Cytotherapy 000 (2024) 1–18

Contents lists available at ScienceDirect

CYTOTHERAPY

journal homepage: www.isct-cytotherapy.org

ISCT Committee Paper

# Considerations for the development of iPSC-derived cell therapies: a review of key challenges by the JSRM-ISCT iPSC Committee

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ARTICLE INFO

*Article History:*
Available online xxx

*Key words:*
regenerative medicine
iPSC-derived cell therapies
the cost of goods (COGs)
quality by design (QbD)
critical quality attributes (CQAs)
good manufacturing practice (cGMP)
quality control (QC)

ABSTRACT

Since their first production in 2007, human induced pluripotent stem cells (iPSCs) have provided a novel platform for the development of various cell therapies targeting a spectrum of diseases, ranging from rare genetic eye disorders to cancer treatment. However, several challenges must be tackled for iPSC-based cell therapy to enter the market and achieve broader global adoption. This white paper, authored by the Japanese Society for Regenerative Medicine (JSRM) - International Society for Cell Therapy (ISCT) iPSC Committee delves into the hurdles encountered in the pursuit of safe and economically viable iPSC-based therapies, particularly from the standpoint of the cell therapy industry. It discusses differences in global guidelines and regulatory frameworks, outlines a series of quality control tests required to ensure the safety of the cell therapy, and provides details and important considerations around cost of goods (COGs), including the impact of automated advanced manufacturing.



## Introduction

In 2007, Shinya Yamanaka demonstrated that adult human fibroblasts could be reverted to an embryonic stem cell (ESC)-like state via a cell reprogramming technique called directed differentiation [1]. These reprogrammed cells were called induced pluripotent stem cells (iPSCs). Since their first derivation, human iPSCs have emerged as a focal point of interest in contemporary regenerative medicine as they hold the potential to replace damaged or diseased tissues [2].Their ability to extensively self-renew in culture and differentiate into over 3 hundred cell types of the human body addresses longstanding limitations in the study of human disease due to the scarcity of human cells, and offers a platform for the development of iPSC-based cell therapies. A limited number of clinical trials utilizing iPSCs have been conducted. However, as yet, no iPSC-based therapies have received approval [3,4]. This is primarily due to significant challenges that

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https://doi.org/10.1016/j.jcyt.2024.05.022

must be addressed before iPSCs can be effectively utilized for large-scale production of cell-based therapies. These challenges include complex manufacturing processes, implementing meticulous quality assurance measures, managing high production costs, and harmonizing regulatory standards [1,2,4,5]. This publication by the Japanese Society of Regenerative Medicine (JSRM) and the International Society for Cell & Gene Therapy (ISCT) iPSC Committee delves into the critical aspects of iPSC-derived cell therapy development, with a primary focus on regulatory perspectives, quality and cost.

Wide adoption of novel cell therapies is highly reliant on a regulatory system that enables safe and fast progression of innovative therapies. An understanding of global regulatory guidance and standards is essential to ensure the safety, efficacy, and quality of iPSC-derived cell therapies. Harmonization of global regulatory standards would simplify and streamline the development and approval of novel cell therapies. We discuss this below, along with an overview of the regulatory landscape in different countries, highlighting similarities and differences between them.

Current global regulations guide clinical-grade manufacturing and product characterization of iPSC-based therapies. The manufacturing workflow for iPSC-derived cell therapies encompasses several stages, each with its unique challenges and opportunities. Quality control (QC) and product characterization are integral to the development and commercialization of iPSC-derived cell therapies. While standards are not defined, there is a general consensus regarding critical quality attributes of iPSCs. Here we provide an in-depth overview of the entire development workflow of iPSCs and iPSC-derived products, and discuss the key assays, methods, and technologies employed to assess and monitor their quality and safety.

Clinical grade manufacturing and characterization impact the Cost of Goods (COGs) associated with the therapy. To make iPSC-derived cell therapies accessible and cost-effective, it is crucial to develop creative manufacturing approaches and optimize manufacturing processes through scale-out and scale-up strategies.

By understanding the factors influencing the cost of iPSC-derived cell therapies, we can identify potential areas for cost reduction and develop robust models to predict and manage manufacturing expenses. We discuss various strategies and technologies available to enhance the production capacity and efficiency of iPSC-derived cell therapies, ultimately reducing COGs.

In summary, this white paper aims to provide a comprehensive overview of the critical elements involved in the development of autologous and allogeneic iPSC-derived cell therapies. By addressing the challenges and exploring the opportunities presented in this field, we aspire to contribute to the acceleration of these promising therapies towards clinical application and widespread adoption.

## Section I. Regulatory Guidance Impacting the Development of iPSC- and iPSC-Derived Cell Therapies

The implementation of a Quality by Design (QbD) approach, coupled with effective cross-functional communication and coordination on issues (like consent, Human Leukocyte Antigens (HLA)-typing, stem cell banking, current Good Manufacturing Practice (cGMP) standardization, information sharing, and cost mitigation) is critical. While regulators prioritize patient safety, all stakeholders share responsibility for efficient translation of iPSC-based therapies through effective planning and communication. It is important to recognize that what is considered suitable for one product, indication, or delivery method may not necessarily apply to another. Standardization is needed in iPSC manufacturing, but it should be within reasonable bounds, acknowledging the need for flexibility to accommodate specific and diverse product requirements. Developers should engage with regulators at early stages with very specific questions, so regulators are exposed to the product attributes and its intended use. Developers play a crucial role in providing essential information to regulators, enabling them to make informed decisions and establish regulations that prioritize patient safety and product efficacy. Developers and regulators share a responsibility to develop a regulatory framework that is practical and devoid of unnecessary obstacles, Active engagement with regulatory agencies to enhance development and understanding of assessment methodologies for cell therapy products should ensure that both safety and potency are adequately evaluated.

There remains jurisdictional variation in how products are assessed, not only staging of clinical trial approval, but also in determination of appropriate compendial methods. Regulatory uncertainty around testing standards results in an inherent risk of overcommitment in overlapping or redundant scientific assays and QC methods that increase production complexity without increasing predictive value related to patient safety or clinical efficacy. Rarely do regulatory agencies advise a sponsor that characterization and safety could be achieved by either simpler or fewer methods. Once a sponsor has begun to use certain testing, it is likely that these habits will be retained through the life cycle of the product. What is often under-appreciated is that testing is a significant driver of the overall product cost, and such testing will then continue to be a long-term cost obligation to the current or future commercial sponsors. In the long run, advancement of iPSC-derived cell therapies will benefit from further development and harmonization of global regulatory standards.

Table 1 provides the broad regulatory frameworks that are applicable for this category of products. It is also evident that even where there is global harmonization (such as mutual recognition under treaty for a foreign recognized medicines agency GMP inspection) this generally excludes cell and gene therapy (as defined as Advanced Therapy Medicinal Products (ATMPs) or in the case of Australia as Biologicals) [6].

## Section II. Characterization of iPSCs and iPSC-Derived Cells Therapies

### *Testing and Characterization Workflow*

Workflow will vary among cell therapy developers, depending on factors such as: starting cell type, reprogramming technique, manufacturing yield, risk tolerance, release criteria, format of the final therapeutic product, use of gene editing, and whether the product is allogeneic (donor-derived) or autologous (patient-derived).

Following patient recruitment and fulfillment of donor selection criteria, the process of all iPSC-derived cell therapy manufacturing begins with a somatic cell type. This somatic cell is then reprogrammed, typically via delivery of the Yamanaka or Thomson factors, to generate iPSCs [1]. It is important to note that the collection of these somatic cells is subject to ethical approval and, in the case of an allogeneic therapy, informed consent from the donor [7,8]. The starting cells must be isolated from the collected sample (e.g., blood-subtypes, or fibroblasts from a skin biopsy), expanded to achieve an optimal growth rate and starting number of cells for reprogramming (Figure 1), and characterized to assess incoming characteristics such as dose, cell identity, genetic stability, sterility, and mycoplasma testing (Table 2). Certain starting cell types may be subject to additional incoming specifications; for instance, fibroblasts isolated from a skin biopsy cannot be passaged beyond a certain passage number before initiating reprogramming [9].Cells obtained for allogeneic therapies are also subjected to rigorous adventitious agent testing to ensure that infectious disease agents are not transmitted from the donor to the patient [8,10]. Authentication via genetic identity testing (e.g., short tandem repeat, STR) is also necessary at this step to ensure the maintenance of the chain of identity from the beginning to end of the manufacturing process. This testing may be done on the somatic cells that are to undergo reprogramming, or on a separate sample (e.g.,

**Table 1**
Global Regulatory guidance governing cell- and iPSC-based therapies.

| Country or Region | Regulatory body | Approval path for clinical trial | Competent section/ office | Relevant law and guidance documents | Roles of the agency or objectives of the laws/ guidance documents | Product jurisdiction[a] |
|---|---|---|---|---|---|---|
| Australia | TGA | Through the CTA Scheme, unless if supporting evidence from a previous clinical trial exists or have obtained approval for an equivalent indication from a national regulatory body with comparable regulatory requirements | TGA Biologicals Section | Schedule 16 of the Therapeutic Goods Regulations 1990<br>Australian regulatory guidelines for biologicals (ARGB)<br>Autologous human cells and tissues products regulation | Helps to understand how to classify biological products<br>Provide information on the supply and use of human cell and tissue-based therapeutic goods<br>Provides regulation for autologous human cells and tissues (HCT) products based on the level of risk to the public | iPSC-derived products are classified as class 4 biologicals (high risk products because the intrinsic function of the donor biological has been changed) |
| European Union | EC | Submission of IMPD to the national competent authorities and ethics committees *via* the Clinical Trials Information System (CTIS) | | The Clinical Trials Regulation (Regulation (EU) No 536/2014)<br>Regulation (EC) No 1394/2007 on Advanced Therapy Medicinal Products<br>Directive 2001/83/EC on the Community code relating to medicinal products for human use<br>Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products<br>Guideline on Good Clinical Practice specific to Advanced<br>Therapy Medicinal Products<br>Directive 2004/23/EC on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells | Oversees the implementation of the Clinical Trials Regulation<br>Provides the overall framework on ATMPs and the definition of 'tissue-engineered medicinal product'<br>Provides the definitions for 'gene-therapy medicinal product' and 'somatic cell-therapy medicinal product'<br>Provide GMP, GCP, and GTP for ATMPs | ATMPs are classified into:<br>• Gene therapy medicinal products<br>• Somatic-cell therapy medicinal products<br>• Tissue-engineered medicinal products<br>• Combined ATMPs |
| | EMA | NA | Committee for Medicinal Products for Human Use<br>Committee for Advanced Therapies | Guideline on human cell-based medicinal products<br>Guideline on safety and efficacy follow-up and risk management of advanced therapy medicinal products<br>Reflection paper on stem cell-based medicinal products<br>Guideline on the risk-based approach according to annex I, part IV of Directive 2001/83/EC applied to advanced therapy medicinal products | Provides scientific recommendations on the classification of ATMPs<br>Gives scientific advice to developers<br>Maintains CTIS<br>Provides authorization for marketing in EU | |
| | National competent authorities and ethics committees of member states | Submission of IMPD to the national competent authorities and ethics committees *via* CTIS<br>Must be conducted in compliance with GCP of ICH | Varies depending on the national competent authorities and ethics committees of the member state | Regulations of each member state according to EC regulations and directives | Supports the development & accelerated assessment or exempt approval of medicinal products for unmet medical needs<br>Oversees clinical trials and production/quality controls of the facilities | |

