accreditation of Cellular Therapy. (2002). *International Standards for Cord Blood Collection, Processing, Testing, Banking, Selection and Release* (2nd ed.). NetCord and the Foundation for the Accreditation of Cellular Therapy.
8. Foundation for the Accreditation of Cellular Therapy. (2019). *NetCord-FACT international standards for cord blood collection, processing, testing, banking, selection, and release* (7th ed.). Foundation for the Accreditation of Cellular Therapy.
9. Foundation for the Accreditation of Cellular Therapy. (2015). *FACT common standards for cellular therapies* (1st ed.). Foundation for the Accreditation of Cellular Therapy.
10. Foundation for the Accreditation of Cellular Therapy. (2017). *FACT standards for immune effector cells* (1st ed.). Foundation for the Accreditation of Cellular Therapy.
11. Maus, M. V., & Nikiforow, S. (2017). The why, what, and how of the new FACT standards for immune effector cells. *Journal for Immunotherapy of Cancer, 5*, 36–40.

12. Foundation for the Accreditation of Cellular Therapy. (2017). *FACT-JACIE international standards for hematopoietic cell therapy product collection, processing, and administration* (Edition 6.1). Foundation for the Accreditation of Cellular Therapy.
13. Ashford, P., Distler, P., Gee, A., et al. (2007). Standards for the terminology and labeling of cellular therapy products. *Transfusion, 47*, 1319–1327.
14. Gratwohl, A., Brand, R., Niederwieser, D., et al. (2011). Introduction of a quality management system and outcome after hematopoietic stem cell transplantation. *Journal of Clinical Oncology, 29*, 1980–1986.
15. Foundation for the Accreditation of Cellular Therapy. (2015). *FACT-JACIE international standards for hematopoietic cell therapy product collection, processing, and administration* (6th ed.). Foundation for the Accreditation of Cellular Therapy.
16. LeMaistre, C. F., Wacker, K. K., Akard, L. P., et al. (2019). Integration of publicly reported center outcomes into standards and accreditation: The FACT model. *Biology of Blood and Marrow Transplantation, 25*, 2243–2250.
17. Center for Blood and Marrow Transplant Research (CIBMTR). Stem Cell Therapeutic Outcomes Database (SCTOD). Last updated April 2020. Available at: https://www.cibmtr.org/About/WhatWeDo/SCTOD/Pages/index.aspx. Last accessed 13 Aug 2020.
18. Warkentin, P. I., Nick, L., & Shpall, E. J. (2000). FAHCT Accreditation: common deficiencies during on-site inspections. *Cytotherapy, 2*, 213–220.
19. Schwartz, J., Keever-Taylor, C., Gastineau, D., et al. (2014). Common citations during FACT inspections – Comparison of third and fourth editions of the cellular therapy standards. *Cytotherapy, 16*, S43–S44.
20. Gastineau, D. A., & Wacker, K. K. (2020). FACT-JACIE standards: common citations and how best to avoid them. Chapter 3. In S. A. Abutalib, A. Padmanabhan, H. P. Pham, & N. Worel (Eds.), *Best practices of apheresis in hematopoietic cell transplantation* (Advances in hematopoietic transplantation and cell therapy) (pp. 11–21). Springer Nature.
21. Caunday, O., Agulles, O., McGrath, E., et al. (2013). Implementation of JACIE accreditation results in the establishment of new indicators that unevenly monitor processes contributing to the delivery of hematopoietic SCT. *Bone Marrow Transplantation, 48*, 604–609.
22. Comarow, A. (2007). *What it takes to be the best*. US News and world Report, 23–30 July 2007, pp. 90–94
23. Chabannon, C., Pamphilon, D., Vermylen, C., et al. (2012). Ten years after the first inspection of a candidate European centre, an EBMT registry analysis suggests that clinical outcome is improved when hematopoietic SCT is performed in a JACIE-accredited program. *Bone Marrow Transplantation, 47*, 15–17.
24. Anthias, C., Ethell, M. E., Potter, M. N., et al. (2015). The impact of improved JACIE standards on the care of related BM and PBSC donors. *Bone Marrow Transplantation, 50*, 244–247.
25. https://www.ebmt.org/jacie-accreditedcentres

AABB Cell Therapy Standards

B. C. Alder

1 AABB: History and Evolution

On November 17, 1947, an executive session of the Blood Bank Institute was convened in response to a request, signed by 67 attendees that urged the Institute to consider the formation of an American Association of Blood Banks (AABB), now known only as AABB. The meeting in Texas resulted in the establishment of a committee, charged with oversight and organizational planning for the soon-to-be formed association.

In the preceding years, the science and practice of transfusion medicine had advanced dramatically. During the Second World War, the US military oversaw the creation of the largest chain of distribution for plasma and other blood components. Attacks on civilians in London placed new and unique strains on civilian blood banks. These attacks helped physicians to appreciate the complexities of the vascular system and the limitations of transfusion, when patients expired, in spite of multiple transfusions.

In the immediate aftermath of the war, blood banking was developing as a medical specialty area. American blood banks that had sprung up during the war were seeking guidance and partnerships from senior professionals in the field. The Blood Bank Institute was the name given to the first meeting of blood banking professionals in 1947. The topics, even then, were familiar and prescient. They included techniques for antigen and antibody determinations and discussions on a nomenclature

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system for antigens. Administrative topics included public relations, hospital-transfusion service relationships, and personnel training.

Another event lending poignancy to the formation of AABB was the Texas City freighter explosion of 1947, an event that at an early time underscored the critical role that a robust healthcare system can play during a calamity [1].

2 Development and Evolution of Standards

In 1958, AABB published Standards for a Blood Transfusion Service, and an independent accreditation program was developed. In 1960, the establishment of the AABB Committee on Inspection and Accreditation represented the official separation between the committees responsible for standards-setting and for inspection program overview. This delineation still exists within AABB.

While initially geared toward education, the standards and accreditation programs evolved continuously, a reflection of the growing challenges and complexities of modern healthcare. By keeping pace with these new challenges, AABB developed a history of helping regulated facilities meet federal, state, and local requirements.

During the late 1980s, the United States Food and Drug Administration's (FDA) application of current Good Manufacturing Practice (cGMP) regulations to blood banks, together with the enactment of Clinical Laboratory Improvement Amendments (CLIA) 1988, requirements in the early 1990s, increased the extent of regulatory oversight of blood banks and resulted in increased cost pressures associated with bank operations. In 1991, AABB's standards-setting philosophy moved toward universal quality management principles based on internationally accepted standards for quality systems. The idea of applying quality management systems business models to the clinical setting was unique at the time and pre-dated by many years the widespread application of the philosophy of quality management to healthcare – a model subsequently embraced by many regulatory and accrediting bodies [2].

The original program designed for blood centers and hospital-based blood banks, and transfusion services grew to encompass standards in five different disciplines, including cellular therapy product services (Table 1). Since 1997, a discipline-specific Standards Committee (SC), acting under an interdisciplinary umbrella committee, called the Standards Program Committee (SPC), has developed each set of Standards. The SPC also includes expertise of a Quality Management Subcommittee, the group responsible for ensuring consistency in quality management concepts across different disciplines. All individuals serving on these committees are volunteers who are active within their fields. These volunteers serve as technical experts, liaisons from other AABB committees, and representatives from other organizations.

The Standards requirements are based on medical practice standard of care, scientific data, and principles associated with good manufacturing practices and

Table 1 From 1991 to 2021: History of AABB involvement in cellular therapy through Standards-setting

Year	Event
1991	Standards for Blood Banks and Transfusion Services 14th edition addresses bone marrow and peripheral blood progenitor cells in dedicated chapter. Chapter includes definition, donor selection, preparation/processing, sterility, and storage.
1994	AABB convenes North American Task Force for the Development of Standards for Hematopoietic Progenitor Cell Transplantation, an inter-organizational task force.
1995	Stand-alone Standards for Bone Marrow and Peripheral Blood Progenitor Cells published (excerpted from 16th edition of Standards for Blood Banks and Transfusion Services).
1996	Standards for Hematopoietic Progenitor Cells published (includes section on quality management).
1997	AABB Quality System Essentials (introduced in Association Bulletin #97-4) published and implemented by accredited facilities.
March 2000	2nd edition of Standards for Hematopoietic Progenitor Cell Services becomes effective.
October 2001	1st edition of Standards for Cord Blood Services becomes effective.
May 2002	3rd edition of Standards for Hematopoietic Progenitor Cell and Cellular Product Services becomes effective.
May 2005	1st edition of newly consolidated Standards for Cellular Therapy Product Services becomes effective. The publication encompasses cord blood products, HPCs, and other somatic cells procured from living and cadaveric donors.
March 2007	2nd edition of Standards for Cellular Therapy Product Services becomes effective. This edition began incorporation of references to regulatory resources and expanded donor eligibility requirements.
September 2008	3rd edition of Standards for Cellular Therapy Product Services becomes effective. This edition harmonized with other standards-setting organizations and regulatory agencies requirements. This was the first edition requiring the use of ISBT 128 terminology for labeling.
March 2010	4th edition of Standards for Cellular Therapy Product Services becomes effective. This edition included incorporating regulatory requirements directly into the standards, rather than just referencing them.
September 2011	5th edition of Standards for Cellular Therapy Product Services becomes effective. This edition implemented efforts to prevent conflicts between standards and regulations of competent authorities (such as US FDA) and incorporated current Good Tissue Practices.
July2013	6th edition of Standards for Cellular Therapy Services becomes effective. The word "Product" dropped from the title, and this edition increased the scope to include standards specific in the area of clinical careand novel and regenerative cellular therapies.
July 2015	7th edition of Standards for Cellular Therapy Services becomes effective. This was the first edition of AABB Standards that became available in the Standards Portal on the AABB website.
July 2017	8th edition of Standards for Cellular Therapy Services becomes effective. This edition expanded requirements to address relevant emerging infectious diseases (due to the Zika virus outbreak) and mandated implementation of full compliance with ISBT 128 labeling.

(continued)

Table 1 (continued)

Year	Event
July 2019	9th edition of Standards for Cellular Therapy Services becomes effective. This edition expanded requirements more specific for Lab and Medical Directors and clarification on their relevant experience. It added Operational Continuity to ensure critical functions continue in all situations and to audit adverse events and complications attributed to procurement and processing. This edition clarified to maintain personnel records for individuals performing critical tasks, to obtain medical orders for distribution, and to control storage areas for temperature and humidity – recording date/time of cryopreservation – and expanded for oxygen monitoring and responding to alarms. ISBT labeling clarified to include Eurocode and should be included on apheresis and marrow products at procurement and at completion of processing.
July 2021	10th edition of Standards for Cellular Therapy Services, scheduled effective date.

quality assurance and applicable regulations. The Standards describe the minimum acceptable requirements for facilities providing these services and may or may not be more stringent than local requirements. When possible, the standards are written to be consistent with the requirements of other standards- setting and accrediting bodies and to recognize regulatory environments different from that of the United States. AABB Standards combine internationally accepted quality management system requirements with relevant technical requirements for each discipline. As such, the Standards can serve as the basis for accreditation anywhere in the world. While some requirements are based on the US FDA's regulations, a committee with international expertise can review requests for variance that involve a departure from US public health priorities.

Any individual AABB member can apply to serve on an SC at any point during the year. Terms of service last for a minimum of one edition of Standards and a maximum of two editions. Committee membership is determined once every 2 years for each edition of Standards and approved by the current or incoming chair of the SC and the AABB Board of Directors.

Each set of Standards is revised on a defined cycle, every 24 months. The Standards for Cellular Therapy Services since the sixth edition are published on a 24-month cycle (Table 1). The ninth edition was effective July 1, 2019, and the tenth edition is schedule to become effective July 1, 2021.

3 Quality Systems Approach to Cellular Therapies

The AABB approach to the field of cellular therapies has aimed to balance flexibility in an outcome-based approach with the need for rigorous evidence-based standards. This approach was formalized in the second edition of Standards for Hematopoietic Progenitor Cell Services (2000) by the use of a quality template. The template, originally designed for consistency with International Standards Organization (ISO) 9000:1994, was also used in the third edition of the Standards

for Hematopoietic Progenitor Cell Services (2002) and in the first edition of Standards for Cord Blood Services (2001).

In 2002, the AABB Board of Directors approved a proposal to consolidate hematopoietic progenitor cells (HPC) and cord blood requirements into a single publication, alongside new requirements for somatic cells such as pancreatic islets and donor lymphocytes. The cellular therapy (CT) SC sought to streamline the formatting of the document, to ensure that product-specific content could be appropriately stratified in an intuitive way. The CT SC recognized that that other AABB Standards Committees relied on the ten-chapter template instead of the twenty chapters that formed the basis for the HPC and Cord Blood Standards and elected to revise the format accordingly. The ten-chapter headings are based on the AABB Quality System Essentials (QSEs), published in 1997 as AABB Association Bulletin #97-4. The 10 QSEs correlate directly with ISO.

Under a quality management system approach, each chapter progresses from general policies to specific procedures. For example, the "Process Control" chapter encompasses most of the work associated with procurement, processing, storing, and distribution of the cellular therapy product. It opens with broad statements requiring that a facility have policies, processes, and procedures to control the work performed. The chapter then addresses several aspects of process control that apply throughout the chain of work, such as change control and process validation, sterility and operational controls, in-process and final inspection of products, and identification and traceability of materials and products. The technical standards then follow a cascading pattern, according to the type of donor or type of product collected. For example, Standard 5.0 addresses general process control requirements. Standard 5.12 follows the general process control requirements and begins the "workflow" section by addressing the determination of donor eligibility. It includes general requirements that apply to all products and all donors, such as confidentiality of the process, donor advocacy, and education. These general donor eligibility standards are followed by more detailed requirements specific to the type of human cells, tissues, or cellular or tissue–based products (HCT/P) donated. Other activities that are covered incorporate suitability determination, clinical evaluation, and testing and screening of donors. Finally, Standard 5.12 and its sub-standards further require that donor qualification be performed and completed in accordance with specific "reference standards." These appear at the end of Chapter 5 and are numbered in a way that links the reader back to the body of Chapter 5. In the example given, since the reference standards are cited in Standard 5.12.1, they appear as Reference Standards 5.12A through 5.12E. These Reference Standards contain the most detailed requirements for donor education, determination of eligibility and suitability, and clinical evaluation and testing of living allogeneic and autologous donors and for cadaveric donors. Additionally, the 5.12 Reference Standards include the qualification of maternal cord blood donors, including the health history risk screening, risk factors for relevant communicable diseases, and screening for a family history of genetic disorders that might affect the therapeutic value of the product [3].

4 Transparency in Standards-Setting

The CT SC deliberates over every requirement in the Standards for Cellular Therapy Services. This process is summarized in Fig. 1.

This deliberative process occurs before a draft is made available to the public for a 60-day comment period. The CT SC then reconvenes to discuss the comments submitted and to determine whether additional changes are required or whether proposed changes should be rescinded. The comment period is an integral part of the process, as it affords the CT SC the opportunity to obtain external feedback in an effort to identify logistical challenges that the CT SC may not have foreseen. It is a vital part of the dialogue between the accreditor and the accreditee and helps to promote transparency in standards-setting. Figure 2 provides an example of how a standard can be developed as a result of public comments.

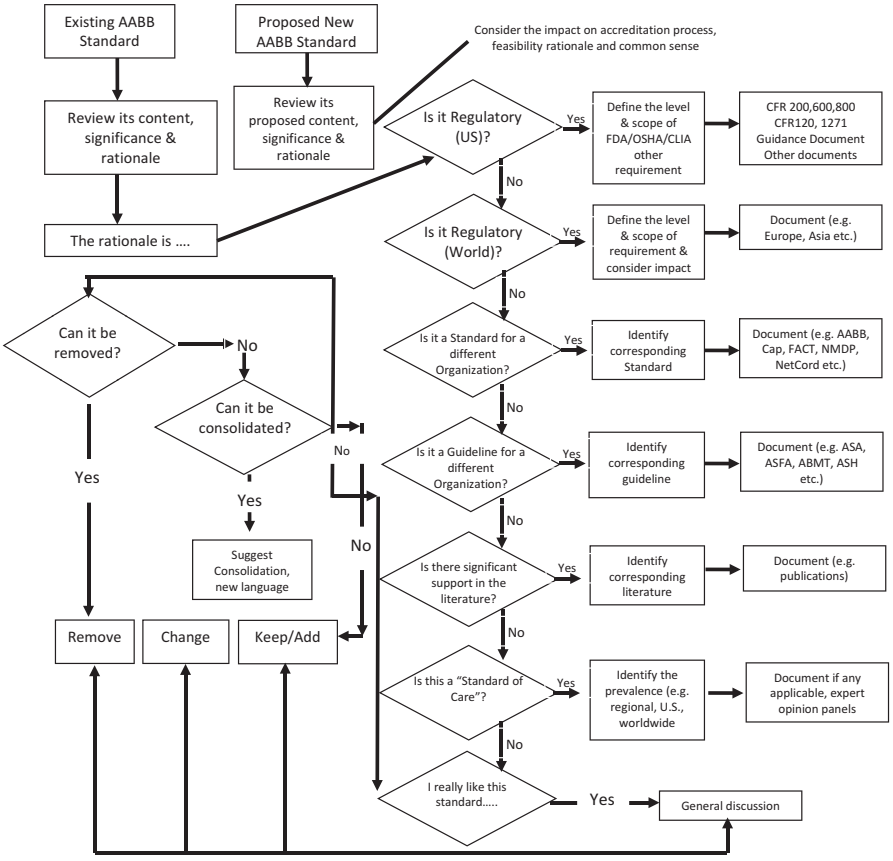

Fig. 1 The Process of review, change, and approval of technical standards within the Standards

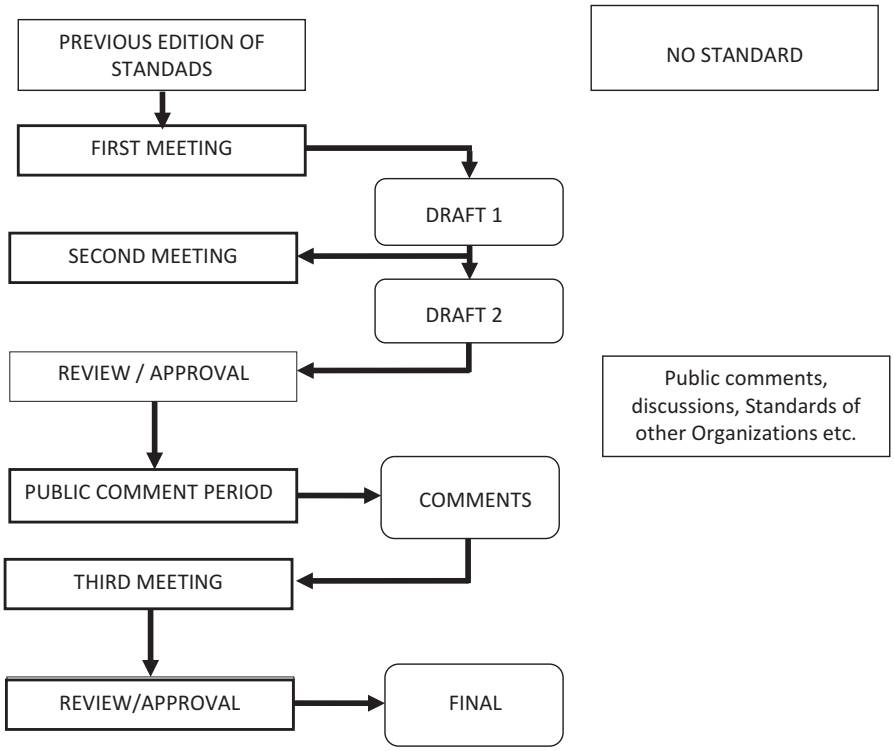

Fig. 2 An Example from *Standards for Cellular Therapy Services* of standards evolution based on the available data and public comments

The process used for developing AABB Standards is designed to ensure compliance with antitrust laws. In addition, representatives from external organizations (such as other standards-setting bodies and the FDA) promote consistency with laws and voluntary requirements. External feedback may also include requests for clarification of a standard, requests for variance from a standard, and reports from the accreditation program on frequent nonconformance from exiting standards. The CT SC relies on these reports to identify areas that may need revision.

In addition to external feedback and reviews, AABB Standards are also reviewed internally for technical accuracy, as well as for legal and regulatory compliance.

The Standards are found on the AABB website in the Standards Portal, which is also where the Guidance and crosswalk between editions are located.

A description of the makeup of the CT SC, who worked on the ninth edition of CT Standards, is presented in Table 2.

Table 2 Organizations and expertise represented on the cellular therapy standards program unit that participated in writing the AABB Standards

Members with expertise in the field of cellular therapy (e.g., donor evaluation, collection, processing, transplantation)
Public member (elected)
FDA liaison(s)
Liaisons from other AABB committees (CT Program Accreditation Unit, Information Systems Committee, Quality Management Subcommittee)
Liaisons from other organizations (e.g., AATB, ACOG, ASFA, FACT, ISCT, NMDP, State of California, CAP, Health Canada, Canadian Blood Services, and Armed Services Blood Program Office (DoD))
AABB BOD representative
Consultants (as deemed necessary)

AABB provides representatives to the Alliance for the Harmonization of Cellular Therapy Accreditation (AHCTA) (www.ahcta.org)

5 Assessing Conformance to Standards

The AABB Accreditation programhas evolved in tandem with the Standards. While education has always been a component of the accreditation process, the ultimate goal of the program is to ensure that AABB-accredited facilities conform to AABB Standards, which, in turn, are developed with the goal of promoting optimal donor care, product handling, and patient treatment.

In the late 1990s, the AABB Standards and Accreditation programs underwent considerable change. The primary goal was to ensure that Standards would focus on endpoints and objectives and contain only requirements, and only the requirements published in Standards are used as the basis for accreditation decisions.

Guidance, or recommendations, on how to achieve those objectives, is available in the Standards Portal on the AABB website, next to the standard to which the guidance relates.

6 Accreditation

The AABB Accreditation program is internationally recognized as a symbol of quality. All policies, processes, procedures, and forms associated with AABB accreditation activities are documented in the Accreditation Information Manual and on the AABB website (http://www.aabb.org/sa/tools/aim/Pages/default.aspx). AABB membership is required for accessing this information on the website.

AABB believes that it serves the best interests of patients and donors to extend the requirements of accreditation to as many facilities as possible. Consequently, the staff work very closely with facilities to help them achieve and maintain their accreditation. Accreditation is granted for a 2-year period. There are a number of events that could trigger a loss of accreditation or a mandatory re-assessment of the

facility. These are defined in the Accreditation Program Policy Manual, a detailed information tool that facilities can use to clarify administrative issues related to their accreditation.

A lead AABB staff assessor, along with a team of volunteers, assesses facilities seeking accreditation for cellular therapy. These "on staff" individuals are full-time assessors, who provide a high level of detail and consistency across assessments. Administrative checks and balances ensure that an assessor does not visit the same facility twice in a row and that the findings of previous assessments are shared with incoming assessors in order to determine whether the root cause(s) of previous nonconformance(s) has/have been eliminated. In some cases, AABB may request proof of implementation before approving a corrective action plan. The recurrence of a previous nonconformance is an immediate decision trigger and results in a facility's status changing from "accredited" to "conditional." Facilities in conditional status are considered to be non-accredited. The facility is promptly removed from the list of accredited facilities on the AABB website. The list of facilities accredited for cord blood, HPCs, and other cellular therapies can be viewed at http://www.aabb.org/sa/facilities/Pages/default.aspx.

Starting in 2007, AABB assessments became unannounced other than a phone call 1 hour before to the AABB assessment team arrives on site. This policy ensures that facilities are always ready for an on-site assessment and decreases the perception that facilities may prepare for an assessment by rapidly bringing systems into compliance shortly before the on-site visit. The AABB team makes an unannounced visit to the facility within a 3-month window for the assessment. Implementation of this practice is designed to increase public confidence in the quality of products and services offered by AABB-accredited facilities. This practice was modified slightly for facilities that are not having a CAP-coordinated assessment or if the Joint Commission does not accredit them. In this case, the notification is given the Friday before the week of the inspection by sending an e-mail to the 3 contacts identified by the facility in the APEX portal.

7 Validation of Assessments

The accreditation program undergoes rigorous and continuous validation of assessments of every type, as AABB participates in both internal and external review. Externally, AABB findings are validated by the Centers for Medicare and Medicaid Services (CMS), because of AABB's deemed status for CLIA. CMS conducts regular validation assessments of all accrediting organizations with deemed status, and that includes the AABB. Since AABB has deemed status with CMS, the Health Resources and Service Administration (HRSA) and the State of California all conduct their own validation assessments routinely.

Internal validation of assessments is performed by AABB as part of the Accreditation Program's quality system. The AABB National Office staff conducts

validation assessments for all activities on an annual basis. These practices help both ensure process control and promote continuous improvement.

The AABB accreditation program and assessor training program are also accredited by the International Society for Quality in Healthcare (ISQua), which gives the program credibility on a global scale. These programs are surveyed every 4 years for reaccreditation.

8 AABB Cellular Therapies Certificate Program

AABB has collaborated with The George Washington University (GW) to offer biomedical healthcare professionals a certificate program in cellular therapies. The program is intended for, but not limited to, individuals with a basic biological or medical science background. The online self-paced program contains professionally narrated presentations, videos, animations, and additional reading resources. It provides 37 credit hours. Scenario-based questions are included in each of the modules to assess students' understanding of the content presented. A review of all slides in each module and a passing score of 80% for all assessments are required. The AABB Cellular Therapies Certificate Program has been recognized as an ASAE 2019 Power of A Gold Award winner, for the value it creates by being a training resource in the cellular therapy communities.

This program includes a learning module on regulations and the content of most of the standards assessed in Section 5 of the latest edition of Standards for Cellular Therapy Services (CT Standards). Completion of this certificate program can help individuals gain a better understanding of the CT Standards, especially those in Process Control Section 5.

9 Circular of Information for the Use of Cellular Therapy Products

The Circular of Information (Circular) for the Use of Cellular Therapy Products is intended to be an extension of the cellular therapy product label and is therefore included in the scope of the assessment.

The AABB Circular of Information for Cellular Therapy Products Task Force has jointly prepared the Cellular Therapy Circular of Information, which includes a collaborative group of multiple nongovernmental organizations that represent the cellular therapy field. The US FDA and HRSA also participated in the development and review process.

The Task Force intentionally limited its scope to include only minimally manipulated cellular therapy products such as peripheral blood progenitor cells, bone marrow, cord blood, and leukocytes. The group recognizes there are multiple cellular

therapy products that could not be adequately covered in the Circular. To accommodate this, the Circular includes multiple blank pages at the end of the document for each facility to add information specific for their cell therapy products that are not classified as minimally manipulated.

10 ISBT 128 Labeling of Cellular Therapy Products

In 2018, AABB mandated implementation of full compliance with ISBT 128 labeling for cell therapy products to be able become accredited and to maintain accreditation.

The goal of ISBT 128 is to globally standardize terminology, coding, and labeling for products of human origin. The standardization of terminology is the first step and is the foundation for standardizing coding and labeling. In order to standardize terminology, the International Council for Commonality in Blood Banking Automation, or ICCBBA, convenes advisory boards composed of experts from around the world. For cellular therapy, this group is the Cellular Therapy Coding and Labeling Advisory Group, or CTCLAG. Closely related to CTCLAG is the Tissue Engineered Products Technical Advisory Group, or TEPTAG, which manages codes for products that are either engineered tissues or that reside in a gray zone between cells and tissues. This group is composed of the chairs of the ICCBBA cellular therapy and tissue advisory groups and other knowledgeable individuals. These advisory groups meet regularly through conference calls and face-to-face meetings to develop new terminology and respond to user requests for additional terminology. Once the advisory groups have reached a consensus for newly developed terminology, it is released for comment. After comments have been taken into consideration, the terminology is finalized and becomes part of the ISBT 128 nomenclature for product description.

ICCBBA establishes computer codes for these product description codes and other information needed to describe products of human origin [4]. Lists of these codes are published in databases or reference tables and can be used to encode information in a variety of ways including in linear or two-dimensional bar codes on labels or electronic data exchange.

Labeling is the last stage in the development of a global standard. Languages, various standards-setting organizations, national and supranational regulations, and national preferences affect labels. Therefore, on an international level, ISBT 128 is flexible about the text that must appear on product labels. It is rigid, however, on how information must be encoded into bar codes and, with linear bar codes, the exact location of the bar codes on the label.

To provide additional guidance and standardization within a country, national consensus documents may be published. These documents blend requirements from regulatory agencies, pertinent standards-setting organizations, national preferences, and the ISBT 128 standard itself. The “United States Consensus Guidance for the Uniform Labeling of Cellular Therapy Products Using ISBT 128” is such a document.

11 Technical Highlights of Each Edition

With each edition of CT Standards, AABB prepares a document that highlights the changes to the updated edition. It will clearly state the implementation date. A summary of changes made to each edition is available on the AABB website. Additionally, a crosswalk is prepared between each edition to indicate the standards that were renumbered or added as new from one edition to the next.

