Manufacturing pluripotent cell therapeutics

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Summary

Here we review strategies for gaining Food and Drug Administration (FDA) approval of allogeneic, pluripotent cell therapies. The crux of the discussion is that when developing a cell therapeutic, it is critical to look as much as a decade ahead to when FDA approval will be sought to commercialize the product through a biologics license application (BLA). While this discussion focuses on FDA approval of cell therapies, it is important to acknowledge the vast number of cell therapy clinical efforts occurring globally, particularly in Europe and Asia, and the specific regulatory requirements for those studies as well. Since many outstanding reviews on cell manufacturing are available [1-6], we specifically focus on the "how and why" of integrating high-quality raw materials and ancillary materials into the manufacturing process of pluripotent cell therapies throughout the product development process. This consideration is especially crucial for pluripotent cell therapies because unlike many other forms of cell therapeutics that are tailored for individual patients and rely on small batch production, pluripotent cell therapies have as one of the first steps the establishment of a master cell bank that must last the lifetime of the product. Thus, raw material quality is paramount from the beginning of the development process. Obtaining high-quality raw materials from suppliers experienced in supporting cell therapy development—from manufacturing to delivery—can increase the probability of success and head off costly surprises that could cause an untimely demise for a promising pluripotent cell therapy candidate.

Introduction

James Thomson's derivation and culture of pluripotent human embryonic stem cells (ESCs) in 1998 [7] and Shinya Yamanaka's discovery of how to transform somatic cells into induced pluripotent stem cells (iPSCs) in 2006 [8] offered tantalizing promises for new transformational therapies. These discoveries established iPSCs as the building blocks and blueprints for generating or regenerating virtually any cell or tissue of the body, and seeded the concept of regenerative medicine via cell and tissue replacement as a lifesaving "third pillar" of healthcare technology, along with drugs and biologics. Now we await results of initial clinical trials using PSC-derived therapies, to validate this vision [9-13].

Unlike therapeutic development of small molecules and biologics, where contract manufacturing organizations (CMOs) and biopharmaceutical companies have driven product development, the field of PSC-derived therapies has been driven mostly by independent investigator sponsors. Of the more than 130 clinical trials involving PSCs as of 2020 [14] using PSC-derived cells or tissues, most are sponsored by academics or clinicians. These pioneering investigators have pushed the field forward, accelerating innovation and providing desperately needed proof of concept that PSC-derived therapies are feasible. However, the development of manufacturing processes for these early prototypes often has not been optimized for cost effectiveness, process reliability, scalability, or future regulatory scrutiny. Cell therapeutics is an intensely process-driven undertaking (i.e., "the process is the product"); consequently, these considerations will profoundly shape regulatory approval for clinical testing and later for licensing to market the product. Thus, the cell therapy field's experiential gap in manufacturing has the potential to slow progress in the best-case scenario and to derail promising therapeutic candidates and companies in the worst case [15].

Making a master cell bank, for example, typically costs hundreds of thousands of dollars—mostly due to required safety testing that is expensive. Should investigators make a master cell bank using a non–GMP-compliant reagent (the importance of which is discussed further in the section "Good manufacturing practices and supplier quality systems"), they could potentially have to go back later and "fix" or compensate for that suboptimal reagent when they begin an investigational new drug (IND) application. Such a delay in clinical development, and the associated cost in money and time, could well lead to failure of the project and the loss of a potentially effective cell treatment from the therapeutic pipeline.

Here we review the factors that can have an impact on the cost, speed, safety, and effectiveness of PSC-derived therapies from the standpoint of FDA regulation, process development, master cell banking, release testing, product lifecycle planning, and the role of suppliers.

Taking these factors into consideration, early and vigilant planning can help companies and academic centers developing such therapies avoid costly mistakes and increase the chances of regulatory approval and commercial success.

FDA regulation of cell therapies: an overview

A cell therapy's ingredients, or material supply chain, are critical to developing a reproducible and robust manufacturing process. Proper sourcing of materials early in development of a cell therapy from reliable suppliers who make products specifically for cell therapeutics can shorten the development timeline, dramatically reduce costs, and improve the likelihood of approval from regulatory authorities. For this concise review, we focus on the US FDA regulations. However, the same overall principle applies to products targeted toward other jurisdictions (Table 1), as similar standards for quality materials and methods for master cell banking will likely apply.

Table 1. Publications providing information on pluripotent cell manufacturing regulations in some non-US jurisdictions.

Publication	Highlights and excerpts
Australia Raising the standard: changes to the Australian Code of Good Manufacturing Practice (cGMP) for human blood and blood components, human tissues, and human cellular therapy products [16]	• The Therapeutic Goods Administration (TGA) regulates manufacture of blood, tissues, and biologicals • Regulations are outlined in the Code of Good Manufacturing Practice (cGMP) • The scope was broadened in 2013 to encompass cell therapies and formalize a risk-management approach
Canada Considering cell therapy product "Good Manufacturing Practice" status [17]	• "… CTPs are drugs that must be manufactured according to GMP requirements aside from specific sample testing and retention requirements", and "Cell therapies will be held to increasingly stringent manufacturing controls as they are developed from early to late stage clinical trials." • "Regulator assessment of cell therapy clinical trial products against GMP principles is performed by Clinical Trial Application reviewers for all stages of clinical trial development. This is done by the Biologics and Genetic Therapies Directorate pre-market review group, who have the authority and specific training to conduct on-site inspections as required, and it does not typically involve Health Canada's establishment licensing group." (This process differs from the US FDA and EMA.) • "Any authorized clinical trial sponsor in Canada claiming to manufacture under GMP … must meet all principles of Division 2—GMPs that apply to fabricating material for use under Division 5—Clinical Trial Applications; however, these establishments will only have evidence of regulatory approval of GMP in the form of a No Objection Letter for a specific clinical trial product manufactured in their facility."
China New regulation for clinical stem cell research in China: expected impact and challenges for implementation [18]*	• "In 2015, the Chinese National Health and Family Planning Commission (NHFPC; the former Ministry of Health, MOH) issued a 'draft' regulation on clinical research and applications that involve human stem cells." • "The standards and technical procedures for the collection, manufacturing and storage of stem cells for clinical use are laid down in the 'Stem Cell Preparations Quality Control and Preclinical Research Guidelines', a supplementary document published by the China Food and Drug Administration (CFDA)." • "Investigators applying for stem cell clinical trials must do so at provincial branches of the NHFPC and CFDA, and register the trials online at the Chinese Medicine Registry and Management System." • "Clinical trials using human embryonic stem cells must harvest and process the cells in line with the 'Guiding Principles for the Ethics for Human Embryonic Stem Cell Research', a joint regulation issued in 2003 by the Ministry of Science and Technology and the MOH."