(*continued on next page*)

**Table 1** (*Continued*)

| Country or Region | Regulatory body | Approval path for clinical trial | Competent section/ office | Relevant law and guidance documents | Roles of the agency or objectives of the laws/ guidance documents | Product jurisdiction[a] |
|---|---|---|---|---|---|---|
| Japan | PMDA | Submission of commercial clinical trial application to PMDA<br>Must be conducted in compliance with GCP of ICH | Office of Cellular and Tissue-based Products<br>Office of Safety II | The Act on Securing Quality, Efficacy, and Safety of Products Including Pharmaceuticals and Medical Devices (PMD Act)<br>The Standards for Biological Raw Materials<br>Good gene, Cellular, and Tissue-based products manufacturing Practice (GCTP)<br>Good Post-marketing Study Practice (GPSP) | Reviews clinical trial applications and manufacturing/marketing authorization applications<br>Offers consultations to give advice on clinical trials and manufacturing/marketing authorization of regenerative medical products, as well as on data for regulatory submissions<br>Oversees manufacturing/quality controls of the facilities | Classified as "regenerative medical products," a category independent of pharmaceuticals and medical devices<br>Regenerative medical products are subdivided into:<br>• Cell-processed products<br>• Gene therapeutics<br>PSC-derived products are classified into the former.Cell-processed products not yet approved by the MHLW, called "specified processed cells," are allowed to be administered to patients at the discretion of medical practitioners or in non-commercial clinical studies as long as the medical practices comply with the Act for the Safety of Regenerative Medicine (ASRM or RM Safety Act for short). |
| | MHLW | | Pharmaceutical Safety Bureau | Guidelines on ensuring the quality and safety of autologous /allogeneic human iPS(-like) cells-derived products<br>Points to consider regarding tests to detect undifferentiated pluripotent stem cells/transformed cells, tumorigenicity tests, and genomic stability evaluation for human cell-based therapeutic products | Issues ministerial ordinances, notifications, and administrative notices on the quality, safety, and efficacy of cell therapy products<br>Provides the conditional and term-limited approval system for regenerative medical products with putative efficacy<br>Provides full or conditional/term-limited manufacturing/marketing authorization | |
| United Kingdom | MHRA | New Clinical Trials of Investigational Medicinal Products (CTIMPs) applications must be submitted *via* the Integrated Research Application System (IRAS) and reviewed by MHRA and the Research Ethics Committee. | MHRA Innovation Office | Regulation (EC) No 1394/2007 on Advanced Therapy Medicinal Products<br>Directive 2001/83/EC on the Community code relating to medicinal products for human use<br>Guidance for Great Britain Marketing Authorisation Applications for Advanced Therapy Medicinal Products (ATMPs) | MHRA is the competent authority for clinical trial authorization for all medicinal products, including ATMPs, and for UK manufacturers or importers of ATMPs.<br>Provides classification opinions and advices about ATMPs<br>Supports the development & accelerated assessment or exempt approval of medicinal products for unmet medical needs | ATMPs are classified into:<br>• Gene therapy medicinal products<br>• Somatic-cell therapy medicinal products<br>• Tissue-engineered medicinal products<br>• Combined ATMPs |

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**Table 1** (*Continued*)

| Country or Region | Regulatory body | Approval path for clinical trial | Competent section/ office | Relevant law and guidance documents | Roles of the agency or objectives of the laws/ guidance documents | Product jurisdiction[a] |
|---|---|---|---|---|---|---|
| United States | FDA | Must be conducted in compliance with 21 CFR Part 312, which was developed in accordance with ICH GCP<br>Compliance with current Good Manufacturing Practice (cGMP) for biologics<br>Compliance with the Current Quality System regulation (QSR) for medical devices<br>BLA or PMA Application must be submitted following clinical trials to obtain marketing approval | Office of Cellular Therapy and Human Tissue CMC<br>in the Office of Therapeutic Products at CBER | Public Health Service Act (PHS Act); Section 351 HCT/Ps | Provides regulations for HCT/Ps with more than minimal manipulation or for non-homologous use (Section 351 HCT/Ps)<br>Provides HDE under the HUD designation program for HCT/Ps that are classified as medical devices | Section 351 HCT/Ps are classified into either:<br>• Biologics (requiring compliance with cGMP and IND approval)<br>• Medical devices (requiring compliance with the current QSR for medical devices and IDE approval) |
| | | | | 21st Century Cures Act, Regenerative Medicine Advanced Therapy (RMAT) designation system | Help accelerate medical product development and bring new innovations and advances to patients who need them faster and more efficiently. | |
| | | | | Code of Federal Regulations Title 21 (21 CFR) | Provides:<br>GCP (Part 312),<br>cGMP (Part 210-211), QSR (Part 820) and HCT/P regulations including cGTP (Part 1271). | |
| | | | | Guidance for Industry: Guidance for Human Somatic Cell Therapy and Gene Therapy<br>Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products; Guidance for Industry<br>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)<br>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 | Describe FDA's interpretation of policy on a regulatory issue that relate to the design, production, labeling, promotion, manufacturing, and testing of regulated products.<br>Guidance documents may also relate to the processing, content, and evaluation or approval of submissions as well as to inspection and enforcement policies. | |
| | | | | Guidance for Industry: Potency Tests for Cellular and Gene Therapy Products<br>Guidance for Industry: Preclinical Assessment of Investigational Cellular and Gene Therapy Products<br>Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products; Guidance for Industry | Provides information about the design of preclinical studies for Section 351 HCT/Ps, potency assays and considerations for the design of early-phase clinical trials of cellular and gene therapy products | |

ATMP; advanced therapy medicinal products, BLA; biologics license applications, CBER; Center for Biologics Evaluation and Research, CTA; clinical trial approval, cGMP; current Good Manufacturing Practice, cGTP; current Good Tissue Practice, EC; European Commission, GCP; Good Clinical Practice, GLP; Good Laboratory Practice, GPSP; Good Post-marketing Study Practice, HCT/P; human cells, tissues, and cellular and tissue-based products, HDE; humanitarian device exemption, HUD; humanitarian use device, ICH; The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use, IMPD; investigational medicinal product dossier, IND; Investigational New Drug, IDE; Investigational Device Exemption, FDA; U.S. Food and Drug Administration, EMA; European Medicines Agency, PSC; pluripotent stem cell, PMA; Pre-Market Approval Application, PMDA; Pharmaceuticals and Medical Devices Agency, QSR; Quality System Regulation, MHLW; The Ministry of Health, Labor and Welfare, TGA; Therapeutic Goods Administration, NA; not applicable.

[a] Depends on the intended use, mode of action, and quality control of the product manufactured from the iPSC; reach out to appropriate regulation body regarding product designations/classifications for definitive clarity.

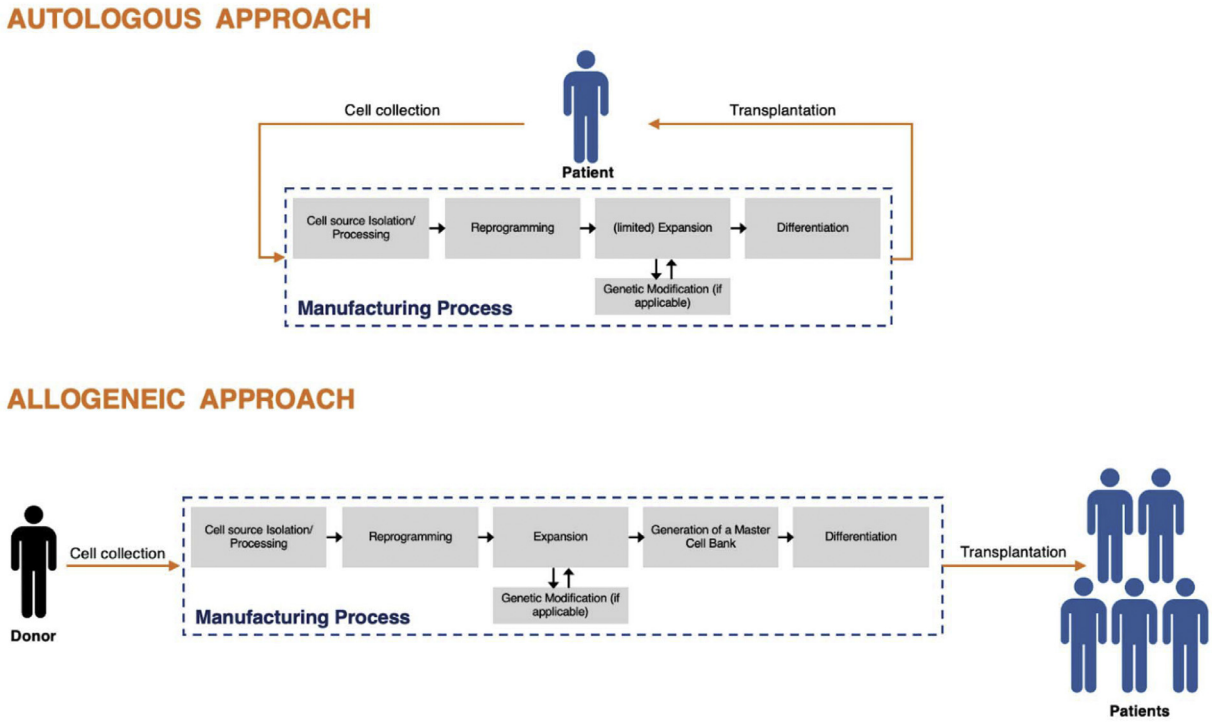


**Fig. 1.** Schema for autologous and allogenic approach for iPSC-based cell therapy.

saliva, hair) collected from the same donor/patient. Many cell therapy developers opt to cryopreserve and bank the starting material to reduce the manufacturing process's risks [7,11]. Strict manufacturing controls are necessary to prevent mix-ups with iPSC lines from the same donor.