The majority of technical standards are found in Chapter 5, Process Control. This section combines standards related to very general concepts (e.g., process control, clinical outcomes, and design control) with standards following the workflow from procurement of the cellular therapy product to administration. The titles of the subsections define the critical steps in this workflow.

There are a number of reference standards associated with each section. As discussed above, reference standards contain the most detailed requirements.

12 Conclusions

The overarching principle of the standards-setting process is to create a framework for provision of the highest quality product, within a well-controlled environment, while minimizing the influence of chance. However, creating the requirements for such an infrastructure must not obscure the most critical test of all – clinical outcome. For this reason, rigorous tracking, trending, and monitoring of patient outcomes is an integral part of the CT Standards. The quality of a cellular therapy product is only one of several variables affecting clinical outcome; nevertheless, it is the one variable over which the laboratory has the most control. Accordingly, accreditation of a cellular therapy program by AABB signifies that the facility has successfully implemented the systems which result in the highest quality products and, consequently, to the best possible clinical outcome [5].

References

1. Starr, D. (1998). *Blood: An epic of history and commerce*. Alfred A. Knopf.
2. AABB. (1997). *Growing, maturing, changing… A history of the American Association of Blood Banks*. AABB.
3. AABB. (2020). *Standards for blood banks and transfusion services* (32nd ed.). AABB.
4. International Council for Commonality in Blood Banking Automation Website: www.iccbba.org. Accessed 10 Sept 2020.
5. AABB. (2019). *Standards for cellular therapy services* (9th ed.). AABB.

USP Standards for Cell-Based Therapies

Fouad Atouf

1 Introduction: Why Standards for Cell-Based Therapies?

Advanced therapies are defined in the United States as gene therapies including genetically modified cells; human cells, tissues, and cellular- and tissue-based products (HCT/Ps) requiring licensure, including allogeneic cord blood units for use in stem cell transplantation; and xenotransplantation products. These are in general products that include somatic, pluripotent, gene-modified, and non-gene-modified cell therapies, as well as gene and tissue therapies. Each of these technologies holds the promise to address unmet medical needs; however, they also come with some unique scientific and regulatory challenges. Cell-based therapies tend to be less stable than traditional drugs resulting in a shorter shelf-life and storage requirements that complicate logistics solutions. Manufacturers must also manage the limited supply of critical materials. For example, viral vectors are essential for making some gene-modified therapies, but only a few companies currently have the infrastructure or expertise to make supplies for clinical trials, and they struggle to meet the increasing demand [1, 2].

Some of the challenges associated with advanced therapies can be overcome by implementing appropriate process controls at every step of the process, establishing a link between process parameters and their impact on the quality of the product to ensure that the end product meets the expected quality profile [3]. These controls allow manufacturers to maintain consistency from the qualification of raw materials to the administration of the product to patients. However, many obstacles remain, and one stands out as the most significant. In a 2014 survey of the industry by the Alliance for Regenerative Medicine (ARM), it was agreed that "Product consistency and lack of standards is possibly the single greatest challenge facing the field" [4]. The use of robust and reliable test methods to measure the critical quality

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attributes of these products is, therefore, essential for getting advanced therapies from development to market, and standardization of these methods is what will ultimately reduce variability and increase confidence in reported values.

This chapter will provide an overview of the United States Pharmacopeial Convention (USP), how its standards evolved, and how it develops standards now. We also review how USP collaborates with stakeholders to build meaningful tools that support the development and marketing of advanced therapies. For this chapter, the term cell-based therapy will be used to imply advanced therapies, as defined above. The standards-setting concepts described herein align with current regulations set forth by the US Food and Drug Administration (FDA) and supplement the approaches described by certification/accreditation bodies such as the Foundation for the Accreditation of Cellular Therapy (FACT) and the American Association of Blood Banks (AABB), as well as standards development organizations and professional societies.

2 The United States Pharmacopeial Convention (USP)

The USP is an independent, scientific, nonprofit organization founded in 1820 by 11 physicians committed to protecting patients in the United States from poor-quality medicines. In 1848, the US Congress passed the Drug Importation Act, which recognized USP standards for strength and purity. Armed with these standards, inspectors could finally identify drugs that were substandard or adulterated. Over the next half-century, the USP's standards for strength, quality, and purity became the de facto reference for defining the adulteration of drugs. When Congress incorporated the USP's standards into key provisions of the Pure Food and Drug Act of 1906, it made official what was already widely practiced. From then on, any drug marketed in the United States was legally required to meet USP standards. Then in 1938, Congress passed the Federal Food, Drug, and Cosmetic Act (FD&C Act) as a response to the more than 100 deaths resulting from poisoning by an antibiotic elixir that had never been tested for safety [5–7]. The FD&C Act further expanded the USP's role by making it the official drug compendium of the United States. Companies were now required to test all new drugs to verify they meet the USP's standards for identity, strength, quality, and purity. The USP compendial standards are continually revised to reflect scientific and technological advances, as well as to adapt to the evolving global regulatory expectations.

The USP has no role in the enforcement of the provisions that recognize its standards, however. That responsibility falls to the FDA, and through a unique partnership, the two organizations have worked together for more than 100 years to help ensure the quality of the domestic drug supply while also promoting the development of next-generation therapies. This commitment was most recently renewed in 2015 at the meeting of the USP Convention, with a resolution of the USP's commitment to align and work with the FDA to increase understanding of the regulatory impact of introducing new standards prior to their establishment.

Although the FDA is the USP's most important partner, it is not the sole stakeholder, especially in an era of globalization. The USP also engages with foreign regulatory agencies as well as with international and regional regulatory bodies such as the World Health Organization (WHO) and the International Council of Harmonization of Technical Requirements for Pharmaceutical for Human Use (ICH). The ongoing dialog with these agencies creates a framework for global regulatory convergence.

USP's partnerships are not limited to regulatory agencies. It also collaborates with a diverse group of industry representatives, academic institutions, and practitioners to evaluate the readiness of innovative technologies for adoption into current industry practices. Through this network, the USP provides the most up-to-date, tested, and trusted public standards that reduced barriers to innovation and support the uptake of innovative technologies used in over 140 countries and legally recognized in over 50 countries.

USP standards help ensure the quality of existing medicines and provide tools that facilitate the development of new medicines, thus promoting broad access to lifesaving therapies. The first standard to cover a biological medicine dates back to 1905 with the introduction of the USP's standard for diphtheria antitoxin [8], which predated the existence of regulatory pathways for licensing this type of medicine. As we will discuss later, the USP standards evolved in scope to ensure the quality of pharmaceuticals throughout the products' lifecycle and to support access to complex products, including emerging therapies such as cell, gene, and tissue therapies.

3 The USP-NF "Book" of Standards

The USP standards for medicines and their ingredients are available in the United States Pharmacopeia-National Formulary (*USP-NF*) book of standards colloquially referred to as the "*USP*." It is essential to highlight, however, that the *USP-NF* is a combination of two compendia, the *United States Pharmacopeia* (*USP*) and the *National Formulary* (*NF*). The *NF* was initially a separate compendium established in 1888 by the American Pharmacists Association (APhA). Its purpose was to provide pharmacists with formulas for small-scale compounding, and over time it grew to include standards for excipients, botanicals, and other related products. Its impact was significant enough that it, too, was included in provisions of the Pure Food and Drug Act of 1906 as well as the FD&C Act of 1938. The USP acquired the *NF* publication in 1975 and combined the two compendia into one book, the *USP-NF,* in 1980. Since 2002, the *USP-NF* has been published annually, and like most publications in the digital age, it has moved exclusively online.

The content in the *USP-NF* consists of documentary standards, which include monographs, general chapters, and General Notices and Requirement (General Notices). Documentary standards for drug substances, dosage forms, and compounded preparations are featured in the *USP* section, while documentary standards for excipients are described in the *NF* section. The only exception is if an excipient

is also used as an active pharmaceutical ingredient (API) in an FDA-approved product marketed in the United States, in which case the documentary standard appears in the USP section instead.

USP-NF monographs, general chapters, and General Notices are distinct from guidelines published by regulatory agencies such as Guidance for Industry documents issued by the FDA. Regulatory guidance provides general statements that can be applied to a wide variety of products, whereas compendial documentary standards offer more specific details that both complement and enhance regulatory guidelines. Monographs, for example, contain test specifications for specific articles (e.g., drug substance, dosage form, and excipient), while general chapters provide detailed information that can be applied across multiple articles. General chapters numbered above <1000> are informational chapters. They do not contain mandatory tests, assays, or other requirements. General chapters below <1000> include mandatory test procedures or requirements and apply to official pharmaceutical articles through reference in General Notices, a monograph, or another applicable general chapter numbered below <1000>. The General Notices contain the underlying assumptions, definitions, and default conditions for the interpretation and application of the USP-NF unless superseded by a general chapter or a monograph.

From a content standpoint and as a general approach, the required chapters tend to cover current technologies, are easy to read and execute, and have clear acceptance criteria. The informational chapters contain best practices and no acceptance criteria, discuss real-world pharmaceutical issues, and provide context for the required chapters.

An essential element of the *USP-NF* is the collection of reference standards cited in monographs and general chapters. The USP reference standards are defined as substances with appropriate qualities to support their intended use. They are highly characterized materials tested through multi-laboratory studies, and their suitability may be demonstrated to support a proper use within a documentary standard or to support other measurements not necessarily prescribed in *USP-NF*. The majority of monographs in the *USP-NF* have at least one reference standard to support the execution of the described test methods. However, rarely is an RS used in conjunction with general chapters.

4 Evolution of Pharmacopeial Standards

The first documentary standards from USP were recipes for making pharmaceutical preparations. Today they encompass over two centuries of accumulated knowledge and provide sophisticated and validated procedures for monitoring the quality of pharmaceuticals during their lifecycle. One of the most important attributes that must be monitored during the lifecycle is the identity of the drug.

A test for identification for a medicinal product will unequivocally confirm that the product conforms to its label. For biologics in general, and advanced therapies in particular, the complexity of these products requires the use of multiple

orthogonal methods, to identify key components of the therapeutic products. Additionally, there are situations where the manufacturer of biologics is required not only to demonstrate the identity of key elements but also to demonstrate the absence of contaminants or purity. Standards for simple or well-characterized active moieties for monitoring identity and purity are described in monographs.

The USP's approach to standards for the first biological medicines included in the compendium, such as small peptides and hormones, relied on the same framework for small molecules. One of the challenges with setting standards for large molecules or complex mixtures relates to how to address impurities since different processes will yield a different set of impurities. As the USP advanced with the development of standards for more complex products such as monoclonal antibodies and cell-based therapies, we recognized the need to evolve the scope and approach to standardization. Biological products are defined by the process used for their manufacture. It is, therefore, important that standards come in the form of tools that ensure the performance of the process or the methods used to measure the outcome of that process, rather than standards that allow the user to demonstrate meeting market specifications. This paradigm shift is an expected evolution for a pharmacopeia like the USP, to adapt to new modalities of treatment such as cell-based therapies. The inherent variability of starting materials for cell therapies (e.g., donor to donor) makes it challenging to create a standard around specifications of a medicinal product. Instead, the developers of these types of treatments would benefit from standards that allow them to ensure that systems, equipment, and methods are being correctly executed to achieve the expected outcomes.

The USP typically initiates standards development after products have been introduced to the market as the scope of a standard was focused on ensuring the products meet market specifications. With the increased complexity of biological medicines such as cell-based therapies, the USP's strategy shifted to standards that focus more on method and process performance, intending to deliver solutions that help with the mitigation of analytical challenges throughout the product lifecycle. Examples of recently developed standards will be described in the sections below, as well as a discussion on a path forward for standards for biological medicines in general.

5 A Public and Science-Based Approach to Standards Development

5.1 Volunteers Are at the Heart of the Standards-Setting Process

The USP relies on its Convention Member Organizations to shape its strategic direction. The convention currently has almost 500 Organizational Members representing a large swathe of the industry. It includes academic institutions, health

practitioners and scientific associations, consumer organizations, manufacturer and trade associations, government agencies, and nongovernmental standards-setting bodies. Member organizations are first recommended by the Council of the Convention and then approved by the USP's Board of Trustees. Next, the new organizational members appoint a Delegate to represent them at the Convention held every 5 years. At the Convention, the Delegates elect the Council of Experts (CoE), which oversees the USP's scientific and standards-setting decisions.

Members of the CoE serve 5-year terms as chair of a USP Expert Committee (EC). The committees are responsible for developing and revising standards that comprise the compendia, which includes the USP and the NF, USP Compounding Compendium, Herbal Medicines Compendium, Dietary Supplements Compendium, and Food Chemicals Codex, as well as USP Reference Standards specified for use with the compendia. Each EC focuses on a specific area of standards for chemical medicines, biologics, excipients, compounded preparations, dietary supplements, and food ingredients. The EC works closely with the relevant USP staff through the development process. Before a standard can become official, it is first published for public comment. The EC reviews the comments, adjusts the standard accordingly, and then votes on adoption. A majority vote of the EC is required to adopt a standard.

All EC members are chosen to serve on a committee by the CoE. These volunteers serve as individual experts, using their best personal, professional, and scientific judgment. To supplement the expertise of the ECs, the USP can also form expert panels on an ad hoc basis to address specialized topics. EPs are advisory to one or more expert committees, but unlike the EC, they are not decision-making bodies. Their recommendations are forwarded to the expert committees, who ultimately have the authority to vote on whether the standard will become official in the USP-NF. The EP is concluded upon completion of its work.

It is critical to the integrity and credibility of the USP's standards-setting activities that they are free of an actual or perceived conflict of interest in the performance of their duties. Therefore, to ensure that outside interests, including their employer, do not influence volunteers, they may only serve on a committee using their scientific experience, as a representative of the USP. In keeping with this policy, experts are barred from using their committee memberships in any way that creates a conflict of interest or confidentiality. Both EC and EP members are required to submit and keep updated statements disclosing interests that may create conflicts, so that any conflicts of interest that exist or emerge may be identified and resolved in a timely way. Expert panel members may participate in deliberations regarding matters in which they have a conflict provided they disclose them. Finally, all members of the CoE, ECs, and EPs are subject to the USP's code of ethics.

Figure 1 shows the process of standards development, including the publication of the draft form of a standard in the Pharmacopeial Forum (PF), the public comments process, and the role of the expert volunteers. The rules and procedures that govern the work of the USP standards-setting bodies are codified in a formal USP document available to the public through the USP website [9].

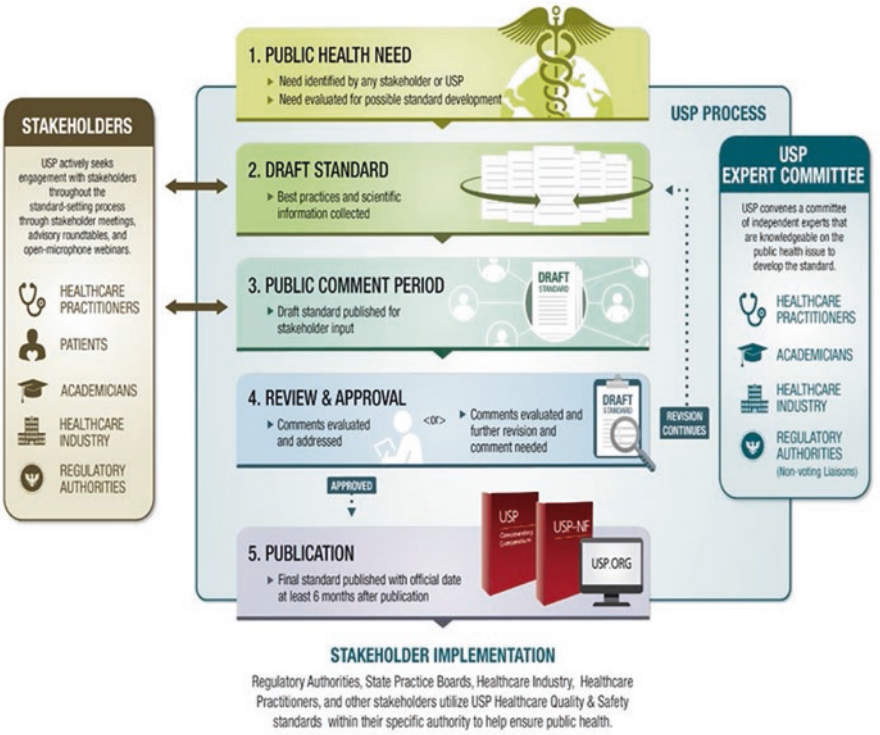

Fig. 1 Process of documentary standards development and role of public input from early engagement, open public comments, and vetting by expert volunteers

5.2 Developing Standards and Biologics

The process begins when a manufacturer or a USP staff member identifies the need for a documentary or reference standard. Initiation of development occurs when the manufacturer of a drug product, also known as the sponsor, provides USP with their specifications, validated analytical procedures, and other supporting data to ensure that both the proposed monograph and Reference Standard candidate meet industry needs. USP scientists then work with engaged industry partners and the EC with appropriate oversight to develop a proposal for use as the public standard. During proposal development, scientists in the USP's laboratories may evaluate one or more procedures. The USP's ultimate goal is to publish a proposal with suitable methods and criteria to ensure quality. While the expert committees and staff are evaluating the documentary standard, sponsors may provide physical materials for consideration as a USP reference standard to support monographs and general chapters. Candidate materials are tested at multiple USP or USP-approved laboratories worldwide. The test results are reviewed by the USP's staff to determine the candidate's suitability as a reference standard. Only after a candidate is found to be

suitable by USP's staff is it submitted to the EC for evaluation, approval, and release to the USP catalog.

After deliberation with the EC regarding the suitability of the information and test methods to be included in a documentary standard, USP staff submit the proposal for publication in the Pharmacopeial Forum (PF). In this free bi-monthly online publication, the first draft of a standard is open for a 90-day public comment period to ensure that the process remains transparent and collaborative. Access to PF online requires a one-time registration and allows the user to access previously published issues of PF. At the end of the comment period, USP staff also review all the public comments, organize the information received, and provide science-based recommendations to the EC. If the comments received from the public are substantial and question the applicability of the standard, the EC may revise the proposal taking into account input from the public comment process and then resubmit it for publication in PF for another round of comments. Once all comments are resolved, the documentary standard may become official in the USP-NF upon approval and ballot by the EC. The standard typically becomes effective 6 months following publication, during which time pharmaceutical companies must prepare for the implementation of the standard "The USP Monograph and Reference Standard development process" [10]. All USP standards that become official go through continuous improvement and revisions [11].

For biologics, including cell-based therapies, the complexity of manufacturing processes and variability in raw materials lead to complex finished products following diverse regulatory pathways. As a result, defining a common set of quality attributes to address in a public monograph becomes challenging. Standards for these types of products require continuous engagement with the FDA, industry, and other stakeholders in addition to the standards development process already described. A vital element of the engagement approach is to gather input early. After receiving a submitted request to develop a standard, the USP will perform an initial outreach to identify impacted stakeholders and evaluate the need for the proposed standard. Outreach includes consulting with practitioners, regulators, and manufacturers before any decision to move forward with the development of a biologic standard. It also includes workshops, roundtables, and stakeholder forums where participants can reach a consensus on the most beneficial standards and methods.

Early engagement with stakeholders also helps to determine which approach is most appropriate. With a growing industry that uses raw materials sourced globally, decentralized manufacturing to deliver products that meet different regulatory expectations, the USP needs to harmonize requirements with other pharmacopeias. If a standard exists in another pharmacopeia, then the USP will attempt to adopt similar approaches, provided that data and information are available to support that decision.

Also, the biologics strategy focuses on developing standards that address key analytical challenges and support biologics testing throughout the product lifecycle. Depending on the need, these standards may or may not be tied to an official documentary standard in the USP-NF. For example, the information and best practices included in a USP standard may support FDA guidance documents or ICH

guidelines to support regulatory harmonization. The USP has adopted ICH text for full implementation, in the case of General Chapters <1225> *Validation of Compendial Procedures*, <1050> *Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin*, and < 1049> *Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products*. When the USP adopts the ICH guidelines, it also creates an extension to these documents with input from stakeholders. For instance, the USP has additional chapters that extend beyond the ICH guideline on the validation of analytical methods, including <1033> *Validation of Biological Assays*, <1223> *Validation of Alternative Microbiological Methods*, and <1223> *Verification of Compendial Methods*.

5.3 Physical Reference Standards

Reference standards (RS) are physical standards developed through a rigorous process requiring collaborations between the USP and external laboratories. The USP program includes standards that are required for the execution of test procedures described in the USP-NF documentary standards, as well as RS without a compendial use. The flow chart in Fig. 2 shows the process used for the development of reference standards. Participation from external laboratories provides an opportunity for early engagement with stakeholders from industry, academia, and regulatory agencies. The USP uses mechanisms such as cooperative research agreements, contracts, and memoranda of understanding to formalize the collaboration with external parties. Qualification of the participant laboratories is conducted to ensure the participants' laboratories have quality systems in place that meet the USP's quality management system for the development and release of reference standards

Fig. 2 Reference standard development process and collaboration with external laboratories

The majority of USP reference standards are developed independently. However, occasionally the USP implements a joint development program with another pharmacopeia. Collaborative development ensures traceability to the World Health Organization (WHO) International Standards or national standards. Calibration against other international or national standards provides opportunities to support global harmonization. The USP calibrates its standards for biologics against international standards provided by the WHO when these are available to ensure traceability to an international unit for medicines dosed in units of activity.

The USP may also develop standalone RS without linkage to a documentary standard. These standards support demonstrating the performance of analytical methods or raw materials. The standards developed in this context use the same controlled process, quality systems, and scientific review by staff and ECs as other USP standards. For biologics, stakeholder engagement continually demonstrates a significant need for performance standards that have broad applications across multiple production sites. Performance standards are used to ensure and describe the method and process performance. They are broadly targeted at product families or classes as opposed to a specific drug substance or drug product. The USP standards are intended to address common quality challenges associated with technologies that cut across different types of products (e.g., system suitability samples, calibrators used to demonstrate the performance of an analytical procedure, process, or equipment). Also, their suitability for use is established using multi-laboratory collaborative studies, and USP scientists continually consult with the ECs to confirm the relevance of these initiatives and to manage the paths to market. The USP's physical reference standards are supported by information packets, which includes the label, packaging, post-packaging quality control, and quality assurance review that will be needed by manufacturers to meet the compliance requirements.

6 Standards Applicable to Cell-Based Therapies

As indicated above, the USP is committed to assisting developers of cell-based therapies by developing standards that provide best practices (documentary standards) as well as reference materials where appropriate to help standardize analytical methods and the quality of raw materials. Developers of cell therapies already benefit from some of the existing general requirements described in USP general chapters, as well as from USP quality standards developed for ingredients used in manufacturing and formulation strategies. Throughout this document, we will reference a series of USP general chapters that outline best practices for several aspects of manufacturing and validating methods applicable to advanced therapies; these chapters are available in the current edition of the USP-NF [12]. For instance, General Chapter <85> *Bacterial Endotoxins Test* is intended to ensure the quality of parenteral drugs, medical devices, raw materials, excipients, water for injection,

pharmaceutical ingredients, and biologics including cell-, gene-, and tissue-based products. General Chapters <87> and < 88> describe in vitro and in vivo biological reactivity tests that evaluate biocompatibility between material or devices used in conjunction with cell-based products.

There are also several monographs in the *USP-NF* that cover quality testing for pharmaceutical products that are used as critical components in the manufacturing, storage, or cryopreservation of cell, gene, and tissue therapies. Examples of these components are dextrose, dextran 40, human serum albumin, and DMSO. While these monographs were originally developed to address the testing for their use as pharmaceutical articles, the quality standards described in these monographs provide a foundation for their qualification for use in direct contact with the cells during manufacturing or storage.

We describe a series of general chapters and applicable standards that have been developed in the last decades to support product development for cell-based therapies. These standards include best practices applicable to the quality of raw materials, manufacturing and testing of cell-based products, and technologies and methodologies used in quality assessment, storage, delivery, and administration of these therapies.

6.1 USP <1046>: Inaugural Cell Therapy Chapter in USP-NF

Cell therapies are unique in many ways and, therefore, require standards specifically developed for them. The first USP documentary standard dedicated to advanced therapies was General Chapter <1046> *Cell and Gene Therapy Products*, published in 2000. This general chapter was the result of a collaborative approach among cell and gene therapy stakeholders who joined a USP expert panel that was initiated by the USP in 1997. At the time, only a few products in this category were undergoing human testing, and it was important for the USP to codify the standards and best practices that would enable further advancement of this field. To that end, the panel was comprised of volunteer expert clinicians, academicians, pharmaceutical and biotechnology scientists, and regulatory scientists from the FDA center of biologics. Upon its completion, General Chapter <1046> described the state of the industry and covered considerations and best practices applicable to manufacturing, administration, analytical methods, stability, labeling, and storage and shipping of cell- and gene-based therapies.

The publication of General Chapter <1046> in the USP-NF was the beginning of a journey to continually explore how standards and best practices are initiated, developed, and published in the compendium to assist the development of advanced therapies from its early stages to licensure and patient access.

6.2 Revision of Chapter <1046> and the Creation of Chapter <1047>

During the 2005–2010 convention cycle, the EC overseeing the cell, gene, and tissue therapies decided to revise General Chapter <1046> to address advances in technologies as well as updates to the regulatory environment. The existing chapter was divided into two new general chapters: <1046> *Cell and Tissue-based Products* and < 1047> *Gene Therapy Products*. The emergence of new forms of cell-based therapies such as cancer vaccines and cord blood-based products triggered further dialog on opportunities for best practices and standardization in this field.

Also, there has been an increase in the number of products that are developed using minimally manipulated human cells-, tissue-, and cellular tissue-based products (HCT/Ps) that are regulated under the FDA's current Good Tissues Practices (cGTP) regulations, 21 CFR Part 1271. The revision of <1046> provided an overview of the type of quality systems, qualifications of materials, manufacturing, as well as release tests for cell- and tissue-based products. During a subsequent revision, in 2016, the expert committee decided to distinguish between the regulatory pathways for cell-based therapies (biologics license application (BLA)) and tissues (good tissue practices (GTPs) only). Also, the description of the products was revised to reflect their current state of regulation, as well as to define decellularization for tissue-based products. In May 2020, this revised general chapter will become official as <1046> *Cell-based Advanced Therapies and Tissue-based Products*.

6.3 Qualifications of Ancillary Materials Used in Manufacturing

While General Chapter <1046> was being drafted, the working group identified the need to develop a second informational chapter that addressed qualification approaches for the raw materials used in the manufacturing of these therapies. Because of the complexity and number of these raw materials, the expert committee decided to focus on raw materials that are not intended to be present in the finished products. The work of the USP biologics expert committee led to the publication of USP General Chapter *<1043> Ancillary Materials Used in Cell, Gene and Tissue-Engineered Products*, which became official in 2003 and has been revised twice since then. One of the key aspects described in <1043> is the approach to qualification of ancillary materials before their use in manufacturing. This approach is intended to assess the potential impact of the ancillary material (AM) on the quality and safety of the finished product. It also includes considerations for addressing lot-to-lot variability as well as establishing traceability of the materials. A typical qualification program will include the following steps:

identification, selection, characterization, vendor qualification, and quality control quality assurance.

The level of testing of AMs is based on the risk associated with the use of these materials. Risk assessments can be managed by the use of quantitative or semi-quantitative tools such as failure mode effects analysis (FMEA) or hazard analysis critical control point (HACCP) systems. These tools allow the assignment of a score that helps the user determine the resources needed to reduce the risk associated with the use of AMs. For example, an AM derived from human or animal tissue and used in a downstream process has a high potential for remaining as a residual in the finished product and therefore scores as a high risk. General Chapter <1043> aids developers in the design of their qualification programs for a variety of AMs. It describes a risk-based classification for ancillary materials as well as guidance to the users of these materials on the type of activities they may need to conduct to reduce the risk associated with the use of these materials. Table 1 describes the various levels of risks.

The subsequent revisions of Chapter <1043> updated the risk-based classification of ancillary materials with examples that reflect the current state of the industry. For instance, the use of "GMP grade" in the earlier version of the chapter was removed to avoid any confusion between material quality standards and a quality system such as GMP. The revision also allowed updating the regulatory considerations related to the use of raw and ancillary materials, as well as to enhance the sections on risk management and provide examples of the impact of residual ancillary materials on product quality and clinical outcomes.