* Based on the 2017 *Guidance for Research and Evaluation of Cellular Therapy Products* (National Medical Products Administration, China), a technical guideline draft for clinical trials of human stem cell–derived cell therapy products was published in August 2020, aiming to provide more targeted recommendations and guidelines for drug registration applicants and researchers conducting stem cell clinical trials [19].

Table 1. Publications providing information on pluripotent cell manufacturing regulations in some non-US jurisdictions. (continued)

Publication	Highlights and excerpts
Europe Cell and gene therapies: European view on challenges in translation and how to address them [20]	• "Advanced therapy medicinal products (ATMPs), covering cell and gene therapy medicinal products and tissue-engineered products in the EU, are regulated in the EU as medicinal products under a specific regulation (EC) No 1394/2007 … " • "The Committee for Advanced Therapies (CAT) at European Medicines Agency (EMA) was established to ensure that the relevant expertise is available in regulatory decision making to support and evaluate these products." • "Pre-clinical investigation for ATMPs is recommended to start with … a risk based approach (RBA) [to enable planning for relevant experiments to establish] safety and efficacy, including first-in-human trial, market authorization and post-authorization follow-up."
Japan New Japanese regulatory frameworks for clinical research and marketing authorization of gene therapy and cellular therapy products [21]	• The Pharmaceuticals and Medical Devices Law and Act on the Safety of Regenerative Medicine were both passed in 2014. • "The SAKIGAKE (meaning a pioneer or forerunner in Japanese) designation system was begun in 2015." • "The MHLW started the "Project for Enhanced Practical Application of Innovative Drugs, Medical Devices and Regenerative Medical Products" to promote personnel exchange and cooperation in writing of guidelines on the evaluation of innovative medical products between the Pharmaceuticals and Medical Devices Agency and academia."
UK Impact of BREXIT on UK gene and cell therapy: the need for continued Pan-European collaboration [22]	• "The development and application of gene and cell therapy have been very high on the EU funding agenda for more than decade. The investment by the EU in consortia working on these approaches to human health has been well documented in the pages of specialist journals. Many of these consortia involve UK-based research laboratories and these have provided substantial benefit to both the United Kingdom and to EU member states. The growth in the field can be credited, to some extent, through such consortia that have provided the breadth of preclinical research required to prove safety, efficacy, and translatability of new approaches." • "The European Society of Gene and Cell Therapy together with the British Society of Gene and Cell Therapy and many other individual member state societies must play a proactive role to ensure that the pillars of scientific collaboration and mobility across geographical Europe are maintained, and even enhanced." • "The forthcoming negotiations are of critical importance for the development of gene and cell therapy. Any reduction in funding, scientific and clinical interactions, and joined-up regulatory systems will have a detrimental impact on development of these innovative new medicines for both the United Kingdom and the EU. Scientists throughout Europe should therefore press the case for continued pan-European collaboration post-Brexit."

For the US market, developing a clinical-grade product and obtaining US FDA licensing and approval to distribute and sell the product involves multiple stages (Figure 1). The first is gaining confidence in the intended clinical product by *in vitro* and *in vivo* studies in an animal model to demonstrate preclinical proof of concept. The next stage is initiating discussions with the FDA about filing an IND application that, if approved, will enable the sponsors to test the safety and limited efficacy of the product in a small phase 1 clinical trial. At the preclinical stage, toxicology and other experiments on animal models will test whether the product is potentially efficacious and safe to introduce into humans. The final stage is clinical testing, which progresses through three escalating phases to ultimately test whether the product is safe and efficacious in a large group of subjects, ideally under double-blind, randomized experimental conditions. The last clinical trial in the escalating phases is considered the registration or pivotal trial.

For complex approaches such as cell or gene therapies, early discussions with the FDA are crucial (Figure 1). The first interaction is an early discussion with the FDA Office of Tissues and Advanced Therapies (OTAT; formerly OCTGT Learn), which is part of the Center for Biologics, Evaluation, and Research (CBER). These types of discussions are designated as Initial Targeted Engagement for Regulatory Advice on CBER Products (INTERACT) meetings and are intended to help innovators more effectively meet the FDA's science-based requirements, help sponsors avoid unnecessary preclinical or other preparatory studies, and plan initial clinical development strategies. After the INTERACT meeting, but before toxicology or IND-enabling studies begin, the FDA recommends having a pre-IND meeting so that sponsors and regulators can agree on the initial studies that will be needed to gain IND approval and start clinical testing.