During reprogramming and expansion, some cell therapy developers opt to cryopreserve and bank at both an earlier passage - often before the reprogramming vector has been cleared - and a later passage - at which point the iPSCs are expected to pass all QC release criteria (Figure 2). The early-passage cryopreserved iPSCs are sometimes named "mini banks" or "seed stocks," whereas the later-passage cryopreserved iPSCs are often named master cell banks (MCB) or working cell banks (WCB). A MCB refers to a fully characterized and released iPSC line, and the WCB refers to research-grade

**Table 2**
Quality control (QC) package for master/working cell banks.[a]

| | | | | Intermediates | | | | Final Product | |
|---|---|---|---|---|---|---|---|---|---|
| Attribute | Tests | Typical testing platforms | Specification | Parent | MCB | WCB | Diff | FIO | Lot Release |
| Dose | Cell Counts & Viability | | | X | X | X | X | | X |
| Identity | Pluripotency markers | Flow, Pluritest | | | X | X | | | X |
| | Differentiation potential | ScoreCard | | | X | X | | X | |
| | Cell specific markers | Flow, Pluritest | | X | X | X | X | X | |
| Purity | Absence of reprogramming vectors | PCR, ddPCR | | | X | | | | X |
| | Absence of contaminating cells | Flow | | | | | X | X | |
| Authentication | Clonality | Visual | | | X | | | | |
| | STR | CE | | X | X | X | X | X | |
| | HLA – Optional | PCR, NGS | | X | X | | | X | |
| | TCR – Optional | NGS | | | X | | | X | |
| Genetic stability | Karyotype | G-Banding | 95% Diploid | X | X | X | | X | |
| | CNV/STR – Alternate Molecular Method | KaryoStat | >80% diploid | X | X | X | | X | |
| Safety | Oncopanel | NGS | Absence | | X | | X | X | |
| | Tumorigenecity- residual iPSC | Flow, ddPCR, Teratoscore, HEC assay | 30 PSC cells? | | | | X | | X |
| Sterility | USP<71> USP <61>, Ph.Eur.2.6.27, and 2.6.1, JP17<4.05> and <4.06>_ | | None detected | X | X | X | X | | X |
| Bioburden | USP<61> <62> | | <1 CFU/mL | X | X | X | X | | X |
| Mycoplasma | USP <63>, Ph.Eur.2.6.7, JP17<G3> | | Negative | X | X | X | X | | X |
| Endotoxin[b] | USP<85>, Ph.Eur.2.6.15, JP17<4.01> | | | X | X | X | X | | X |
| Viral Testing[c] | USP<1237>, Ph.Eur.2.6.16, JP17<G3> | | | X | X | | | | X |

CNV, copy number variant; ddPCR, digital droplet; PCR, FIO, For information only; HEC, highly efficient culture; STR, short tandem repeat; HLA, Human leukocyte antigen; MCB, Master cell bank; NGS, next generation sequencing; PCR, polymerase chain reaction; TCR, T cell receptor; WBC, white blood cell count.

[a] Tests, specifications, and timing of tests listed in this table are examples only and that tests and their timing for individual products should be determined in accordance with the principle of the risk-based approach.

[b] Only if product is exposed to animal origin products.

[c] Depends on exposure of product to high risk components/raw materials.

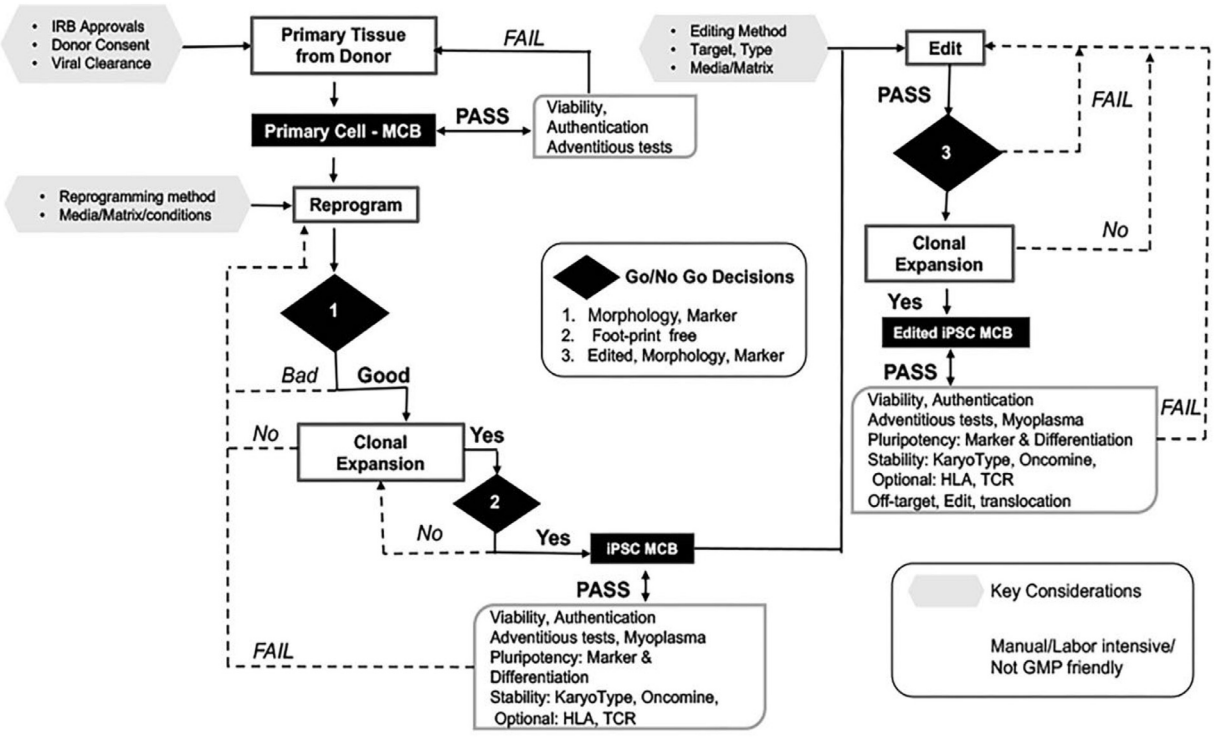


**Fig. 2.** Detailed process flow diagram with Go/No Go Steps for generation of iPSC Banks. MCB: master cell bank.

daughter cells generated from expanding the original MCB. An important consideration is that, for certain regulatory agencies (e.g., the FDA) the term "master cell bank" requires additional adventitious agent testing that is appropriate for allogeneic, but unnecessary for autologous products. Before cryopreservation of the later-passage MCB/WCB, in-process characterization is performed to assess clearance of the reprogramming vector. Sterility and mycoplasma testing may also be performed during reprogramming and expansion as an in-process control. If an iPSC line or clone has cleared the reprogramming vector, it can be expanded and cryopreserved to generate the MCB/WCB. The full characterization to release the iPSC MCB/WCB is optimally performed on a thawed cryopreserved vial, and assesses the attributes listed in Table 2 [8]. Please note that the tests, specifications, and timing of tests listed in this table are only examples. These should be selected and refined for individual products in accordance with the principle of the risk-based approach.

Some products may require gene-editing, either for the development of a hypo-immune product, the introduction of a therapeutic protein, or the correction of a disease-causing genetic mutation [12,13]. In these processes, gene editing is commonly performed as a unit operation after generating high-quality iPSC lines**.** The gene-edited iPSC line is then made clonal, expanded, and characterized for the attributes listed in Table 2**,** as well as additional characterization such as whole genome sequencing (WGS) to assess efficacy of editing and confirm absence of unwanted off-target edits or translocations [14–16].

The characterized iPSC lines are then differentiated to the final therapeutic cell type. The use of cryopreservation in this stage is highly dependent on the final cell type and therapy developers' approach. Processes with longer differentiation timelines may benefit from cryopreserving at an immature or progenitor cell stage, with a subset of characterization at this point [11]. Other processes may include cryopreservation only at the end of the differentiation process; however, the ability to cryopreserve at this point is highly dependent on the format of the therapeutic product – the more sensitive the cell type or complex the 3D structure, the more challenging it may be to cryopreserve and thaw.

Before transplantation into the patient, the final product undergoes thorough characterization to assess dose, identity, purity, authentication, potency, and safety. Genome integrity and karyotypic stability are important to evaluate, as iPSC are known to harbor specific mutations that confer a growth advantages (Table 3). Further testing and clarification are needed in some cases to scientifically interpret assay data, indicating the presence or absence of genetic modifications, and correlate these data with the actual risk of tumorigenesis.

*Attributes, Tests, and Platforms*

The critical attributes of iPSCs will depend on the therapeutic product being developed. However, there is general consensus on critical quality attributes (CQAs) for iPSCs. Some quality tests have guidance from pharmacopeias, while others currently lack standards. When pharmacopeial guidance is absent, developers must undertake efforts to make such testing both consistent and informative. QbD approach ensures that the manufacturing strategy is specifically designed to be fit for purpose for the therapeutic product for a particular indication. Detailed CQAs for the iPSC intermediate and iPSC-derivatives are defined based on the needs of a given project [25–28]. As the pharmaceutical space is rapidly evolving, guidelines will continue to progress. Periodic revision of CQAs, testing methods and pharmacopoeias will be necessary.

The iPSC-derivative produced as a bulk in GMP-complaint suite is termed drug substance (DS), which together with the necessary preservative and components such as scaffolds are administered to the patient as drug product (DP). The primary objective is assurance of safety to the patient by ensuring detailed characterization and conformance of the product to the CQAs. Some of the CQAs become part of the product release while others are for information only [29]. Often, early developers take a step-by-step approach to product development, focusing on short-term goals like regulatory approval rather than having a clear long-term strategy. This can lead to problems down the road. By not defining product requirements upfront,

**Table 3**
Known genomic anomalies associated with research-grade iPSCs.

| Type of aberration | Frequency of occurrence | Origin | Impact | Reference |
|---|---|---|---|---|
| Anueploidy - Gain in chromosomes 1,12, 17, 20 and X | ~32-34% in hESC; ~20% in hiPSC | During culture adaptation or expansion | Multiple genes affected | [17–19] |
| Subchromosomal Copy number variation - Amplification of 20Q11.21; 12p13.31 | 0–25% of hESCs – Amplification of 20q11.21; 13% of hESCs- Amplification of 12p13.31 | Selection of rare populations in parental cells during reprogramming or culture | BCL2L1, NANOG., associated with pluripotency pseudo-genes, cancer-related genes, & genes within common fragile sites | [20,21] |
| SNV | | Parental cells | No correlation with formation of abnormal tissue | [22] |
| CNV >3 | Hotspots - 14q32.33 and 17q12 loci | During expansion and manipulation of cells during reprogramming | formation of abnormal tissue after transplantation (86% predictability) | [22] |
| Oncogene - dominant negative TP53 mutations | 6% of unique hiPSC lines | Extended culture | seen in human cancer, promotes cancer stemness | [23,24] |

CNV, copy number variant.

quality may suffer. For example, inappropriate raw materials may prevent clinical translation or increase costs.