Table 1 Risk-based approach and classification of ancillary materials per USP Chapter <1043>

Level risk	Criteria that define the level of risk
Tier 1: low risk	Intended for use as licensed drugs, biologics or medical devices. Suitability for use as a manufacturing components is required because the formulation, stability profile, and other quality aspects of these materials may change once the material has been introduced in the manufacturing process
Tier2: low to moderate risk	*Intended to be used as ancillary materials*. These materials are well-characterized and produced under *quality systems well-suited for biological manufacturing*, but the *material is not a licensed* medical product. Many are produced specifically for the manufacture of biological products
Tier 3: moderate risk	These are *research-grade materials not intended* for use in biological manufacturing; *sometimes approved by regulatory agencies as part of an in vitro diagnostic device*. Tier 3 requires more qualification than Tier 1 or Tier 2 materials
Tier 4: high risk	These are materials produced as industrial or research-grad materials and may contain harmful impurities. They may also contain animal- or human-derived components with potential contaminants. This tier requires extensive qualification before use as component in biological product manufacturing

6.4 *From General to Specific Requirements: Standards for Ancillary Materials*

While Chapter <1043> does not prescribe testing beyond the supplier's certificate of analysis, the USP developed a series of documentary standards that provide test procedures applicable to specific AMs, such as bovine serum, cytokines, and enzymes used in cell processing. These test methods can offer valuable tools to the developers of cell-based therapies to ensure the quality of these materials and to meet regulatory expectations for the qualification of raw materials used in manufacturing. The procedures applicable to specific ancillary materials are supported by reference standards that can be used as calibrators or comparators when these ancillary materials are qualified and to ensure consistency in manufacturing. Figure 3 shows the approach to developing standards for ancillary materials going from general to a specific approach, as well as the types of ancillary materials that have been included in the USP-NF. These specific chapters describe test methods and supporting reference standards for materials such as fetal bovine serum (FBS), recombinant interleukin-4 (IL-4), recombinant trypsin, and collagenase I and II.

The FBS standard was developed in collaboration between suppliers and end-users, working within a USP expert panel and with support from the International Serum Industry Association. In addition to addressing the identification of the FBS and some of the general requirements, such as pH, osmolality, hemoglobin content, and total protein, the USP standard describes functionality tests in the form of growth promotion and clonality assays. The full set of test specifications is described

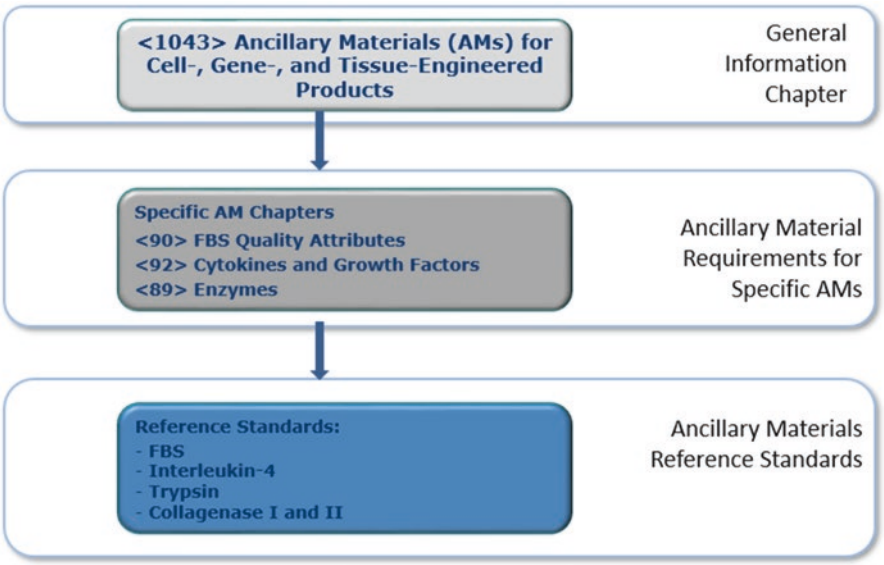

Fig. 3 From general to specific: The USP's approach to ancillary materials standards

in USP Chapter <90> *Fetal Bovine Serum—Quality Attributes and Functionality Tests*. A reference standard developed in conjunction with this chapter has been demonstrated to be suitable for use in the identification test, as well as with the growth promotion assay.

Chapter <89> *Enzymes used as Ancillary Materials in Pharmaceutical Manufacturing* describes test procedures and acceptance criteria for some of the enzymes used on cell culture and tissue processing; these are trypsin, collagenase I, and collagenase II. In addition to modern identification tests, each of the documentary standards describes enzymatic assays that allow the measurement of activity and assignment of units of enzymatic activity per mass of protein. Enzymatic preparations that have been calibrated against a validated standard, such as the reference standard provided by the USP, will now have better accuracy in activity assignment and will yield consistent results when used to process cells and tissues.

6.5 *Flow Cytometry: A Workhorse Technique in Cell-Based Therapy Applications*

Flow cytometry lends itself not only to quantitative applications, but it also is a technology that allows the user to measure multiple quality attributes such as identity, purity, as well as a surrogate for the potency of a cellular product. The advances in flow cytometry technology have allowed developers of cell and gene therapy products to adopt these methodologies for product characterization and product release testing. To support the increased use of these technologies in the testing of cells for the development of clinical diagnostic and therapeutic applications, the USP introduced General Chapter <*1027*> *Flow Cytometry*. This chapter addresses some of the practical aspects of characterization and phenotyping of well-characterized cell-based products. The chapter also provides best practices from sample preparation to data management and controls and touches on approaches of validation of these test methods.

One of the advanced applications of flow cytometry is the enumeration of CD34+ stem cells in different types of blood cell samples. Counting cells with accuracy is important as the number of cells correlates with engraftment of stem cells when cells derived from bone marrow, cord blood, or peripheral blood are used to treat a number of conditions. Transplantation of hematopoietic stem cell-based therapies has been in practice globally for decades now; however, there are only a few CD34+ cell-based products that have received market authorization or undergoing clinical assessment. To support advancing this type of product to licensure and ultimately to the patients, the USP adopted and validated a method based on the *ISHAGE guidelines* for CD34+ cell enumeration, as established by the International Society of Cell Therapy. This method was validated through an international collaborative study and included in USP Chapter <127>. The study also established a stable formulation of a freeze-dried cell preparation as a reference standard to support the

enumeration of CD34+ cells in a clinical or QC laboratory. This RS can be used to calibrate the flow cytometry instrument, qualify the assay reagents, and set proper controls for the identification and quantification of cell populations of interest.

6.6 Cryopreservation of Cells

General Chapter <1044> *Cryopreservation of Cells* was recently published in the USP-NF to address best practices for cryopreservation of a wide range of cells, including those used for cell therapy products or as cell substrates for the production of biological therapeutic products. The chapter discusses critical steps in a cryopreservation process for different cell types, including hematopoietic stem cells, mesenchymal stem cells, lymphoid cells, and human pluripotent stem cell lines. The necessary steps discussed in this chapter are the pre-freeze process, containers, cryoprotectant solutions, cooling, cryogenic storage, safety, and transport, in addition to thawing and post-thaw steps.

6.7 Sterility Assurance

One of the most critical tests required by the FDA and other regulatory bodies relates to sterility assurance, demonstrating the absence of bacteria, mycoplasma, and fungi. The conventional growth-based microbial tests described in the USP <71> *Sterility Tests* and other pharmacopeias are often time- and labor-intensive. The newly published USP General Chapter <1071> *Rapid Sterility Testing of Short-Life Products: A Risk-Based Approach* provides best practices on how manufacturers can assess suitable testing technologies applicable to their products, based on their user requirement specifications. The next steps for the USP are to describe in an extension to this chapter some of the test methods that have the potential to move to the compendium.

6.8 Product-Specific Standards: Challenges and Opportunities

For advanced therapies, the USP's focus has been the development of overarching and universally applicable standards that support the assessment of key quality issues, as well as the development of measurement tools that support classes and families of products. However, in the early 2000s, the USP experienced a spike in the number of requests to develop monographs for cell- and tissue-based products. The requests were predominantly from companies seeking reimbursement from the Center of Medicaid and Medicare Services (CMS), via a pass-through application that allows these products to receive a code for payment. The CMS currently

requires the submission of a copy of the USP monograph (or a letter stating that the product has been approved for inclusion in the USP) for non-implantable biologicals if the product has not received FDA approval as a biologic [13].

Developing monographs for such products turned out to be challenging. Many of these were either cleared as devices by the Center for Devices and Radiological Health (CDRH) at the FDA or were marketed without prior authorization through the FDA current Good Tissues Practices (cGTP) regulations, 21 CFR Part 1271. Both pathways are much less stringent than those for a Biological License Application (BLA). The information provided by sponsors for these monographs focused on release testing for the intended use of these products and not on methods for testing quality attributes. Continued dialog with this segment of the industry has resulted in the elaboration of monographs to include more relevant information on analytical testing.

The USP expert committees in the past two decades convened workshops and expert panels to gain a better understanding of quality aspects for tissue-based products. The USP, supported by the relevant expert committees, developed a pathway for including these monographs in the USP-NF [14] and scientific approaches to the assessment of test methodologies to be included in the monographs. For cell-based products, the product-specific standards tend to address typical quality attributes such as identity, purity, and potency, with most the tests relying on flow cytometry to determine the composition of cell-based products as well as identifying the main cellular components. For decellularized and some of the tissue-engineered products that may be presented as scaffolds or a combination of scaffolds and cells, the critical tests seem to focus on physical and mechanical properties, in line with the intended use for these products.

7 Importance of Standards and Need for Collaborative Efforts

There has been remarkable progress in the area of cell-based therapies over the past two decades, especially with the recent approvals of a few chimeric antigen receptor (CAR) T-cell products. The manufacturing and testing approaches for these therapies are challenging and rely on the use of different types of biological assays, among other sophisticated test methods to measure critical quality attributes (CQAs). For example, a recent study investigated the kinds of CQAs that apply to the manufacturing and release of tumor-associated antigen-specific T cells (TAA-T) [15]. The final list of CQAs included the supplies and reagents used in the manufacturing; in-process testing, such as the phenotype of the original starting product as determined by pre-processing flow cytometry; complete blood counts (CBCs); and the ratio of T cells to antigen-presenting cells.

In a workshop organized by the Forum on Regenerative Medicine [16], participants agreed that defining and measuring the CQAs for cell-based therapies is one

of the biggest challenges facing the field of regenerative medicine. While regulatory guidelines provide general guidance and define the types of quality attributes to be addressed in a regulatory filing, the diversity of cell-based therapies under development makes it challenging to develop common assays for the characterization of these products. The ability to measure CQAs and to ensure product consistency will undoubtedly benefit from the use of standardized methods and supporting reference materials. An essential utility of standards is to enhance the user's confidence in measurements to control the quality of their products, and it is, therefore, crucial that manufacturers have a good understanding of the intended use of the quality attributes of their products. Analytical requirements will depend on the type of quality attribute being measured and on whether the method is qualitative or quantitative.

Validation of analytical methods is a critical step toward the implementation of testing approaches in a quality-controlled environment. In the context of advanced therapies, and given the variability of starting materials, the complexity of assays, it is important to engage stakeholders at early stages and initiate collaborations through round-robin studies to assess methods' performance using different types of sample matrices, platforms, and equipment. Standards that are based on multi-stakeholders' input will ensure harmonization across industry and allow developers to focus on developing innovative therapies. The USP supports these efforts and further facilitates the implementation and adoption of new methods through its science-based and transparent process.

A recent report from the Standard Coordinating Body identified over 200 existing standards relevant to regenerative medicine, including more than 40 supportive standards applicable to one or more of the cell therapy, gene therapy, or tissue engineering sectors [17]. The diverse scope of these standards and the difference in approach among organizations that developed these standards highlight the challenge with the applicability of these standards. There is a need for better coordination of efforts to ensure the standards are complementary.

The USP is committed to working with regulators and developers of novel products and other stakeholders to address challenges through standardization of analytical methods and by supporting the development of reference standards that can be used to demonstrate performance for methods and processes. As USP documentary standards advance to the compendium, they provide best practices that integrate information aligned with FDA guidance documents and other regulatory guidelines.

The USP also supports the work of industry members associated with societies such as the International Society of Cell Therapy (ISCT). Our collaborations with the Alliance for Regenerative Medicine (ARM) encompass organizing workshops where stakeholders debate the common analytical tools used across industry and explore areas where standardization can help achieve consistency in manufacturing. We also recognize the need for better coordination around standards development for better management of the resources, to that end; we are very supportive and will align our work with the Standard Coordinating Body (SCB), the National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL), and the National Institute of Standards and Technology (NIST).

8 The Path Forward and Future of Standards at the USP: Concept and Examples

For advanced therapies, as discussed above, the USP will focus mainly on developing best practices and informational chapters describing analytical methods with broad applicability. We will also seek to develop reference standards to support method development and validation. The USP will continue to partner with stakeholders from the cell and gene therapy industry to initiate and deliver tools and standards to advance product development. The following are a sample of areas of focus for the USP:

- **Ancillary materials will remain a focus:** Some of these materials have the risk of being carried throughout the process and becoming impurities in the finished product. The quality of ancillary materials is critical to ensuring the safety of the finished therapeutic products [18]; the USP will, therefore, prioritize high-risk materials and develop methods for measuring their residual levels, as well as providing best practices for their effective removal.
- **Gene editing and multi-component products:** The focus here will be on common components used in these therapeutic products (e.g., enzymes). Developing assays and reference materials to ensure their quality so that developers can focus on the unique elements used in these therapies.
- **Raw and starting materials:** Focus on plasmid DNA, cell culture media, and apheresis products.
- **Impurities:** Host cell proteins and host cell DNA from cell lines used for the production of viral vectors.
- **Flow cytometry standards**: Focus on markers for MSCs and for characterization of starting materials.
- **Viral vectors used in gene-modified cell products:** Standards for measuring viral titer and vector copy number.

9 Conclusions

Manufacturers of cell-, gene- and tissue-based products must be sure that every component used in manufacturing meets all of the appropriate qualifications. Most importantly, any specifications of quality should, at the very least, confirm the purity, safety, and potency of the finished products. Also, the performance of methods and processes should be accounted for with validated control strategies that secure, verify, and confirm consistency in the manufacturing of cell-, gene- and tissue-based products. Pharmacopeial standards such as those developed by the USP provide manufacturers with the critical tools they need to ensure the quality of their finished products. In addition, compendial standards have proven to be immensely valuable for managing regulatory expectations, which makes them an indispensable part of any manufacturer's toolkit.

References

1. van der Loo, J. C., & Wright, J. F. (2016). Progress and challenges in viral vector manufacturing. *Human Molecular Genetics, 25*(R1), R42–R52. https://doi.org/10.1093/hmg/ddv451. Accessed 28 Aug 2020.
2. May, M. (2020, February). Gene therapy dollar is waiting on viral vector dime. *GEN, 40*(2), 36–38.
3. Lipsitz, Y. Y., Timmins, N. E., & Zandstra, P. W. (2016). Quality cell therapy manufacturing by design. *Nature Biotechnology, 34*(4), 393–400. https://doi.org/10.1038/nbt.3525. Accessed 28 Aug 2020.
4. Alliance for Regenerative Medicine. (2014). *Pharma and biotech survey*. Alliance for Regenerative Medicine.
5. Zoon, K. C., & Yetter, R. A. (2012). The regulation of drugs and biological products by the Food and Drug Administration. In J. I. Gallin & F. P. Ognibene (Eds.), *Principles and practice of clinical research*. Elsevier.
6. Meadows, M. (2006, January–February). *Promoting safe and effective drugs for 100 years*. U.S. Food and Drug Administration.
7. Paine, M. F. (2017). Therapeutic disasters that hastened safety testing of new drugs. *Clinical Pharmacology and Therapeutics, 101*(4), 430–434. https://doi.org/10.1002/cpt.613. Accessed 28 Aug 2020.
8. Anderson, L., & Higby, G. J. (1995). *The spirit of voluntarism: A legacy of commitment and contribution: The United States pharmacopeia 1820–1995*. United States Pharmacopeial Convention. NLM 9507777.
9. USP. (2018). *Rules and procedures of the 2015–2020 council of experts*. United States Pharmacopeial Convention. https://www.usp.org/sites/default/files/usp/document/about/policies-rules/2015-2020-coe-rules-procedures.pdf
10. United States Pharmacopeia. (2020). *The USP monograph and reference standard development process*. https://www.usp.org/chemical-medicines/the-reference-standards-and-monograph-process. Accessed 28 Aug 2020.
11. United States Pharmacopeia. (2020). *USP's commitment to stakeholder engagement related to biologics licensed under the Public Health Service Act (PHSA)*. https://www.usp.org/biologics/development-process. Accessed 28 Aug 2020.
12. USP. (2020). *United States Pharmacopeia and National Formulary 2020 (USP43-NF38)*. United States Pharmacopeial Convention.
13. Centers for Medicare and Medicaide Services (CMS). (2019). *New requirements for the hospital outpatient prospective payment system (OPPS) drug and biological pass-through application*. https://www.cms.gov/Medicare/Medicare-Fee-for-Service-Payment/HospitalOutpatientPPS/Downloads/drug-application-requirements.pdf. Accessed 28 Aug 2020.
14. USP. (2020). *Guideline on drugs approved for inclusion*. United States Pharmacopeial Convention. https://www.usp.org/donate/submission-guidelines/guideline-on-drugs-approved-for-inclusion. Accessed 28 Aug 2020
15. Tanna, J. G., Ulrey, R., Williams, K. M., & Hanley, P. J. (2019). Critical testing and parameters for consideration when manufacturing and evaluating tumor-associated antigen-specific T cells. *Cytotherapy, 21*(3), 278–288. https://doi.org/10.1016/j.jcyt.2019.02.004. Accessed 28 Aug 2020.
16. National Academies of Sciences, Engineering, and Medicine. (2017). *Navigating the manufacturing process and ensuring the quality of regenerative medicine therapies: Proceedings of a workshop*. The National Academies Press.
17. Standards Coordinating Body (Nexight Group). (2019, Fall). *The regenerative medicine standards landscape*. https://www.ncbi.nlm.nih.gov/pubmed/30929992. Accessed 28 Aug 2020.
18. Atouf, F. (2016). Cell-based therapies formulations: Unintended components. *The AAPS Journal, 18*(4), 844–848. https://doi.org/10.1208/s12248-016-9935-9. Accessed 28 Aug 2020.

The Role of the National Institute of Standards in Measurement Assurance for Cell Therapies

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1 The Roles of NIST as the National Measurement Laboratory

As part of the Department of Commerce, the National Institute of Standards and Technology (NIST) has a unique mission to advance measurement, standards, and technology to foster innovation and to promote industrial competitiveness. We carry this mission out through our robust laboratory programs, broad stakeholder engagement, and global standards leadership. NIST is the National Metrology Institute for the USA, which makes NIST responsible for advancing measurement science by developing, improving, and validating measurement technologies, and promoting global harmonization of measurements and standards. Our work in the biosciences at NIST is focused on building confidence in quantitative biology in support of a growing bioeconomy, including the cell therapy industry.

Cellular therapies encompass a large range of therapeutics, and as the science and technology continue to evolve, the NIST laboratory program also evolves in close collaboration with stakeholders. In addition to our technical programs, NIST efforts include convening workshops and consortia, leading and contributing to documentary standards development, and funding public-private partnership to advance manufacturing such as the National Cell Manufacturing Consortium Roadmap [1] and NIIMBL, the Advanced Manufacturing Institute for Biopharmaceutical Manufacturing (https://niimbl.force.com/s/).

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2 Measurement Assurance and Standards for Cell-Based Therapies

2.1 The Role of Standards

As the cell therapy industry matures, standards are becoming increasingly important for accelerating research and development and for product approval and commercialization. Various types of standards are useful for different purposes. A standard can help to streamline manufacturing processes, enable interoperability and integration of processes and data, ensure quality and consistency of products, and improve confidence in a measurement method. A documentary standard on ancillary materials can help cell therapy developers select the appropriate ancillary materials for their specific product. An analytical method standard can greatly compress the time it takes to validate a method for evaluating critical quality attributes and streamline regulatory review. A reference material is another type of standard that can help to benchmark a method or be used for comparison of results in manufacturing and testing.

The need for standards has been strongly endorsed by many organizations including the National Cell Manufacturing Roadmap [1] and the industry organization Alliance for Regenerative Medicine [2]. The 21st Century Cures Act [3] refers to the development of standards for regenerative medicine therapies and directs the US FDA, NIST, and stakeholders to coordinate the development of standards. An outcome of this legislation was the formation of the Standards Coordinating Body for Gene, Cell, and Regenerative Medicines and Cell-Based Drug Discovery (SCB, https://www.standardscoordinatingbody.org/), which is collocated with NIST and aims to engage, coordinate, and educate the development and implementation of standards. The SCB is a clearinghouse for standards relevant to cellular therapies that have been developed and are in the process of being developed, and the SCB website is a formidable and up-to-date resource. The US FDA describes the importance of standards [4] in "The Guidance Document on Standards development and the Use of Standards in Regulatory Submissions Reviewed in the Center for Biologics Evolution and Research," which specifies "preferential use (of) International harmonized standards." Ideally, standards are designed to enable innovation, but this requires careful consideration to ensure that they are not overly prescriptive and are supported by data.

2.2 Community Engagement

NIST has been closely engaged with the regenerative medicine and cellular therapies' community for more than a decade. An important contribution has been the organization of workshops geared at companies, other agencies, and academic researchers that promote the concepts of measurement assurance. Table 1 lists

Table 1 Workshops organized by NIST and links to workshop announcement and reports

March 2019	NIST-FDA-SCB Workshop: Realizing the Benefit of 21st Century Cures through Standards Development
	https://www.standardscoordinatingbody.org/march2019workshop
April 2018	NIST-FDA Workshop: Genome Editing
	https://www.nist.gov/news-events/events/2018/04/nist-fda-genome-editing-workshop
Oct 2017	NIST-FDA Flow Cytometry Workshop: Building Measurement Assurance in Flow Cytometry
	https://www.nist.gov/news-events/events/2017/10/nist-fda-flow-cytometry-workshop-building-measurement-assurance-flow
April 2017	NIST-FDA Cell Counting Workshop: Sharing practices in cell counting measurements
	https://www.nist.gov/news-events/events/2017/04/nist-fda-cell-counting-workshop-sharing-practices-cell-counting
	https://www.sciencedirect.com/science/article/pii/S1465324918304705
May 2016	Genome Editing Standards Workshop
	https://www.nist.gov/system/files/documents/mml/bbd/GenES-Workshop-5-2-16-agenda.pdf
	https://www.nist.gov/system/files/documents/2017/06/30/genome_editing_standards_workshop_5-2-16_report_3.pdf
Feb 2016	NIST CAR-T Biomanufacturing Symposium
	https://www.ibbr.umd.edu/NISTCART
	https://www.liebertpub.com/doi/10.1089/humc.2016.29014.com
May 2015	NIST Workshop: Strategies to Achieve Measurement Assurance for Cell Therapy Products
	https://www.nist.gov/mml/bbd/biomaterials/workshop-measurement-assurance-strategies-cell-therapy-products
	https://stemcellsjournals.onlinelibrary.wiley.com/doi/full/10.5966/sctm.2015-0269

relevant workshops and workshop reports. NIST workshops are often held in collaboration with partners, such as the FDA, SCB, and professional organizations including the American Society for Gene and Cell Therapy and the International Society for Advancement of Cytometry. In addition to partnering with FDA on workshops, we also host FDA researchers in our laboratory space. NIST also participates in the NIH Regenerative Medicine Innovation Catalyst and in the National Academies Forum on Regenerative Medicine. NIST was a charter member of MATES, the Multi-Agency Tissue Engineering Science working group, a long-standing means by which Federal agencies involved in tissue engineering and other aspects of regenerative medicine and cell therapies stay informed of each other's activities and coordinate their efforts. NIST works closely with the Department of Defense-funded Advanced Regenerative Manufacturing Institute by providing subject matter expert support and plays a similar role for the Department of Commerce-funded NIIMBL.

2.3 Assisting Measurement Assurance

In addition to our activities in measurement technologies and standards, one of the most important and unique contributions that NIST provides to the community is educating and advocating about measurement assurance. Testing methods that ensure that cells are suitable for a particular application are required to enable confidence in the performance of these cell-based products. Measurements associated with assay characteristics, such as reproducibility, uncertainties and limits of detection, and dynamic range, provide benchmarks that define a specified range within which measurements must fall to be accepted. These characteristics and acceptability limits for them provide confidence to manufacturers that assays are performed appropriately and are required for demonstrating measurement quality as part of regulatory approval. Our laboratory programs provide an essential bridge between the concepts of measurement acceptability and harmonization and the implementation of these concepts. An example of how to introduce quality metrics into a cell viability assay is described in [5–7]. At the outset of the study, collaborating laboratories were experiencing large variability in assay results between their laboratories. A cause-and-effect analysis and a series of control measurements resulted in identification of important sources of variability in the assay protocol. The control measurements were used to develop specifications that must be met to provide evidence for confidence in the test result.

3 Laboratory Technical Activities in Support of Cell-Based Therapies

NIST laboratory research programs are critical in that they inform and take guidance from the measurement challenges in producing cellular therapies. Our laboratory activities include the development of reference materials, improvement of the quantitative value of existing techniques, and the development of new measurement technologies. Much of this work is undertaken with industry. Some of these activities are detailed below.

3.1 Cell Counting

3.1.1 Importance of Cell Enumeration

Cell counting (or cell enumeration) is one of the most fundamental measurements in cell biology, and many cell-based bioassays, including activity and potency, must be normalized to the cell number to allow data inter-comparability. For example, the number of cells within a bioreactor may serve as a quality assurance metric in a manufacturing process, and cell number is critical for determining the proper dose of a cell-based therapy. Counting the number of cells in a cell preparation accurately and reproducibly for cell-based biotechnology has posed significant challenges in technology transfer, scale-up, and manufacturing. These difficulties arise from the heterogeneity and dynamic nature of cells, as well as from challenges in sampling and sample handling. Several standards and methods exist for comparison of cell counting methods within a lab, for example, when a lab desires to move from manual hemocytometer counting to a more automated or semiautomated method for cell enumeration. However, there has remained a need to assess the quality of a single counting process without the comparison to another method and in the absence of appropriate reference materials.

3.1.2 Establishing a Performance Metric

We have developed a method to assess the overall quality of a cell counting measurement process through an experimental design and series of statistical metrics. This method does not require a reference material or “ground truth” cell number value and can be broadly applied to different cell types and measurement techniques. Through appropriate experimental design and statistical analysis, a performance metric can be calculated based on the deviation of cell count values (i.e., residuals) from a modeled ideal proportional response. When dilution is well controlled, proportional response of cell count to changes in dilution serves as an internal reference, where a deviation from proportionality indicates a systematic error in

the counting method. Combined with evaluation of measurement precision, fundamental requirements of a cell counting method are evaluated and can be further compared across methods. The metrics derived from the experimental and statistical framework characterizes the entire cell count measurement process, including the measurement platform, method-specific factors such as dilution steps and sampling, and the specific cell preparation measured [8]. This approach for evaluating the quality of a cell counting measurement in the absence of a reference material can aid in the selection, validation, and optimization of a cell counting measurement process. The method is incorporated into an international standard adopted by the International Standards Organization (ISO) Technical Committee (TC) 276 – Biotechnology, in "Cell Counting Part 2: Experimental design and statistical analysis to quantify counting method performance." A general guide for cell counting is also available from ISO/TC 276 entitled "Cell counting – Part 1: General guidance on cell counting methods." Recent studies performed by NIST in collaboration with Lonza utilize the Cell Counting Part 2 experimental design and statistical framework to present a case study [9].

An additional illustration is provided in "Standards Landscape in Cell Counting: Implications for Cell and Gene Therapy" [10], which also provides a summary of the current standards available in the area of cell counting.

3.1.3 Counting Viable and Non-viable Cells

Percent viability of a cell sample is defined as the percentage of live cells to total cells in a sample. Based on this definition, it is crucial to be able to identify and distinguish non-viable cells from living, healthy cells. Cell viability evaluation is widely and routinely used in basic research. In recent years, evaluating the proportion of viable to non-viable cells has become critically important in the cellular therapy and regenerative medicine field, as patient therapies increasingly rely on viability measurements to ensure product quality or as release criteria for a cell therapy product. There is a variety of viability assays or cell health assays which identify the state of health of a population of cells or of individual cells. Common methods for identifying cell viability include assessing membrane permeability via dye exclusion assays (e.g., trypan blue stain or DNA stains such as DAPI or propidium iodide), metabolic activity assays such as MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) or ATP assays, and assays for apoptotic markers such as caspases or annexin. These methods assess different biological properties associated with cell health and are therefore not necessarily equivalent or correlated. There is often confusion in comparing % cell viability values when the definition of viable and non-viable cells is unclear. NIST is contributing to the development of a standard within ISO Technical Committee 276, which provides guidelines for describing critical quality attributes, such as cell viability, using a fit-for-purpose approach (ISO/CD 23033 Biotechnology – Analytical Methods – General guidelines for the characterization and testing of cellular therapeutic products). Additionally, NIST is establishing experimental approaches to identify

biological properties related to cell health that are most relevant for specific applications. Cell viability measurements are often based on the semi-automation of instrumentation such as microscopes or spectrophotometers, including aspects of data analysis. NIST is working closely in collaboration with instrument manufacturers to address ways to improve viability measurements by verifying some of the assumptions made when using a cell counting instrument to evaluate cell viability. The introduction of reference or benchmarking materials to a sample helps to reduce the uncertainty about instrument performance. For example, a homogeneous and stable reference bead spiked into a cell sample can provide insight as to whether focus and brightness levels are appropriately set on an instrument, and by varying magnification and verifying bead diameter, the field of view can be verified, helping to confirm assumptions about sample volume. These and other techniques help us to gain insight into some of the assumptions being made when a cell counting measurement is conducted.