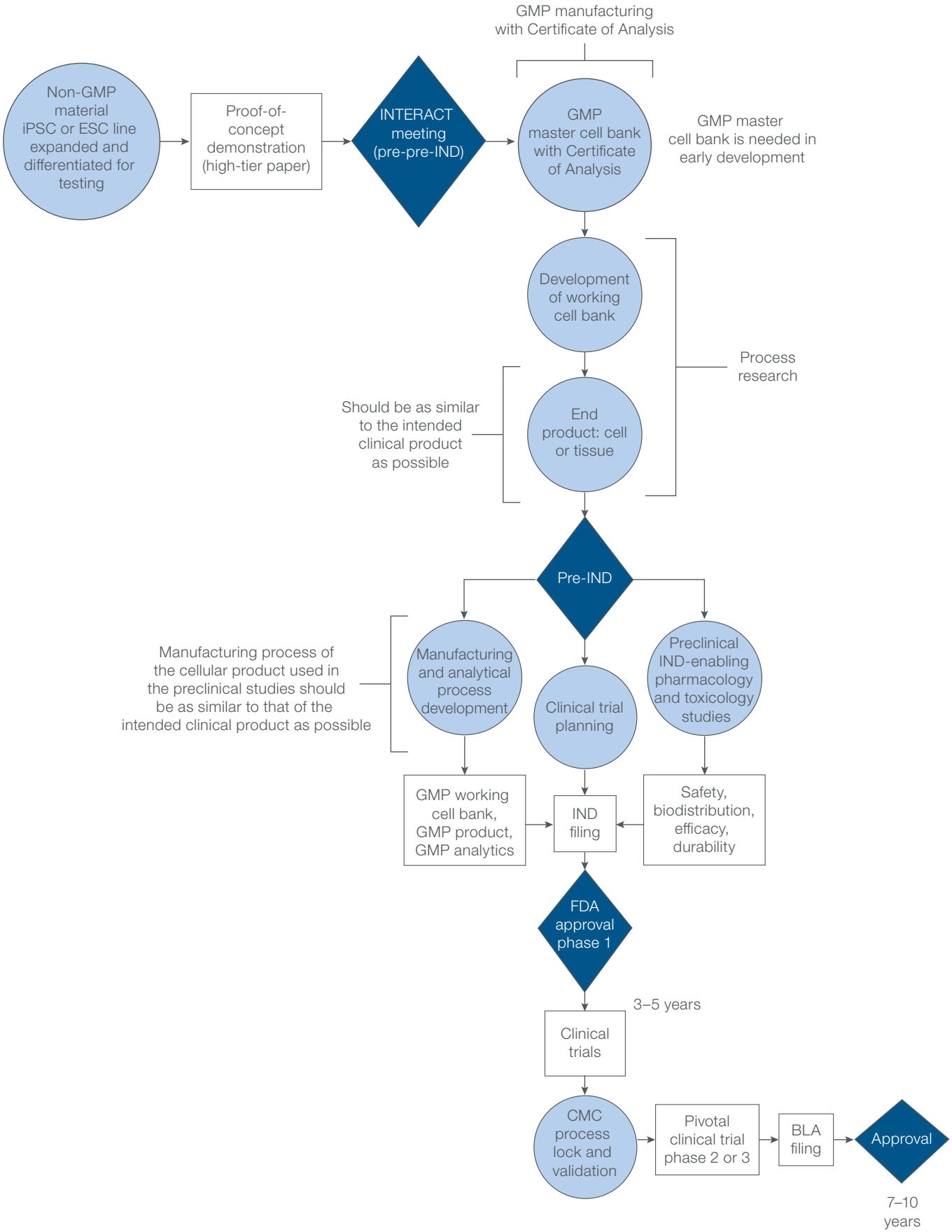


Figure 1. Overview of the approval process for clinical-grade cell therapy products.

Cell-based therapies are regulated as biological products that must comply with applicable sections of the Code of Federal Regulations Title 21, Part 211 (21 CFR 211) and Part 610 (21 CFR 610) [23], and should follow the various FDA, International Organization for Standardization (ISO), and International Conference on Harmonisation (ICH) guidance and regulations (Table 2). Given the youth of the cell therapy field, the rapid pace of advances, and the meticulous nature of regulatory science, the availability of practical information is something of a moving target. The organizations that regulate cell therapies or set standards acknowledge that their guidelines and regulations are likely to change regularly as the field evolves. Moreover, at the time of this writing, the FDA has not yet issued guidance for clinical iPSCs. Nonetheless, a clear understanding and adoption of the most current regulatory framework and guidance are essential for researchers advancing a cell product so that they have the highest likelihood of meeting the minimum requirements when the time comes for the FDA to evaluate their product. Table 2 provides a summary of standards and guidance available as of the date this article was published, and a link to the FDA website that can be referenced for future updates.

Table 2. Publications with information on manufacturing of cell therapeutics.*

Publication	Description
Guidance for Industry: Current Good Tissue Practice (CGTP) and Additional Requirements for Manufacturers of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps) [24]	Provides recommendations for complying with CGTP requirements under Title 21 Code of Federal Regulations, Part 1271 (21 CFR Part 1271).
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) [25]	Discusses the challenges associated with the manufacturing of human somatic cell therapies and provides recommendations and the current (at that time) thinking of the FDA on how to address hurdles such as sources of cells, the potential for contamination, and product release. This specific document is meant to inform a sponsor on how to develop an IND submission, so it contains sufficient information for the FDA to determine the safety, identity, purity, and potency of the investigational product.
FDA Guidance for Industry: Q7 Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients [26]	Provides good manufacturing practice (GMP) guidance for the manufacturing of active pharmaceutical ingredients with an appropriate system for quality management. One of its goals is to help manufacturers ensure they are meeting quality and purity characteristics that the drug is purported to possess. Specific guidance for pharmaceuticals manufactured by cell culture or fermentation are described in Section XVIII.
FDA Guidance for Industry: Quality Systems Approach to Pharmaceutical cGMP Regulations [27]	Provides information to help manufacturers who are implementing modern quality systems and risk management approaches to meet the cGMP regulations. They describe a comprehensive quality systems model for manufacturing human and veterinary drugs, including biological drug products.
FDA Guidance for Industry: Analytical Procedures and Methods Validation for Drugs and Biologics [28]	Provides recommendations for analytical procedures and methods validation to support the documentation of the identity, strength, quality, purity, and potency of drug substances and drug products, including biologics.
USP General Chapter <1046>: Standards for Cell and Tissue Therapies [29]	Founded in 1820, the USP helps protect the safety and quality of the global food, medicine, and dietary supplement supply by setting enforceable standards for the FDA and many state authorities and foreign governments. This chapter describes "issues related to the manufacturing, sourcing of components, and characterization of cellular or tissue-based products to ensure their safety and efficacy."
USP General Chapter <1043>: Ancillary Materials for Cell and Tissue-Based Products [30]	Discusses qualification of ancillary materials, including identification, selection, characterization, vendor qualification, quality assurance, and quality control. It also provides reference standards and specific product chapters (e.g., bovine serum, cytokines, and growth factors).
International Stem Cell Banking Initiatives white paper: Quality control guidelines for clinical-grade human induced pluripotent stem cell lines [2]	The product of community engagement and consensus building by the Global Alliance for iPSC therapies (GAiT), whose goal is to support the global application of pluripotent cell therapies by setting the acceptable standards for physical, chemical, and biological properties of iPSCs in the development of clinical-grade cell lines.
CFR Title 21 Part 610: General Biological Product Standards [23]	Provides federal regulations governing biological products: specifically, release requirements, general provisions, testing for mycoplasma and relevant transfusion-transmitted infections, dating period limitations, and labeling standards.
CFR Title 21 Part 211: Current Good Manufacturing Practice for Finished Pharmaceuticals [23]	Provides federal regulations governing the minimum cGMP for preparation of drug products (including biological products such as human cells, tissues, and cellular and tissue-based products) for administration to humans or animals. Specific requirements can be found on: general provisions, organization and personnel, buildings and facilities, equipment, control of components and drug product containers and closures, production and process controls, packaging and labeling control, holding and distribution, laboratory controls, records and reports, and returned and salvaged drug products.

Table 2. Publications with information on manufacturing of cell therapeutics.* (continued)

Publication	Description
ICH Harmonised Tripartite Guideline: Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin Q5A(R1) [31]	Outlines testing and evaluation of the viral safety of biotechnology products derived from cell lines of human or animal origin, as well as data that should be submitted in the marketing application/registration package. The document covers products derived from cell cultures from characterized cell banks, as well as other biological products.
ICH Harmonised Tripartite Guideline: Comparability of Biotechnological/ Biological Products Subject to Changes in Their Manufacturing Process Q5E [32]	Provides principles for assessing the comparability of biotechnological or biological products before and after changes are made in the manufacturing process. It provides recommendations for relevant technical information that will serve as evidence that the manufacturing process changes will not have an adverse impact on the quality, safety, and efficacy of the product.
ICH Harmonised Tripartite Guideline: Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products Q5D [33]	Discusses appropriate standards for the derivation of human and animal cell lines and microbial cells to be used to prepare biotechnological or biological products, as well as for the preparation and characterization of cell banks to be used for production.