A QbD approach emphasizes understanding sources of variability and mitigating risks early on. This enhances manufacturing efficiency and ensures consistent product quality. Ultimately, QbD reduces the chances of batch failure or product recall, making translation faster, cheaper, and more successful. Taking a holistic view starting from the early developmental stage and covering whole life cycle of the products, rather than moving one step at a time, leads to better outcomes [30]. The methods and tests identified and used for quality assessment must be consistent, indicative and appropriate for the risk being measured. Here we define what CQAs should be taken into account for iPSC intermediates and for DS and DP for autologous and allogeneic therapies. For DS and DP, these CQAs may help define efficacy readouts for later stages of a trial.

*Sterility*

the iPSC manufacturing process is lengthy, making sterility of cells a relevant concern. Loss of culture sterility compromises the quality of the cell therapy product and harm patients. Key contaminants that compromise cell culture sterility are bacteria, fungi, and mycoplasma. These tests are often performed as quick tests, and long-term cultures for slow growing bacteria or fungi. For slow-growing species, tests are performed for both aerobic and anaerobic species and typically such tests take up to 14 days [31]. Mycoplasma tests are often quicker and require qPCR-based methods that are extremely sensitive. It is important to note that before these tests are executed in a manufacturing process, they need to be validated and tested for inhibition by media components that go into manufacturing of iPSCs and their derivatives [32].

Sterility tests may be performed in live cultures after the reprogramming process and/or initial clone picking process is complete – particularly because at these stages the cultures go through extensive manipulation, and it is good to confirm their sterility before spending time and resources on further expansion. It is certainly critical to perform sterility of intermediate iPSC banks when the banking is complete. Since allogeneic banks require significantly more expansion of cultures than autologous banks, checking sterility before vialing and after banking is recommended.

Sterility testing for DS is often done in a similar manner to what is suggested for iPSC banks. For instance, for autologous banks, it may be done after aliquoting cryovials, whereas for allogeneic banks it may be done before and after aliquoting cryovials. Guidance for sterility testing can be found at USP <71> and <61>, and Ph.Eur. 2.6.27 and 2.6.1.

*Endotoxins*

These are lipopolysaccharides derived from the cell walls of Gram-negative bacteria. While endotoxins do not harm cultures, when introduced, along with a transplant into patients, can cause severe inflammatory reaction at the site of administration. Most frequent sources of endotoxins are glass- and plastic-ware used in the manufacturing process. Endotoxin presence is often tested using a calorimetric assay and currently assays do not provide presence or absence – they rather show that the endotoxin levels in a culture may be below the detection limit of the assay (for instance 0.01 endotoxin units (EU)/ mL). Depending upon the route of administration, endotoxin acceptable level limit may vary (for instance for the eye it is 0.2 EU/mL, but it may be significantly higher for other organs) [32]. For live products, the DP may not be released without a final endotoxin test, whereas for cryopreserved products and intermediate banks, endotoxin test may be performed post-vialing. Guidance for endotoxin testing may be found at USP <85> and Ph.Eur.2.6.14.

*Product identity*

Matching the intermediate and the final product to its donor is a regulatory requirement to rule out any inadvertent mixing of cells between different donors [33]. Although this requirement is more critical for an autologous product, providing single donor identity of banks is important even for allogeneic products. Tests for this may be performed by evaluating HLA, STR polymorphism, or single nucleotide polymorphism [34,35]. For an autologous product, these tests may be performed at multiple stages throughout the manufacturing process including the final product, whereas for an allogeneic product testing at the intermediate banks may be sufficient.

*iPSC purity*

Complete reprogramming of the source material and appropriate expansion of reprogrammed clones should yield highly pure iPSCs. Complete reprogramming to the iPSC stage can be tested by analyzing the expression of key pluripotency markers (e.g., OCT4, TRA1-81, SSEA4, NANOG), analyzed by flow cytometry or qPCR-based assays [36], or by running PluriTest® [37]. While iPSC purity is not a regulatory requirement for any intermediate stage bank, it is often informative of the manufacturing process, and may be indicative of the success of DP manufacturing. For allogeneic banks, a fully reprogrammed bank potentially increases its utility, enabling to generate cell therapy products from all germ layers. In the case of an autologous product, this test may improve confidence in manufacturing. To save resources, this test may be performed both before and after vialing.

*Product purity*

Residual iPSCs in final products are considered contaminants that increase the risk of teratoma formation [38]. In general, iPSCs rarely survive the culture conditions used during the differentiation process. Nevertheless, depending on the therapeutic dose of cells, the risk is greater for conditions that require infusion of larger number of

cells. Traditional methods for detection of residual iPSCs rely on markers associated with undifferentiated state; using flow cytometry to detect protein expression, and ddPCR to measure gene expression at greater sensitivity. Recently, a method called highly efficient culture (HEC) assay was reported to detect as low as 5 iPSCs when spiked in 1 million cardiomyocytes [39]. While there is no clear regulatory guidance on acceptable level of residual iPSCs, a risk-based approach is critical depending on the indication, site of delivery and dose.

*Loss of reprogramming systems*

The key concern with residual reprogramming factors is the carryover of potent factors into the DP, leading to its instability and potentially increasing the risk of tumorigenicity. Therefore, a complete loss of reprogramming systems is a regulatory requirement for both autologous and allogeneic products alike. In both cases, this test may be performed at the intermediate banking stage [40]. qPCR-based assays are the most used for detecting residual reprogramming vectors. One key regulatory requirement is to determine the lower limit of detection (LoD) and determine the sensitivity of the assay to meet that LoD. If the LoD is not low enough (e.g., less than one copy per cell), regulatory agencies may challenge the utility of this assay.

*Genomic stability*

Genomic instability of reprogrammed cells is a major concern of derived cell therapy products. Karyotypic abnormalities, copy-number variations (CNV), and point mutations can be acquired during the reprogramming or the expansion phase of iPSC manufacturing [11,23,41]. These abnormalities may change the tumorigenic potential of the final cell therapeutic product and are a big concern for allogeneic products that are grown to large banks, hence could be in culture for prolonged periods of time. Abnormalities can be detected using G-banding, targeted sequencing for or a panel of relevant genes (e.g., oncogenes or other disease-or lineage-specific genes of interest). These tests may be performed at the MCB stage for allogeneic iPSCs and the intermediate "banking" stage for autologous products. However, there is no regulatory guidance in the US for CNV and genomic sequencing of iPSC-derived products.

*Dose/viability*

To maintain optimal culture conditions and ensure that a consistent dose is given to the patient, it is important to measure cell viability (number of viable cells per volume of cell suspension). Dye exclusion assays such as Trypan blue can be used to quantify cell viability. This assay is based on the principle that viable cells have an intact, impermeable membrane that excludes Trypan blue dye which, on the other hand, can easily enter the damaged membrane of death cells. Flow cytometry can also be used to measure the proportion of live and dead cells in a population [42]. Guidance may be found at Pharmacopeial methods USP<1046>, Ph.Eur.2.7.29.

*iPSC potency*

In this context, potency refers to differentiation potency, the capability of iPSCs to differentiate into the 3 germ layers – endoderm, mesoderm, and ectoderm – thereby demonstrating the potential to give rise to over 3 hundred cell types found in the human body [13]. Evaluating this fundamental biological property of iPSCs is crucial to mitigate the risk of treatment inefficacy, ensuring timely patient care. Methods for assessing iPSC pluripotency typically involve embryoid body formation or directed differentiation of monolayer cultures. Positive expression of markers indicative of ectoderm, mesoderm, and endoderm confirms pluripotency. Of note, culture conditions impact the differentiation potential of iPSCs and should be reported with pluripotency data to regulatory agencies.

*Product potency*

Potency testing for product release is acknowledged by many agencies as one of the larger challenges for developing CQAs for iPSC products [43–45]. One of the most influential policies was FDA's specific guidance in 2011 which acknowledges that "The complexity of CGT (cell and gene therapy) products can present significant challenge(s) to establishing potency assays" [44]. Notwithstanding this, all product developers need to establish assays to determine whether a released lot has the potency sufficient to achieve the desired clinical effect. However, quality cannot be tested into a product, in the same manner that sterility assurance depends not only upon the lot releases testing but also depends upon numerous other controls: such as control of the environment, control of starting materials, or aseptic validations. Similarly, potency assurance for a product should identify the product attributes which influence potency, such as manufacturing steps or key materials. This evolution of potency from a testing-based approach to a potency assurance model is delineated in the draft FDA Guidance Document [43] which is intended to address the inherent uncertainty around developing measures of product effectiveness. Whilst there is general uniformity for the requirements for any approved products, with iPSCs the challenge lies more in the uncertainty around requirements for investigational products at different phases of development and how stringently and variably these requirements are applied. All agencies expect that the methods for characterizing potency evolve during product development. For instance, the characterization of biological activity, is expected to be developed prior to any First in Human studies [45], but this method should allow for quantitation- thus a candidate potency assay. It is allowable that in some cases surrogate assays will be permitted, but during clinical development agencies expect that an ideal candidate method is not only developed, but is also validated. Likewise, there is an expectation that methods are validated prior to any "confirmatory" trials (EMA) whilst FDA specifies that as the purpose of Phase 3 or pivotal trials is to 'gather meaningful data about product efficacy' and without this a trial will be placed on clinical hold. Indeed, it is self-evident that without potency and biological characterization methods, it would be impossible to subject products to further development during the product evolution whilst ensuring comparable or superior activity, thus potency testing is a key tool to establish comparability.

*Manufacturing approaches for autologous and allogeneic iPSC-derived cell therapies and cost of goods considerations*

The manufacturing approaches for autologous and allogeneic iPSC-derived cell therapies require slightly different processes [46]. Both processes require similar workflows including cell source isolation and processing, reprogramming, expansion, genetic modification, and differentiation but have different regulatory requirements at each step (Figure 1, Table 4). In either case, developing a scalable manufacturing process is essential to achieving economies of scale, reaching the critical quality attributes and reducing cost.