As part of an ASTM working group, NIST is also involved in developing and validating a system for assessing cell viability in scaffolds [11]. The assay system includes human cells encapsulated in an ionically cross-linked polysaccharide hydrogel scaffold that can be disassembled by gentle pipetting action for further analysis and confirmation of in situ results. ATP is measured by a luciferase luminescence assay, and DNA is measured by a fluorescent dye. Through the use of standard curves, the assay yields SI-traceable units of moles of ATP per gram of DNA. A series of carefully designed experiments are being conducted to validate that the method yields reliable results for cell encapsulated in a scaffold, and an interlaboratory study is being organized to assess reproducibility and robustness. This work will be used to support an ASTM standard test method for measuring cell viability in scaffolds.

3.2 Flow Cytometry

3.2.1 Challenges to Comparability

Although flow cytometry has a long history in clinical applications and is heavily relied on for evaluating cell populations in pre-clinical and clinical trials for therapies, there are many challenges to achieving confidence in the measurement result. Measurements made on different instrument platforms at different times and places often cannot be reliably compared because of hardware differences between instruments and the lack of well-characterized process control materials. Discrepancies between and among measurements introduce uncertainty in diagnostic and therapeutic decisions and impede advances in basic science. We collaborate with researchers at other federal agencies, and in industry, academia, professional societies, and standards organizations, to accelerate the standardization of flow cytometry measurements through establishing measurement traceability and using reference controls and standards. An ultimate goal of our flow cytometry standardization

effort is to obtain comparable assay results across different instrument platforms at different times and locations with sufficient measurement assurance.

3.2.2 Equivalent Number of Reference Fluorophores

To establish traceability and assist comparability, we have developed the unit of equivalent number of reference fluorophores (ERF) for fluorescence value assignments of microsphere calibration materials [11, 12]. We work with microsphere and instrument manufacturers within the Flow Cytometry Quantitation Consortium [13] to provide an ERF value assignment to commercial calibration microsphere products to enable more confident quantification and comparison of fluorescence intensity signal and hence the standardization of the intensity scale and performance characteristics of flow cytometers.

3.2.3 Quantifying Biomarkers

Through collaboration with the FDA, international metrological institutions, and industry, we are developing human peripheral blood mononuclear cell (PBMC)-based cell reference materials. These materials enable quantitative measurements of cell functional markers such as cytokines and chemokines and disease biomarkers such as CD20, ZAP-70, and CD38. Multiplexed cytometric assays are being developed to take advantage of the known CD4 expression on healthy human T lymphocytes as a reference biomarker control [14, 15]. The CD4 expression levels on T cells from healthy individuals and a commercially available lyophilized PBMC product have been quantitatively measured using flow and mass cytometry and are expressed in the unit of antibodies bound per cell (ABC) [16, 17]. These ABC biomarker expression results (as shown in Fig. 1) are instrument independent and are therefore quantitative and comparable across different instrument platforms and locations. We are expanding this work to quantify CD19 using CD4 as a reference value for clinical applications.

3.3 Quantitative Microscopy

Cells are frequently examined by light microscopy since the spatial resolution of the light microscope is compatible with the spatial scale of the major structural features of cells. In addition, the temporal resolution with which data can easily be collected is well-suited to many biological processes that are associated with fluctuations in cell phenotype. Increasingly there is interest in taking advantage of the quantitative static and dynamic features of cells that light microscopy can characterize in order to identify and measure relevant cell features that serve as indicators to help direct the manufacturing process.

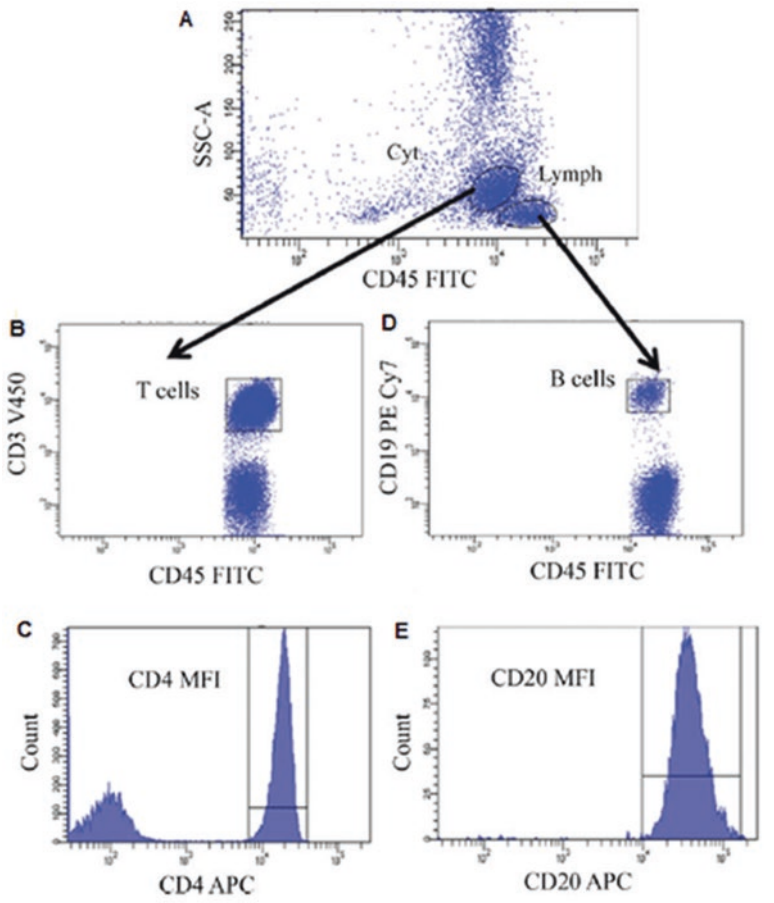

Fig. 1 Use of CD20 as a very useful biomarker for B-cell chronic lymphocytic leukemia (CLL) The quantification of CD20 is based on known CD4 expression on T helper cells from Cyto-Trol control cells, both stained with APC. The whole blood sample was stained with CD45 FITC, CD19 PE-Cy7, and CD20 APC, and Cyto-Trol was stained with CD45 FITC, CD3 V450, and CD4 APC in two separate sample tubes. After staining and washing, the two samples were combined in a single tube and run on a calibrated flow cytometer. (**a**) Two individual lymphocyte gates (CD45+ and Low SSC) were drawn as "Cyt" for Cyto-Trol cells and "Lymph" for unknown whole blood sample in CD45 FITC vs. SSC-A; (**b**) gated on "Cyt," T cells were identified in a dot plot of CD45 FITC vs. CD3 V450; (**c**) under T-cell gate, CD4 histogram shows the positive CD4+ gate, which was used to obtain respective mean fluorescence intensity (MFI) value of CD4; (**d**) gated on "Lymph," B cells were identified in a dot plot of CD45 FITC vs. CD19 PE-Cy7; (**e**) gated on B cells, CD20 histogram shows the positive CD20+ gate that was used to obtain the MFI value of CD20. With measured MFI values of CD20 and CD4, CD20 expression in ABC can be obtained. The use of CD4 expression as the reference control drastically reduces the variability of CD20 expression measurements and enables quantitative measure of CD20 expression that is independent of cytometer platforms used

3.3.1 Measurement Assurance in Imaging

Applying imaging to cell-based therapies, particularly in manufacturing applications, requires methods for establishing confidence in the quantitative interpretation of the data. Over many years, we have published reports on experimental methods for reliable quantification of cellular parameters such as size, morphology and signal intensity from single cells over time [6, 18–20], and a documentary standard guide on quantifying cell morphology [21]. One of our activities in measurement assurance in fluorescence microscopy has been the development of a turn-key benchmarking protocol using a commercially available glass as a reference material and a software routine that operates within the open-source program MicroManager [22]. This simple software routine enables users to easily evaluate instrument performance including limit of detection, linearity, and saturation [23]. This easy-to-use protocol can be employed to provide evidence that an instrument is operating consistently from day to day and allows comparison of different instruments. Materials for implementing the protocol, including the MicroManager plugin, spreadsheet scripts for automated charting of microscope performance, a video with step-by-step instructions, and a list of commercially available, inexpensive, and photostable fluorescence reference materials, are available [24]. In addition, we have worked with the ASTM community to establish a documentary standard for performing quantitative fluorescence intensity measurements in cell-based assays with widefield epifluorescence microscopy [25].

3.3.2 Live Cell Imaging

Live cell imaging provides "high-resolution" measurements in the sense that we collect time-dependent data from large numbers of individual cells. We then use these data to assess if a cellular activity or feature at a particular point in time can reliably serve as critical process control measurement during manufacturing. For example, our ongoing work using large fields of view and micropatterned grids is allowing quantification of the temporal and spatial details of CAR-T cell target recognition, the time required for killing of target cell, the ability of a T cell to kill multiple cells, and the frequency of these events.

The collection of dynamic microscopic data on live cells provides a window into the mechanisms by which cells achieve a stable, yet heterogeneous range of phenotypes within a population of cells [18, 19, 26]. The temporal data from cell populations allows the developments of predictive models of population behavior based on the stochastic and deterministic components of gene expression [26–28]. These kinds of data provide more informed interpretation of biomarkers and can be used in predictive modeling of populations.

While the technology to record live cell images from cellular populations has been available for some time, only recently has it become routine to derive quantitative data from these image sets using image analysis. We have focused on developing live cell imaging tools to monitor large numbers of single cells [29] and to

quantify changes in morphology and gene expression using fluorescence protein reporters. Collaboration with NIST's Information Technology Laboratory has resulted in the development of strategies for visualizing and analyzing large image datasets [30], enabling the use of such data in developing predictive models. The handling and processing of large image datasets has required the development of software that is accessible to and continues to be developed with a user community including researchers at the NIH National Center for Advancing Translational Sciences. The Web Image Processing Pipeline created by NIST [31] allows users to process and extract quantitative data from large datasets and to visualize gigabyte-size composite images from many stitched fields of view through pan and zoom functions.

3.3.3 Label-Free Imaging

While fluorescence microscopy of labeled cells can provide critical insight for developing and assessing control of a manufacturing process, in-process applications on real samples will require label-free methods. Methods such as chemical imaging of complex biological and materials systems using broadband coherent anti-Stokes Raman scattering (BCARS) imaging, high-resolution surface plasmon resonance (SPR) imaging for imaging cell-material interactions, and quantitative phase imaging (QPI) are under development.

Our BCARS program has advanced vibrational spectroscopic imaging to new levels. Vibrational spectroscopy methods can identify many biomolecules and materials based on their vibrational signatures and deduce aspects of their molecular structure and local environment. BCARS microscopy is a special form of Raman spectroscopy, developed at NIST that uses pulsed laser systems to probe intramolecular vibrational modes with orders of magnitude increase in acquisition rate [32, 33]. This method has demonstrated an unprecedented combination of speed, sensitivity, and specificity compared to other vibrational methods. We have demonstrated the potential of this powerful technique with a variety of biologically relevant materials such as tissues [33, 34] and to distinguish markers of differentiation in stem cell cultures [35]. Advances in this technology will make it possible to someday acquire spatially and temporally resolved functional molecular information in living cells [32].

Quantitative phase imaging (QPI) enables label-free measurement of the optical pathlength difference induced in light as it passes through a cell [36]. Unlike conventional Zernike phase contrast microscopy which only qualitatively measures the phase change of light through cells, QPI provides quantitative phase measurements and the opportunity to have comparable measurements between experiments, instruments, and laboratories. NIST has utilized QPI as an orthogonal technique to follow induced pluripotent stem cell (iPSC) proliferation [37]. Current efforts are underway to build measurement confidence using the transport of intensity (TIE)-based QPI technique [38] to enable easy integration of QPI with other imaging modes such as fluorescence microscopy. NIST is evaluating microspheres and developing

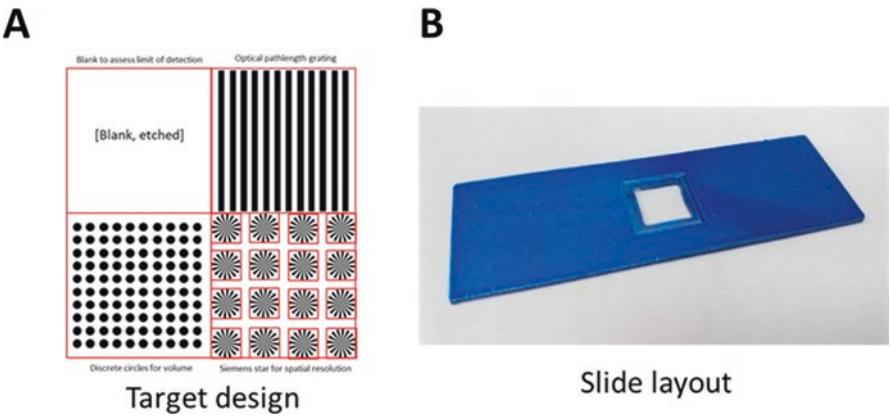

Fig. 2 Design and application of a quad-pattern glass etched phase target to benchmark quantitative phase microscop**y**
(**a**) The quad-patterned test target is designed as a 12 mm × 12 mm glass coupon with four etched features: (1) a featureless flat region of glass, (2) a lateral grating, (3) a square grid of cylindrical features with discrete optical volumes, and (4) a Siemens star resolution target to cover the N.A. of a range of air objectives. (**b**) Test target sample holders are designed to be the same size as a microscope slide with a window to accommodate the glass coupon

custom reference materials to calibrate and provide traceability to QPI measurements [37]. We have developed a prototype reference material for benchmarking QPI methods that consists of a glass etched target with discrete segmentable features of defined size and optical phase characteristics similar to those in mammalian cells (see Fig. 2).

Another label-free imaging method that allows quantitative imaging of spatially resolved refractive index changes is surface plasmon resonance imaging (SPRI). SPRI can be used for the dynamic tracking of cell-secreted proteins and subcellular components [39–41] and the quantification of the mass of subcellular features such as focal adhesions [42]. We continue to study how subcellular mass and density fluctuations at the cell-substrate interface report on cellular functions such as T-cell activation.

3.3.4 Image Analysis and Machine Learning

A challenge associated with imaging that is often taken for granted is the need for reliable algorithms for analysis of cell features, intensities, lineage tracking, division time, and other features in static and dynamic imaging conditions. Our imaging programs involve studies on comparison of imaging algorithms [20, 43], as well as the development and assessment of deep learning methods for challenging problems such as segmentation and tracking of single iPSC. The application of quantification of cell features to characterize iPSC colonies [30] and retinal pigment epithelial cell therapies [44] has been demonstrated.

While imaging is a powerful tool for quantifying many aspects of cells, image analysis algorithms can be a source of uncertainty, bias, and irreproducibility in quantifying image features. Even relatively simple segmentation operations of high signal to noise fluorescence images to identify cells and quantify morphology can produce different results due to the choice of algorithm [20]. Details of image analysis algorithms should be reported, and when possible, algorithms should be tested with reference data to determine that the software used is providing reliable results.

Assessing reliability of image analysis routines becomes even more critical and challenging with very large image datasets and the use of sophisticated computational tools for image analysis that are based on machine learning, deep learning, or artificial intelligence approaches. These tools can be very powerful methods for identifying images with features that correspond to desired features, even when a human observer may not be able to distinguish them. An example is the application of convolutional neural nets to GMP-grade tissue-engineered retinal pigment epithelium (RPE) which allowed assessment of the quality of the RPE from noninvasive brightfield imaging [44, 45]. Optical absorbance was determined by normalizing the brightfield images to a blank brightfield image, providing a quantification of melanin, the presence of which accompanies maturation of the cells (see Fig. 3). The optical images and corresponding data from orthogonal measurements of transepithelial resistance (TER) and polarized secretion of VEGF in the samples were used to train an algorithm to predict TER and VEGF secretion function from image data. The microscope system was designed at NIST and implemented in the GMP facility at NIH to collect data that were subsequently analyzed at NIST. The approach potentially provides a label-free, noninvasive method to predict potency of manufactured RPE.

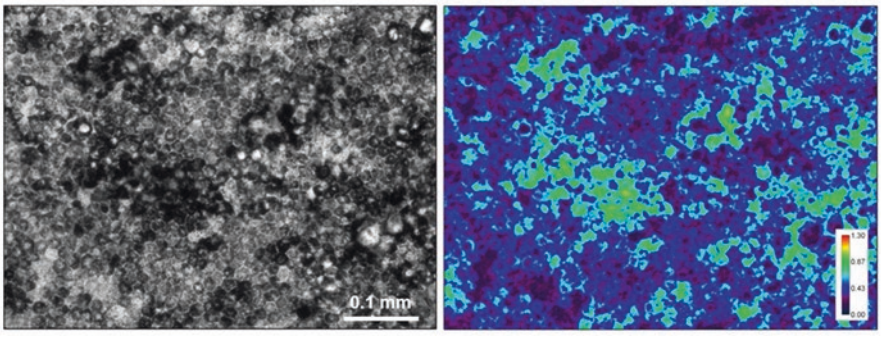

Fig. 3 RPE absorbance imaging
Left: Transmitted light brightfield image of tissue-engineered retinal pigment epithelium (RPE). Mature RPE expresses melanin, a pigment that absorbs light, yielding the darkened regions in the image. Individual cells appear as small, circular shapes about 0.01–0.02 mm in diameter. *Right:* The corresponding quantitative absorbance image. A calibrated scale is on the bottom right for absorbance units. Artificial intelligence algorithms detected subtle patterns in the pigmentation and cell features that enabled the prediction of RPE tissue quality. (Photo Credit: Nicholas Schaub)

3.4 Genomic Measurements and Gene Editing

The use of genomic sequence identity becomes necessary during the development and manufacturing of cellular therapies for verifying genomic editing of cells, for quality control of processed cells from patients, for definitive identification of the source of cells, or for identifying disease-specific genomic variants. NIST's efforts in genomic measurements are focused on establishing confidence in genome editing and interpretation of genomic sequence data.

3.4.1 Genome Editing Consortium

The NIST-led Genome Editing Consortium [46] aims to address the measurements and standards needed to increase confidence of utilizing genome editing technologies in research and commercial products. By working collaboratively with experts in industry, academia, and other government agencies, NIST's focus is providing measurement solutions and standards to support innovation and products in genome editing technology. Pre-competitive areas identified for standardization and being pursued by this consortium are (i) clear communication about the field via a standard genome editing community lexicon, (ii) shared understanding and interpretation of genome editing data via standard metadata formats, benchmark datasets, and metadata repositories representative of genome editing, and (iii) tools for understanding the reproducibility, performance, and comparability of key assays and measurements for detecting DNA sequence changes after genome editing, some of which will be used to characterize product safety, via shared control samples and shared paradigms for qualifying assays.

3.4.2 Genome in a Bottle Consortium

NIST led the development of the world's first and only whole human genome reference materials with the Genome in a Bottle Consortium (GIAB) [47]; these authoritatively characterized human genomes help enable laboratories to accurately "map" DNA for genetic testing, benchmark sequencing technologies and bioinformatics, validate medical diagnostics, and develop companion diagnostics for customized drug therapies [48, 49]. The US FDA used this resource to approve one of the first commercially available next-generation DNA sequencers for clinical use, making precision medicine more accessible. Seven human genomes used to benchmark sequencing and bioinformatic analysis pipelines [50] are provided as NIST Reference Materials. The Reference Material DNA is derived from Personal Genome Project cell lines that are broadly consented and available for commercial use and redistribution, as well as iPSC development. They are being used by the NIST Genome Editing Consortium as well-characterized genomic backgrounds to develop controls for off-target genome edits.

4 Conclusions

The primary mission of NIST is measurement science. The development of the complex therapies that incorporate living cells presents many measurement challenges. NIST works to address those challenges by actively engaging with the communities that research, fund, regulate, and manufacture cell therapies. This involves a portfolio of activities including in-house basic research, development of reference materials, providing calibration services, engaging in laboratory collaboration with stakeholders, contributing to the development of consensus standards, and organizing workshops for information exchange. As the field of cell therapies continues to mature and evolve, measurement technologies will need to evolve. NIST will continue to welcome the input and participation of all stakeholders in our efforts.

Acknowledgments Certain commercial equipment, instruments, or materials are identified in this chapter to foster understanding. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology nor does it imply that the materials or equipment identified are necessarily the best available for the purpose

References

1. National Cell Manufacturing Consortium. (2017). Achieving large-scale, cost-effective, reproducible manufacturing of high-quality cells [Online]. Available: http://cellmanufacturingusa.org/ncmc. Accessed 17 July 2020.
2. Alliance for Regenerative Medicine. https://alliancerm.org/manufacturing/. Accessed 17 July 2020.
3. U.S. CONGRESS. (2016). 21st Century Cures Act H.R.34. Washington DC. Federal Register. https://www.federalregister.gov/documents/2020/05/01/2020-07419/21st-century-cures-act-interoperability-information-blocking-and-the-onc-health-it-certification. Accessed 17 July 2020.
4. U.S. Food and Drug Administration. (2019). Standards development and the use of standards in regulatory submissions reviewed in the center for biologics evaluation and research. Guidance for industry. March 2019. https://www.fda.gov/media/124694/download. Accessed 17 July 2020.
5. Rosslein, M., Elliott, J. T., Salit, M., Petersen, E., et al. (2015). Use of cause-and-effect analysis to design a high-quality nanocytotoxicology assay. *Chemical Research in Toxicology, 28*, 21–30.
6. Elliot, J. T., Tonya, A., & Plant, A. L. (2003). Comparison of reagents for shape analysis of fixed cells by automated fluorescence microscopy. *Cytometry, A52*, 90–100.
7. Elliot, J. T., Rosslein, J. T., Song, N., Toman, B., et al. (2017). Toward achieving harmonization in a nano-cytotoxicity assay measurement through an interlaboratory comparison study. *ALTEX, 34*, 201–218.
8. Sarkar, S., Lund, S. P., Vyzsatya, R., Vanguri, P., et al. (2017). Evaluating the quality of a cell counting measurement process via a dilution series experimental design. *Cytotherapy, 19*, 1509–1521.
9. ISO. (2019). Cell counting – Part 2: Experimental design and statistical analysis to quantify counting method performance. ISO 20391-2:2019(en).

10. Sarkar S. Pierce L, Lin-Gibson S. Lund SP (2019) Standards landscape in cell counting: Implications for cell & gene therapy. Cell & Gene Therapy Insights 5, pp 117-131
11. ASTM. (2018). New test method for measuring cell viability in a scaffold. In ASTM Subcommittee: ASTM 2018. F04.43 (Ed.), WK62115.
12. Wang, L., Derose, P., & Gaigalas, A. K. (2016). Assignment of the number of equivalent reference fluorophores to dyed microspheres. *Journal of Research of the National Institute of Standards and Technology, 121*.
13. Wang, L., & Gaigalas, A. K. (2011). Development of multicolor flow cytometry standards: Assignment of ERF units. *Journal of Research of the National Institute of Standards and Technology, 116*.
14. Federal Register. (2016). Flow cytometry quantitation consortium. *Office of the Federal Register*, 46054–46055. https://www.govinfo.gov/app/details/FR-2016-07-15/2016-16761. Accessed 17 July 2020
15. Degheidy, H., Abbasi, F., Mostowski, H., Gaigalas, A. K., et al. (2016). Consistent, multi-instrument single tube quantification of CD20 in antibody bound per cell based on CD4 reference. *Cytometry Part B: Clinical Cytometry, 90*, 159–167.
16. Wang, L., Degheidy, H., Abbasi, F., Mostowski, H., et al. (2016). Quantitative flow cytometry measurements in antibodies bound per cell based on a CD4 reference. *Current Protocols in Cytometry, 75*, 1.29. 1-1 29.14.
17. Wang, L., Abbasi, F., Ornatsky, O., Cole, K. D., et al. (2012). Human CD4+ lymphocytes for antigen quantification: Characterization using conventional flow cytometry and mass cytometry. *Cytometry, A81*, 567–575.
18. Wang, M., Misakian, M., He, H. J., Bajcsy, P., et al. (2014). Quantifying CD4 receptor protein in two human CD4+ lymphocyte preparations for quantitative flow cytometry. *Clinical Proteomics, 11*(p), 43.
19. Halter, M., Sisan, D. R., Chalfoun, J., Stottrup, B., et al. (2011). Cell cycle dependent TN-C promoter activity determined by live cell imaging. *Cytometry A, 79A*, 192–202.
20. Halter, M., Tona, A., Bhadriraju, K., Plant, A. L., et al. (2007). Automated live cell imaging of green fluorescent protein degradation in individual fibroblasts. *Cytometry A, 71*, 827–834.
21. Dima, A. A., Elliott, J. T., Filiben, J. J., Halter, M., et al. (2011). Comparison of segmentation algorithms for fluorescence microscopy images of cells. *Cytometry A, 79*, 545–559.
22. ASTM. (2014). Guide for using fluorescence microscopy to quantify the spread area of fixed cells. In ASTM (Ed.), *ASTM 2014* (pp. F2998–F2914)
23. Edelstein, A. D., Tsuchida, M. A., Amodaj, N., Pinkard, H., et al. (2014). Advanced methods of microscope control using muManager software. *Journal of Biological Methods, 1*, 2, p e10.
24. Halter, M., Bier, E., Derose, P. C., Cooksey, G. A., et al. (2014). An automated protocol for performance benchmarking a widefield fluorescence microscope. *Cytometry A, 85*, 978–985.
25. National Institute of Standards and Technology (NIST). (2017). Fluorescence microscopy benchmarking [Online]. NIST. https://www.nist.gov/programs-projects/fluorescence-microscopy-benchmarking-overview. Accessed 17 July 2020.
26. ASTM. (2018). Standard guide for performing quantitative fluorescence intensity measurements in cell-based assays with widefield epifluorescence microscopy. In ASTM (Ed.), *Book of standards* (Vol. 13.02).
27. Sisan, D. R., Halter, M., Hubbard, J. B., & Plant, A. L. (2012). Predicting rates of cell state change caused by stochastic fluctuations using a data-driven landscape model. *Proceedings of the National Academy of Sciences of the United States of America, 109*, 19262–19267.
28. Lund, S. P., Hunnard, J. B., & Halter, M. (2014). Nonparametric estimates of drift and diffusion profiles via Fokker-Planck algebra. *The Journal of Physical Chemistry B, 118*, 12743–12749.
29. Hubbard, J. B., Halter, M., & Plant, A. L. (2019). Properties of a multidimensional landscape model for determining cellular network thermodynamics. *bioRxiv*. https://doi.org/10.1101/682690. Accessed 17 July 2020.
30. Chalfoun, J., Majurski, M., Dima, A., Halter, M., et al. (2016). Lineage mapper: A versatile cell and particle tracker. *Scientific Reports, 6*, 36984.

31. Bhadiraju, K., Halter, M., Amelot, J., Bajcsy, P., et al. (2016). Large-scale time-lapse microscopy of Oct4 expression in human embryonic stem cell colonies. *Stem Cell Research, 17*, 122–129.
32. Bajcsy, P., Chalfoun, J., & Simon, M. (2018). *Web microanalysis of big image data*. Springer.
33. Camp, C. H., & Cicerone, M. T. (2015). Chemically sensitive bioimaging with coherent Raman scattering. *Nature Photonics, 9*, 295–305.
34. Camp, C. H., Lee, Y. J., Heddlestone, J. M., Hartshorn, C. M., et al. (2014). High-speed coherent Raman fingerprint imaging of biological tissues. *Nature Photonics, 8*, 627–634.
35. Camp, C. H., Lee, Y. J., & Cicerone, M. T. (2016). Quantitative, comparable coherent anti-stokes Raman scattering (CARS) spectroscopy: Correcting errors in phase retrieval. *Journal of Raman Spectroscopy, 47*, 408–415.
36. Leey, Y. J., Vega, S. L., Patel, P., Aamer, K. A., et al. (2014). Quantitative, label-free characterization of stem cell differentiation at the single-cell level by broadband coherent anti-Stokes Raman scattering microscopy. *Tissue Engineering Methods (Part C), 20*, 562–569.
37. Mir, M., Bhaduri, N., Wang, R., Zu, R., et al. (2012). Chapter 3: Quantitative phase imaging. In E. Wolf (Ed.), *Progress in optics*. Elsevier.
38. Kwee, E., Peterson, A., Stinson, J., Halter, M., et al. (2018). *Large field of view quantitative phase imaging of induced pluripotent stem cells and optical pathlength reference materials*. SPIE.
39. Paganin, D., & Nugent, K. A. (1998). Noninterferometric phase imaging with partially coherent light. *Physical Review Letters, 80*, 2586–2589.
40. Peterson, A. W., Halter, M., Tona, A., Bhadriraju, K., et al. (2009). Surface plasmon resonance imaging of cells and surface-associated fibronectin. *BMC Cell Biology, 10*(p), 16.
41. Bhadriraju, K., et al. (2010). Using surface plasmon resonance imaging to probe dynamic interactions between cells and extracellular matrix. *Cytometry A, 77*, 895–903.
42. Peterson, A. W., Halter, M., Tona, A., & Plant, A. L. (2014). High resolution surface plasmon resonance imaging for single cells. *BMC Cell Biology, 15*(p), 35.
43. Peterson, A. W., Halter, M., Tona, A., Plant, A. L., & Elliott, J. (2018). *Mass measurements of focal adhesions in single cells using high resolution surface plasmon resonance microscopy*. SPIE.
44. Bajcsy, P., Cardone, A., Chalfoun, J., Halter, M., et al. (2015). Survey statistics of automated segmentations applied to optical imaging of mammalian cells. *BMC Bioinformatics, 16*(p), 330.
45. Schaub, N. J., Hotaling, N. A., Mabescu, P., Padi, S., et al. (2020). Deep learning predicts function of live retinal pigment epithelium from quantitative microscopy. *Journal of Clinical Investigation, 130*, 1010–1023.
46. Padi, S., Manescu, P., Schaub, N., Hotaling, N., et al. (2020). Comparison of Artificial Intelligence based approaches to cell function prediction. *Informatics in Medicine Unlocked, 18*, 100270.
47. NIST. (2020). NIST genome editing consortium. https://www.nist.gov/programs-projects/nist-genome-editing-consortium. Accessed 17 July 2020.
48. NIST. (2020). Genome in a bottle. https://www.nist.gov/programs-projects/genome-bottle. Accessed 17 July 2020.
49. Goldfender, R. L., Priest, J. R., Zook, J. M., Grove, M. E., et al. (2016). Medical implications of technical accuracy in genome sequencing. *Genome Medicine, 8*(p), 24.
50. Zook, J. M., McDaniel, J., Olson, N. D., Wagner, J., et al. (2019). An open resource for accurately benchmarking small variant and reference calls. *Nature Biotechnology, 37*, 561–566.