* All FDA guidances relating to cell therapy with updated information can be found at: **https://www.fda.gov/biologicsbloodvaccines/guidancecomplianceregulatoryinformation/guidances**

GMPs and supplier quality systems

A critical element of manufacturing a pluripotent cell product is the principle of cGMP (Table 3). cGMP refers to the "minimum requirements for the methods, facilities, and controls used in the manufacturing, processing, and packing of a drug product" to ensure the product is safe and is of the correct potency and composition. The recent FDA Guidance on Chemical, Manufacturing, and Control (CMC) Information for Human Somatic Cell Therapy IND Applications provides an outline of recommendations [25], the Code of Federal Regulations (CFR) Part 210 and 211 provide cGMP requirements for drug manufacturing, and the FDA guidance on cGMP for phase 1 investigational drugs includes a short section on cGMP standards for biological and biotechnological products, including cell therapy products [34].

Table 3. Essential components of GMP manufacturing.

System	Example
Control of materials	Warehouse with incoming goods quarantine and release process
GMP analytics	Quality control laboratory with quality assurance oversight
Standard operating procedures	Batch record documentation with process for deviations and CAPA*
Qualified operators	Required training for gowning, safety, and operations
Controlled access with environmental control	Keyed access to ISO 7** environment with ISO 5** space for aseptic processing
Environmental monitoring and cleaning cycles	Drop plates, swab testing, particle monitoring, and regular disinfection and cleaning

* Corrective and preventive action.
** Cleanroom classifications.

The general cGMP requirements entail specifications for the following: personnel; quality control plans and functions; facilities and equipment; control of components, and containers and closures; manufacturing and records; laboratory controls; packaging, labeling, and distribution; and record keeping. The FDA acknowledges that cell therapeutics represents a "special manufacturing situation" because "it can be difficult to distinguish changes in quality attributes or predict the impact of observed changes in quality attributes on safety." Because it may not be possible to follow the cGMP guidelines for drugs to the letter, the FDA recommends that the investigator include justification for alternative approaches in the records on the investigational product. They also recommend observance of a series of steps to ensure aseptic conditions, and internal performance reviews when multiple batches of a product are made. Suppliers for cGMP-compliant materials to be used in cell therapies are obliged to manufacture them in designated cGMP facilities designed according to FDA guidance on cGMP for phase 1 investigational drugs [34] that provide space for manufacturing, storage for materials, intermediates, and final products, and support laboratories [35].

cGMP standards emphasize comprehensive quality systems and harmonize with other quality systems in wide use, including ISO 9000, non-US pharmaceutical quality management requirements, and the FDA's device quality system regulations. Guidance for implementing the quality systems and risk management model is outlined in the FDA Guidance for Industry: Quality Systems Approach to Pharmaceutical cGMP Regulations, which was co-released by the Center for Biologics Evaluation and Research, and thus encompasses biologics as well as pharmaceuticals. The guidance stresses that "quality should be built into the product, and testing alone cannot be relied on to ensure product quality."

The four major elements of the FDA model are management responsibilities, resources, manufacturing operations, and evaluation activity, with each discussed in detail in the guidance literature. Proper implementation of these practices will lead to consistent manufacturing of a quality product, management of risk, and may allow for changes in facilities, processes, and equipment with the need for prior approval with accompanying regulatory submissions. Moreover, the reduced risk of manufacturing problems may lead to fewer and shorter FDA inspections.

The ISO and ICH also provide guidance on raw materials that, although more geared toward small molecules and biologics, provides a foundation for the evaluation, selection, and qualification of materials for cell therapeutics. ISO 9001 discusses quality control system requirements, and ISO 13485 provides regulatory guidance for medical device management systems. The ICH has produced a set of guidances for biotechnology products, including on quality (ICH Q5A/D), comparability when changes are introduced to manufacturing processes (ICH Q5E), test procedures and acceptance criteria (ICH Q6B), a GMP guidance (ICH Q7), and quality risk management (ICH Q9).

The importance of early planning

The FDA's catchphrase "the process is the product" encapsulates their approach to complex biological products and stems from the regulatory strategy for large molecules like antibodies and proteins. Manufacturing such biologics differ from the chemical synthesis of small molecules, where two identical products can be made from a different sequence of steps and then be proven identical at the atomic level to confirm drug substance and product comparability. For large molecules, biologics, and cell and gene therapy manufacturing, however, changing the sequence of process steps can introduce changes to the end product that affect its safety and efficacy but may not be easily detected. Consequently, their regulation focuses on the manufacturing process as the "product" rather than just the final release specifications that typically define a drug.

GMP operations in an ISO 7 suite at the Gene and Cell Therapy Laboratory at the University of Washington.

At a practical level, "the process is the product" means decisions made early in process development of allogeneic cell therapies are essentially "hardwired" into the manufacturing process. As a result, process development becomes increasingly more expensive and difficult to change as the product winds through clinical trials to a BLA. As discussed in the introduction, this aspect of cell manufacturing is mainly the consequence of the need early in the process for a master cell bank, which is costly and time-consuming to make, and must meet stringent testing requirements.

When changes are required along the path of preparing a regulatory submission, which is not unusual in typical drug development, such changes must be well-documented and supported by data showing the impact the change will have on the final product, something that can only be had with the proper foresight and procedures in place during every stage of product development. By contrast, in the development of cell therapies, introducing changes to the starting cellular material along the way to regulatory submissions is much costlier and more time-consuming because the starting cell supply (i.e., master cell bank) may no longer be the same as the intended product, and would usually have to be remade to meet the FDA standards of "comparability" for human biological products [32].

Comparability is defined within the regulatory approval process as the need to demonstrate the equivalence of a product following a process change in manufacturing. The concept came when FDA realized that for biological products, changes in the manufacturing process, equipment, or facilities could lead to changes in the final product that may require additional clinical studies to demonstrate the product's safety, identity, purity, and potency.

Tests of comparability for a biologic may include a combination of analytical testing, biological assays, pharmacodynamics, animal toxicity, and clinical testing for safety and efficacy. Comparability is acknowledged as difficult for cell-based products because "the process is the product", and the final product cannot be fully characterized as a small molecule can be. For example, a sponsor that introduced changes in starting materials, reagents, or manufacturing after validation would be required to provide a demonstration of comparability [36]. As a result of these regulatory pressures, investigators developing a pluripotent cell therapy product would be prudent to utilize and adhere to the most stringent level of GMP guidance at every step, with full foresight of the envisioned process from master-bank development through to marketing authorization. In a competitive environment where time and money are limited, and delays can result in loss of funding, competitive position, and investor confidence, changes should be minimized, risks mitigated, and clinical-grade and GMP-compliant materials should be used as soon as an IND filing is predicted, even for stock solutions such as phosphate-buffered saline (PBS).