Autologous iPSC-derived cell therapies involve isolation and sometimes expansion of the starting cell material for each patient [10], therefore the manufacturing process requires individualized processing, and QC measurements must be repeated for each patient. The cell source isolation process and the choice of starting material have significant implications on cost [11,32] and likelihood of ultimately producing a therapeutic product. On the other hand, allogeneic iPSC-derived cell therapies use a single donor to generate iPSC-derived cell products that can be used to treat multiple patients [47]. The donor must be screened for communicable diseases [7] and, if possible, have a homozygous HLA type. Thus, to ensure all safety specifications are met, allogeneic cell therapies involve significantly more donor testing than autologous cell therapies, however, differently from autologous testing, the costs are only incurred once. Lastly,

**Table 4**
Differences in manufacturing workflow between autologous and allogeneic iPSC-derived cell therapy products, and the associated cost of good considerations.

| Stage of Manufacturing | Autologous | Allogeneic |
|---|---|---|
| Cell source isolation and processing | • Starting cell material is isolated and expanded (if needed) from each patient, and does not require adventitious agent testing. Any starting cell source QC (e.g., sterility) is repeated for each patient.<br>• Variables impacting cost include the requirements (e.g., cleanroom vs. lower-grade facility, etc) and reproducibility of the cell source isolation process and personnel for manufacturing. The chosen starting material also has implications for cost; fibroblasts, for example, require more time for isolation and expansion than blood-derived cells that may not require expansion | • A single donor may be used to generate iPSC-derived cell product for many patients. It is necessary to identify a suitable donor - one free of communicable diseases & ideally w/ a defined homozygous HLA type. Adventitious agent testing may be performed on the donor material but is not a requirement.<br>• Variables impacting cost include the donor recruitment, screening, and qualification process, including costly adventitious agent testing.<br>• Inability of a given iPSC line to not cells of all germ layers necessitates the needs of multiples iPSC banks. |
| Reprogramming | • Reprogramming to generate iPSCs and subsequent characterization is performed for each patient's source material.<br>• Variables impacting cost include: the reproducibility and scale-out ability of the reprogramming process, and consistency and costs of reprogramming reagents, including license fees. | • Reprogramming is performed once on the donor material.<br>• Variables impacting cost include: ability to successfully reprogram selected donor material, costs of reprogramming reagents, including license fees, and inability of a specific IPSC bank to make cells of all 3 germ layers. |
| Modification via gene editing (if applicable) | • Many autologous iPSC-cell therapy programs do not employ gene-editing; however, genetic modification may be required depending on the disease indication. | • Multiple gene-edits may be used in the effort of engineering universal, hypo-immune iPSC lines. Each gene edit requires the modification, clonalization, expansion, and characterization of multiple iPSC clones. The yield of each step significantly impacts cost. Non-hypo-immune allogeneic iPSC lines do not require any gene editing. |
| Expansion (Seed/Master Cell Bank Establishment) | • Limited expansion is required, as each iPSC line is used to generate a therapeutic dose for only one patient. | • Significant expansion is required to generate a Master Cell Bank, as a single iPSC line is used to generate therapeutic doses for many patients.<br>• This expansion relies on scaled-up approaches which may employ 2D or 3D culture systems, and additional characterization is required to ensure continued genomic stability of cells post-expansion.<br>• The yield of the scale-up expansion process is a significant factor that impacts cost. |
| Cell banking | • A formal "Master Cell Bank" or "Working Cell Bank" is not required for autologous iPSC-derived cell therapies due to regulatory considerations that are most relevant to allogeneic (21 CFR 1271.90 (a)). However, the iPSCs typically undergo minimal expansion to generate a small Intermediate "bank" of cryopreserved iPSCs. | • Generation of an allogeneic Master Cell Bank carries specific regulatory expectations that include adventitious agent testing (21 CFR 1271.90 (a)).<br>• Test results must be negative for successful use of that bank in a cell therapy product |
| Quality control | • Quality control for the loss of reprograming plasmid, genomic stability, sterility, and identity back to the patient is required for every run.<br>• Sterility, Mycoplasma, Endotoxin test.<br>• All of these QCs impact costs as they need to be repeated for every patient's cells. | • Quality control for the loss of reprograming plasmid, genomic stability, sterility, and identity back to the donor are required.<br>• A set of viral negative tests.<br>• Sterility, Mycoplasma, Endotoxin test.<br>• STR<br>• QC cost is incurred one time only |
| Differentiation | • Differentiation to generate therapeutic cells and subsequent characterization and product release QC testing is repeated for each patient.<br>• Variables impacting COGs include: the reproducibility and scale-out ability of the differentiation process, efficiency and consistency of the differentiation process, and costs of differentiation reagents. | • Differentiation is performed in large batches to generate multiple therapeutic doses per batch.<br>• Variables impacting COGs include: yield and scale-up ability of the differentiation process, costs of differentiation reagents, need to make an intermediate drug substance bank or a final drug product bank. |

allogeneic cell therapies may incur additional costs than autologous, due to the more stringent regulatory requirements.

To achieve economies of scale and reduce COGs, both autologous and allogeneic iPSC-derived cell therapies require a scalable manufacturing process [46]. This may involve scale-up to achieve economy of scale for allogeneic approaches or scale-out for autologous manufacturing approaches, depending on the cell culture format used. Different platforms and technologies are available for each approach, including closed, automated systems, multi-layered systems, hollow fiber systems, and bioreactors. The choice of approach and platform will depend on the specific manufacturing process requirements and cost and value drivers for specific therapeutic product.

Generation of iPSCs for use in clinical workflows requires a series of upfront choices that can have a profound impact on their use in the intended application. Figure 1 provides an overview of the workflow commonly used in the development of iPSC-based therapies, which comprises of 5 major steps, each with various considerations, choices, and testing requirements. Before initiating a manufacturing run, it is important to have a clear understanding of the licensing terms associated with donor material and/or iPSC lines for research, clinical and commercialization stages. Equally important are the traceability measures.

1. After consideration of the indication, and the therapeutic product to be used to treat it, the next step is to select a donor cell type, with the necessary consent and donor screening data [48,49]. Donor material should be appropriately sourced and tested so that products made from the resulting iPSC lines are fit-for-purpose.
2. Additional testing of the starting cell type depends on the starting materials used - common choices for starting somatic cells include fibroblasts and blood-derived cells such as CD34+ hematopoietic stem cells and erythroblasts. Blood cell types require less expansion and testing than fibroblasts prior to reprogramming. Conventional stem cell transplantation (bone marrow) uses CD34+ cells and standards and procedures are well established for this cell type [50]. Fibroblasts may be more prone to somatic mutations that are generated in culture and are generally at higher risk of pre-existing mutations due to UV exposure based on the site of skin biopsy collection.

3. The choice of starting primary cells also determines which reprogramming method is best suited to consistently generate iPSCs. Initial methods that used integrating vectors [1,51] are less favorable due to random integration of the transgene into cells with potential risk of malignant transformation and reactivation of the transgenes [52,53]. The predominant non-integrating methods used are episomal vectors, negative strand RNA Sendai virus (SeV) and mRNA. Each method has its distinctive features, and comparative studies between these methods document the relative advantages and disadvantages of each [54,55]. For the generation of iPSCs under appropriate current cGMP manufacturing conditions, robustness and reproducibility has been observed with Sendai virus based and episomal methods [11,56,57].
4. The emerging iPSC clones are then clonally picked and expanded in a scale specific manner. Isolation of individual clones is preferred to achieve a homogenous culture, as some clones may clear the reprogramming factors at different rates or may harbor an abnormal karyotype. In some cases, monoclonality can be detected using visual methods. Identification of the right clone to pick and further expand is a crucial part of the workflow and several strategies are used to assist with this step [58–62].
5. Expansion primarily uses culture in 2D format, though 3D expansion systems are rapidly evolving to enable scaling-up to much larger culture volumes and cell numbers. In some cases, established iPSC lines may be further modified to knock out or express specific genes.
6. The resulting iPSCs are typically cryopreserved, either as an intermediate product for autologous therapeutic development, or for the generation of a qualified MCB for allogeneic therapy development. The extent of characterization is different for the 2 processes and is described in greater detail in subsequent sections. The goal is to use iPSCs as a starting material for differentiation into the therapeutic cell type(s) of interest [28]. Method and scale of production can vary widely depending on the cell type(s) of interest and is beyond the scope for this paper.

Appropriate, consistent, and indicative testing of iPSCs in addition to go/ no go steps need to be instituted to ensure establishment of robust intermediates and iPSC banks. Figure 2 provides a detailed process flow diagram. Below are examples of such steps in the iPSC generation workflow, some of which are described in more detail in the Section II under Attributes, Tests and Platforms.

- In case of using skin biopsy-derived fibroblasts as a starting material, a fibroblast cryo 'bank' may first be established as a source material for reprogramming. Age of the donor, site of collection of skin biopsy, tissue dissociation method, fold expansion of fibroblasts and cryopreservation method can all impact the efficiency of reprogramming and quality of the resulting iPSC clones [63]. The initial establishment of a small well-characterized cell bank of both primary cells and the resulting iPSC clones can be helpful in ensuring that the cells are devoid of undesirable somatic mutations, either in the tissue source itself, or during clonal iPSC line establishment and/or expansion. For allogeneic MCBs, a well-characterized seed bank of iPSCs may be desired. However, when minimally manipulated or expanded cells such as blood cell types are used as the source, iPSC clones are characterized with retained primary cells. A risk-based approach should be carefully considered and implemented irrespective of the starting cell type. At this point, a pass/ fail decision needs to be made based on whether the starting cell material has met all required specifications.
- Choice of reprogramming method and assessment of the resulting iPSCs is a gating stage with a go/ no go decision based on the quality of the iPSCs [54,64,65]. Newer methods specifically designed for clinical applications, avoid oncogenic reprogramming factors such as c-Myc and replace it with L-Myc [66]. Confirmation that the lines are indeed pluripotent stem cells is crucial and may be carried out through a combination of genetic testing, biomarker expression analysis and functional pluripotency confirmation [11]. A limited cell bank or seed stock may be established at this stage with minimal characterization.
- The lines that are confirmed to be pluripotent are further tested for expansion capacity to the required scale. Additional screening of lines to determine that they are foot-print free and lack the exogenous genetic elements and reprogramming factors is a critical safety test, and leads to another critical go/no go step [11]. When selecting suitable iPSC lines as raw materials for a particular cell therapy product, it is necessary to ensure efficient differentiation capacity into the desired cell type [67,68].
- In some instances, the intermediate cell banks may be further subjected to genome modification to generate a separate bank of modified cells. In such cases, a third go/ no go decision might be 'presence of required gene edits and absence of off-target edits. Regardless of whether genomic modifications are done, it is also critical to ensure the chromosomal and genomic stability of the resulting lines.
- Finally, sufficient numbers of cells must be generated and cryopreserved for both characterization studies needed to qualify the cell bank as well as for later stages such as differentiation.