National Science Foundation Engineering Research Center for Cell Manufacturing Technologies (CMaT)

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1 Introduction

Cell therapies, especially stem cell and immune cell therapies, could revolutionize treatments of unsolved and chronic medical conditions, thus making a transformative impact on patient health and the healthcare economy. They hold promise in treating many cancers and autoimmune diseases and potentially alleviating diabetes, stroke, heart diseases, spinal cord injury, neurodegenerative diseases, Crohn's disease, and many other devastating disorders. Recent FDA approval of chimeric antigen receptor T cell (CAR T) products (Kymriah®, Yescarta®, Tecartus®, Breyanzi®, and Abecma®) for the treatment of different blood cancers has garnered

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much excitement and investment in research and clinical trials for effective cell therapies [1, 2]. However, with the commercial launch of these products, manufacturing shortfalls regarding scalability, product reproducibility, quality, and mechanisms of action have become critical challenges in cell therapy developments [3].

Although the first two cell-based immune therapy products were approved by the FDA in 2017, and several others were approved in the past few months, no concerted national effort has been made to enable broad innovations in scalable bioprocessing of therapeutic cells, with standardized characterization, end-to-end and continuous quality control, and quality-by-design (QbD)-driven manufacturing. This has hindered broad translation of many cell therapies into clinical and industrial practice and hence to patients. As approved products have moved from small-scale clinical trials to the open market, some have faced manufacturing failures and significant reimbursement challenges, and overall sales has been lower than expected. It is now well accepted that in order for these potentially life-saving therapies to become widely accessible, commercially and clinically, the biomanufacturing community must develop (a) new tools, software, technologies, and bioprocesses, to reproducibly manufacture high-quality cells at a large scale and at a lower cost; (b) robust supply chain, storage, and distribution logistics; and (c) a well-trained, diverse cell manufacturing workforce. These are the goals of the National Science foundation (NSF)-funded Engineering Research Center for Cell Manufacturing Technologies (CMaT, www.cellmanufacturingusa.org) and the philanthropically funded Marcus Center for Therapeutic Cell Characterization and Manufacturing (MC3M, www.cellmanufacturing.gatech.edu).

2 Need for a Comprehensive, International Effort in Technology Development for Cell Manufacturing

Clinical trials have shown that cell therapies can be functionally "curative" for some conditions, while in other cases they might accelerate healing, improve quality of life, and reduce hospital stays and chronic care. Despite their immense promise and

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transformative potential to "cure the incurable" and reduce suffering and cost, access to cell therapies is currently limited to a small number of patients and is available only at leading clinical centers. At present, therapeutic cells can only be processed at small scales and high cost due to the lack of integrated manufacturing innovations and advanced bioprocessing technologies. Although more than 1000 clinical trials are ongoing, and a growing number of cell therapy businesses have started, little effort has been made to enable scalable manufacturing of therapeutic cells as a reproducible, safe, and affordable pharmaceutical product with quality control and standardized characterization [4]. This has slowed down their broad translation into clinics and industry [5, 6] and has restricted access to a few, even in the most advanced countries, let alone in developing nations.

Manufacturing cells as a therapeutic product poses complex challenges, different from those currently experienced by the pharmaceutical and biotech industry. First, the product (cells) is a "living, breathing" entity whose properties and function can change with every manipulation requiring a whole new paradigm for large-scale manufacturing and quality control. Second, very little standardization exists across the field for cell collection, characterization and processing, cell identity markers, potency assays, and storage solutions. In addition, for many cell-based therapies, even with promising preclinical and clinical data, little is known about their critical quality attributes (CQA), i.e., biomarkers or properties that render them safe and effective for specific disease indications. Thus, quality-by-design (QbD) [7], the fundamental premise of current manufacturing practice, has not been implemented in cell manufacturing and without which, large-scale, reproducible, low-cost production of high-quality and safe cells cannot be achieved, and the promise of cell therapies cannot be realized.

Cell therapies are living drugs with beneficial implications for treating various diseases. Unlike pharmaceutical drugs, cell therapies are live cells that are responsive to their surrounding conditions and possess specific attributes corresponding to the donor [8, 9]. Consequently, strategies used to evaluate therapeutic potency, identify mechanisms of action, and predict outcomes for each patient of a cell therapy product remain a significant challenge for regulatory agencies [10]. Although current evaluation standards are sufficient to ensure safety (e.g., mycoplasma, adventitious virus testing), improved methods for rapid testing of therapeutic performance or potency, and manufacturing by monitoring critical process parameters (CPPs) informed by CQAs will substantiate evidence and ultimately approvals for these novel therapies [8, 10, 11]. While researchers and cell therapy developers recognize the need and utility for identifying CQAs, approaches to achieve this goal remain underdeveloped. By utilizing high-content analyses (i.e., *multi*-omics and multidimensional measurements) for comprehensive characterization and targeted/personalized performance assays of cell therapy products, thousands of attributes can be correlated to performance using computational predictive modeling approaches to greatly increase the probability of identifying novel CQAs that are indicative of function at the cellular and molecular levels [6]. Deep characterization of cell therapy products is necessary to (a) better understand batch-to-batch, donor-to-donor, and site-to-site variability of cell therapy products and (b) develop correlative

models that can identify a set of CQAs for each cell product that are predictive of their efficacy when targeting specific clinical conditions [3, 10]. Initial multi-omics characterization and interrogation of cell therapy products are time-consuming and expensive; however, strategic design from the start of this study will generate the high-content data for correlation analysis, and once that has been established, focus can be placed on those multivariate characteristics that are predictive of performance.

Approaches to improve clinical study design and cell manufacturing rely on the identification of CQAs of cell therapy products. For cell therapies, allogeneic cells are a readily available source for deployable treatments; however dosages are on the order of millions to billions of cells [12]. Cell manufacturing to achieve high yields of allogeneic cells requires extensive screening and interrogation before and after the manufacturing process. Beyond QA/QC purposes, identification of CQAs would greatly enhance selection and release criteria and in-line monitoring and greatly expand our current knowledge of donor-specific differences and critical process parameters of cell manufactured products [9, 10]. Not only would the cell manufacturing industry be positively impacted, prospective CQAs specific to both allogeneic and autologous cell therapy products have a great potential to guide clinicians and inform patients to achieve more effective outcomes.

3 Vision and Mission

Manufacturing cells as a therapeutic product poses complex challenges, different from those currently experienced by the pharmaceutical and biotech industry. First, the product (cells) is a "living" entity whose properties and function can change with every manipulation, requiring a whole new paradigm for large-scale manufacturing and quality control. Second, very little standardization exists across the field for cell collection, characterization and processing, cell identity markers, potency assays, and storage solutions. In addition, for most cell-based therapies, even with promising preclinical and clinical data, little is known about their CQA, i.e., the set of properties (multivariate) that render them safe and effective for specific disease indications; the critical process parameters (CPPs), i.e., the set of manufacturing or process variables that, when controlled, yields a consistent and reproducible product of appropriate CQAs; and mechanism of action (MOA) in a particular disease or patient population. Thus, quality-by-design (QbD), a fundamental premise of current industrial-scale manufacturing practice, has not been implemented in cell manufacturing.

The FDA defines QbD as a systematic approach to pharmaceutical and biopharmaceutical development and manufacturing that emphasizes product and process understanding and control. The manufacturing must be based on sound science and quality risk management that allows the developer to define *quality* of the product based on sound understanding of product and patient data. The agency further stipulates that it is critical that companies understand the process so deeply that one can

design and *control* the process to achieve a defined quality. Without the ability to implement manufacturing processes based on such principles, large-scale, reproducible, low-cost production of high-quality and safe cells cannot be achieved and the promise of cell therapies will remain elusive to a large population around the world.

The value and need for supporting organizations like CMaT and MC3M are even greater now than before given the tremendous growth in this sector both clinically and in industry. Multiple new products and companies have formed, new cell therapies and indications are being tested, and industry is reporting major barriers despite significant progress – understanding CQAs and lack of robust methodologies to evaluate CQAs; understanding CPPs that control the CQAs; understanding MOAs for specific patient populations; lack of real-time or robust and low-cost process analytical technologies (PATs) that can monitor cell/process quality during manufacturing; robust, more physiologically relevant, rapid, and predictive potency tests and product release tests; feedback-controlled automation; poor scalability of products; lack of best practices, standards, and clear metrics for product comparability; a nascent supply chain and risky logistics model; and a shortage of trained workers. CMaT is working to solve these problems through a national, convergence science effort where diverse engineering experts are working with industry partners, workforce experts, clinicians, cell biologists, and standards agencies, within a framework of regulatory processes, social policy, and healthcare economics, to enable both fundamental engineering innovations and workforce innovations to transform the manufacture, cost, availability, and efficacy of cell therapies, propel the growth of an emerging industry with high-value jobs, and eventually help address the unsustainable escalation of healthcare costs.

CMaT's vision is to transform the manufacture of cell-based therapeutics into a large-scale, lower-cost, reproducible, and high-quality engineered system for broad industry and clinical use. CMaT will become a visionary and strategic international resource and an exemplar for developing new knowledge, transformative technologies, an inclusive and well-trained workforce, and for enabling standards for cell production and characterization processes.

To achieve this vision, CMaT is enabling convergence of three synergistic research and technical innovation thrusts:

- Multi-omics and characterization-based discovery of CQAs and CPPs (Thrust 1)
- Tools and technologies for rapid and reliable assessment of process and product (cell) quality, i.e., potency and safety, preferably in real time (at-line or in-line) (Thrust 2)
- Supply chain, process and systems engineering for scale-up or scale-out manufacturing, and reliable production, storage, and distribution (Thrust 3)

Each thrust area is then applied to three Engineered Systems (Test-Beds):

- Mesenchymal stromal cells (MSCs) to repair, regenerate, and restore diseased tissues/organs
- T-cell and immune immunotherapies to cure cancer

- Induced pluripotent stem cell-derived cardiomyocytes (iPSC-CM) to treat heart diseases

The vision, mission, and goals of the MC3M are very similar to that of CMaT, except the MC3M is more clinically and translationally focused, working closely with clinical trials to characterize cell therapies and translating findings from the CMaT ecosystem. The vision of MC3M is to bring together bioengineers, manufacturing engineers, and industrial engineers to work closely with cell biologists, clinicians, and industry partners to make cell therapy manufacturing a well- characterized, quality-controlled, efficient, and highly reproducible process for broad clinical use.

The goals of the Marcus Center are to:

- *Collaborate* with clinical partners to identify the characteristics of cells that are associated with therapeutic potency in specific treatments
- *Innovate* and develop new methods for rapidly validating function, potency, and safety of manufactured cells
- *Invent* new tools and technologies for scale-up and scale-out of therapeutic cells (stem and progenitor cells, as well as immunotherapeutic cells)
- *Develop* technologies for cell purification, separation, delivery, packaging, and storage while maintaining cell purity, numbers, and quality
- *Enable* low-cost, highly reproducible, large-scale production of highly potent therapeutic cells by incorporating industrial design principles, automated robotics-based systems, supply chain management, and manufacturing process flow concepts
- *Translate* our engineering methodologies and new technologies to preclinical and clinical applications in collaboration with clinicians and industry partners
- *Help* build a strong, well-trained workforce in cell manufacturing

CMaT and MC3M fundamentally believe that QbD-driven cell manufacturing can ultimately improve cost and access. Cell therapies are an extremely high-risk product for industry, but we can reduce this risk significantly by reducing batch failures; understanding CQAs, CPPs, and MOAs; predicting efficacy and safety and which patient population would be most benefited from a given therapy; ensuring reproducibility and robust quality control; and making a well-trained workforce available on demand. We believe that quality will be a bigger driver of manufacturing, than cell numbers or scale. Why do we need billions of cells? If cell subpopulations that are more efficacious and safe and can be identified, then the need for scale goes down, and cost goes down. We also must reduce cost of goods and services (COGS) – by improving reproducibility, automation, process control, and a trained workforce. Certainly, allogeneic cells will help to reduce these costs and improve access on demand. If we are able to define CQAs, CPPs, and MOAs – the question of comparability and how to assess comparability becomes easier, thus reducing risk and cost and improving global access. Finally, we believe that development of industry-wide consensus standards and best practices would lead to precompetitive advancements and reduction of cost.

CMaT's vision for enabling transformative changes in cell manufacturing, healthcare, economy, and the manufacturing workforce of the nation and to have a

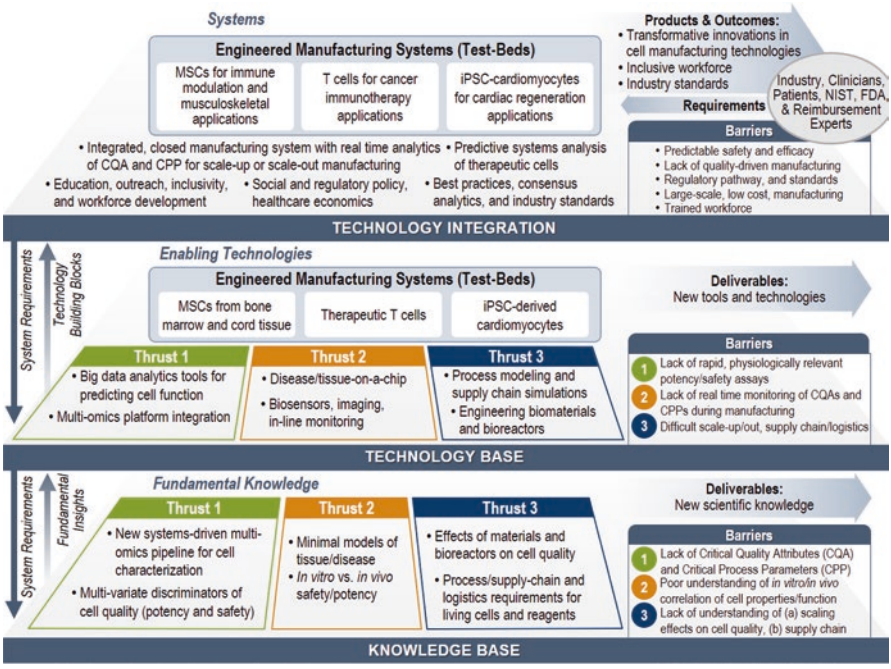

Fig. 1 Three-plane strategy chart for CMaT

global impact is illustrated in the three-plane chart (Fig. 1). First, new fundamental knowledge is being gained about how manufacturing processes affect cell quality to catapult scalable cell manufacturing, especially in areas of CQA, i.e., the set of properties of a given type of cell that is "predictive" of its safety, efficacy, or potency or the minimal set of properties that manufacturers must control and keep consistent in order to ensure reproducible product performance. This is different from "release criteria"– typically used to satisfy regulatory requirements which are often a set of cell surface markers and/or lack of microbial agents in the product. There is also little correlation between *in vitro* and *in vivo* potency-safety (i.e., quality) measurements. Furthermore, not much is known on how scaling of the manufacturing process affects cell function.

Second, CMaT is developing new process analytical technologies (PAT) and improved product analytical and potency measurement tools that would be broadly applicable to all Test-Beds as well as other cell therapies and biomanufacturing platforms. Such tools and technologies include rapid in-line testing of critical cell and process attributes, integrated sensors and imaging probes, and organ/disease-on-a-chip models for rapid, low-cost, potency testing; big data analytics to model cell quality; as well as engineered biomaterials and bioreactors coupled with advanced process modeling and supply chain innovation. Third, at the systems level, CMaT is addressing key barriers in each Test-Bed: predicting safety and efficacy on industry-relevant Test-Beds; large-scale, low-cost manufacturing; lack of

industry standards and a trained workforce; improved supply chain logistics and distribution models; and regulatory and social policy as related to large-scale cell therapies. Finally, CMaT is nurturing an inclusive, industry-academia-clinician-government ecosystem to achieve its goals. This broad vision, driven by needs from industry, academics, clinicians, patients, reimbursement experts, and regulatory agencies, will collectively enable large-scale, low-cost, reproducible manufacturing of high-quality therapeutic cells.

CMaT's technology development strategy builds on a strong foundation of fundamental knowledge in manufacturing technologies, process engineering, computational modeling, and cell engineering to develop and integrate an array of enabling tools and technologies that will lead to high-quality, scalable, cost-effective manufacturing systems for three transformative cell therapy platforms (Test-Beds). Barriers at the fundamental knowledge, enabling technologies, and systems levels have been identified though stakeholder engagement. Specifically, unlike many other Engineering Research Centers (ERCs), CMaT had the distinct advantage of a completed and updated technology roadmap before it started. The NIST-AMTech roadmap developed by the National Cell Manufacturing Consortium and led by CMaT leadership serves as the fundamental roadmap for all CMaT activities. This comprehensive 60-page national roadmap (see www.cellmanufacturingusa.org/ncmc/), originally issued by the Office of Science and Technology Policy of the White House in 2016, was further updated by industry, clinical, standards, and regulatory stakeholders in 2017. In 2019, CMaT convened the academia-industry-government stakeholder group again for a road mapping workshop at our annual retreat, which has now led to an updated 10-year roadmap to 2030, available through our website (see http://cellmanufacturingusa.org/ncmc). This new roadmap, with more quantitative goals, will guide CMaT for the next few years. In addition, CMaT is continually engaged with our advisory boards and stakeholders to update the roadmap, identify threats and barriers, and modify/correct our goals and tasks.

One way to envision the integrated goals for CMaT is to achieve a fully closed-loop, integrated, and scalable manufacturing platform that incorporates real-time monitoring of CQAs and CPPs, rapid assessment of physiologically relevant potency and safety parameters, feedback-controlled automation to adjust process parameters and achieve consistent product quality regardless of input material and ancillary material variations or scale of manufacturing, and a robust supply chain, storage, and logistics platform. This is shown in Fig. 2. It should be noted that we envision the closed manufacturing system to be cell type (i.e., Test-Bed) specific with a basic structure and tools that are common across all Test-Beds (cell types). It is also worth noting that this vision of the manufacturing system is agnostic to the size/scale of the manufacturing platform and holds true for both benchtop small footprint systems (bedside/local manufacturing) and larger, more centralized manufacturing systems (regional/distributed and centralized manufacturing). It is also applicable to both autologous and allogeneic cell platforms, but will of course be product-specific.

The three proposed Engineered System Test-Beds reflect current clinical and commercial interests (T- and NK-cell immunotherapy, MSCs for regenerative medicine), as well as longer-term emerging therapies (induced pluripotent stem cell-derived cardiomyocytes [iPSC-CM] for cardiac repair). We have chosen to focus on

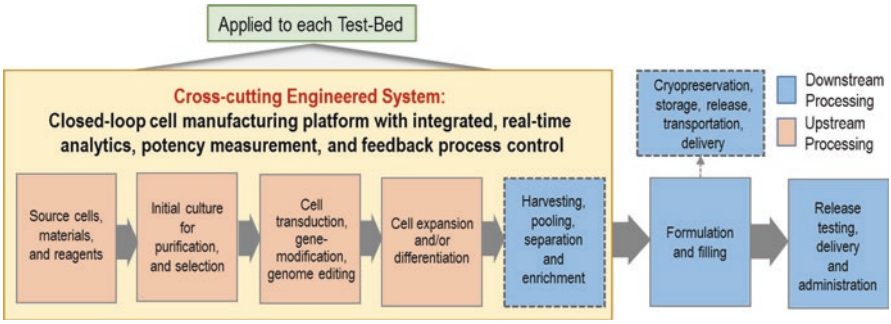

Fig. 2 Engineered system vision in CMaT and MC3M

chimeric antigen receptor (CAR)-T cell and MSC (from bone marrow or cord tissue, BM-MSC/C-MSC) manufacturing systems because their large-scale production is limited by barriers in current manufacturing technologies and our team has deep expertise in these areas. We anticipate the most rapid translation of CMaT research into these two Test-Beds given access to clinical data and industry partners working with these cells. Our choice of iPSC-CMs as the third Test-Bed is due both to our collective expertise in this area and because of rapidly growing interest and transformative clinical potential of reprogrammed and differentiated, patient-matched, adult cells for regenerative medicine.

Our work on iPSC-CMs will impact how manufacturing develops in early-stage clinical implementation of these cells and other cell types that require differentiation during manufacturing. Importantly, although we have chosen three cell therapy Test-Beds based on current clinical needs and emerging innovation opportunities, the platform technologies, models, and knowledge developed in CMaT are adaptable to other cell systems.

Crucial to the CMaT vision is the recognition that cell manufacturing and all related engineering innovations must be embedded within the social and regulatory policy environment. To help cell manufacturing reach its potential, CMaT has included faculty in relevant social sciences (e.g., public policy and ethics) and will support high-quality research that addresses critical barriers (e.g., regulatory policy harmonization, intellectual property, and global access to therapies).

4 Structure

4.1 Academic and Clinical Partnership

MC3M and CMaT, are both led by the Georgia Institute of Technology (Georgia Tech). MC3M was established in 2016 through a $15.75 million philanthropic grant from the Billie and Bernie Marcus Foundation and augmented by a $1.25 million commitment from the Georgia Research Alliance and a $7.25 million commitment

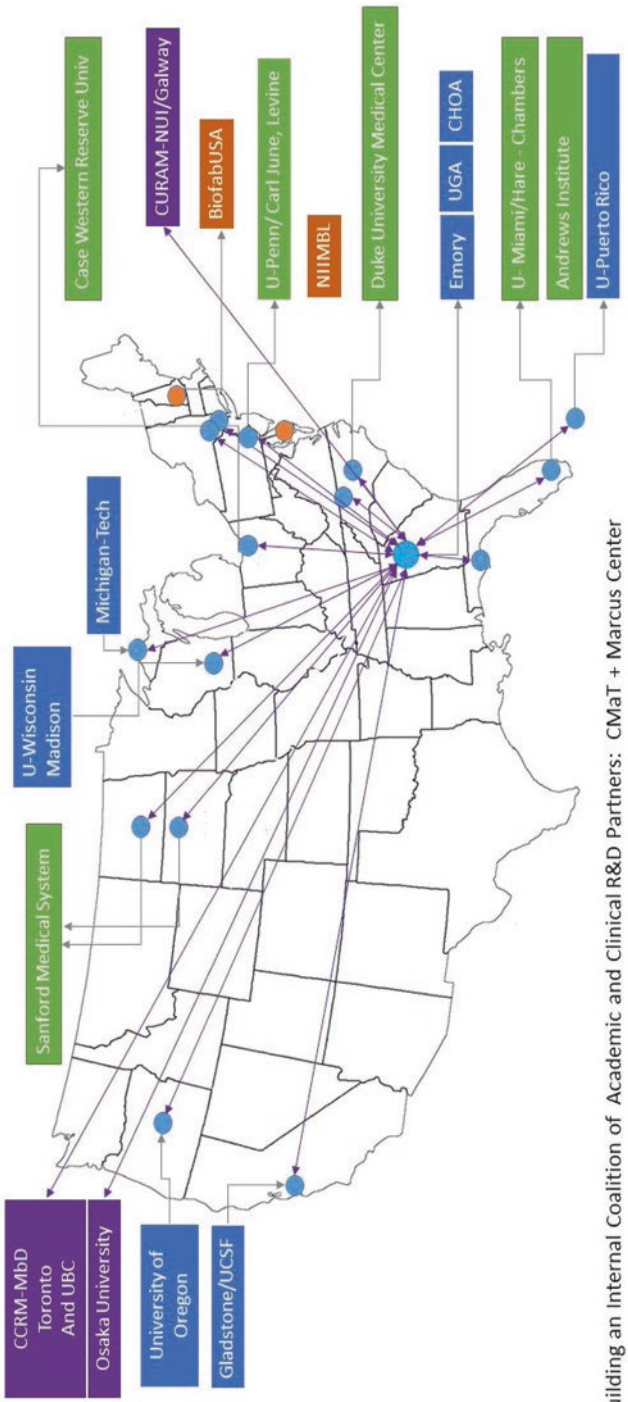

Fig. 3 Current national and international partners of CMaT and MC3M

from Georgia Tech, which included a $5 million commitment towards faculty hire in the area of cell manufacturing technology development. MC3M partnered with several other universities to apply for an Engineering Research Center (ERC) grant from the NSF and in 2017 successfully received an ERC award to establish a new national center on Cell Manufacturing Technologies (CMaT).

CMaT currently includes nine US academic research organizations and four international partners. Georgia Tech is the lead Institute of CMaT and the University of Wisconsin-Madison (U-Wisc), the University of Georgia (UGA), and University of Puerto Rico at Mayagüez (UPRM) are the major partners. Other US-based organizations in CMaT include Emory University, The Gladstone Institutes at the University of California, San Francisco (UCSF), the University of Pennsylvania, the University of Oregon, and the Morgridge Institute for Research, affiliated with U-Wisc. Current international partners include researchers from the Center for Commercialization of Regenerative Medicine (CCRM) and the University of British Columbia in Canada, Osaka University in Japan, National University of Ireland in Galway, and Queen's University in Belfast, Northern Ireland.

MC3M also partners with several clinical centers in the USA, including Emory University, Duke University, University of Miami, the Sanford Medical System and Hospitals in North and South Dakota, the Andrews Institute in Florida, and the Children's Healthcare of Atlanta (CHOA).

Figure 3 shows the rich ecosystem of academic and clinical institutions in CMaT and MC3M, which are working together to advance cell therapies.

4.2 Industry Partners

A key aspect of CMaT is its partnership with a broad group of stakeholders across the cell manufacturing value chain, including industry. CMaT, as of June 2021, has 28 industry partners, including big pharma, tools companies, mid-size biotechs, and start-up companies. A key aspect of all ERCs, including CMaT, is its close collaboration with industry, to understand the challenges and barriers faced by the industry and to work on projects relevant to industry translation of cell manufacturing technologies. These companies not only participate in CMaT projects by providing valuable and timely input, they also form CMaT's Industry and Practitioners' Advisory Board (IPAB) to advise researchers and CMaT leadership on project selection, project progress, industry relevance, and emerging gap areas. A list of current industry partners can be found at www.cellmanufacturingusa.org.