Raw materials, starting materials, ancillary materials, and excipients

Raw materials are defined as the "starting materials, reagents, and solvents used in manufacturing therapeutic products" [37]. Cell therapy manufacturing requires that materials used in manufacturing meet certain standards, as defined in the FDA Title 21, USP Chapter <1043> [30], and USP Chapter <1046> [29] (reviewed by Atouf et al. [37] and Solomon et al. [1]). Regulatory authorities will evaluate all materials used in cell processing, thus they should be fully defined, sourced from multiple suppliers to reduce supply chain risk, and be as simple as possible, since each additional product introduces its own risk, including to the supply chain. The qualification of materials becomes more comprehensive as the product progresses through the development pathway towards clinical application. Information about the qualifications of materials is submitted to the FDA in the IND application, which requests permission for a clinical trial.

Cell therapy manufacturers must qualify all components according to regulations and available published and draft guidance. In the case of proprietary reagents and materials, confidential information regarding the components and manufacturing can be submitted by suppliers in the form of a drug master file that can describe, among other elements of the manufacturing process, the drug substance (in this case, cell product), intermediates, materials, and excipients [1]. However, regulatory officials do not review these documents unless referenced in an IND application and, as such, it is the cell manufacturer's responsibility to ensure all materials coming in contact with the cell product are evaluated, qualified, and documented.

Some starting materials remain part of the final product, either as active components of the final product or as inert components that do not exert an activity. Cryoprotectants are common excipients in cell manufacturing that are typically inert but are a critical part of the IND application. The IND must list all excipients and raw materials that may remain in the final product along with their concentration, source, and information regarding their qualifications [17].

Ancillary materials are used in the manufacturing process but are not intended to be part of the final product. It should be noted that terminology can differ between jurisdictions. Ancillary materials, often referred to as "raw materials" in Europe, include, but are not limited to, cytokines and growth factors, culture media, buffers, monoclonal antibodies, cryopreservation agents, disposables such as plasticware, and cell separation reagents and devices [1,29]. Because they come in contact with the cells, they can affect the purity, potency, and safety of the cell product. Their potential antigenicity requires that their removal is assessed and, in some cases, limits are set for an acceptable residual amount in the final product.

Guidance for the use of ancillary materials in cell manufacturing is available from different national and international organizations such as the US FDA, ISO, USP, ICH, and European Medicines Agency (EMA) [1]. The FDA CMC information contains extensive regulatory guidance on raw material qualification. However, raw materials are not usually regulated, and the cGMP requirements vary significantly from supplier to supplier depending on their cGMP quality system.

The United States Pharmacopeia (USP) requires a risk reduction protocol for ancillary materials based on a four-tiered system where the risk of each material is ranked, and ancillary materials in each tier require specific activities of the cell manufacturer to mitigate risk [29]. These activities are phased, with a subset required of all products (e.g., a requirement for a Certificate of Analysis and lot-to-lot testing), and others phased in as the risk associated with the ancillary material increases (e.g., safety testing for residual materials containing animal products or gene therapy vectors). Risk escalates as the product moves into later phases of testing. As such it is essential to work with a supplier who can meet both current and future quality needs.

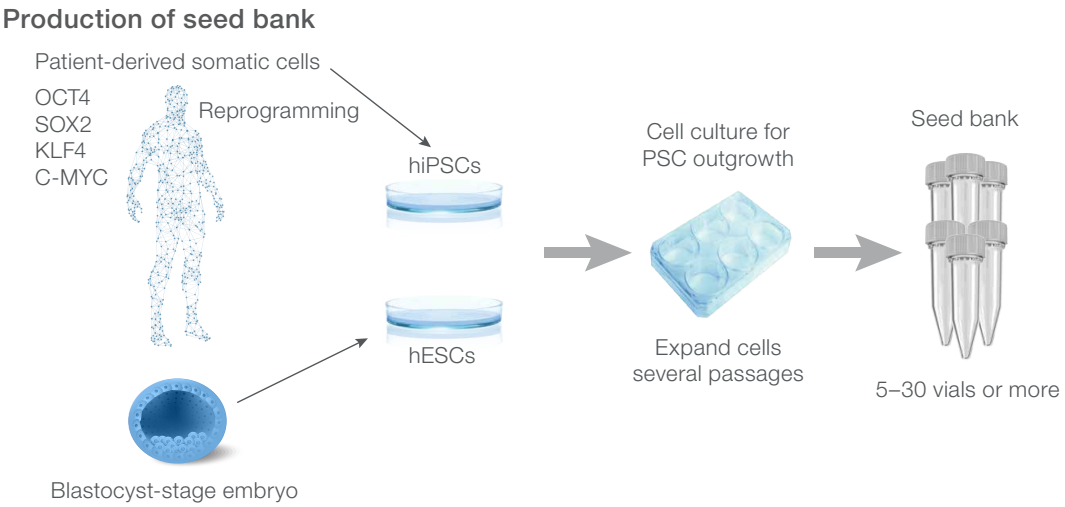
Master cell banks

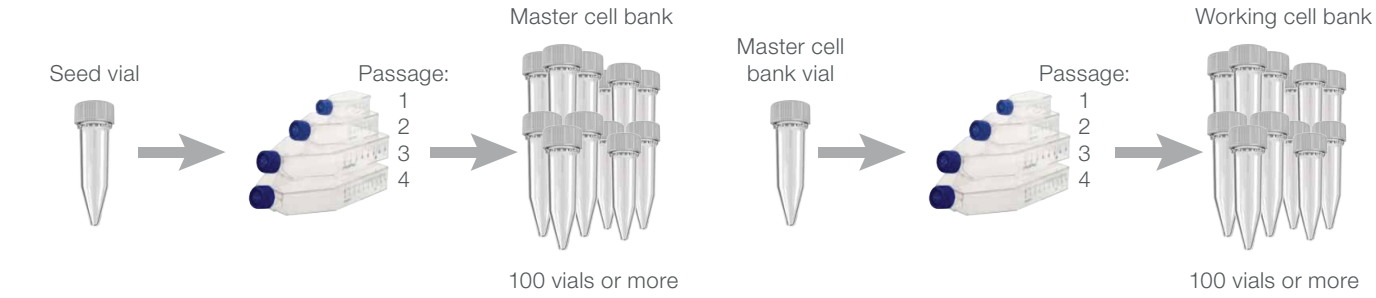
The establishment of the master cell bank is a crucial step in developing an allogeneic cell therapy because it should ideally be the same from preclinical testing to the commercialization of a successful product and forms the basis of all derived products across the lifetime of the therapy. The requirements for cGMP-banked cell lines can be found within the ICH Q5A [31] and ICH Q5D [33], which the FDA has adopted, as well as the FDA memorandum "Points to Consider on the Characterization of Cell Lines Used to Produce Biologics" [38]. These requirements stipulate that the master cell bank must be sterile; have a stable karyotype; and be devoid of bacteria, fungi, and mycoplasmas, as well as adventitious agents and a panel of viruses. The compendial assays and other tests required for release of each lot of cells represent an expensive, rigorous undertaking by which the sunk costs lead to a lock of the process at the earliest stage. Therefore, cell therapy developers must plan carefully for success at this stage, or pay later in time and money. An essential element of the IND application for the FDA will be documenting the components and materials that will be used in the manufacturing of the product, starting with the master cell bank. This confirmatory testing is expensive and time-consuming.