A novel risk that has not been encountered in other pharmaceuticals and medical devices and needs to be evaluated in the development of iPSC-derived products is tumorigenicity. Tumorigenicity could be caused due to a variety of reasons [25] such as residual undifferentiated iPSC in the final product, reactivation of reprogramming factors such as c-myc [69], risk of modulating p53 [70], persistence of EBNA1 in the case of episomal reprogramming system [71], or somatic mutations that originate from the parental cell during the process of reprogramming or differentiation [17,23,72]. Since this risk is a new challenge not faced in conventional drug development, there is an urgent need to develop and standardize new methods to evaluate the tumorigenic potential of iPSC-derived products, including in vitro assays for sensitive detection of cellular impurities and for the evaluation of genomic instability [38]. Regardless of whether one opts for an autologous or allogeneic approach, the potential risk of tumorgenicity remains a concern.

The complexities associated with immune suppression and engraftment should not be underestimated in the field of regenerative medicine, often receiving insufficient attention in the literature. The autologous approach is advantageous in mitigating concerns related to immune rejection [11,73]. In the case of allogeneic approaches, the use of immunosuppression [74] or matching HLA haplotypes [50,75] can effectively reduce the risk of immune rejection. A more recent innovative approach involves engineering iPSCs with inactivated HLA genes that avoid self-killing via immune cloaking [76,77]. However, it is important to note that this approach comes with the added risk of genome instability due to extended culture and modifications. The instability of iPSCs at both genetic and epigenetic levels is challenging to foresee and the testing methods often lack standardization.

To address this, it is advisable to collaborate with the International Consortium on Tumorigenicity [38]. Sharing relevant pre-clinical data and adopting the Consortium's strategies could offer significant value in addressing these complexities. Additionally, engaging with clinical immunologists and transplantation experts is recommended. Their insights can offer a more comprehensive understanding of the intricacies of immune modulation. Given that the immune system is pivotal in eliminating transformed cells in vivo (oncovigilance), such considerations should underpin the development of therapeutic products.

In the development of iPSC products intended for allogeneic use, it is crucial to perform HLA haplotyping at a high resolution, as outlined by the European Federation for Immunogenetics (EFI). One strategy to potentially mitigate immune activation involves considering female donors with homozygous HLA-haplotype. Another avenue worth exploring is the genetic modification of stem cell lines to induce immune tolerance. However, it is important to recognize that such engineered cells fall under the category of genetically modified organisms (GMOs). Therefore, it is essential to understand the regulatory implications associated with this approach. Thorough research should be conducted, and all necessary data should be provided to the relevant regulatory body and authority.

## Section III: cost of goods for iPSCs and iPSC-derived cell therapies

In addition to regulatory and characterization considerations, cell therapy developers must consider their manufacturing strategy and associated costs. This section provides guidelines on the analysis of COGs for manufacturing iPSCs and iPSC-derived cell product, including starting material procurement; materials and reagents, equipment, personnel, and facility operation for each stage of manufacturing; distribution and storage, and product release QC. We briefly highlight the differences between autologous and allogeneic products, list several costs to consider in the development of a manufacturing cost model, highlight costs from iPSC-derived cell therapy developers in a series of case studies, and lastly address opportunities to reduce COGs through advancements in automation and characterization. Understanding and planning for COGs during process development could reduce costs, allowing for more affordable pricing and broader patient access to iPSC-derived cell therapies.

For autologous iPSC-derived cell therapies, the reprogramming and differentiation steps will need to be performed for each patient's cells, and the reproducibility, efficiency, and consistency of these processes are therefore of chief concern. In contrast, reprogramming is performed only once on the donor material for allogeneic cell therapy development, and the donor selection and ability to bank and scale up expansion of the resulting iPSCs are key concerns. Because individual batches of allogeneic therapies are administered to a larger number of patients, the required characterization is higher to ensure that communicable diseases are not transferred from donor to patient. The manufacturing process for allogeneic iPSC-derived therapies also involves significant expansion to generate a MCB, as a single iPSC line is used to generate therapeutic doses for many patients. These manufacturing differences are outlined in Table 4 and affect the COGs of the manufacturing process for each type of therapy. For example, the costs of repeated QC characterization and individualized processing for each patient significantly impact the COGs of autologous iPSC-derived cell therapies [3,78]. On the other hand, the costs of donor recruitment, screening, qualification, adventitious agent testing, and scale-up processes significantly impact the COGs of allogeneic iPSC-derived cell therapies.

### *Section III.A. Breakdown of costs in an iPSC-derived cell therapy manufacturing process*

#### *Section III.A.1 Considerations in developing a manufacturing cost model*

Developing a manufacturing cost model for iPSC-derived cell therapies is a critical step in optimizing production processes and minimizing COGs. Renske et al. [79] provides a comprehensive framework and methodology for understanding the cost structure of iPSC-derived cell therapy manufacturing. Costs can be calculated for each stage of the manufacturing workflow, and further broken down into the following general categories: materials & reagents, equipment, labor, and personnel, facility operating costs, and product characterization. Note that the primary cost drivers will vary significantly across the phases of therapeutic development (e.g., pre-clinical, Ph1/2a, Ph2b, Ph3, and commercialization) [80], with the expectation that COGs per therapeutic dose decreases over time as economies of scale are reached [3]. Table 5 lists costs to consider in the development of a manufacturing cost model for an iPSC-derived cell therapy product.

In developing such a model, it is helpful to distinguish between direct and indirect costs. Direct costs consist of raw materials, labor, equipment, and consumables, while indirect costs encompass facility and infrastructure, QC and validation, regulatory compliance, research and development, and overhead and administration. Distinguishing between fixed and variable costs is also essential. Fixed costs, which typically include facility and infrastructure, equipment depreciation, salaries, and non-royalty-based licensing fees, remain constant irrespective of the production volume. In contrast, variable costs, which typically include raw materials and consumables, utilities, overtime and temporary labor, maintenance and repair, royalty-based licensing fees, and costs associated with characterization, typically fluctuate with production levels. In addition, transportation and ID verification costs associated with supply chain control and vendor qualification costs should be included on a case-by-case basis. By categorizing these costs, manufacturers can better allocate resources and identify opportunities for optimization and cost reduction.

Scenario and sensitivity analyses can be used to evaluate the potential effects of changes in input costs, production volumes, or other factors on the overall cost structure. By understanding the nuances of a manufacturing cost model, manufacturers can identify opportunities for cost reduction, streamline production processes, and ultimately make iPSC-derived cell therapies more accessible to patients in need.

#### *Section III.A.2 Case studies: cost of goods breakdown for various iPSC-derived cell therapy programs*

Please note that all costs referred to below are in US dollars, and do not factor in the effect of inflation.

*Case study 1: generation of allogeneic iPSC-derived cell therapy products, Cell Therapies Pty Ltd., Australia.* In Table 6 we outline key costs in the generation of allogeneic clinical-grade iPSC MCB and differentiated iPSC-derived cell products for Cell Therapies Pty Ltd. Labor, equipment, materials, facility operation, quality control, tech transfer, and consumables costs are included.

The biggest COG-related challenges highlighted by Cell Therapies Pty Ltd. include (i) reduction of high-cost material use (cytokines). and (ii) elimination/ reduction of unnecessary processing steps and/ or testing, to reduce the high cost of labor. These require specific studies to demonstrate effective differentiation at reduced material doses, with lower cost materials or testing, simplified processing, or automation.

*Case study 2: COGs comparison between a quality-by-design (QbD)-based automated system CellQualia and manual operation, Cyto-Facto Inc., Kobe, Japan.* The CellQualia is not a robotic system that can mimic human operation, but a QbD-based process monitoring cell manufacturing system. The CellQualia system proposes to reduce costs through reducing personnel requirements, enabling data integrity, eliminating human variation and error, and providing a digital process and QC information to make an electronic batch record, which enables one to link the supply chain and clinical data, and improve the process and product.

Each iPSC expansion run starts with 4 million cells and takes 6 days to expand to 800 million cells at harvest, which in turn are cryopreserved at 1 million cells per vial to obtain 800 tubes in total. The QC tests take 15 days in addition to a 6-day cultivation period. At the Kobe facility, 1 iPSC clone is generated per month on average, with approximately 11 iPSC clones generated per year, and 1 month dedicated to maintenance. This yields approximately 8800 vials of iPSCs annually.

**Table 5**
Costs to consider in the development of a manufacturing cost model.

| Cost category | Resources |
|---|---|
| Materials and reagents | • Reagents and raw materials: cell media (for starting cell source, IPSCs, and differentiation); matrices; reprogramming vectors; growth factors, cytokines, supplements and signaling molecules; genome editing tools; buffers; cryopreservation media; ethanol; DI water; etc.<br>• Cell culture consumables: tissue culture vessels (flasks, well plates, etc.), liquid handling (conical tubes, pipette tips, etc.), cryovials, miscellaneous (cell scrapers, strainers, coverslips, etc.)<br>• PPE: gloves, cleanroom suits, etc. |
| Equipment | • Cell culture equipment: incubators, biosafety cabinets, pipetters, plate tilters, centrifuges, water baths<br>• Storage & handling: refrigeration, freezers, cryo storage<br>• Characterization equipment: brightfield & fluorescence microscopy, qPCR, plate reader, flow cytometer, confocal imaging, sequencing equipment, etc.<br>• Equipment associated w/ automated and/or closed manufacturing, and scale up (e.g., large-scale bioreactors) or scale out processes (e.g. single-patient closed systems). Note that the use of such equipment may incur additional costs for tool-specific consumables such as tubing or bags.<br>• Additional costs to consider: depreciation of equipment over time, one-time installation costs, regular servicing costs, environmental monitoring costs |
| Labor and personnel | • Fixed: CMC directors, program managers, project managers, product managers, facility supervisors, quality assurance, administrative support<br>• Variable: process development scientists, associates & technicians; cell therapy processing/ manufacturing associates & technicians; assay development scientists; quality control technicians |
| Facility operating costs | • Base lab buildout cost if setting up own facility, or lease and service fees if renting cleanroom space<br>• Environmental & equipment monitoring (temperature, humidity, pressure, gas, etc.)<br>• Utilities costs associated w/ operating ISO 5, 6, 7, and/or 8 cleanroom environment |
| Product characterization, QC testing | • Characterization and QC testing costs vary highly depending on whether the assays are validated and performed internally, or outsourced.<br>• Computation services (e.g. whole genome sequencing analysis services) |
| Storage, distribution and packaging | Cryotanks for storage<br>• LN2<br>• Process for ensuring adequate LN2 supply and temperature monitoring<br>• Maintenance and calibration of tanks and monitoring system<br>• Process for complete documentation of temperature<br>Cryoshippers for distribution<br>• LN2<br>• Temperature monitoring system for cryoshippers<br>• Maintenance and calibration of cryoshippers and monitoring system<br>• Courier or other shipments fees<br>• Process for complete documentation of temperature |
| Other | License fees associated with manufacturing may include licenses to use specific reagents (e.g. reprogramming vectors), tools or technologies (e.g. intracellular delivery tools, etc), or processes (e.g. differentiation protocols). These fees may have a significant impact on the costs of commercializing a cell therapy. Fees may include upfront payments & fees, royalties, milestone payments, and/ or profit sharing. |

CMC, chemistry, manufacturing and control; iPSC, induced pluripotent stem cells; PPE, personal productive equipment; qPCR, quantitative PCR; PCR, polymerase chain reaction; LN2, liquid nitrogen.