4.3 Organizational and Management Structure

All of CMaT's activities are organized into four synergistic and interdependent focus areas – research, education and workforce development (EWD), innovation ecosystem (IE), and diversity and inclusion (D&I). The three pillars of research,

Fig. 4 The CMaT structure

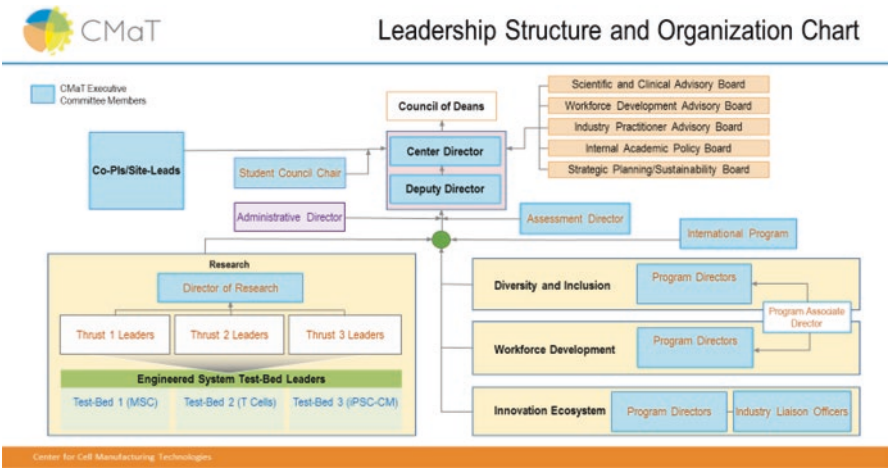

Fig. 5 CMaT structure

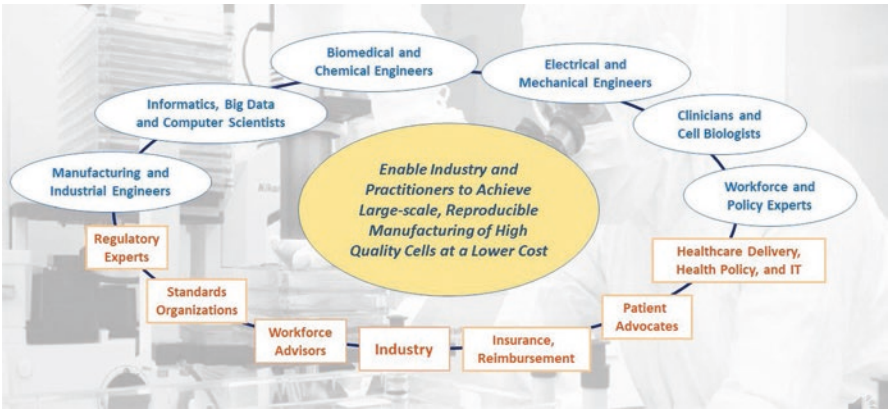

Fig. 6 Bringing all stakeholders on deck

Fig. 7 Images of researchers in MC3M during daily operations

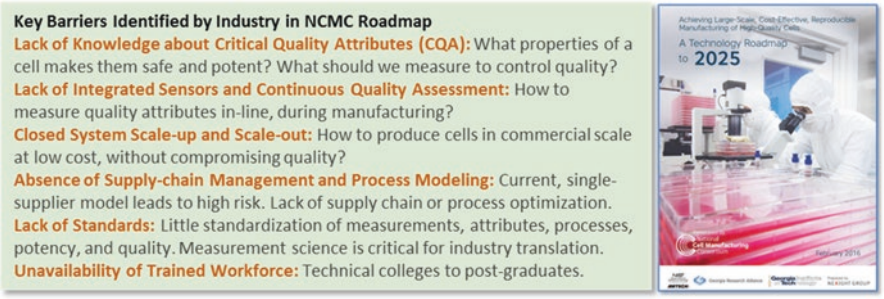

Fig. 8 Key barriers identified by industry in NCMC roadmap

EWD, and IE are all deeply rooted in and supported by the fundamental foundation of diversity and inclusion (D&I), which ensures that CMaT (a) is an exemplar and leader in creating and championing an inclusive culture and (b) continuously recruits and retains a diverse group of trainees from high schools, 2-year colleges, and undergraduate and graduate programs, as well as a diverse group of faculty and research professionals, to enrich the cell manufacturing ecosystem and enable better innovation and intellectual process. Figure 4 represents this collective vision. Figure 5 shows the overall organizational and management structure in CMaT.

At U-Wisc, the site PI/Associate Director for Research/Co-Lead Test-Bed 3 (Sean Palecek) is joined by six other faculty. Particular research strengths at U-Wisc are in iPSC culture and differentiation, biomaterials, and gene editing (Fig. 6).

At UGA, the site PI (Steven Stice) is joined by seven other faculty. Particular research strengths at UGA are in iPSC culture and differentiation, image analysis, and NMR analysis. In terms of leadership roles, Arthur Edison is the Co-Lead of Thrust 1. James Warnock (Chair, Biomedical Engineering) is Co-Workforce Development Director.

At UPRM, the site PI (Madeline Torres-Lugo) is joined by six other faculty. Particular research strengths at UPRM are in big data analytics, iPSC culture and differentiation, and biomaterials synthesis. In terms of leadership roles, Maribella Domenech is the Co-Lead Test-Bed 3, due to her expertise in working with pluripotent stem cells (Fig. 7).

Personnel in each of our affiliated universities (Emory, Gladstone Institutes, the Abramson Cancer Center at the University of Pennsylvania, Michigan Tech, and University of Oregon) bring key expertise in cell manufacturing research and clinical translation, especially through its GMP facility (EPIC). The University of Oregon brings expertise in sensor development and mesenchymal stromal cell culture. The Abramson Cancer Center at U-Penn is one of the world's foremost authorities in T-cell immunotherapies. The Gladstone Research Institute is a renowned Center of Excellence in stem cell research and biology and provide complementary strengths to CMaT (Fig. 8).

4.3.1 Management Structure

Research

CMaT Project leads report to Thrust leaders, who are ultimately responsible for synthesizing materials for reports and presentations as needed. All Thrust leaders report to the Associate Director for Research. Test-Bed Leaders organize discussions within their Test-Bed as needed, but do not report directly to Thrust leads.

The research Test-Beds and research Thrusts work closely with the pillars of Engineering Workforce Development (EWD) and Innovation Ecosystem (IE) to ensure (i) training of a diverse, inclusive, and highly multidisciplinary cell manufacturing workforce for the emerging industry and clinical manufacturing centers, from the technician levels to managers and leaders, and (ii) translation of CMaT discoveries and inventions to industry and clinical practice – not only through licensing and best practice dissemination but also by nurturing trainees, faculty, and technologies to support entrepreneurship.

Executive Committee

The CMaT Executive Committee (EC) includes the Director, Deputy Director, and Associate Director for Research, Workforce Directors, Innovation Directors and Industry Liaison Officers, Diversity Directors, the Assessment Director, the International Program Director, and the Student Council Chair. Continual assessment and quantitative feedback-based strategic realignment is key to CMaT's success, so we have included the Director of Assessment in the Executive Committee to assure frequent communication with center leadership. Ultimately this group advises the Director and is essential in shaping changes in policy and procedures for the center.

4.4 Student Leadership Council and Advisory Boards

4.4.1 Student Leadership Council

There are currently 16 members of the Student Leadership Council (SLC), with members from each major partner in CMaT. The SLC communicates directly with the Executive Committee, as the SLC Chair is a member of the Council and thus participates in the biweekly meetings. The SLC works with the Education team to help guide the development of the graduate student experience in CMaT. Leadership in the SLC rotates annually between each of our university partners.

4.4.2 Scientific and Clinical Advisory Board

The Scientific and Clinical Advisory Board (SAB) consists of world-renowned scientists, engineers, and clinicians from all fields related to CMaT's objectives, including GMP manufacturing facility directors, standards experts, patient advocates, and those involved in the reimbursement industry. A full list of SAB members can be found in the CMaT website (http://cellmanufacturingusa.org/scientific-and-clinical-advisory-board). The SAB meets twice yearly (once virtually and once in person at the annual retreat) and is involved in review and sunsetting of projects. In addition, CMaT has established a formal Private-Public Partnership with the Center for Biologics Evaluation and Research (CBER) at the US Food and Drug Administration (FDA). A CBER member joins CMaT's SAB as a Liaison to provide input to the SAB and to CMaT leadership and to understand the latest developments in research and education in this field.

4.4.3 Workforce Development Advisory Board

The Workforce Development Advisory Board (WDAB) consists of renowned education and accreditation experts from around the country, each focusing on different educational levels, from technical college through postgraduate. A full list of WDAB members can be found in the CMaT website. This board advises CMaT on best practices, appropriate directions, and optimal allocation of resources, as well as help with continual assessment of workforce activities. The WDAB meets twice yearly (once virtually and once in person at the annual retreat).

4.4.4 Industry/Practitioner Advisory Board

The Industry/Practitioner Advisory Board (IPAB) is comprised of one representative from each of our member companies (see http://www.cellmanufacturingusa.org/industry). IPAB members complete a yearly SWOT analyses. In addition, they review 6-month and 12-month project reports from each project, which, in addition to the presentations at the CMaT retreat, informs the SWOT analysis. The IPAB meets twice yearly (once virtually and once in person at the annual retreat) and is involved in review and sunsetting of projects and providing project directions.

4.4.5 Sustainability Advisory Board

This board brings tremendous experience and expertise in industry/manufacturing, federal program review, state-level research enterprise sustenance, and venture capitals and is tasked with developing a strategic plan for CMaT's long-term sustainability.

5 Facilities

MC3M has 4000 ft^2 of BSL2 Laboratory space, including an ISO 8 Analytics Laboratory and isolated ISO 7 suites. The facility is state of the art and also fully ADA compliant, including the GMP suites. It fosters faculty, students, and industry collaborations to pilot new process technologies for cell manufacturing needs and also addresses the need to train the next generation of cell manufacturing workforce, including those from technical colleges and veterans. Students and faculty learn proper gowning procedures and operational requirements like material and supply quarantine processes and environmental monitoring. The facility has a GMP-grade autoclave, a direct ducted biosafety cabinet for parallel process of virally transduced product, an automated cryoswitch for automatic monitoring of liquid nitrogen storage units, and an interlock system to maintain pressure differentials. A major focus of the Marcus Center is development of in-line sensors and continuous monitoring of cell quality during manufacturing. The equipment purchased for the MC3M ISO 7 and ISO 8 labs has been sourced for the most part from Thermo Scientific, for IQ/OQ/PQ [Installation Qualification (IQ)/Operational Qualification (OQ)/Performance qualification (PQ)] and capability and GMP/ISO 7 compatibility. The centrifuges, CO_2 incubators, reach-in incubator, refrigerators, and freezers are all purchased from Thermo Scientific. All storage equipment systems are continuously monitored and independently logged every 24–48 h to confirm appropriate storage conditions. As part of an environmental monitoring program, particle counts are recorded on a weekly basis for both dynamic (personnel working) and static (empty lab) readings.

MC3M has GMP-certified portal pass-through to the ISO 7 labs and in-line analytical testing ability such that each GMP suite has a dedicated analytical suite to maintain isolation of products (in-line microscopes, mass spectrometer). This type of in-line analytics is currently unavailable in cell therapy characterization and manufacturing and was identified as the most critical need for cell therapies in the National Cell Manufacturing Roadmap led by Georgia Tech (see below). The facility itself is not GMP certified for the production of commercial product, rather operates under these guidelines to ensure quality and reproducibility of results while providing resources for training for CMaT, Georgia Tech, and others.

6 The Roadmaps

Between 2014 and 2016, the Georgia Research Alliance (www.gra.org) and Georgia Tech led the nationwide consortium (NIST-AmTech Cell Manufacturing Consortium) on cell characterization and manufacturing and developed the national roadmap for the USA to become a world leader in this space. Georgia Tech and the GRA conducted several national workshops to identify challenges faced by the cell manufacturing industry and academic/clinical GMP facilities. This roadmap forms the basis of research and development activities of the Marcus Center. The activities

of the Center will be focused on collaborations across the USA with industry partners and clinicians and academic GMP facilities working on cell therapies. When appropriate, international partners will be included. Many of these partnerships are already established through the Cell Manufacturing Consortium.

The MC3M and CMaT team members helped establish and led the National Cell Manufacturing Consortium (NCMC), an industry-academia consortium funded by the Advanced Manufacturing Technologies program (AMTech) of the National Institute for Standards and Technologies (NIST). AMTech's goal is to establish industry-academia partnerships in key areas of manufacturing and to put together 10-year national roadmaps. The PI, Co-PIs, as well as Thrust and Test-Bed Directors worked in close collaboration with more than 30 industry partners; 15 academic/clinical centers, including Good Manufacturing Practice (GMP) facilities who conduct cell therapy clinical trials; and government agencies (FDA, NIH, NSF, NIST, and DOD), to create and distribute an industry-driven, 10-year national roadmap titled: "Achieving Large-Scale, Cost-Effective, Reproducible Manufacturing of High-Quality Cells: A Technology Roadmap to 2025." This roadmap, completed in February 2016 and also highlighted by the White House (Fact Sheet and Organs Summit, June 13, 2016), is a product of 4 national workshops, with 70+ thought leaders focused on visioning, challenges and gaps, solution concepts, and integration. The roadmap clearly identifies current challenges (translational barriers) and outlines clear, stakeholder-driven, quantitative engineering milestones (categorized by short-, medium-, and long-term needs) that must be achieved to make manufactured cells a viable, reproducible, and reliable product. We (and the NCMC) believe that it is time to implement this roadmap, across all technology readiness levels (TRLs). CMaT will be the "sandbox" for new, discovery-driven preclinical engineering innovations (TRLs 1-5), where industry and academia will seamlessly collaborate, making fundamental discoveries and inventing transformative technologies within an ecosystem of world-class convergent expertise, innovation, infrastructure, and workforce development. These roadmaps are accessible via the CMaT website at http://cellmanufacturingusa.org/ncmc.

Fig. 9 NCMC cell manufacturing roadmaps

The 2016 National Roadmap (and the subsequent 2017 and 2019 update, Fig. 9) from the National Cell Manufacturing Consortium (a public-private consortium of industry, government, clinical, and academic leaders) identified lack of skilled cell manufacturing workforce and lack of appropriate training in data analytics and data sciences as two major barriers for the translation of promising therapies into products and suggested that these areas need immediate national attention. We, at the recently established NSF Engineering Research Center (ERC) for Cell Manufacturing Technologies (CMaT), are partnering with engineers and scientists, clinicians, industry practitioners, workforce development experts, education leaders, diversity and inclusion experts, social scientists and policy experts, as well as reimbursement, regulatory, and standards development experts to build the future workforce, and retrain the current workforce, for this emerging industry. The 2017 roadmap update specifically identified four critical areas for immediate attention: process automation and data analytics, especially for identification of critical quality attributes (CQAs) of therapeutic cell products and related critical process parameters (CPPs); supply chain and transport logistics; workforce development; and standardization and regulatory support. The first two areas, which are the most critical research and development areas identified by industry, academic, and government stakeholders, require significant skills in data analytics, artificial intelligence (AI), and predictive modeling. The third, workforce development, must integrate the necessary research skills for the emerging cell manufacturing industry into the training of the future jobseekers and develop innovative ways to retrain current workers to meet the challenges and needs of the industry. CMaT, along with industry and government partners, is developing key data science and AI concepts for the biomanufacturing industry while identifying the necessary skill sets for the emerging cell and gene therapy manufacturing jobs. It is clear that a convergence of expertise spanning cell culture and cell biology, bioprocess engineering, sensors and automation, manufacturing and supply chain sciences, regulatory knowledge, and most importantly data sciences, AI, and predictive analytics is needed for the success of this industry.

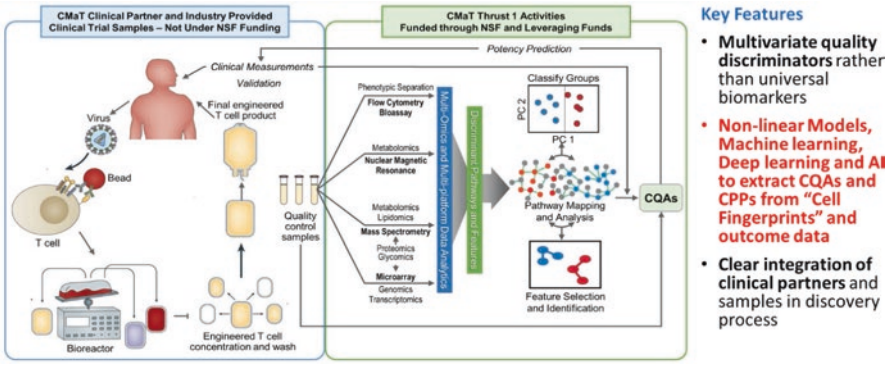

Fig. 10 Data analytics, AI, and predictive modeling to identify CQAs, CPPs, and patient attributes for successful outcomes

However, such a multidisciplinary training platform does not currently exist and requires a focused, well-resourced, national convergence science approach involving stakeholders across academia, industry, and the government.

One of the key challenges identified by industry in the NCMC roadmap is this lack of understanding of what the CQAs are for a given product and disease indication, as well as the corresponding CPPs that would allow manufacturers to control and ensure quality. It is also unclear as to which patient population or disease subtypes would benefit from particular cell therapy products and how to integrate patient-specific data into the cell manufacturing process to generate the most effective and safe cells. Unlike single molecule therapeutics (small molecule drugs or protein/nucleic acid biologics), cells are extremely complex and are "living drugs" whose properties change during manufacturing.

Cells can be characterized using a wide variety of measurements – from transcriptome and epigenome to proteome, metabolome, surface markers, secretome, cell morphometry, biophysical properties, etc. – and a combination of these variables is likely to provide predictive quality attributes for the product. However, very little has been done to combine these multi-omics data sets with biophysical or morphometric properties and preclinical or clinical outcomes and patient-specific data to identify cell CQAs and related CPPs. This is a major thrust area in CMaT (Fig. 10) where we are already applying complex data analytics, AI and machine learning methods, and cloud-based, HIPAA-compliant, data management systems (in collaboration with Amazon Web Services, AWS).

Another major barrier identified by industry is the lack of a robust supply chain for the manufacturing process and a nascent, poorly scalable logistics/distribution system – especially in the context of a highly temperature sensitive product (i.e., living cells or tissues). Therefore, computational models of supply chain for autologous and allogeneic cell therapies that addresses these issues to build optimal supply chain and logistics platforms are required, regardless of the manufacturing paradigm (e.g., centralized vs. distributed manufacturing, bedside manufacturing, etc.). This is also a major focus area in CMaT where data analytics, machine learning, and AI-based models are being employed. The goal is to not only design fail-safe supply chains but also to be able to predict the impact of a variety of disruptions to distribution and logistics in manufacturing. Risk mitigation strategies to cope with these disruptions, including interactions with reagent suppliers, manufacturing facilities, and clinics, are also being modeled. Efforts are underway to integrate actual costs, regulations, ethical considerations of access, and standards into the model to better understand the impact of supply chain and manufacturing process design on various stakeholders.

A third challenge identified by industry and clinical manufacturers is in the area of in-line monitoring and automation. A product as complex as a living cell requires in-line monitoring of the manufacturing process and continuous or at least regular

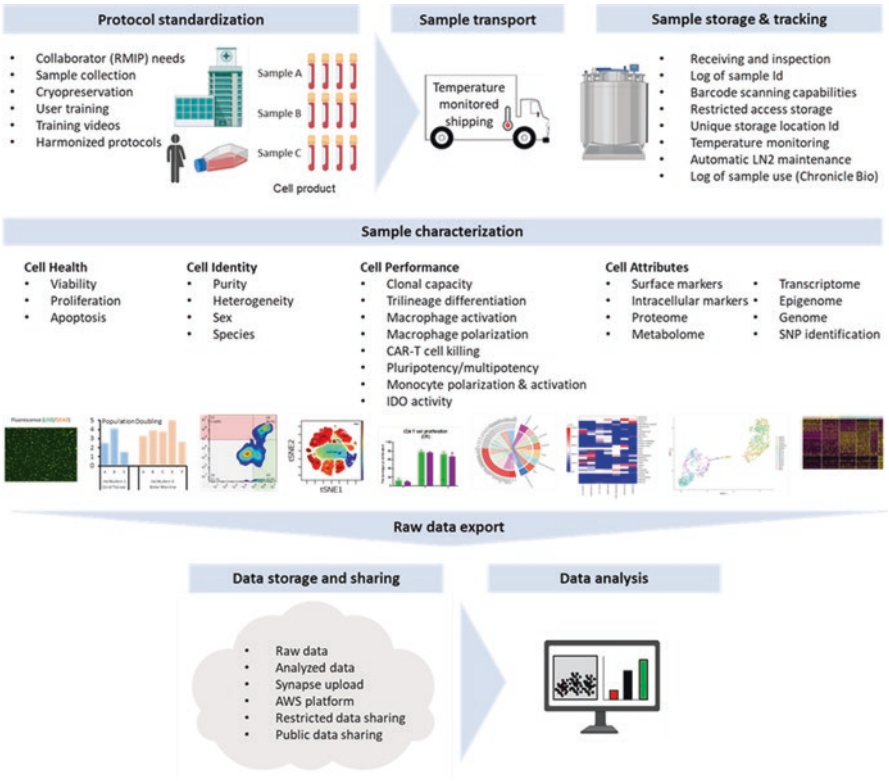

Fig. 11 Workflow for standardization and harmonization of protocols and data within CMaT and MC3M

quality assessment and quality control. Ultimately, industry prefers a quality-driven manufacturing paradigm with feedback-controlled process automation. Therefore, process sensor or continuous product quality measurement-based intelligent automation will become an integral part of biomanufacturing in the future. In the biologics manufacturing field (e.g., monoclonal antibodies), this concept of continuous manufacturing and process control is slowly being implemented and will also become critical for cell and gene therapy manufacturing in the future.

7 Harmonization and Standards Development

A key best practice in a large center like CMaT is protocol and data format standardization across the different labs and institutions, as well as keeping detailed batch records of experiments in a standardized format. These should be kept in a seamless cloud-based data management system, accessible from anywhere in the CMaT ecosystem. CMaT partnered with Amazon Web Services (AWS) to set up a cloud-based

data management system for all our omics data – this became operational in the second year and is being tested in a few CMaT labs on a pilot basis. To enable the customizable and eventually standardized batch record system, CMaT is working with Cytiva (formerly known as GE Healthcare), to implement their new Chronicle™ LIS/data management system, which is GMP compatible and operates on the AWS platform. Figure 11 shows a workflow for standardization and harmonization of protocols and data within CMaT and MC3M.

Protocol standardization: protocols for sample processing and preservation are essential for obtaining high-quality data quality downstream. We create harmonized protocols, as well as user training for sample handling, based on RMIP researcher needs. Sample collection should be done with consideration to downstream characterization needs, such that preservation method, cell number, and replicates of each sample are appropriate for desired characterization work.

Sample transport: transport should be done in a temperature-monitored fashion, to ensure that sample quality is maintained.

Sample storage and tracking: storage and tracking of each sample is done in an auditable fashion, such that each sample vial is matched to a unique storage location, as well as to user, assay protocol, material lot, and resulting data. Storage temperature is automatically maintained, and storage access is restricted, in order to minimize temperature fluctuations.

Sample characterization: characterization is performed using validated methods and equipment, according to SOPs.

Data storage and sharing: raw data is exported onto a cloud-based platform, enabling easy access for further analysis. Data sharing can be set up with user restrictions or as a publicly available project.

Data analysis: raw data is made available in user-friendly format, for ease of use in analysis. We have the capability to perform further data analysis, and this can be provided where appropriate.

8 Workforce Development

CMaT's workforce development program is designed to recruit, inspire, and train a highly inclusive group of next-generation engineering innovators and leaders with broad, convergent expertise in biomanufacturing and, specifically, cell manufacturing technologies. Drawing on evidence-based approaches and established best practices, the Engineering Workforce Development program trains technically skilled, globally competitive, and culturally aware engineers prepared to advance cell manufacturing.

The program addresses critical workforce needs identified by our industry partners in the 10-year National Cell Manufacturing Consortium (NCMC) roadmap and reflects the latest NCMC roadmap update undertaken in August 2019. We are

Table 1 Regional strategies

Region	Strategy
Georgia	We are building on state investments to support advanced bioscience training by prioritizing our partnership with the Technical College System of Georgia (TCSG) through summer research experiences for both instructors and students and long-term internships for students. We partner with TCSG instructors to develop new curricular materials for 2-year colleges to help meet the workforce needs identified by our industry partners.
Wisconsin	We are prioritizing work with an existing stem cell technologies certificate offered by the Madison Area Technical College and are working to integrate cell manufacturing content into this successful program.
Puerto Rico	We are focusing on our partnership with the local public school system, especially at the high school level. These efforts aim to engage students in the fields of cell and biomanufacturing both to build a pipeline of students interested in these areas and to help prepare students to contribute to rebuilding the island's pharmaceutical and biomanufacturing sector following Hurricane Maria.

developing new research, training, and outreach experiences designed to achieve the following outcomes:

- A diverse group of undergraduate and graduate engineers with a unique set of key technical and professional skills necessary to transform the cell manufacturing industry
- Students from 2-year technical colleges prepared for careers in biomanufacturing
- Increased student interest in bioengineering and cell manufacturing at the precollege and college levels, especially among underrepresented groups in engineering
- Teachers and instructors at all levels – from K-12 to graduate education – who use new curricular materials, new pedagogical approaches, and new strategies for broadening participation
- An ecosystem of sustainable partnerships that link industry, global institutions, K-12 schools, technical colleges, and universities to address the current and future needs of the cell manufacturing workforce

Both our university education and our precollege education programs include targeted recruiting and outreach to underrepresented students – including students of color, women, English learners, and students with disabilities – to broaden participation and develop a culture of inclusion within the cell manufacturing stakeholder community. We are leveraging existing successful partnerships and local and state resources (Table 1) to bring talented undergraduate and high school students to CMaT labs at each partner institution for hands-on training experiences. We are connecting all CMaT trainees with their peers at other institutions, CMaT faculty, and industry-clinical stakeholders through collaborative research projects, education programs, annual retreat, weekly meetings, and a trainee exchange program. Finally, we are working closely with the industry/practitioner advisory board (IPAB)

and professional associations to identify key continuing education needs and to develop professional education programs to support the industry.

9 Regional and CMaT-Wide Synergies

While CMaT's EWD efforts are center-wide, each CMaT institution has its own unique strengths and is located within the context of a larger regional economy. As a result, we are prioritizing and tailoring EWD activities across the CMaT ecosystem to maximize their impact.

10 Innovation Ecosystem

The vision of CMaT's Innovation Ecosystem (IE) is to enable a vibrant cell manufacturing industry capable of producing large-scale, low-cost, reproducible, and high-quality cell-based therapeutics to reduce human suffering, improve quality of living, and lessen the financial burden on the healthcare system. The mission is to pioneer a dynamic set of mutually beneficial partnerships with the cell manufacturing value chain to accomplish the strategic IE goals while simultaneously achieving global intellectual leadership, national industrial competitiveness, lasting economic impact, and broader access to cell therapies for patients. The five strategic IE goals are to:

- Engage a diverse group of innovation leaders, entrepreneurs, industry, and practitioners in all aspects of the ERC to enhance impacts, effectiveness, and efficiency
- Partner with nontraditional stakeholders (standards bodies, regulatory experts, policy forums, reimbursement industry, clinicians, and others) to ensure that engineering innovations happen in the context of standardization, regulatory policies, and reimbursement framework and they are relevant and translatable
- Nurture the ecosystem through a series of enriching meetings and programs that foster a two-way exchange of knowledge and value across all stakeholders
- Empower students and faculty with knowledge, connections, and skills necessary to be entrepreneurs and create new start-ups
- Develop sustainable partnerships and business models for CMaT to flourish beyond NSF support

Industrial and practitioner partnerships are established throughout the cell manufacturing value chain to create a vibrant community of stakeholders. This includes pharma, biotech, cell therapy, contract manufacturing (CMO), process automation, and sensor companies; tools and reagents companies; government agencies, regulatory bodies, and standards organizations; private foundations, consulting, and specialty firms for transition to GLP/GMP; and the health insurance/reimbursement

industry. The CMaT approach is to recruit and retain a diverse portfolio of industry members across the value chain and also engage a broader community of practitioners and stakeholders. The current cadre of industry member companies spans the complete cell manufacturing value chain and represents a mixture of small to large companies. The Industry/Practitioner Advisory Board (IPAB) is comprised of one representative from each of our member companies. CMaT has established a fee-based membership program where participating industry members contribute financial and in-kind support. The fees and benefits are tiered to facilitate attracting companies from the whole value chain, of diverse sizes, differing capacity for participation, and varied interests. Creating a diverse ecosystem of companies is highly important to CMaT to cultivate relationships between researchers, students, and companies, as well as exposing students/trainees/PIs to the different needs and priorities of companies at various stages of formation, growth, and development.

IPAB members are expected to (1) be actively engaged in CMaT; (2) help solicit, review, and select research projects, provide insights on research gaps, and lend expertise in manufacturing, validation, design, regulations, economic evaluation, and technology transfer; (3) contribute to workforce programs by providing guidance on the desired skill sets needed for success in industry and, when appropriate, teaching; (4) provide students with industry perspective by mentoring and hosting internships; and (5) assist in establishing a culture of innovation and inclusion. The IPAB recognizes that access to highly skilled human capital is a key benefit derived from ERC participation. As such, they are eager to contribute to workforce initiatives. Tight coupling of industry with workforce programs will contribute to CMaT's long-term sustainability.

There are several ways that company representatives engage with CMaT. All company representatives are invited to join weekly all CMaT community web-based video conference calls. These video conference calls focus on one Thrust area each week and report out the progress of one to two projects within the Thrust area. Part of the on-boarding process for new member companies includes an introduction and company overview during one of the CMaT weekly calls. CMaT weekly calls consistently have broad participation from researchers and member companies, with all participants providing feedback and perspectives in an open forum. As member company representatives identify specific projects of interest, the Industry Liaison Officers (ILOs) facilitate direct connections to the research teams so that companies are able to engage more deeply in areas of interest. This engagement does not just focus on the research outputs but all components of CMaT – course development and desired workforce skills feedback, diversity and inclusion workshops, and tools.