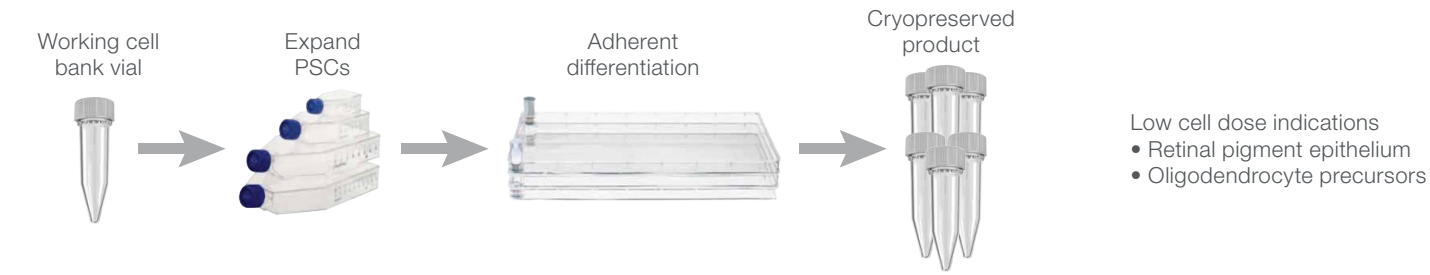
Furthermore, as discussed above, changes to the master cell bank by remaking it from a common seed material or starting with a new donor can introduce significant variability in final product differentiation and manufacturing that in turn will require substantial investments of time and money to reoptimize, qualify, or validate the manufacturing process.

All working cell banks must derive from the same master cell bank and undergo a consistent and standardized passaging protocol for cell line expansion. Phenotypic drift can occur during each population doubling, so the sponsor should ensure that each lot of the final product intended for humans has undergone the same number of population doublings as the cells used for the IND-enabling studies. Consequently, a GMP master cell bank should be produced in sufficient quantity and should have undergone appropriate testing so it can meet the projected demand for treating the target indication within a preset number of population doublings over the lifecycle of the product.

As an example of this type of planning, consider a cell therapy product for type I diabetes. The prevalence and incidence of the disease can be used to calculate the size of the master cell bank needed to ensure that every patient who needs the therapy within the region where it will be marketed can be clinically administered the cells using the precise cell therapy regime used in the preclinical phase of product development. The master cell bank should have sufficient quantity to justify the cost to produce it and ethically fulfill the obligation to treat every patient who needs it (Figure 2). If this hypothetical master cell bank consists of 100 vials of a predetermined number of cells of a given potency and passage and each vial can produce 100 vials of a working cell bank, the master cell bank can support manufacturing up to 10,000 lots of cells (using the FDA's agreed-upon number of cell passages for the working cell bank). If each manufactured lot of cells generated from one working cell bank vial is sufficient to treat 1,000 patients, the master cell bank will be adequate to treat the approximately one million patients in the US currently diagnosed with type I diabetes [39]. The remaining cells would cover the new cases diagnosed in the US each year [40] for about 65 years or would allow expansion into non-US markets under a shorter product lifespan. While on the surface it seems premature to undertake such planning even before an IND is filed, the importance of such early lifecycle planning for successful, patient-centered commercialization cannot be overstated.







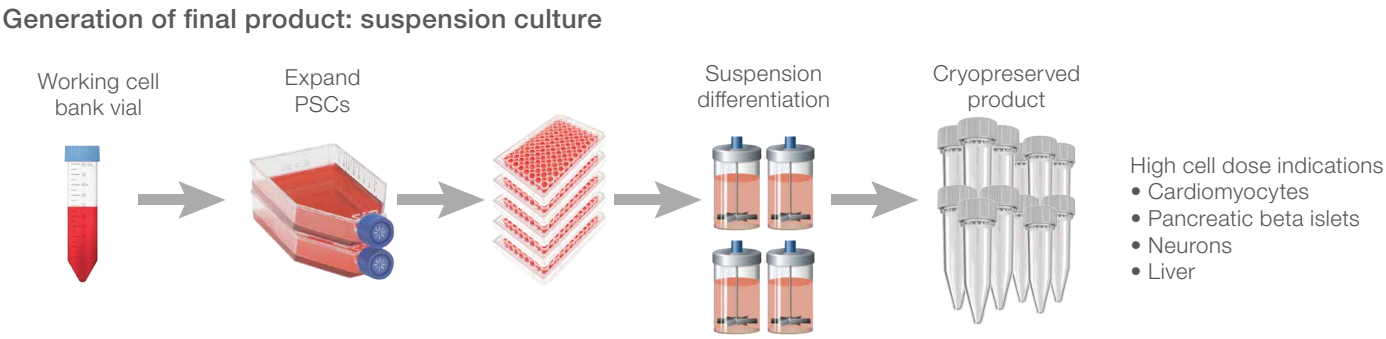


Figure 2. Process overview for development and manufacturing of a cell therapy product.

Tissues acquired for cell products must be sourced following appropriate Health Insurance Portability and Accountability Act (HIPAA) guidelines and with institutional review board (IRB) oversight governing donor eligibility, consent, and privacy, and risks [41]. In the specific context of cell manufacturing, cell donors for allogeneic cells must be screened and tested to ensure they are free of pathogenic viruses including HIV and hepatitis virus using detection assays that are FDA-licensed, cleared, or approved, and typing for HLA matching and polymorphisms is likely to be required for all allogeneic transplants. The impact of HLA mismatches on patients receiving transplants of pluripotent cell derivatives is poorly understood, and a tremendous (and unfeasible) number of cell lines would be needed to match the HLAs of the human population [42,43]. Taylor et al. proposed that establishing hESC banks from individuals homozygous for certain HLA alleles would decrease the required number of banked lines [43]. However, the number of homozygous HLA types needed to match a population differs depending on the genetic heterogeneity of the population. A bank of 150 pluripotent cells is estimated to provide a full match at HLA-A, HLA-B, and HLA-DR for <20% of recipients, a beneficial match for 37.9%, and an HLA-DR match of 84.9% [43]. Pappas et al. estimated that fewer than 80 lines of a homozygous, custom haplotype could match >50% of the Californian population as a model of a genetically admixed population representing five principal ancestries [44]. Investigators would be prudent to obtain as detailed genetic information as possible to inform future studies and help clinicians target patients with specific HLA types who might especially benefit from the transplant or be at less risk for adverse events resulting from as yet undefined immune responses.