Table 7 summarizes the breakdown of COGs with respect to facility operations, consumables, sterility, and raw materials. These costs are summed to attain the total manufacturing cost, and together with labor are used to calculate the cost of manufacturing of each vial of iPSCs. Note that the costs highlighted are specifically for the expansion of iPSCs, and do not include viral tests or lab tests to characterize the starting material, and do not include the cost for reprogramming or generating the primary stock of clinical grade iPSCs. However, the costs for conducting copy number variant (CNV) and karyotype analysis; flow cytometry measurement of TRA-1-60, SSEA-3 and SSEA-4; liquid chromatography-mass spectrometry characterization of kynurenine and 2-AAA; lactate production measurement in medium; and testing of mycoplasma, sterility and endotoxin are included as a regular QC test set for shipping (Table 7). Additionally, as WGS is currently not mandatory for going into the clinic, the cost for WGS is not included.

*Case study 3: generation of autologous iPSC-derived retinal pigmented epithelium (RPE) (National Eye Institute, NIH, USA).* The key NEI-NIH costs in the manufacturing of autologous iPSC lines were included in Kim et al [3]. In Table 8 we summarize the costs for differentiating the iPSCs to RPEs, including materials and reagents, as well as characterization. NEI was able to leverage in-house analyses to minimize characterization costs. Several advantages for establishing autologous versus allogeneic iPSC lines are noted in Kim et al [3].

*Case study 4: characterization of allogeneic clinical-grade iPSC MCB (CiRA Foundation, Japan).* At CiRA, a single iPSC MCB comprises 300 vials, with 2.5 × 10^5 cells per vial. The performed characterization assays are listed in Table 9. Costs associated with characterizing the MCB are outlined in Table 10. As shown, viral testing comprises the most significant characterization cost for allogeneic iPSC MCB generation.

### *Section III.B. Strategies and opportunities to reduce cost of goods for clinical-grade iPSC generation*

The cost of iPSC generation and subsequent differentiation into therapeutic cell types is currently high. High cost will initially limit the application of these therapies to life-threatening or severely-disabling conditions for which no effective alternative therapies are available.

However, as experience is accumulated, as infrastructure and supply chains mature, and as regulatory pathways becomes more defined, the cost and risk of iPSC-derived cellular products will fall. Reduced costs will inevitably open opportunities to extend iPSC-

**Table 6**
COGs for case study 1—generation of allogeneic iPSC-derived cell therapy products.

| Generation of allogeneic iPSC MCB | | |
|---|---|---|
| Category | Cost | Notes |
| Personnel and facility costs | $400,000 - $700,000 | For 4-6 months of work, including GMP documentation, training, n=2 confirmatory runs, and GMP manufacturing runs |
| Release testing, including characterization | $80,000 - $100,000 | From an outsourced vendor |
| Materials and reagents | $5,000 - $10,000 | |
| **Generation of differentiated iPSC-derived cell product** | | |
| Category | Cost | Notes |
| Tech transfer (example case 1) | $1,500,000 - $2,500,000 | Example case 1: well-optimized, 2-week manufacturing process with streamlined tech transfer |
| Ongoing product manufacturing runs (example case 1) | $100,00 - $150,000 per run | Example case 1: well-optimized, 2-week manufacturing process with streamlined tech transfer |
| Tech transfer (example case 2) | $2,500,000 - $4,500,000 | Example case 2: 40-day manufacturing process requiring significant process development |
| Ongoing product manufacturing runs (example case 2) | $200,000 - $300,000 per run | Example case 2: 40-day manufacturing process requiring significant process development |
| Release testing | $10,000 - $30,000 per run | Outsourced |
| Capital equipment | Example: $100,000 - $120,000 per bioreactor | Equipment may be required and depends on the target total number of differentiated cells to be yielded at the end |
| Materials and reagents | Highly variable | Dependent on process and growth factors required to differentiate the cells |

**Table 7**
COGs for case study 2—a comparison between and quality-by-design (QbD)-based automated system CellQualia and manual operation for expansion of clinical-grade iPSC clones.

| Expansion of clinical-grade iPSC clones | | | |
|---|---|---|---|
| ***Category*** | *Cost* | | *Notes* |
| | CellQualia | Manual | |
| Facility – floor rent, equipment lease | $567,138 | $290,215 | |
| Facility – floor manager, HVAC, EMS equipment maintenance | $257,705 | $119,243 | |
| Facility Consumables | $144,360 | $144,360 | Including electricity, $LN_2$, $CO_2$ |
| Sterility Maintenance | $85,403 | $224,538 | Including sanitation, cleaning, wear, disinfection, cleaning, germ identification |
| Raw Materials | $627,000 | $451,000 | Including cell media, QC tests, plastic consumables, QC reagents |
| Annual manufacturing cost | $1,681,606 | $1,229,356 | |
| Annual labor cost | $1,910,400 | $3,175,200 | |
| Annual total cost | $ 3,592,006 | $4,404,556 | |
| Per iPSC vial cost | $408 | $501 | The total cost of producing 1 vial of iPSCs via the automated CellQualia system vs manual operations |

derived cellular therapies to additional clinical settings and therapeutic needs. Below we highlight several opportunities for reduction in COGs for iPSC generation.

*Optimization and innovation of reagents*

Reprogramming tools or kits (viral and non-viral), cell adhesion substrates (e.g., Laminin 521 or 511), specialized media for expansion or differentiation (e.g., E8), and soluble growth factors (e.g., FGF-2) represent the largest fraction of reagent costs. Market competition is already reducing the cost of these existing reagents, but these forces will be unlikely to transform the field in the near term [81].

More likely opportunities to reduce cost will be innovation in reagents that improve efficiency and effectiveness of biological process outcomes. For example: (i) reprogramming reagents that increase the probability of reprogramming and decrease the incidence of incomplete reprogramming), (ii) media compositions that increase expansion rate and decrease the risk of premature spontaneous differentiation, or (iii) durable biomaterial surfaces that enable iPSC attachment and proliferation of stable iPSC populations, replacing expensive and fragile laminin surfaces, thereby reducing variation and simplifying workflow. The resulting improvements in process outcomes have the potential to increase the yield of batches meeting the required quality specifications, thereby reducing the cost per batch.

**Table 8**
COGs for case study 3—generation of autologous iPSC-derived retinal pigment epithelium (RPE).

| Generation of autologous iPSC-derived retinal pigment epithelium | | |
|---|---|---|
| Category | Cost | Notes |
| Media and reagents | $25,000 - $30,000 | Bulk purchasing was critical to minimize costs |
| Sterility testing | $200 - $500 per run, x 5 | Approximately $2,000 in total for in-house analyses; outside analytical lab quoted approximately $1000 per run |
| Flow cytometry | $250 - $300 per run, x 3 | Approximately $1,000 in total for in-house analyses, not including the one-time capital equipment cost |
| Viability | <$200 | In-house analyses, not including the one-time capital equipment cost |
| Cytokine analysis | $400 - $500 per run | In-house analyses |
| Maturity | $2,500 one-time cost | For equipment |
| Identity | $500 per run | |

**Table 9**
COGs for case study 4—quality testing for iPSC MCB at CiRA foundation.

| Status | Test items | Methods | Criteria |
|---|---|---|---|
| Release assay | Morphology | Microscopy | Comparable to embryonic stem cells |
| | HLA | PCR-SBT | Match with origin |
| | STR | PCR and capillary electrophoresis | Match with origin |
| | Viability | Cell count | For information only |
| | Karyotype | Conventional Giemsa staining<br>G-banding | No chromosomal abnormality |
| | Sterility test | BacT/ALERT | Negative |
| | Endotoxin | Kinetic turbidimetric | ≦5 EU/mL |
| | Mycoplasma | PCR | Negative |
| | Viral testing (HIV, HCV, HBV, HTLV, Parvo B19) | PCR | Negative |
| | Residual vector | PCR (Taqman qPCR) | No residual vector |
| Reference assay | Doubling time | Cell count | For information only |
| | Pluripotency-associated marker expression (TRA-1-60, SSEA4, TRA-2-49, OCT3/4) | Flow cytometry<br>Microarray | For information only |
| | Genome analysis | | |
| | SNV/Indel | WGS/WES | (Result: No genetic mutation at region related to cancer) |
| | CNV | WGS/SNP array | |

EU, endotoxin units; HLA, human leukocyte antigen; SNP, single nucleotide pleomorphism; STR, short tandem repeats; PCR, polymerase chain reaction; WGS, whole genome sequencing; WES, whole exome sequencing.

*Automation of iPSC manufacturing*

Automation is always high on the list strategies to optimize the scalability, yield, and cost of manufacturing. Reducing the time, burden, variation and contamination risk of manual processing represents a first order benefit (Table 11). However, a much greater potential benefit of automation would come through the opportunity for great improvement in the quantity, quality, repeatability and reproducibility of quantitative in-process data capture (image-based morphometrics "radiomics", expansion kinetics, secretome, cell "biopsies") which can be used to transition from traditional skilled but subjective measures of "heathy", "good-looking" or "happy" cells to quantitative metrics Critical Process Parameters (CPPs) or intermediate Critical Quality Attributes (iCQAs) for decision-making. Such metrics can be used to set clear "guard rails" that define the potential range variation that is still predictive of the pathway to success. In addition, automated iPSC generation processes can provide an "early warning system" that can flag process variation. This could be used to flag and exclude bad batches early and reduce waste of effort, time and reagents on failing, "off target," process. Even better, it might be possible to identify early process variation which enables corrective action to salvage processes that might otherwise fail. Of even greater potential value and impact, automation and associated opportunities for robust and continuous in-process monitoring creates opportunities in large-scale data systems suited for training of artificial intelligence and machine learning (AI/ML) algorithms which may greatly accelerate both understanding and control of in-process data to improve scalability and yield while minimizing cost [82,83].