The IPAB meets twice each year with the purpose of reviewing Center progress. One meeting takes place during the Annual CMaT Retreat, where the entire CMaT community meets in person to share project results, deepen connections and collaborations, participate in CMaT-wide training courses, and connect with the broad industry member community. Member companies are invited to include multiple participants, and the Annual Retreat helps connect trainees directly with industry as well as strengthen the ties between research teams and the industry members

interested in specific projects. Other activities at the Annual Retreat include opportunities for trainees to engage with industry through career panels, organized lunch discussions, and poster sessions with industry judging.

CMaT's technology transfer strategy for moving ERC-developed technologies to market has two sequential approaches. The first approach is to license ERC-generated intellectual property (IP) to CMaT member companies. The second is to either commercialize technologies through new company formation or license to non-CMaT companies. This second approach is only available if and when all CMaT member companies pass on a license to ERC-developed IP or if the member companies choose to license a particular invention only in fields of use not overlapping with the targeted field of use of a licensee or start-up company being spun out of the ERC.

CMaT's IP policy provides priority access to IP based on membership tier level. In Year 2, Full (Tier 1) members get 30 days to review the IP before Associate (Tier 2), Affiliate (Tier 3), and Start-Up (Tier 4) members. In all instances, nonconfidential notices of the invention disclosures are provided, and members must opt-in to see the confidential/enabling information. Full members expressing interest in supporting patent filing can secure a noncommercial, nonexclusive, royalty-free license (NERF) at a minimum. They also get a 6-month option period to negotiate an exclusive commercial license. After the 30-day Tier 1 priority review period, Tier 2 and 3 members can decide if they want to support patent filing and receive a NERF. They also have the option to negotiate an exclusive/commercial license if no Full members exercise their option. In the event that no member companies opt to license the IP, the inventions revert back to the originating academic members to be marketed by their respective tech transfer offices.

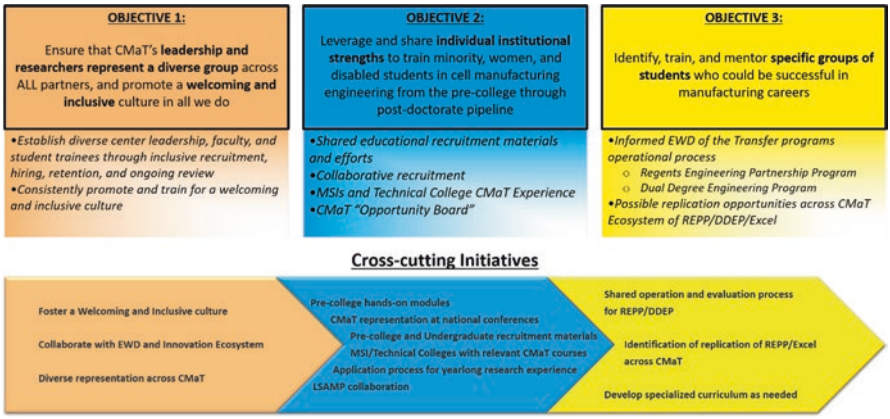

Fig. 12 Diversity and inclusion objectives

11 Diversity and Inclusion

The mission of CMaT's diversity and inclusion pillar is to be the "best-in-class" engineering research consortium by infusing the values of diversity and inclusion at each educational level and across all program components. CMaT aims to establish and embody a new set of norms and values throughout the emergence of the cell therapy research, development, and manufacturing industry. As an ERC, CMaT has the opportunity to substantially impact the representation and culture within our own center and to promote these values throughout the CMaT ecosystem to positively influence the emerging cell manufacturing industry.

All CMaT institutions have a strong history and a deep commitment towards diversity. Higher Education Magazine in 2015 ranked GT #1 in graduating Minority Engineers at the BS and PhD levels, including #1 in African American and Hispanic doctorates and #2 in African American bachelor's degrees. U-Wisc and UGA have increasing populations of underrepresented groups. The goal is to significantly increase diversity among all CMaT participants, but particularly at these universities.

Diversity and inclusion are at the core of CMaT and are central to achieving CMaT's goals and objectives (Fig. 12). These initiatives facilitate an ecosystem-wide culture of inclusion and are integral to CMaT's workforce development and engagement. CMaT's diversity and inclusion strategy is centered around a collaborative and multiple touch point model to achieve the engagement of underrepresented groups within CMaT.

- *Collaborative*: Peer/group, team, and tiered strategies are utilized with students, faculty, labs, and industry for effective mentoring/modeling.
- *Multiple touch points*: These include ongoing communication via e-mail, workshops, social media, and newsletters and engagement in multiple CMaT initiatives/activities for recruitment, retention, and training.

These strategies guide CMaT's focus on the following three objectives across CMaT.

- *Objective 1*: Ensure that CMaT's leadership, researchers, and students represent a diverse group across *all* institutional partners and promote a *welcoming and inclusive culture* in all we do.
- *Objective 2*: *Share and leverage strengths* (SLS) from CMaT unique geographical positioning to the individual institutional diversity and inclusion programs across partners to train minority, women, and disabled students in cell manufacturing engineering from the precollege through postdoctorate pipeline.
- *Objective 3*: Identify, train, and mentor specific groups of students, such as veterans, disabled students, or other nontraditional students, who could be successful in manufacturing careers with targeted CMaT industry training.

12 Conclusions

Cell-based therapies have already shown to have a transformative effect on the practice of medicine and patient outcomes in hematological cancer. In the coming years, it is expected that cell therapies will revolutionize the treatment of wide variety of chronic and acute, previously incurable diseases. Despite their promise, scalable manufacturing of cells with a desired set of quality attributes remains a significant challenge. CMaT is developing the next generation of cell manufacturing tools, from process analytical technologies and improved cell bioprocessing methods to mathematical algorithms for predicting cell function or supply chain logistics. Such public-private international partnership that brings together stakeholders and researchers from a wide array of disciplines and creates a convergent ecosystem is critical to solve the complex challenges in cell manufacturing.

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References

1. Mullard, A. (2017). FDA approves first CAR T therapy. *Nature Reviews Drug Discovery, 16*(10), 669.
2. Mullard, A. (2017). Second anticancer CAR T therapy receives FDA approval. *Nature Reviews Drug Discovery, 16*(12), 818.
3. Piscopo, N. J., Mueller, K. P., Das, A., Hematti, P., et al. (2018). Bioengineering solutions for manufacturing challenges in CAR T cells. *Biotechnology Journal, 13*(2).
4. Campbell, A. T., Brieva, L., Raviv, J., Rowley, K., et al. (2015). Concise review: Process development considerations for cell therapy. *Stem Cells Translational Medicine, 4*(10), 1155–1163.
5. Ahrlund-Richter, L., De Luca, M., Marshak, D. R., Munsie, M., et al. (2009). Isolation and production of cells suitable for human therapy: Challenges ahead. *Cell Stem Cell, 4*(1), 20–26.
6. Dodson, B. P., & Levine, A. D. (2015). Challenges in the translation and commercialization of cell therapies. *BMC Biotechnology, 15*, 70.
7. Lipsitz, Y. Y., Timmins, N. E., & Zandstra, P. W. (2016). Quality cell therapy manufacturing by design. *Nature Biotechnology, 34*(4), 393–400.
8. Riviere, I., & Roy, K. (2017). Perspectives on manufacturing of high-quality cell therapies. *Molecular Therapy, 25*(5), 1067–1068.
9. Galipeau, J., & Sensebe, L. (2018). Mesenchymal stromal cells: Clinical challenges and therapeutic opportunities. *Cell Stem Cell, 22*(6), 824–833.
10. Haddock, R., Lin-Gibson, S., Lumelsky, N., McFarland, R., et al. (2017). *Manufacturing cell therapies: The paradigm shift in health care of this century* (Discussion Paper. NAM Perspectives). https://nam.edu/manufacturing-cell-therapies-the-paradigm-shift-in-health-care-of-this-century/. Accessed 4 Dec 2020.
11. Moll, G., Hoogduijn, M. J., & Ankrum, J. A. (2020). Editorial: Safety, efficacy and mechanisms of action of mesenchymal stem cell therapies. *Frontiers in Immunology, 11*, 243.
12. Pittenger, M. F., Discher, D. E., Peault, B. M., Phinney, D. G., et al. (2019). Mesenchymal stem cell perspective: Cell biology to clinical progress. *NPJ Regenerative Medicine, 4*, 22.

Financial Considerations for Academic GMP Facilities

Adrian P. Gee

1 Whether or Not to Build a cGMP Facility

Current successes in cellular therapy, e.g., chimeric antigen receptor (CAR) T cell therapies for leukemia, have re-excited interest in the field to the extent that several academic institutions are considering building and operating new cGMP facilities, much as many countries feel that they must have a national airline. Realistically this choice has to be made very carefully. Among the questions to be asked are as follows: (i) Is there a foundation of faculty and staff with an interest in using such a facility, and, if not, what should be the relative timing of recruiting such people and building a facility? (ii) Will the faculty be able to attract competitive funding to help support the facility? (iii) Where will the funding be obtained for building and, more importantly, operating such a facility regardless of the level of activity? These are vital considerations since, at best, the facility may just break-even. (iv) Does the facility intend to support only internal projects or will contract manufacturing be performed? (v) Does the patient referral pattern and catchment area support the need for such a facility, or are there pre-existing facilities that would be potential competitors?

Given that cellular therapies are currently attracting attention, it may be possible to obtain partial or entire foundation or charitable support to build a facility, e.g., the Gates Biomanufacturing Facility at the University of Colorado. There are few opportunities to obtain building costs through governmental agencies in the United States, although the California Institute of Regenerative Medicine (CIRM) provided in 2008 $272 million in funding for building 12 stem cell research facilities [1], which included $20 million for constructing the GMP facility at the University of California Davis in Sacramento. In Canada, funding has been available through national funding agencies to construct cGMP facilities, e.g., the Center for the

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Commercialization of Regenerative Medicine (CCRM) facility in Toronto, which was designed to bridge the gap between academia and industry to bring promising new therapies to the market. Governmental funding has not generally been as available to support operating costs. In Europe national funding has been available for the construction of several facilities, e.g., the Catapult cell and gene therapy GMP facility in Stevenage in the United Kingdom [2] was built using a grant of $87 million and works in collaboration with Guy's Hospital in London to develop and commercialize cell and gene therapies. The Centre for Cell Manufacturing was established at the National University of Ireland in Galway in 2014 by the Irish Ministry of Research and Innovation [3]. It is licensed for the manufacture of stem cells for use in human clinical trials. In the United States, commercial entities have supported building cGMP facilities, e.g., Novartis funded construction of the new $27 million, 23,610 ft^2 Novartis-Penn Center for Advanced Cellular Therapeutics CGMP facility at the University of Pennsylvania [4].

2 What to Build

In the United States, the processing of minimally and more-than-minimally manipulated products is regulated differently [5] from that of minimally manipulated products [6]. Some institutions handle these two classes of products differently. The former are produced in Class 100 (ISO5) biological safety cabinets placed in unclassified space, while the latter are manufactured in a clean room environment. In other facilities both types of products are manufactured in clean rooms. Clean room space is more expensive to construct, and, therefore, this choice is an important one.

The decision of the amount of space to build is also critical. Probably the best choice is not only to build sufficient clean room space to meet anticipated demand for the next 2–3 years but also to provide additional shell space to expand the facility in the future. This can be used for offices and storage in the interim but is constructed so that it can be easily refitted with HVAC, etc. to inexpensively convert it to additional clean room space.

Another important choice is the construction method. The most expensive option is bricks and mortar with separate air handling capacity. Alternatives include prefabricated modular clean rooms [7] that can be located in shell space. These vary in complexity to full facilities with multiple rooms and linking corridors, storage, and gowning areas. They are available from multiple manufacturers. Cheaper options consist of a clean room "tent" or enclosure that is placed in unclassified space. For institutions that are not contemplating new construction, it is possible to locate isolators [8] into exiting unclassified space. These are clean room workstations that basically consist of self-contained biological safety cabinets linked to incubator space. Manufacturing is performed entirely within this space, which is accessed from the outside by means of glove ports. This option restricts the number of products that can be manufactured simultaneously, unless multiple isolators are purchased.

Facility design varies from the use of multiple smaller manufacturing suites, each of which is dedicated to a particular product type, to large manufacturing areas containing multiple biological safety cabinets. The FDA has approved both designs, but in both cases, there must be written changeover (line clearance) procedures if multiple products are handled in the same space. Most academic facilities favor the former design to allow more flexibility in the range of products that can be prepared within a single facility.

3 Operational Costs

It is easy to focus initially on the construction costs of a cGMP facility; however, in the longer term, it is the running costs that may make or break the budget [9]. There are certain expenses that must be met regardless of whether or not manufacturing of products is taking place. These include utilities (HVAC costs are high), cleaning, equipment calibration and maintenance, environmental monitoring, and staff costs to provide these services. Normally these costs can be incorporated into manufacturing costs, but if the facility is not busy, there must be an alternative funding source to cover these expenses. One approach to addressing this problem is to use the facility to manufacture billable therapeutic cell products, e.g., hematopoietic stem cells, in addition to non-chargeable investigational products [10, 11]. This helps to bring in a regular income and provide a baseline level of use of the facility at all times.

The primary source of income for academic cGMP facilities is peer-reviewed grants which incorporate a clinical trial (Fig. 1). In the United States, the primary sources of these funds are the National Cancer Institute; the National Heart, Lung, and Blood Institute; and other institutes and the Leukemia and Lymphoma Society and the American Cancer Society, the Alliance for Cancer Gene Therapy, the California Institute for Regenerative Medicine, and some of the professional societies. These may support both viral vector manufacturing and the preparation of the cellular therapy products and their testing. Multiple such grants are normally required to support facility operations, since phase 1/early-phase 2 trials usually require preparation of limited numbers of products. National funding agencies are becoming more used to budgets including manufacturing and testing costs in addition to some cGMP operational costs.

4 Contract Manufacturing

Another option is contract manufacturing for biotech companies (Fig. 1). An example of which is EATRIS, the European Infrastructure for Translational Medicine, which is a consortium of 80 European research institutions including academic and hospital-based GMP facilities [11] in Finland, the Netherlands, and Italy. This group

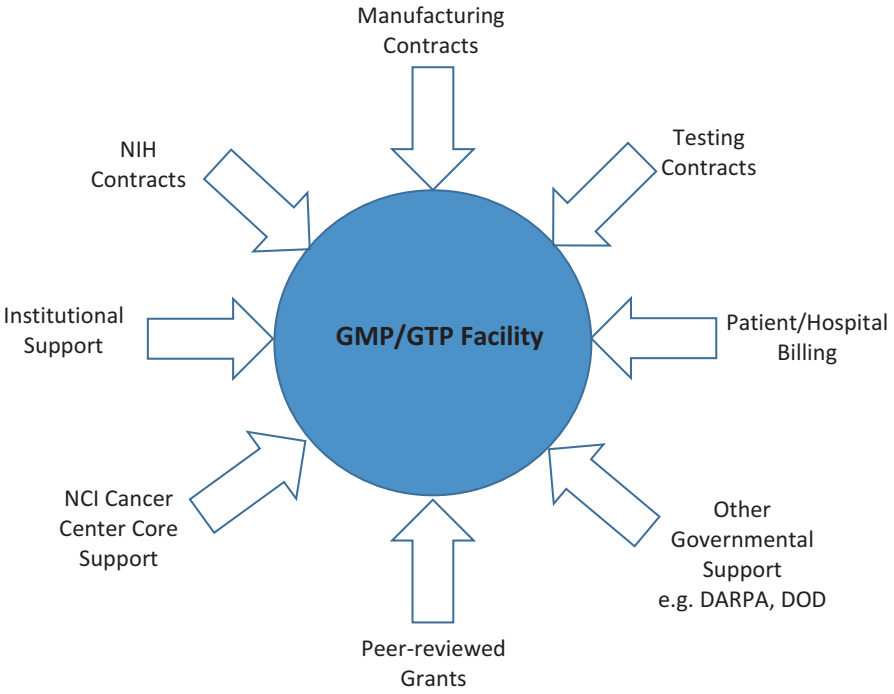

Fig. 1 Sources of income for GTP/GMP facilities
NIH US National Institutes of Health, *NCI* US National Cancer Institute, *DARPA* US Defense Advanced Research Projects Agency, *DOD* US Department of Defense

provides translational, manufacturing, testing, and tissue banking services for advanced therapy medicinal products. Other arrangements are generally of two types. A contract may use the facility's existing staff to prepare the products, e.g., EATRIS; alternatively, the company may rent facility space from the academic institution and use their own staff for manufacturing [12]. Both arrangements involve liability and supervisory issues to ensure that cGMP regulations are being followed. Contract manufacturing usually requires the development of good working practices between the two entities. There is a tendency for the contractee to assume that their work takes top priority and to request changes and additional services at little or no cost during the course of the contract. At the same time, the contractor must ensure that expectations, e.g., timelines, are met routinely. The establishment of a good working relationship may take some time, but this usually pales by comparison to the time required to develop the initial legal contract. Many facilities include a quality control laboratory, which performs product testing and environmental monitoring. These services may be of interest to other laboratories within the institution and provide an additional source of income. The same is true for other services, such as regulatory assistance for clinicians preparing an Investigational New Drug (IND) application.

There are somewhat limited opportunities to obtain generic, rather than product-specific funding from national entities, but these are described in separate chapters in this volume. One example was the Production Assistance for Cellular Therapy contract sponsored by the National Heart Lung and Blood Institute. This program, which has been funded for 15 years, provides support to selected academic cGMP manufacturing facilities to prepare cell therapy products, and during the first 10 years, it included some infrastructure support [13]. These products were used exclusively for early-phase clinical trials [14]. During the third iteration, products were originally prepared for translational studies, but the scope was subsequently broadened to include clinical products. State entities such as CIRM and the Cancer Prevention and Research Institute of Texas (CPRIT) [15] have provided funding for core activities, e.g., CPRIT has funded cGMP manufacturing activities for manufacturing cell therapy products and vectors for use in both adult and pediatric cancer treatments.

5 Other Funding Sources

Institutional support may be an option in some cases. If an institution is a US National Cancer Institute Comprehensive Cancer Center, some funding may be available if the facility is named as a shared resource for the center. In addition, if the hospital or medical center is promoting the availability of cellular therapy at its institution, they may be willing to provide some support to the facility as an unrestricted gift or grant (Fig. 1).

Although the vast majority of products manufactured in an academic cGMP facility will be for research use, it is possible to charge for investigational drugs under the FDA cost recovery program. This is described in a guidance "Charging for Investigational Drugs under an IND – Questions and Answers" published in June 2016 [16]. This program allows for the recovery of only the direct costs associated with making the drug product available to the patient in the clinical trial.

6 Conclusions

Development of an academic cGMP facility is fraught with difficulties. Firstly, it should not be anticipated to generate income for the institution (unless it has a number of very profitable manufacturing contracts) [17, 18]. This is a consequence of (i) the existence of continuous running costs, regardless of the level of manufacturing activities, and (ii) the inability to charge patients for the research products manufactured for their treatment. This means that the primary source of income comes from peer-reviewed grant funding, at a time when competition for such funds is intense. Alternatives, such as contract manufacturing, are also hard to obtain and may restrict

some operational flexibility. These difficulties are preceded by the expense of building the facility and determining its size and scope of operations.

These considerations raise the question as to whether cGMP manufacturing should be available at most academic facilities or whether it should be regionalized or localized so that it is predominantly performed by a small number of experienced facilities as a fee-for-service operation.

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References

1. California Institute of Regenerative Medicine. (2008). CIRM major facilities speed stem cell science and create jobs. https://www.cirm.ca.gov/our-progress/video/cirm-major-facilities-speed-stem-cell-science-and-create-jobs. Accessed 9 July 2020.
2. The Cell and Gene Therapy Catapult. A Center of Excellence. https://ct.catapult.org.uk/about-us. Accessed 9 July 2020.
3. National University of Ireland Galway. The Centre for Cell Manufacturing Ireland. http://www.nuigalway.ie/stem-cells/about/. Accessed 9 July 2020.
4. Penn Medicine. (2016). Novartis-Penn Center for Advanced Cellular Therapeutics Unveiled at Penn Medicine. https://www.pennmedicine.org/news/news-releases/2016/february/novartispenn-center-for-advanc. Accessed 9 July 2020.
5. Code of Federal Regulations. Drug Good Manufacturing Practices (GMP). Title 21, Parts 210 and 211. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=211. Accessed 9 July 2020.
6. Code of Federal Regulations. Human Cells, Tissues, and Cellular and Tissue-based Products, Part 1271. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=127. Accessed 9 July 2020.
7. Industrial Quick Search. Benefits of Modular Clean Rooms. https://www.iqsdirectory.com/resources/benefits-of-modular-cleanrooms/. Accessed 9 July 2020.
8. Pharma Manufacturing. Isolator Systems Take on Cleanrooms. https://www.pharmamanufacturing.com/articles/2003/34/. Accessed 9 July 2020.
9. Bersenev, A., & Fesnak, A. (2020). Place of academic GMP facilities in modern cell therapy. In S. Katz & P. Rabinovich (Eds.), *Cell reprogramming for immunotherapy. Methods in molecular biology* (Vol. 2097). Humana, New York.
10. NSight. (2016). NB Research Report Manufacturing Matters in Cell Therapy. https://www.nelsenbiomedical.com/app/uploads/2016/10/NB160308_1_research_report.pdf. Accessed 9 July 2020.
11. EATRIS European Infrastructure for Translational Medicine. https://eatris.eu/. Accessed 9 July 2020.
12. Hildreth, C. (2017). *Academic and medical CDMOs serving the cell therapy industry*. BioInformant. https://bioinformant.com/academic-medical-cdmos-serving-cell-therapy-industry/. Accessed 9 July 2020.
13. Production Assistance for Cellular Therapy. https://www.pactgroup.net/. Accessed 9 July 2020.
14. Reed, W., Noga, S. J., Gee, A. P., Rooney, C. M., et al. (2009). Production Assistance for Cellular Therapies (PACT): Four-year experience from the United States National Heart,

Lung, and Blood Institute (NHLBI) contract research program in cell and tissue therapies. *Transfusion, 49*(4), 786–796.
15. Cancer Prevention and Research Institute of Texas. Core Facilities. https://www.cprit.state.tx.us/our-programs/academic-research/core-facilities. Accessed 9 July 2020.
16. U.S. Food and Drug Administration. (2016). Charging for Investigational Drugs Under an IND – Questions and Answers Guidance for Industry. Guidance for Industry. June 2016. https://www.fda.gov/media/85682/download. Accessed 9 July 2020.
17. Abou-El_Enein, M. (2014). Manufacturing Clinical-Grade Cell and Gene Therapy Products. Economic Implications for Academic GMP Facilities. Südwestdeutscher Verlag für Hochschulschriften.
18. Abou-El_Enein, M, Romhild, A., Kaiser, D., Reinke, P., et al. (2013). Good Manufacturing Practices (GMP) manufacturing of advanced therapy medicinal products: A novel tailored model for optimizing performance and estimating costs. *Cytotherapy, 15*(3), 362–383.

Governmental Support Opportunities for Cellular and Gene Therapies in the United States

Lisbeth A. Welniak

1 Introduction

The US Government is the principal funder of academic center and nonprofit institution-based basic and translational biomedical research in the United States even though industry is major investor in overall US medical and health research and development. Industry's investment was 66.7% of the total estimated spending in 2018 compared to 22.2% for federal agencies [1]. For developers of cell-based therapies, federal funding can provide the support necessary to demonstrate proof of concept of therapeutic benefit in preclinical animal models and move the cell-based therapy through late-stage translational research and early clinical trials prior to seeking commercialization and investment by biopharmaceutical partners.

2 Federal Funding Agencies

2.1 National Institutes of Health (NIH)

The NIH is the largest public funder of biomedical research in the world, investing more than $31.7 billion a year in extramural research funding in fiscal year 2019 [2]. The NIH is made up of 21 research institutes and 6 centers. Each of the

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institutes and five of the centers have research agendas focused on different biomedical disciplines, while the Center for Scientific Review is responsible for receiving grant applications to the NIH and conducting the scientific peer review of approximately 70% of the submitted grant applications assigned to the various institutes and centers. Because the majority of institutes are defined by either a disease or anatomical organ or physiological system, funded research in the areas cell and gene therapies, regenerative medicine, and tissue engineering are found across the NIH institutes. For examples, the National Cancer Institute (NCI) has a scientific interest in cellular therapies that target cancer and have funded seminal studies that led to the development of chimeric antigen receptor (CAR) T-cell therapies [3]. Cell and gene therapies for neurology indications have led the number of submissions to the Food and Drug Administration (FDA) for Regenerative Medicine Advanced Therapy Designation (RMAT) designation as of 2019 [4], demonstrating the advancements in regenerative medicine for neurological diseases. The National Institute of Neurological Disorder and Stroke (NINDS) funds basic, translational, and clinical research that have been foundational to this advancement. With scientific interest that include the biology and treatment of infectious diseases and immunological disorders, the National Institute of Allergy and Infectious Diseases (NIAID) has funded and supported the development of cell and gene therapies targeting infectious diseases, including HIV, as well as cell and gene therapies for a range of immunological diseases, including primary immune disorders like severe combined immunodeficiency (SCID) and Wiskott-Aldrich Syndrome. The National Eye Institute (NEI) funds translational research and clinical trials utilizing either gene therapy or cell therapy for a number of eye diseases. The National Heart, Lung, and Blood Institute (NHLBI) supports a wide variety of research grants and research programs in cell and gene therapies, regenerative medicine, and tissue engineering focused on addressing heart, lung, blood, and sleep disorders.

2.1.1 NIH-Wide Programs

The majority of extramural funding is managed under the auspices of 24 of the institutes and centers. Under the Office of the NIH Director Common Fund programs, the NIH addresses emerging scientific opportunities and pressing challenges in biomedical research that no single NIH Institute or Center (IC) can address on its own but are of high priority for the NIH as a whole. The Common Fund is a unique resource at the NIH, establishing programs to support high-risk, innovative endeavors with the potential for extraordinary impact on the selected field of study. Common Fund programs are short-term, goal-driven targeted initiatives, with deliverables intended to catalyze research across multiple biomedical research disciplines.

In 2020, the Common Fund has several active programs that provide funding to support aspects of regenerative medicine and cellular therapies. Most of the active programs that include awards for development of novel therapies fund investigators pursuing highly innovative high-risk projects in any area of the NIH mission.

Common Fund programs include the Extracellular RNA Communication program, which contains an arm focused on exploring the clinical utility of extracellular RNAs, exosomes, and extracellular vesicles [5], and the Regenerative Medicine Program (RMP), which has produced induced pluripotent stem cell (iPSC) lines, including both a cGMP CD34+ cord blood-derived iPSC master cell bank and a research cell bank, which are available upon request [6]. Additional information on the RMP-generated induced pluripotent stem cell (iPSC) lines is available on the NIH Common Fund webpages. The third program is Somatic Cell Genome Editing program which supports the development of the tools and delivery systems needed to advance the use of gene editing in gene and cellular therapies [7].

The 21st Century Cures Act was signed into law on December, 13, 2016 [8]. The legislation mandates and provides funds for four scientific innovation programs at the NIH: the All of Us Research Program, the Brain Research through Advancing Innovative Neurotechnologies (BRAIN) initiative, the Cancer Moonshot, and the Regenerative Medicine Innovation Project (RMIP). The Cures Act specifies funding for each of these programs. The Cancer Moonshot has funding through fiscal year 2023 [9], and RMIP was funded for 4 years, with the last funding opportunity accepting applications in the last quarter of 2020 [10]. The goal of RMIP is to accelerate progress in the field of clinical research on adult stem cells toward the development of safe and effective regenerative cell therapy products.

2.2 NIH Grants Process

The NIH provides a large variety of resources for investigators who are new to the NIH grants application process or who are seeking funding support and resources in a new scientific area. The NIH website provides detailed information and tutorials on each step of the grant application process. In addition to reviewing the materials on the NIH website, investigators are highly encouraged to seek advice from an NIH program officer prior to writing and submitting a grant application. Program officers can provide more tailored guidance based on the investigator's need and the scope of the research project. Program officers work at each of the institutes and at the centers that fund extramural research. The NIH has developed a tool to aid investigators as they search for the institute and program officer that best aligns with the scientific goals of their project. The tool is called Matchmaker, and it is available at the NIH RePORTER website (https://projectreporter.nih.gov/reporter_matchmaker.cfm).

The NIH Research Project Grant Program (R01) is the oldest and most commonly used grant program at NIH. The proposed work will be evaluated on review criteria of significance of the problem or critical barrier to be addressed, the qualifications of the investigators to carry out the proposed work, innovation, research approach to accomplish the aims of the project, and the scientific environment. Well-conceived and well-written project proposals to conduct discovery science and/or proof of concept studies that a new cell therapy has the potential to advance

human health can excel in the NIH peer review for R01 grants. However, proposals to conduct the translational development studies necessary to obtain Investigational New Drug (IND) approval are not well suited to the traditional R01 as the required work is often viewed as incremental, building on the initial discovery, and lacks innovation in the required studies to move production from the research laboratory, into cGMP manufacturing suites and Phase I clinical trial safety studies. To address this concern, NIH institutes may have specific translational and clinical programs available to investigators to support the development of cell therapies from the research laboratory and into clinical trials. Table 1 provides a list of examples that were active at the date of this writing.