The FDA requires information and documentation on the history, source, derivation, and characterization of both the master cell bank and the working cell bank, as well as the frequency of the testing. For the master cell bank, compendial assays must be performed to establish the identity of the cells, the purity of the cell bank along with descriptions and quantification of contaminating cells, activity of the cells (if activity is relevant to the therapeutic application of the product), and processes critical to product safety, such as culture conditions, cryopreservation protocols, and genetic and phenotypic stability of the cells. The characterization of the working cell bank is typically less extensive than the master cell bank: the FDA recommends testing for *in vitro* and *in vivo* adventitious viral agents, bacterial and fungal sterility, mycoplasmas, identity, and cell lineage. Most of the cell line characterization and qualification work is typically performed by an experienced contract research organization (CRO), although sponsor-specific tests such as flow cytometry may be done in-house.

For storage of banked cells, cryopreservation in vapor-phase liquid nitrogen is a standard method that maintains viability and lessens the risk of genetic mutations and development of subpopulations upon thawing [45]. Correct cryopreservation procedures are essential for maintaining the retention of the desired cell properties and thus an essential element of cell therapy development, and one that must be tailored to the particular intended clinical use and product characteristics. Best practices for the banking, testing, and storage of hESCs have been reviewed by the International Stem Cell Banking Initiative [46], and also apply to iPSCs. Optimization of conditions such as cell status and growth conditions prior to freezing and after thawing should be carried out early in product development with qualified reagents and avoidance of animal-derived components. Various tests must be performed to show the cryopreserved cells are viable, retain their self-renewal or other desired characteristics, and are free from adventitious agents.

Final product release testing and stability

Similar to the requirements for master cell banks, lot release of cell-based products must adhere to stringent guidelines and require a series of compendial assays and critical quality specifications that are established through the development process (Table 4). Documentation of the history of the cells and all activities they have undergone, from the cell bank to release, should be kept according to standards for quality management systems. An identity test must be performed to unequivocally identify the product, which can include differential surface markers or cell morphology to distinguish between different cell types. A purity test must be used to quantify the amount of the intended active product, with tests for impurities dictated by the safety risk of the particular impurity or raw material residue. Since cell products cannot be sterilized before release, they must be tested to ensure they meet acceptable criteria for sterility and absence of mycoplasmas and endotoxin contamination. Potency must also be evaluated using *in vitro* or *in vivo* bioassays or a combination thereof, with reference materials as positive controls for the assay. To precisely measure the amount of product in each lot, a dose-defining assay must be performed to enumerate cell populations within the lot.

Table 4. Some criteria for the final release of cell-based products.

Test	Source	Purpose	Examples for PSC products
Sterility and adventitious agents	21 CFR 610.12 USP <71>	Each lot of a manufactured product must be tested in a manner appropriate to the material being tested through a validated test with validated and written procedures in place.	• Bacteria and fungi • Human and simian viruses and retroviruses • Murine, porcine, and bovine pathogens
Mycoplasma	21 CFR 610.30 USP <63>	The presence of the mycoplasma must be determined by culturing test samples with control samples according to procedures stipulated in 21 CFR 610.30 to show there is no growth in test samples.	
Endotoxin	USP <1046> and USP General Chapter 85	A number of different methods are described for measurement of endotoxins, all based on the Limulus amebocyte lysate (LAL) assay.	
Identity	21 CFR 610.14	"The identity test shall be specific for each product in a manner that will adequately identify it as the product designated on final container and package labels and circulars, and distinguish it from any other product being processed in the same laboratory. Identity may be established either through the physical or chemical characteristics of the product, inspection by macroscopic or microscopic methods, specific cultural tests, or *in vitro* or *in vivo* immunological tests."	• Surface markers • Isoenzyme analysis • Genetic fingerprint • Morphology • Bioassay • Biochemical marker(s)
Purity	21 CFR 600.3	"Purity means relative freedom from extraneous matter in the finished product, whether or not harmful to the recipient or deleterious to the product. Purity includes but is not limited to relative freedom from residual moisture or other volatile substances and pyrogenic substances."	• Percentage of viable cells • Percentage of cells that express markers of interest • Lack of (or within defined limits for) undesired cell types • Limits on process contaminants (e.g., feeder cells)
Potency	21 CFR 600.3	"The specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, to effect a given result."	• Viable cell number • Colony-formation assay • Change in expression of specific genes • Secretion of desired macromolecule • Induction of secondary effect • Evidence of metabolic activity • Evidence of cell function
Dose	USP <1046>	"An assay that precisely measures the amount of product. Cell therapy products may be dosed on the basis of enumeration of one or more cell populations."	• Viable cell number • Enumeration of specific cell population • Total DNA • Total protein
Other		Depends on desired properties of cell line being released.	• Appearance • Morphology • Size • Teratoma assay • Genomic and epigenetic stability

The stability of the product will vary widely depending on its intended use, specific composition and attributes, and its requirements for storage, packaging, and shipping. As part of preclinical qualification, the product must undergo stability testing (ideally following the principles described in ICH guideline Q5C) to ensure that the storage conditions maintain the product's quality attributes of viability and efficacy during manufacture, storage, and delivery.

Planning for success

Process consistency and product lifecycle planning are crucial for launching and marketing a therapeutic, which means that developers must make the leap of faith that their product will be successful and plan for that reality by ensuring their process can be scalable and cGMP compliant even before they file for an IND. For instance, the reagents they are using in preclinical development must be available at a scale needed for manufacturing once the investigational product is approved and marketed, because reagent and supplier changes (if possible at all) can involve great expense and delays after an IND application is filed.

Thus, the material supply chain is vital for creating a product with longevity and reliable supply, which can be a matter of life and death to patients who might depend on a particular product. Suppliers experienced in the "art" of cell therapy, including GMP manufacturing and supply chain management and in manufacturing cell therapy–specific raw or ancillary materials, can play a crucial role in product lifecycle planning from the earliest stages. Selecting appropriate vendors experienced in supply chains for cell therapy development can provide procedures for document retention, cGMP-compliant materials with traceability, and multiple sources for components in case of breaks in the supply chain (Table 5).

The role of suppliers

Qualifying the materials used in cell manufacturing can be challenging and complex, and it is critical for the investigator to understand the guidance that suppliers are following for each material that comes in contact with the cells. If the materials are produced for research use only (RUO), additional testing may be required beyond what suppliers provide in their Certificates of Analysis.