**Table 10**
COGs for case study 4—characterization of allogeneic clinical-grade iPSC MCB at CiRA Foundation.

| Characterization of allogeneic clinical-grade iPSC MCB | | |
|---|---|---|
| Category | Cost | Notes |
| Viral testing | $76,000 - $152,000 | Performed by external contractors |
| Genomic analysis | $7,600 | Approximately equal to the cost of the reagents |
| Karyotyping | $3,000 | Performed by external contractors |
| Total cost of QC | $92,000 - $168,000 | Approximately total cost of characterizing allogeneic iPSC MCB; costs are dominated by viral testing |

*Quality control and validation*

Methods for characterization of the final cell therapy product represent a large component of the cost of manufacturing [78]. One aspect of these measures is risk mitigation: confirmation of sterility and testing for tumorigenicity, residual vector, karyotype abnormality or known oncogenic mutations. Other metrics assess cell identity, cell state or cell function. These include: imaging morphometry, flow cytometry, bulk RNAseq, single cell RNAseq (scRNAseq), "score card" assessment (response to directed differentiation or embryoid body formation), secretome analysis and genomic sequencing. These assessments add up to a substantial investment in any individual batch or lot of iPSCs.

In the current environment of development, regulatory review and clinical trial introduction, there is general consensus that large investments in quality metrics is essential. Over time, and with increased clinical experience, these metrics are likely to evolve. Data will demonstrate the relative value of individual metrics as a screening tool for safety, efficacy and variation. Cost reduction will be achieved by narrowing the focus of analysis to critical attributes, eliminating the use of redundant or ineffective measures.

**Table 11**
Automated iPSC manufacturing systems.

| Approach | Cell culture format | Relevant platforms and technologies |
|---|---|---|
| Scale-out | 2D adherent | **Semi-closed standard tissue culture systems (Conring)**<br>**Closed, automated systems** (Miltenyi Prodigy, Lonza Cocoon, Cellino, iPeace) |
| | 3D suspension | **Closed, automated systems** (Miltenyi Prodigy, Lonza Cocoon)<br>**Mini 3D bioreactors** (PBS Mini Bioreactor (0.5L), Eppendorf DASbox Mini Bioreactor (60-250 mL), Chemglass (100 mL), Biotts (30 mL), Sartorius Ambr 250) |
| Scale-up | 2D adherent | **Multi-layered systems** (Corning HYPERStack vessel, Corning CellSTACK, Corning HYPER-Stack, Corning CellCube)<br>**2D Bioreactor** (Corning Ascent Fixed Bed Reactor system, Sinfonia CellQuali system)<br>**Hollow fiber systems** (Terumo Quantum, FiberCell Systems) |
| | 3d suspension | **3D Bioreactors** (Eppendorf BioBlu (3-250L), Pall Allegro STR, PBS Biotech Bioreactors (3-80L), Sartorius, MIllipore Mobius (3L), Cytiva Xcellerex (10-2k L)) |

Standardization and scaling of availability of effective measures will further reduce costs. scRNAseq stands out as a powerful quantitative technological tool capable of characterizing the identity, homogeneity, batch-to-batch consistency of iPSCs and iPSC-derived cell products, including the response of populations to activation or differentiation signals. Bulk RNAseq and karyotyping are far less capable of detecting the presence of small populations of heterogeneous cells that may have biological impact or risk [84].

Large-field-of-view imaging is re-emerging as another powerful tool for assessment of cell state, biological stability over time, and screen for small area variation (i.e. heterogeneity) in final products. Imaging tools are far less costly than scRNAseq or other genomic/proteomic methods, and are being accelerated into a phase of quantitative measurement science through application of radiomic analysis tools and AI/ML algorithms [85].

*Cell source materials – HLA-matched or engineered iPSC banks*

The development of HLA-matched homozygous banks of iPSCs, or iPSC lines that are engineered to exclude major histocompatibility complex (MHC) antigens, may lower the barrier of cost and risk associated with providing iPSC products to large sections of many regional populations. However, these banks will be most effective in more homogeneous genetic populations (e.g., native Japanese) and will be less effective in highly heterogeneous populations (e.g., North America, South America, or Europe) [51]. Furthermore, in the context of donor-donor immune compatibility, we should not underestimate the difficulty to circumvent the immune system. The complexity of the immune system presents challenges beyond HLA matching. Factors such as T cell responses against minor histocompatibility antigens, natural killer (NK) cell reactivity against iPSC derived tissues, and pre-formed HLA donor-specific antibodies further complicate immune compatibility [86,87]. Generating iPSC lines that are 'invisible' to the immune system (universal donors) may enable universal immune compatibility, however, this approach also carries the risk of stripping away the body's primary defense against cancer, as any aberrant behavior from these cells could go unnoticed. Addressing this issue, the literature proposes integrating suicide gene systems into iPSC lines. Nevertheless, major genetic engineering increases regulatory hurdles, emphasizing the need for thorough preclinical testing and early engagement with regulators to assess novel technologies' risks in clinical applications. Eventually, successful immune control will likely require a multifaceted approach involving [1] HLA matching, [2] immunosuppression, [3] genetic modification to reduce immune responses, and [4] tolerance induction with emerging strategies such as regulatory T cell infusion.

## Discussion

iPSC-derived cell therapies possess considerable potential for the replacement of degenerating or lost tissues, the arrest or reversal of disease progression, and the enhancement of the quality of life for individuals on a global scale. In order to expedite the advancement of these iPSC-derived cell therapies and increase patient accessibility, it is judicious for the field to converge upon harmonized regulatory standards, streamline efforts pertaining to product characterization and manufacturing, and minimize COGs. In this comprehensive review we delineated the subsequent steps necessary to propel iPSC-derived cell therapies towards clinical realization.

The development of iPSC-derived cell therapies entails a meticulous and intricate process that involves manipulating the fate and characteristics of cells. Every step in this process, along with the parameters and materials utilized, significantly influences the cell product CQAs. Several pivotal decisions need to be made during the course of process development, encompassing the selection of starting materials, reprogramming methods, clone selection, and cell banking. In conjunction with precise control over process-specific parameters and raw materials, these decisions exert a profound impact on both the iPSCs and the resulting iPSC-derived cell therapy. This development process is not undertaken blindly but incorporates critical checkpoints to evaluate the quality of cells prior to process completion. Upon completion, extensive characterization is employed to ensure the purity, safety, and quality of iPSCs and iPSC-derived cell therapies.

The regulatory standards in the cell therapy space are critical for product development. Cell therapy developers are in need of clear guidelines for both the autologous and allogeneic cell products they are manufacturing to ensure their universal safety and global adoption. Moreover, cell therapy developers should seek an active role in establishing such regulatory frameworks by synthesizing a product-relevant, simple to execute and scalable strategy for cell therapy testing.

Compendial methods are release criteria safety tests for pharmaceutical drug product manufacturing. Examples of such tests include USP <71> product sterility testing and USP <63> mycoplasma testing. It will be essential to adapt compendial methods for cell therapies, to account for their shorter shelf life and smaller product volumes than pharmaceutical drug products. As such, alternative methods known as rapid microbial test methods (RMM) have been developed, which once sufficiently qualified for performance, are supported by regulatory agencies such as the FDA and EMA [88]. The next steps would involve achieving jurisdictional alignment. Harmonization of the tests for USP <71> from the European Pharmacopoeia and Japanese Pharmacopoeia has largely been achieved, exemplifying the progress in this area. Wider global harmonization to standardize regulations surrounding iPSC-derived cell therapies, and avoiding over-specification of products would facilitate efficient testing, avoid unnecessarily inflated manufacturing costs and optimize manufacturing cycles, to ultimately maximize the therapeutic benefits for patients. To that end, since 2007 ISCT has provided a forum for regulators, policy makers and medicinal inspectors from representative agencies from our membership to meet with industry and academia to inform and educate one-another using close to real world case-studies. Furthermore, since 2004, ISCT has been the host organization bringing together over 20 stakeholders to inform the FDA of specific concerns or developments to mutually advance the field [89]. Nevertheless, developers need to grasp the opportunity to inform their regulatory strategy, and propose a regulatory path for their products, as sponsors have the most information about their product's risks.

Optimization and reduction of costs in the development and manufacturing of cell therapies is essential for expediting product cycle, improving patient access, and promoting widespread adoption. The COGs related to iPSC-derived cell therapies encompass the direct expenses incurred throughout the manufacturing process. The total cost model including the direct and indirect costs is important to understand and plan for early in the development process, whether for autologous or allogeneic approaches, and for each clinical stage. When considering a manufacturing cost model, it is imperative to consider QC and characterization which make up a large portion of the costs. Such comprehensive understanding of the cost variables within the process would open up opportunities for cost reduction, such as obtaining competitive supply agreements from vendors or pursuing proactive strategies that target process efficiency and efficacy, through further innovation and process automation.

*Conclusion*

Sixteen years have elapsed since the discovery of human iPSCs, yet the necessity for extensive discussions persists regarding the harmonization of international regulatory frameworks, CQAs, and manufacturing methods for autologous and allogeneic products. We hope that this white paper by the members of the JSRM-ISCT iPSC

Committee will serve to promote further discussion among the iPSC-based cell therapy communities and industry, while bridging the gap between academia and industry toward effective and affordable iPSC-based therapies.

## Declaration of Competing Interest

Dominic M. Wall: Employment, Peter MacCallum Cancer Centre, Cell Therapies Pty Ltd, Shareholder Cell Therapies Pty Ltd, Currus Biologics, Research Funding Therapeutic Innovation Australia. Nathan Smith: Employment Cell Therapies Pty Ltd.

## Author Contributions

Marinna Madrid: COGS/ Section III Outline, Figures and Tables, part of draft, Uma Lakshmipathy: Outline, Section II, Figures and Tables, Xiaokui Zhang: COGS – part of draft, Kapil Bharti: Section II, COGS data, Dominic M. Wall, Yoji Sato: Regulatory, George Muschler: COGS – part of draft, Anthony Ting: COGS – part of draft, Nathan Smith: COGs contribution, Shuhei Deguchi: COGS data, Shin Kawamata: COGS data, Jennifer C. Moore: COGS, Bar Makovoz: Discussion section draft, whole manuscript alignment and final proof reading and edits, Stephen Sullivan: whole manuscript alignment, regulatory considerations, QbD, Veronica Falco: whole manuscript alignment, comments on QbD, regulatory considerations, CQAs and automation, “dose/viability” paragraph, iPSC potency paragraph, HLA-matching considerations, Arwa Z Al-Riyami: referencing, drafting regulation table, manuscript alignment and merging.

## Acknowledgments

Felix Grignon and Andy Naqati of ISCT headquarters, Takako Yamamoto, Kobe Univ. Dept. Science and Technology and Innovation for manuscript preparation and editing.

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