Applications to NIH are submitted to grants.gov which is an online portal that is used by all US federal grant-making agencies or to eRA Commons which is a system managed by NIH. Applications are submitted by the authorized organization representatives from the academic institution or business entity. All organizations must be registered with grants.gov in order to submit an application. Once NIH

Table 1 Examples of NIH programs to support translational research

Program	Program goals	References
NHLBI Catalyze Program	Offers funding, technical support, and mentorship to help transform basic scientific discoveries into viable therapeutics, devices, and diagnostics to treat heart, lung, blood, and sleep diseases and disorders. Supports product definition studies, preclinical research, and the development of cutting-edge platform technologies.	[11]
NINDS Cooperative Research to Enable and Advance Translational Enterprises for Biotechnology Products and Biologics (CREATE Bio) Program	The program is dedicated to biotechnology product- and biologics-based therapies to treat neurological disorders. It is a two-track program that supports optimization in order to obtain a candidate appropriate for entering second track which supports IND-enabling studies for the candidate, as well as early-phase clinical trials.	[12]
NHLBI Gene Therapy Resource Program	The program supports gene therapy research primarily for heart, lung, and blood diseases. Resources are provided, at no cost to the investigator, as services for preclinical vector production, pharmacology/toxicology testing, immunology testing, and regulatory support.	[13]
NIAID Immune Tolerance Network (ITN)	Goal is to accelerate clinical development of immune tolerance therapies trough the development, funding, and conduct of clinical trials in immune tolerance. The network may also support limited product development essential for the subsequent evaluation of these approaches in clinical trials.	[14, 15]
NCI Immuno-Oncology Translational Network (IOTN)	Goal is to accelerate the development of improved immunotherapeutic strategies capable of eliminating established cancers or preventing cancers before they occur.	[16]

receives the application via the grants.gov system, it is reviewed by the NIH Division of Receipt and Referral for completeness and compliance and assigns the application to a specific NIH institute based on the area of research. Funding decisions for unsolicited applications to parent funding opportunities are based on funding guidance set by each institute every fiscal year.

2.3 Other Department of Human Health and Services Funding Organizations

The US Department of Human Health and Services (HHS) is composed of 11 agencies. These agencies oversee and support public health and some also support biomedical research in addition to the NIH. These include the Food and Drug Administration (FDA), which provides funding opportunities each year to address human health issues as well as food and animal feed safety, as well as the Centers for Disease Control (CDC). The NIH, FDA, and CDC all participate in an annual solicitation for small business innovation research grant applications, and the FDA occasionally issues other funding opportunities. The NIH Guide for Grants and Contracts publishes not only NIH funding opportunities but also serves as a resource for funding opportunities from the HHS sister agencies such as the Agency for Healthcare Research and Quality (AHRQ), CDC, FDA, Health Resources and Services Administration (HRSA), Substance Abuse and Mental Health Services Administration (SAMHSA), as well as the National Aeronautics and Space administration (NASA) which is an independent agency of the US Federal Government. Interested parties can subscribe to the weekly service that provides updates on new funding opportunities. A searchable NIH Guide for Grants and Contracts is available online at https://grants.nih.gov/funding/searchguide/index.html#/.

2.4 National Science Foundation (NSF)

While NIH is the largest funder of biomedical research from the US Government, there are other funding agencies outside of NIH and HHS that provide funding support for research that is within their scientific mission. In particular, regenerative medicine and cellular therapies often cut across federal agency scientific agendas.

The NSF is an independent federal agency. Its mission is to support all fields of fundamental science an engineering, except for medical science. Because it does support biological sciences and bioengineering and biomanufacturing, the NSF does have a scientific interest and supports research in the area of cellular therapies, regenerative medicine, and tissue engineering. Unlike NIH which will accept applications to develop novel cellular therapies, applications to NSF typically are more focused on novel engineering, bioprocesses, and biomanufacturing approaches.

NSF accepts applications through the grants.gov portal, the NSF FastLane System, or Research.gov. Interested parties can find information on how to submit a proposal in the relevant NSF funding opportunity announcement.

2.5 Department of Defense (DoD)

The Department of Defense funds biomedical research that is intended to advance healthcare for active US military service members, veterans, and the American public. It is charged to meet its mission "by funding high impact, high risk and high gain projects that other agencies may not venture to fund" [17]. The DoD has solicited and funded research for regenerative medicine and rehabilitation that aim to directly benefit wounded warriors. The Department of Defense administers the Congressionally Directed Medical Research Programs (CDMRP). CDMRP was initiated in 1992 and continues to receive congressional appropriates to fund biomedical research in response to the needs of the US military and the American public [17]. The CDMRP has a long list of research programs that supports with the goal of prevention, control, and cure of a wide range of specified diseases and disorders that are published on the CDMRP website [18].

3 Federal Funding for Small Business Research

The Small Business Innovation Research (SBIR) program [19] was established by Congress under the Small Business Innovation Development Act of 1982 (Public Law 97-219). The goal of the legislation was to strengthen the role of innovative small business concerns in Federally-funded research and development. The Small Business Technology Transfer (STTR) program was modeled on the SBIR program and was established by Congress as a pilot program by the Small Business Technology Transfer Act of 1992 (Public Law 102-564, Title II). Both programs provide research and development funding to small US businesses through these highly competitive funding solicitations. The goal is to advance the technological potential of the small businesses' research and to incentivize commercialization of these technological and scientific advances.

Congress has consistently reauthorized and extended the SBIR/STTR programs. Eleven different federal agencies have SBIR programs, and five of the eleven have STTR programs. Federal agencies with extramural research and development budgets that exceed $100 million per year are required by law to set aside 3.2% of their budget to SBIR, and those with research budgets over $1 billion are required to set aside 0.45% of funds for STTR. Each agency runs its own SBIR/STTR program although all must follow the guidelines established by Congress. Each agency designates research and development topics in their SBIR and STTR solicitations. The following agencies have had solicitations that included regenerative medicine and

cell and/or gene therapies: Department of Health and Human Services (DHHS), participating DHHS include NIH, the Centers for Disease Control and Prevention, and the US Food and Drug Administration; National Science Foundation; and Department of Defense. All submitted applications undergo a proposal evaluation and awards are made on a competitive basis. Interested parties can search for funding opportunities and topic areas through each agency's website, or they can search across all funding agencies at the SBIR.gov website.

The SBIR/STTR grant and contract opportunities are based on a phased program. In Phase 1, the main objective is to establish the technical merit and feasibility of the proposed research and development efforts. In Phase 2, the objective is to advance the technology toward ultimate commercialization. Only US small businesses are eligible to participate in the SBIR/STTR programs. In addition, for STTR grants, the partnering nonprofit research institution must be located in the United States.

4 US Federal–Private Partnerships to Advance Cellular Therapy Manufacturing in the United States

Manufacturing USA® was established in 2014 and is comprised of 14 public–private institutes and their federal sponsoring agencies. They are made up of over 1900 member organizations representing manufacturers of all sizes, academia, and other entities. Each institute focuses on a different advanced manufacturing technology area but works toward the same high-level goal: "to secure America's future through manufacturing innovation, education, and collaboration" [20]. Two of these institutes are directly relevant to the area of cell therapies. To advance their missions, these institutes frequently release funding opportunities for proposals to innovate and enhance the manufacturing platforms for biomanufacturing as well as addressing the need for workforce development.

4.1 The National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL)

NIIBML is a public–private partnership with a mission to accelerate biopharmaceutical innovation, support development of related manufacturing standards, and advance education and training of the US biopharmaceutical manufacturing workforce. NIIMBL is funded through a cooperative agreement with the National Institute of Standards and Technology (NIST) in the US Department of Commerce with significant additional support from its members [21]. Focus areas for NIMBL have included existing biopharmaceutical products such as monoclonal antibodies,

engineered antibodies, proteins, vaccines, and virus-like particles as well as emerging products in the fields of gene and cell therapies.

To advance its mission, NIIMBL issues frequent project calls. As of November 2020, NIMBL has issued three project calls that provide funding to conduct work in technology development, workforce development, and Global Health Fund projects. Examples of topic areas from project call 4.1 in the area of new technologies included (1) analytical technologies for vector manufacturing; (2) cell processing technologies and analytical technologies for live cell products (cell therapies); and (3) technologies for intensified processing of therapeutic proteins [22].

4.2 Advanced Regenerative Manufacturing Institute

The Defense Department funded an 87-member coalition to develop next-generation manufacturing techniques for repairing and replacing cells, tissues, and organs for wounded service members, in December 2016. This agreement, awarded by the Army Contracting Command, provides for 7 years of operation with financial support supplied by a combination of $80 million in DoD funds and more than $214 million in non-federal cost sharing [23]. The aim is for this organization to become self-sustained. The coalition, named BioFabUSA, is sponsored by the Advanced Regenerative Manufacturing Institute (ARMI), a nonprofit organization located in Manchester, New Hampshire. ARMI's mission is make practical the large-scale manufacturing of engineered tissues and tissue-related technologies, benefit existing industries, and grow new ones. To that end, the technical scope for BioFabUSA work includes innovations across five principal areas: (1) cell selection, culture, and scale-up; (2) biomaterial selection and scale-up; (3) tissue process automation and monitoring; (4) tissue maturing technologies; and (5) tissue preservation and transport. The goal of the program is to integrate biomanufacturing and bioprocessing advances to create disruptive research and development tools and FDA-compliant manufacturing processes that can meet the emerging large volume needs [24]. Like NIIMBL, ARMI issues project calls.

References

1. America R. (2019). U.S. Investments in Medical and Health Research and Development, 2013–2018.
2. Databook N. (2019). NIH Extramural & Intramural Funding: FY 2019 Operating Plan. https://report.nih.gov/nihdatabook/report/283. Accessed 9 Jan 2021.
3. Grupp, S. A., et al. (2013). Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *The New England Journal of Medicine, 368*(16), 1509.
4. Regenerative Medicine Advanced Therapy. (2019). RMAT Update Cell Therapy Liaison Meeting. 21 November 2019. https://cdn.ymaws.com/isctglobal.org/resource/resmgr/regulatory/nov_2019_ctlm/ctmrmat_bryan.pdf. Accessed 9 Jan 2021.

5. National Institutes of Health (NIH). Extracellular RNA Communication (ERC) program. https://commonfund.nih.gov/exrna. Accessed 9 Jan 2021.
6. National Institutes of Health (NIH). Regenerative Medicine Program. https://commonfund.nih.gov/stemcells. Accessed 9 Jan 2021.
7. Perry, M. E., et al. (2018). Genome editing to 're-write' wrongs. *Nature Reviews Drug Discovery, 17*(10), 689–690.
8. Library of Congress. Public Law 114–255, S.E.D. (2016). To Accelerate the Discovery, Development, and Delivery of 21st Century Cures, and for Other Purposes. https://www.congress.gov/114/plaws/publ255/PLAW-114publ255.pdf. Accessed 9 Jan 2021.
9. National Cancer institute (NCI). The Cancer Moonshot. https://www.cancer.gov/research/key-initiatives/moonshot-cancer-initiative. Accessed 9 Jan 2021.
10. National Institutes of Health (NIH). Regenerative Medicine Innovation Project. https://www.nih.gov/rmi. Accessed 9 Jan 2021.
11. National Heart, Lung, and Blood institute (NHLBI). Frequently Asked Questions (FAQs) About Catalyze. https://www.nhlbi.nih.gov/grants-and-training/funding-opportunities-and-contacts/NHLBI-Catalyze-Program. Accessed 9 Jan 2021.
12. National Institute of Neurological Disorders and Stroke (NINDS). CREATE Bio Program Overview. https://www.ninds.nih.gov/Current-Research/Research-Funded-NINDS/Translational-Research/CREATE-BIO. Accessed 9 Jan 2021
13. National Heart Lung and Blood Institute (NHLBI). What is the NHLBI Gene Therapy Resource Program? https://www.gtrp.org/Default.aspx. Accessed 9 Jan 2021.
14. National Institute of Allergy and Infectious Disease (NIAID). Apply To Be Part of the Immune Tolerance Network. https://www.niaid.nih.gov/grants-contracts/itn-opportunity. Accessed 9 Jan 2021.
15. National Institute of Allergy and Infectious Disease (NIAID). Partner with the ITN. https://www.immunetolerance.org/professionals/about-us/partner-itn. Accessed 9 Jan 2021.
16. National Cancer Institute (NCI). (2017). Immuno-oncology Translational Network (IOTN) Advancing Research Progress to Improve Immunotherapy and Immunoprevention. https://www.cancer.gov/research/key-initiatives/moonshot-cancer-initiative/funding/upcoming/iotn-foa-video. Accessed 9 Jan 2021.
17. Congressionally-directed Medical Research Programs (CDMRP). About us. http://cdmrp.army.mil/aboutus.html. Accessed 9 Jan 2021.
18. Congressionally-directed Medical Research Programs (CDMRP). Research Programs. https://cdmrp.army.mil/researchprograms. Accessed 9 Jan 2021.
19. Small Business Innovation Research (SBIR). Small Business Technology Transfer (STTR) programs. https://www.sbir.gov/about. Accessed 9 Jan 2021.
20. Manufacturing USA, M.U.A. https://www.manufacturingusa.com/about-us. Accessed 9 Jan 2021.
21. The National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL). About NIIMBL. https://niimbl.force.com/s/about-niimbl. Accessed 9 Jan 2021.
22. The National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL). (2020). Project Call 4.1T. https://niimbl.force.com/s/project-call-41. Accessed 9 Jan 2021.
23. Soares, J. (2017). DOD-funded Advanced Regenerative Manufacturing Institute opens its doors to the future. U.S. Army. https://www.army.mil/article/192860/dod_funded_advanced_regenerative_manufacturing_institute_opens_its_doors_to_the_future. Accessed 9 Jan 2021.
24. Advanced Regenerative Manufacturing Institute (ARMI). About us. https://www.armiusa.org/about-us. Accessed 9 Jan 2021.

Correction to: GLP Regulations for Nonclinical Studies

Aisha Khan, Yee-Shuan Lee, and Joshua M. Hare

Correction to:
Chapter 5 in: A. P. Gee (ed.), *Cell Therapy*, https://doi.org/10.1007/978-3-030-75537-9_5

In the original version of this chapter, figure 4 on page 88 was not complete. The full figure is given below:

Fig. 4 Sample of a study protocol

The original version of this chapter can be found at https://doi.org/10.1007/978-3-030-75537-9_5

A. P. Gee (ed.), *Cell Therapy*, https://doi.org/10.1007/978-3-030-75537-9_42

Fig. 4 (continued)

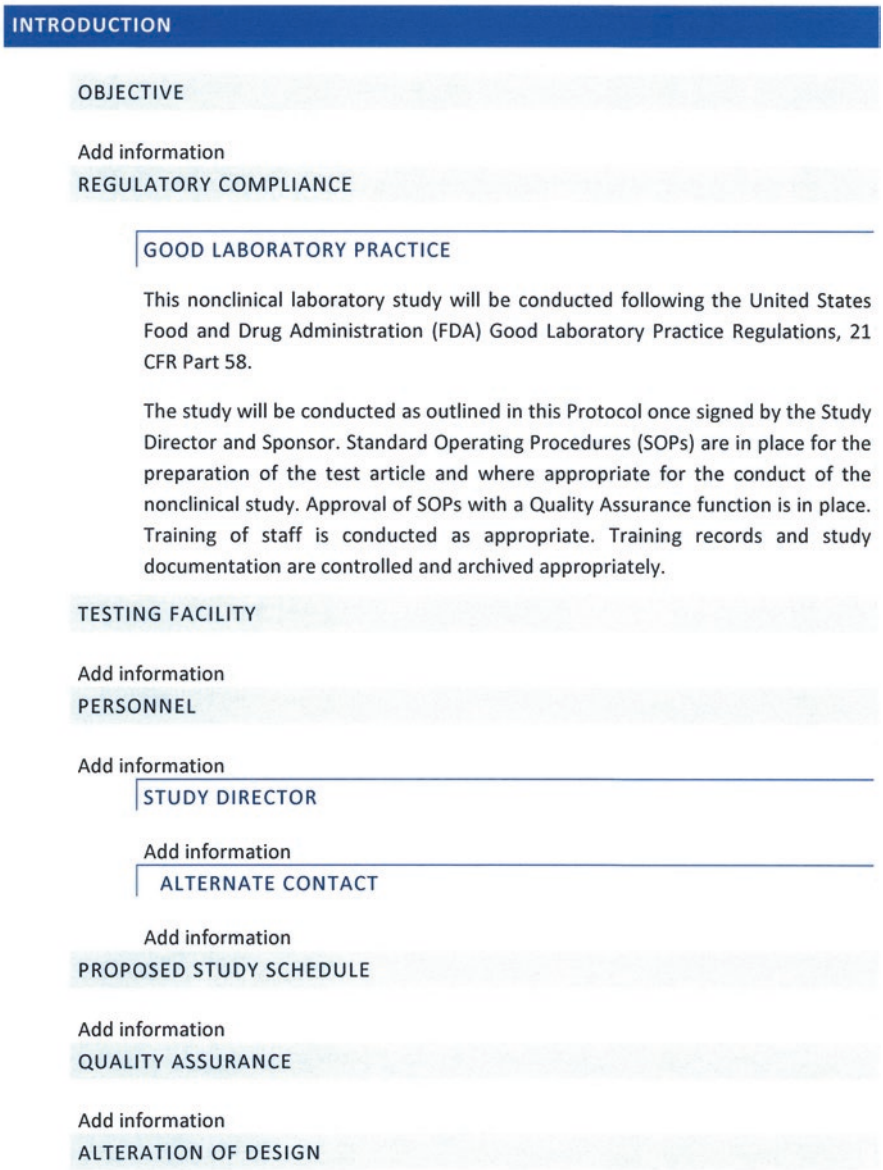

Fig. 4 (continued)

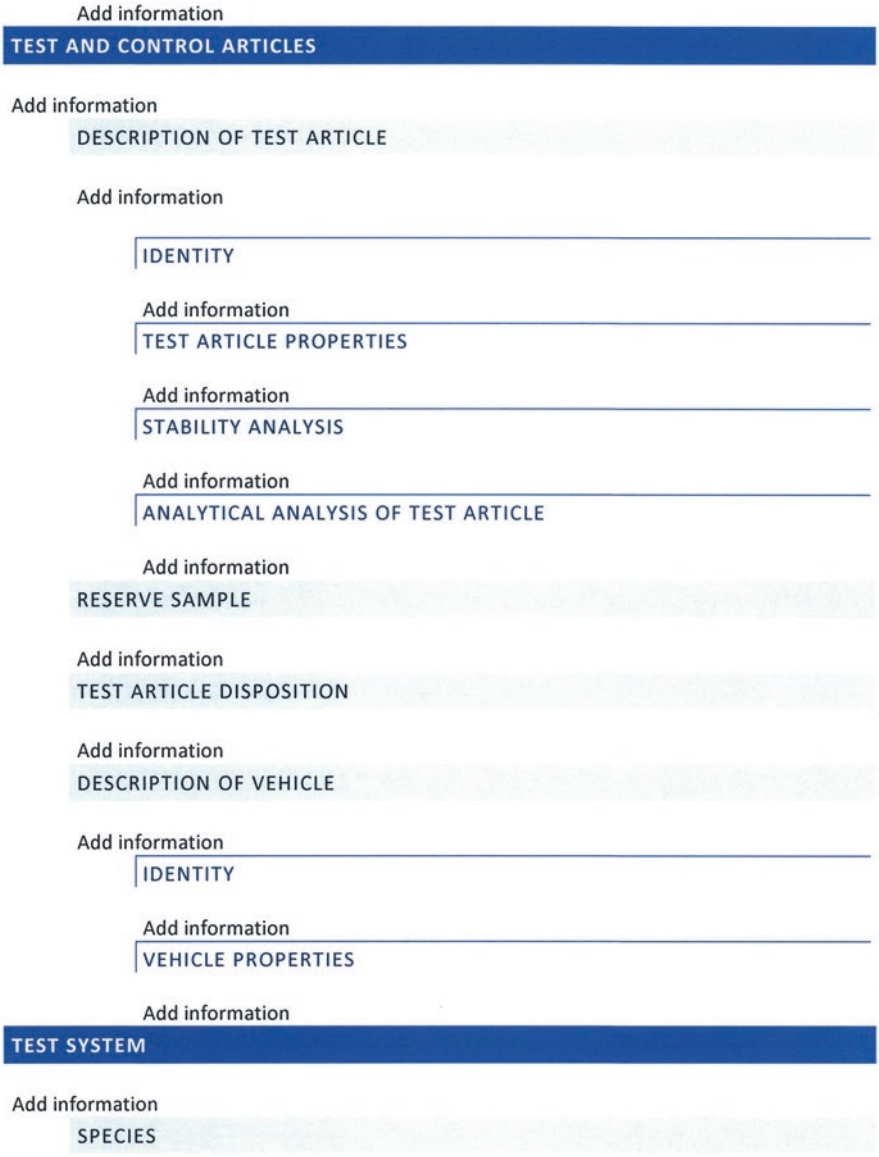

Fig. 4 (continued)

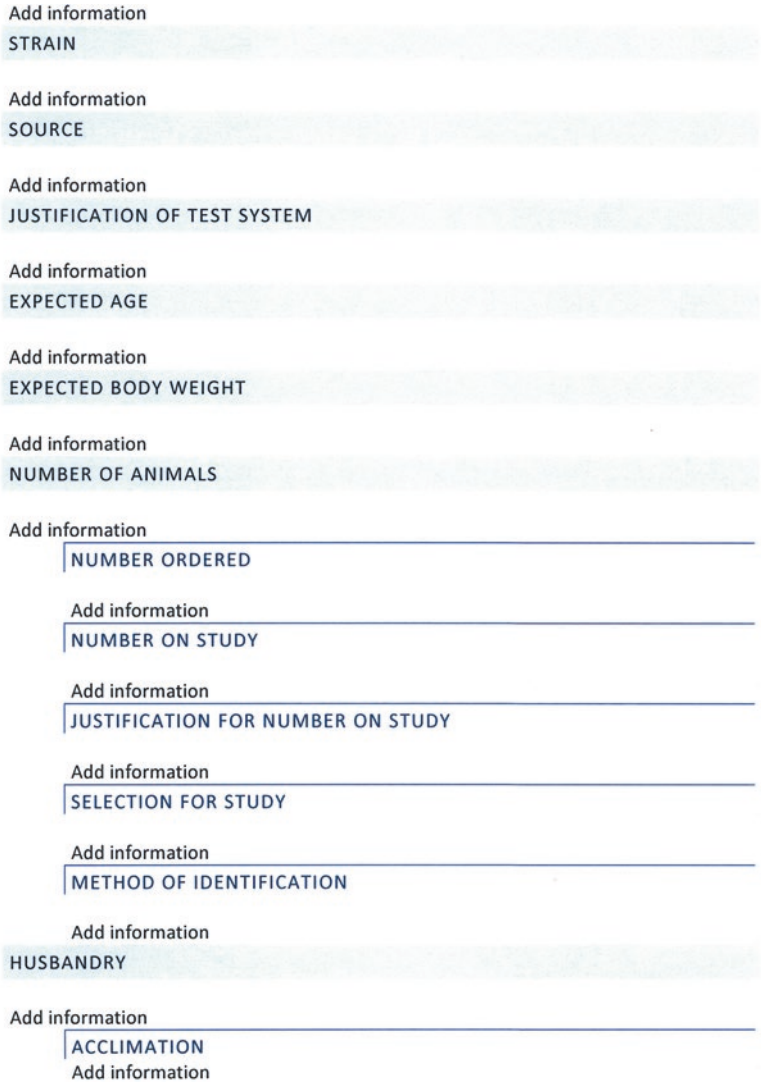

Fig. 4 (continued)

HOUSING
Add information

ENVIRONMENTAL CONDITIONS
Add information

DIET AND DRINKING WATER
Add information

BASAL DIET
Add information

BASAL DIET CONTAMINANTS
Add information

WATER
Add information

WATER CONTAMINANTS
Add information

STUDY DESIGN

Add information

STUDY GROUPS/ NUMBER OF ANIMALS

Add information

STUDY TREATMENT AND TIMELINES:

Add information

STUDY EVALUATIONS

Add information

BODY WEIGHTS

Add information

CAGE-SIDE OBSERVATIONS

Fig. 4 (continued)

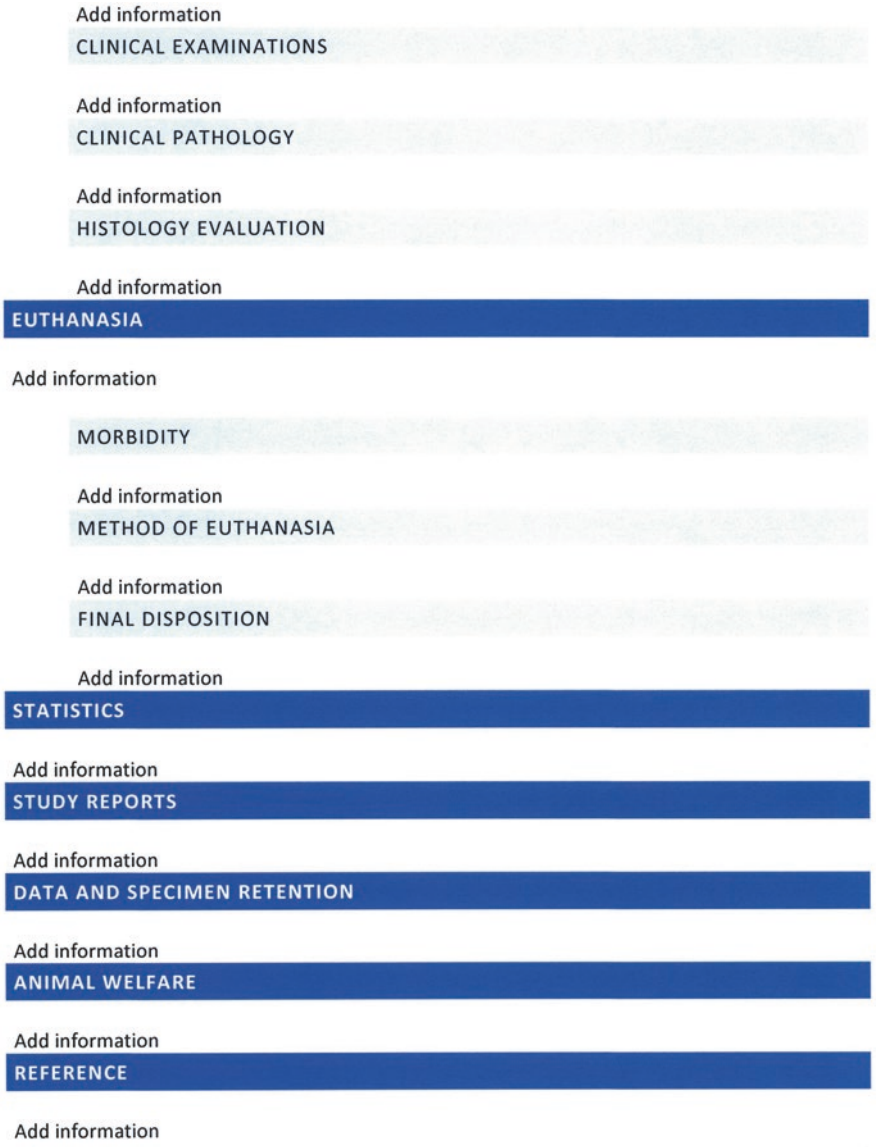

Fig. 4 (continued)

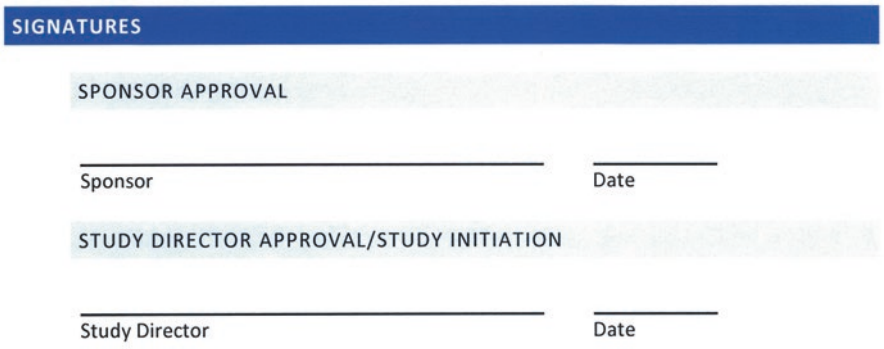

Fig. 4 (continued)

Index

A. P. Gee (ed.), *Cell Therapy*, https://doi.org/10.1007/978-3-030-75537-9