One challenge of cell therapeutics is that regulators still view it as the responsibility of cell manufacturers to work with the suppliers to ensure all compliance requirements are met, as defined by the most current and relevant guidance [1]. The inherent characteristics of cells as a therapeutic product present additional challenges that are not always addressed by the current development processes for drugs or even biologics: these include their lability, production protocols that require small batches and biological components, and difficulty of analytic methods in characterizing the final cellular product. Potency assays are especially challenging because they are hardest to design and different for every product type. Thus, having access to high-quality materials from a supplier who understands these issues potentially can provide a crucial competitive advantage to a manufacturer and even make the difference between success and failure. The demands on cell therapy developers are high to ensure lot-to-lot consistency, validation of test methods, provision of Certificates of Analysis, and adequate and reliable supplies of materials. Establishing continual communication with a supplier experienced in providing materials for cell manufacturing early in the process, when filing an IND is first envisioned, can alleviate some of this pressure by aligning expectations and providing an additional layer of quality and document control.

Table 5. Gibco™ Cell Therapy Systems™ (CTS™) products for iPSCs.

iPSC workflow	Research use ancillary materials	CTS ancillary materials
Culture somatic cells	KnockOut Serum Replacement	CTS KnockOut SR XenoFree Medium
Reprogram	CytoTune-iPS 2.0 Sendai Reprogramming Kit	CTS CytoTune-iPS 2.1 Sendai Reprogramming Kit
Expand	Essential 8 Medium	CTS Essential 8 Medium
	Vitronectin Recombinant Human Protein, Truncated	CTS Vitronectin Recombinant Human Protein, Truncated
Passage	TrypLE Select Enzyme	CTS TrypLE Select Enzyme
	Versene Solution	CTS Versene Solution
	RevitaCell Supplement	CTS RevitaCell Supplement
Bank/recover	Synth-a-Freeze Medium	CTS Synth-a-Freeze Medium
	PSC Cryomedium	CTS PSC Cryomedium
	PSC Cryopreservation Kit	CTS PSC Cryopreservation Kit
	RevitaCell Supplement	CTS RevitaCell Supplement
Differentiate	Essential 6 Medium	CTS Essential 6 Medium
Other reagents	DPBS	CTS DPBS
	KnockOut DMEM/F-12	CTS KnockOut DMEM/F-12
	KnockOut DMEM	CTS KnockOut DMEM
	Hibernate-E Medium	CTS Hibernate-E Medium
	Hibernate-A Medium	CTS Hibernate-A Medium

Thermo Fisher Scientific is one option for a supplier with extensive experience in cell therapy development by offering CTS products. These products have been developed to ease the transition from stem cell therapy research to clinical applications by providing high-quality, GMP-manufactured, commercial-scale ancillary materials with a high degree of qualification, traceability, and regulatory documentation (Tables 5 and 6). CTS products have been used in commercially approved cell therapies as well as over 200 clinical trials and are backed by professional regulatory support and over 30 years of GMP manufacturing experience.

Table 6. Criteria for CTS products.

cGMP manufacturing	Testing and documentation	Proven use
• Manufactured in conformity with GMP for medical devices, 21 CFR Part 820, following USP <1043> and Ph. Eur. 5.2.12 • Manufacturing sites that are FDA-registered and ISO 13485–certified and regularly audited	• Traceability documentation, including Drug Master Files (DMFs) and/or Regulatory Support Files (RSFs) and Certificates of Origin • Product safety testing, including sterility, endotoxin, and mycoplasma on media and reagents	• Used in FDA-approved and EMA-approved CAR T cell therapies [47,48] and the first FDA-approved therapeutic cancer vaccine [49] • Used in over 200 clinical trials

Glossary

Ancillary materials: Components, reagents, or materials used during manufacturing that can exert an effect on the cell product but is not intended to be part of the final cell product.

Biological drug product: Generally a large, complex molecule such as a protein or antibody that is made through biotechnology from a living system.

Biologics license application (BLA): A request for permission to introduce, or deliver for introduction, a biologic product into interstate commerce (21 CFR Part 601.2). The BLA is regulated under 21 CFR Parts 600–680.

Chemistry, manufacturing, and control (CMC): Principles applied to the manufacturing of drugs, biologics, and cell therapies to assure the identity, quality, purity, and potency of the investigational product.

Cellular product: "Living human or animal cells or tissue that have been manipulated or are used in ways that result in their regulation as somatic cellular therapies as defined by the US FDA" (USP <1046> definition).

Certificate of Analysis: A document that should be issued for each batch of intermediates or active pharmaceutical ingredient (API) upon FDA request that lists each test performed following compendial or customer requirements signed by authorized personnel and indicating quality units and information on the original manufacturer [23].

Clinical-grade cells: Cells that are optimally defined in terms of quality and safety such that they are suitable for use in cell transplantation for humans.

Combination product: A product that involves a medical device and/or a drug and/or a biologic—combining any two of these product categories and sometimes even all three (from FDA CFR).

Compendial assays: Standardized methods and specification testing for raw materials and finished products that are a basic requirement for most regulatory submissions worldwide.

Cell Therapy Systems (CTS) products: GMP-manufactured products that have been specifically designed to meet cell therapy quality, safety, and regulatory requirements in order to reduce risk and support cell therapy developers from research through clinical translation and commercialization. Go to **thermofisher.com/cts** for more information.

Current Good Manufacturing Processes (cGMP): "cGMP refers to the Current Good Manufacturing Practice regulations enforced by the FDA. cGMPs provide for systems that assure proper design, monitoring, and control of manufacturing processes and facilities. Adherence to the cGMP regulations assures the identity, strength, quality, and purity of drug products by requiring that manufacturers of medications adequately control manufacturing operations. This includes establishing strong quality management systems, obtaining appropriate quality raw materials, establishing robust operating procedures, detecting and investigating product quality deviations, and maintaining reliable testing laboratories" (FDA definition).

Excipient: Any component that is intended to be part of the final product, such as human serum albumin or dimethyl sulfoxide (DMSO), but that is not intended to exert an effect.

International Conference on Harmonisation (ICH) E3: A document that makes recommendations on information that should be included in a core clinical study report of an individual study of any therapeutic, prophylactic, or diagnostic agent conducted in human subjects.

Investigational new drug (IND) application: A request a sponsor of an investigational product makes to the FDA to test its therapeutic potential in humans after its therapeutic activity and acute toxicity potential have been tested in animals.

Master cell bank (MCB): A culture of fully characterized cells that are distributed by aliquots into containers in a single operation, processed together to ensure uniformity, and stored in a manner that maintains their stability (e.g., usually at –70°C or lower).

Potency: "The potency, that is, the therapeutic activity of the drug product as indicated by appropriate laboratory tests or by adequately developed and controlled clinical data (expressed, for example, in terms of units by reference to a standard)" (FDA definition).

Raw materials: "A general term used to denote starting materials, reagents, and solvents intended for use in the production of intermediates or active pharmaceutical ingredients" (ICH Q7).

Seed train: The process by which an adequate number of cells to inoculate a production bioreactor is generated.

Working cell bank (WCB): Cells derived from the expansion of one aliquot of the master cell bank that will be used in the manufacturing process.

Qualification: Data establishing the source, identity, purity, biological safety, and overall suitability of a product has been collected and evaluated.

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